TRANSACTIONS
of the
NATIONAL ACADEMY OF SCIENCE AND TECHNOLOGY
Republic of the Philippines

1991
Volume XIII

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National Academy of Science and Technology
Bicutan, Taguig, Metro Manila
Philippines

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Welcome Address

Dioscoro L. Umali
President and National Scientist
National Academy of Science and Technology
Bicutan, Taguig, Metro Manila

On behalf of my fellow Academicians, I would like to extend to all of you my most cordial welcome to the 13th Annual Scientific Meeting (ASM) of the National Academy of Science and Technology (NAST) of the Philippines. You have given importance to this scientific meeting whose theme is "Managing Ecosystems for Long-Term Human Survival."

Secretary Follosco, I take pride in letting you know that the participants are scientists of research institutions from all corners of our country.

We invited them to this occasion not only to listen to and learn from one another, but also to get acquainted with one another and collectively assess our ecosystem, as well as chart the common strands of action in managing it for long-term human survival.

Human efforts to secure sufficient food, shelter, fuel and income for our people have destroyed most of our vast forest, degraded the land and contaminated the air and water resources.

The livelihood security of our rural population is especially affected by the deterioration of basic life support systems. There is a direct and compelling linkage between environmental deterioration and long-term human welfare, especially as they relate to the increase and perpetuation of human poverty.

From history we know that population can balloon to a point where demands begin to exceed sustainable yield. When that point is reached, needs begin to consume the very resource base itself.

Population growth worsens poverty. The impoverished have few options. One of them is to exploit whatever resources they can reach. In so doing, they can inflict damage on the very system that could unshackle them from life sentences of deprivation. They are thus both victims and perpetuators of environmental deterioration. This phenomenon is aptly called "environmentally destructive poverty."

Therefore in dealing with conservation of our ecosystem, it is apparent that it cannot be done in isolation from its sister problems of population explosion and poverty. I discussed quite substantively these three issues in my keynote address last April at the International Conference on Environment in New York City.

One hopeful sign is the growing realization that building a just and humane society is the only solution in achieving ecological recovery and development. Responding to this call is not only the obligation of the government but also a collective task for all of us.

But lately we have been confronted with an environmental catastrophe. Mt. Pinatubo, after centuries of slumber, went raging mad. Its volcanic debris made a wasteland of thousands of hectares of farms. With Pinatubo's fury, more than 300 people died. It deprived thousands of people of their livelihood, jobs, farm lot, farm animals and houses, without which life is not worth living.

But the disaster victims and areas are not beyond salvation. Right now scientists have many bright ideas and findings providing light at the end of the tunnel. Secretary Follosco just released a Flash Bulletin enumerating the uses of volcanic debris. Our people have enough resiliency, versatility and dynamism in coping with the catastrophe that devastated the physical environment. I am sure we will overcome this havoc wreacked by nature with the force of the people -- and that is People Power -- as we did before.

What gives significance to this occasion is the fact that we have with us this morning two great exponents of environmental component in all its major thrusts. In implementing DOST's action programs at the countryside, Secretary Follosco was able to rally more than 40 universities, colleges and research institutions. Before, these institutions competed with one another or duplicated one another's activities, but now they also work together like a good orchestra with Secretary

Follosco conducting. We are indeed grateful to Secretary Follosco. You found the time to be with us in spite of your very heavy involvement during this science week.

We have with us also Secretary Factoran -- I refer to him as one of the animated green patriots of this planet. You read everyday in our newspapers his agenda for action being implemented. I am sure the participants are quite pleased to see you and be able to talk to you and ask you some questions.

We would like to acknowledge with gratitude the financial contribution of various institutions and persons who made possible the holding of this annual meeting.

I bid welcome with open arms the participants to this meeting and I formally declare this annual scientific meeting of NAST open.

Thank you.

Opening Remarks

Ceferino L. Follosco
Secretary, Department of Science and Technology
Bicutan, Taguig, Metro Manila

Ladies and gentlemen, good morning.

The honor is mine for being invited to address the Academy's venerable members, who compose the select circle of our country's most outstanding scientists.

The annual celebration of the National Science and Technology Week (NSTW) is incomplete without the annual meeting of the respectable members of the National Academy of Science and Technology (NAST). Science and technology (S & T), like the role of every scientist and technologist, is very utilitarian in nature. Although scientists in our country are not given much prestige, which is not the case in other countries, the wheels of progress definitely would not turn sans the scientists and technologists.

Yesterday, during the keynote speech of President Aquino at the NSTW opening ceremonies, I shared her empathy for the scientists whom she regarded not only as builders of this nation but also as saviors of many people who have been given scientific alerts in the course of natural phenomena that occurred in the country. But one thing makes me sad, -- and I'm sure this makes many of you academicians who are full-blooded scientists sad as well -- that this unscience-cultured society only recognizes and gives attention to scientists in times of eclipses, typhoons, earthquakes and volcanic eruptions. Come what may, though, the scientists' role in the progress of this nation, in the lifestyle of every Filipino, could never be taken for granted.

The direction of science and technology (S & T) today is clear and challenging. The Science and Technology Master Plan launched last year has already brought in so many bright prospects to the S & T national agenda. Its three major strategies with various programs are now in place and moving successfully in the countryside. As this Master Plan is a collaborative

accomplishment of many sectors, I am indeed delighted and appreciative of the support being provided by the private agencies, financing groups, the academe, entrepreneurs, farmers' groups and several other organizations, to the fruits of science and technology.

As scientists, your primary task is bringing S & T in the service of the people. And this is being done by the S & T Master Plan, which focuses on the modernization of the production sector through massive technology transfer and commercialization. The Comprehensive Technology Transfer and Commercialization Program (CTTC), as centerpiece of this first strategy, offers livelihood opportunities to help alleviate the poverty-related problems.

As scientists, your search for knowledge should be eternal to effect progressive innovations that would lead to industrialization. That is why the S & T Master Plan's Strategy II aims at upgrading research and development capability. This strategy directs goals for providing the missing link between our scientific base and innovation and productivity.

No other group except that of scientists should initiate and strive hard to create a national consciousness on the importance of science and technology. This target is being pushed by the S & T Master Plan's Strategy III, which bats for development of S & T infrastructure, including manpower development and creation of a science culture.

We have as bottomline the S & T Master Plan. We tapped many sectors, as well as several organizations and civic groups. We have strengthened our information dissemination. Today, we can truly claim that there were a lot of times when we hogged the limelight through the tri-media, not only as an aftermath of disasters, but also with the interesting science projects that we have launched and will still be launching.

Strategy III has offered the country's first science centrum, which we opened last year, as exploratorium of science for children and adults. We were able to tap a million-dollar funding from the World Bank for our manpower development program. The scientific career system for you scientists is now in effect. This gives you the compensation you deserve with equivalent rates of up to the salary of an undersecretary.

There are other significant S & T records -- too many to mention -- which our scientists should be proud of. Still we expect other milestones to take place within the next few months. I hope this annual meeting of the Academy will further

pave the way for implementaiton of the Science and Technology Master Plan's programs and directions.

The challenge of our country's industrialization bid rests heavily on the scientists' shoulders. There should be a truly hospitable science environment and culture, coupled with a science-sensitive value system, to make science and technology our country's twin keys to industrialization.

I wish your Academy a very fruitful meeting.

Thank you and good day.

Keynote Address

Fulgencio L. Factoran Jr.
Secretary
Department of Environment and Natural Resources
Visayas Avenue , Quezon City

On behalf of the Department of Environment and Natural Resources, I would like to thank the National Academy of Science and Technology for inviting me here today.

For the greater part of the evolution of our planet, time has always been on its side. It took some three to four billion years to cool and settle this molten sphere into a suitable balance that would allow the elements to form into the basic building blocks of life. And, through all this time, nature generously allowed billions of years to pass, much like an oenologist waiting decades for a bottle of wine to age into perfection.

The next billion years or so saw the face of the earth in vigorous ferment, with life taking form and shape in ever higher degrees of evolution. Single-celled beings became multi-celled, taking on more complex forms. Living organisms sufficiently evolved, moved on to land from their previously waterborne existence. And, in the last 30,000 years, our forebears appeared to walk, and to settle the breadth of this planet.

The larger brain capacity of man, and his mobility, made it easy for him to adapt to various climes, and to think of ways and means to adapt to his environment. And, where he could not adapt, he devised measures to fend for, and defend, himself. He fashioned shelter and clothing from materials provided by his environment. He hunted food, then moved on to domesticating animals. And, where he finally found an environment that provided well enough for him, he settled down and planted favored crops.

From the very moment that man sought to adapt to his environment, science and technology came into being, for these two represent man's attempt to explain and to categorize the phenomena that occur in him and his environment, and his

adaptive reaction to them. But because man also styles himself as a creator, he has also used science and technology to bring into existence materials and processes previously unavailable to him through natural means. And, where he thought it necessary, man also created new environments.

It is this creative impetus which has given us the renaissance, the age of exploration and discovery, the industrial revolution, and the modern era that we now both enjoy and dread.

We enjoy because never before in the history of mankind have we attained such material progress. We have eradicated diseases, bridged oceans, created land and gone to the moon. And all these even as a man-made space craft hurtles along toward the outer reaches of the solar system and the milky way galaxy.

We dread, however, because where man has created wealth, he has also created inequity, deprivation and destruction. Billions of people living in the least developed countries live marginalized lives, far from the material comforts of the wealthiest countries, and far from the benefits of modern technology. They also live under the constant threat of deprivation caused by natural phenomena, such as drought, storms, earthquakes and other cataclysms. The recent cyclone episodes in Bangladesh and the eruption of Mt. Pinatubo have shown us just how vulnerable people are in a setting of underdevelopment and want.

Man has also used science and technology in order to put in his hands the means to destroy his planet and his race. The stock of nuclear arms possessed by the so called "atomic club" and the billions of dollars spent each year for conventional arms by the less developed countries have given nations the ability to wipe mankind out of the face of creation. The recent war in the gulf is a grim reminder of man's willingness to use these weapons against himself. That oil spills were deliberately staged in a sort of "eco-warfare" is an even more chilling specter of just how far man can take this aggression.

However, the greatest destruction unleashed by man is a destruction that happens quietly, and often under our very noses. But because this happens without the sound of gunfire, nor the exchange of words by world leaders, it does not get as much attention. And yet, it is among the most violent of man's destructive acts. Consider the following facts:

1. The earth is losing as much as 20.4 million hectares of its tropical forests annually due to improper logging, shifting cultivation and land conversion.

2. Animal and plant species are becoming extinct at a natural rate of about one species for every 1 1/9 years. But because of destructive human activities, the extinction rate is hundreds of times higher. Thus, there is a distinct possibility that species which may not have been discovered yet may become extinct.

3. In 1987, human activities released about 8.5 million mt of carbon dioxide, 255 million mt of methane and 770,000 mt of chlorofluorocarbons into the atmosphere, thus adding to the heat trapping phenomenon known as the greenhouse effect.

And so on. These grim statistics have far more terrifying consequences than Saddam's oil-fired eco-war. They show just how much and how rapidly man is changing the face of the earth, and changing it through destruction. Unfortunately, he is also changing it much faster than he can gain knowledge to understand the earth more fully. Thus, mankind stands at a point of risk and uncertainty -- continually prodding and testing the limits of nature and the environment, and yet not really knowing how nature and the environment will react when pushed too far. This state of affairs is rather like being given a bomb and being taught how to set it, without really knowing that bombs can, and do explode.

And, even if we can generate environmental statistics like those I cited earlier, they still cannot mask the general lack of understanding, a "knowledge deficit", if you will, that countinues to afflict the fields of ecology and environmental science. Perhaps, we can say that we know a lot, and yet understand little. This is tragic when one considers that the proper management of our ecosystems absolutely depends on our clear understanding of the myriad of interactions and linkages that sustain life.

Of course, this reflects the unfortunate priorities established in many of the scientific and technological communities around the world -- priorities driven by commercial demand, and not by real human and natural needs. If this gripe sounds familiar, it is reminiscent of the old criticism against the scientific community, which has put a man on the moon, and yet has not found a cure for the common cold. And yet, it is a complaint that becomes even more valid now, where, even as the earth is threatened by

ecological doom, we still find the time and the resources to develop more weapons of destruction, and material whimsies that cater only to the wants of the more advanced consumer societies.

These unfortunate priorities, and the general lack of knowledge, really become more obvious when the setting becomes the third world.

For example, in the Department of Environment and Natural Resources, there is a dearth of environmental scientists. Admittedly, this is not an acceptable situation for a government organization that has made the environment the centerpiece of its efforts. And yet, the reality of it is that we are not able to recruit environmental scientists because there are so very few of them around, and those who are there rarely want to take on the vicissitudes of public service.

This "knowledge deficit" can be seen in many other aspects of our work. We have made, for example, the bold decision to stop logging in the virgin forests of our country. The reason here is to conserve biodiversity, with the knowledge that future Filipinos will reap the benfits of improved agricultural crops, medicine and other products that can be derived from such a move. In a sense, we have asked the current generation to make a sacrifice for succeeding generations.

The problem, however, is that while we know that biodiversity conservation is good, we do not know yet the potential species within our virgin forests that could become a boon for us in the near and far future.

There are still a lot of unknowns even in just the identification of floral and faunal species. In a very short botanical expedition of a Swedish group in Palawan, for example, it was able to discover three plant species never before known to science. The group members, as well as other plant scientists, have assured us that there are still many yet undiscovered. However, the tragedy of deforestation is such that we may have already lost many species without even knowing what, where and how important they were.

But even if we already know these species and their potentials, we still have to hurdle another level of scientific and technological limitation. I refer to the ability to transform the genetic material or the chemical product from these species into

forms of concentrated utility to man, such as in improved varieties of food crops and medicines.

It is said that there are only about 2,500 experts on this worldwide. Unfortunately, most are in the developed countries and in the multinational corporations. Thus, without a truly aggressive effort on our part to develop such capabilities, we may end up having the raw materials and yet still be dependent on the developed countries for their processing, marketing and use.

The management of ecosystems also requires an understanding of the socio-cultural dynamics of people dependent or linked to these ecosystems. While sociology or cultural anthropology are sometimes considered the poorer cousins of the hard sciences, such as biology and physics, it is increasingly realized that they do point out how best to strengthen man-nature interrelationships.

A case in point is the relocation of the Aetas displaced by the eruption of Mt. Pinatubo, which would have been much easier had we possessed a better understanding of their ways, value systems and livelihood technologies. While consultations may help resolve this, such consultations gain in value only if government and/or the assisting donor groups understand the Aeta perspective.

There is also an increasing awareness that we can learn a lot about sustainable management of natural resources from indigenous societies, which have been very close to nature and have peacefully co-existed with their environment. Indigenous or tribal people can identify more plant species and their uses than many of our best botanists. Such indigenous knowledge should be supported and utilized. We should support efforts at this new field of study called "Ethnoecology" so that we may learn to work in closer partnership with nature, just as our forebears, and our indigenous cultural communities, have.

Admittedly, we are entering the realm of ecology and environmentalism rather belatedly. The more advanced countries, and even some of the more enlightened developing countries, took up the cudgels of formal environmental education at least a couple of decades ago. Even the more prestigious universities of this country have just recently begun including ecological and environmental sciences in their academic offerings. Therefore, the local academic and scientific communities have much catching

up to do, if only to bring our body of ecological knowledge within reasonable distance of the frontrunners.

Our efforts at building our body of eco-knowledge, however, have given many of us a valuable insight, which may do us all well to remember in the future.

During the stages of human development, man was thought to be constantly awed by the power of untamed nature. He was brought to his knees by lightning. The power of earthquakes shook his very consciousness. And the might of volcanic eruptions made him worship. But when man began to develop his body of knowledge -- his sciences and his technologies -- he began to think that he could, after all, become the master of nature.

And yet, after years of trying, after years of building his mastery, man continues to be under the dominion of nature. He is still prone to earthquakes, to floods, to storms and to volcanic eruptions. And, he is still subject to the hidden wrath of nature, a wrath unleashed by his disrespect of the elements, and of natural rules. Thus, man is now victim to such unheard of phenomena as black rain, acid rain and global warming.

But, because of all these, man has again realized, and is willing to admit that, even after all these years, and even after all his claimed, and ignorant supremacy, he is still awed by nature and its forces.

For, the more he learns about nature, the more he is impressed by its diversity, its relatedness, its might and most importantly, its wisdom.

Thank you and good day.

PLENARY PAPERS

PLENARY SESSION I

Plenary Paper:	**Conservation and Management of the Philippine Marine Ecosystem**
Chairman:	Carmen C. Velasquez
Plenary Speaker:	Edgardo D. Gomez
Rapporteur:	Leopoldo S. Castillo
Discussants:	Angel C. Alcala
	Cirilo B. Serna

I. SUMMARY/HIGHLIGHTS OF DISCUSSIONS

A. On Coral Reefs

Dr. E.D. Gomez identified the major problem of destruction of coral reefs through relentless exploitation by an ever-increasing number of people. Dr. A.C. Alcala, president of the Silliman University, reiterated the view that the destruction was man- made. However, he also averred that there were stresses caused by natural occurrences, such as typhoons and volcanic eruptions. He also mentioned that human-induced stresses were geometrically increasing in severity without let-up, giving no time for the reefs to recover. Time was when the reefs were teeming with fish. Before, it took a fisherman only several minutes to gather a kilogram of fish. But now a trip of 2 - 3 hours will enable the fisherman to harvest only 1.6 - 2.0 kg of fish, as shown in his studies of a protected island. And for the aquarium trade some species are in much diminished numbers if not totally absent.

Dr. Alcala agreed with Dr. Gomez that the strategy of limited access to the coral reefs would allow the recovery of its productivity. The former also suggested the combination of this strategy with the establishment of protected areas (parks and reserves). With this, the degraded coral reefs would return to their normal condition within a fairly short period of 5 - 10 years, as he had demonstrated in his experiments at Sumilon Island. Dr. Alcala also asserted that the presence of strong enforcement mechanism was important in sustaining the positive effects of protection.

With regard to reef systems away from human population centers, such as the Tubbataha National Marine Park in the Sulu Seas, the enforcement measures should involve a strong political will on the part of the government and non-government organizations.

Dr. Cirilo B. Serna, director of the Environmental Management Bureau of DENR, agreed with the views of Dr. Gomez and Dr. Alcala. He warned, however, that these were easily said than done. He further averred that the approach through local autonomy might be quixotic considering the deeply ingrained Filipino value of *pakikisama.*

B. On Seagrass Ecosystem

The paper of Dr. Miguel D. Fortes on "Seagrass Ecosystem and Resources in the Philippines" gave a clear picture of the nature and variety of seagrass ecosystem components. This was affirmed by Dr. Serna, who pointed out the apparent role of seagrass in preserving two of the important endangered species, namely, the green sea turtle (*pawikan*) and the dugong.

The views of Dr. Alcala corroborated the finding on the valuable role of seagrass for important marine species, as well as for producing organic matter. Seagrass, like the mangrove, will contribute substantial amounts of carbon important to the food chain. He also observed that migratory birds found the seagrass locale as feeding areas.

II. RECOMMENDATIONS

1. Components on education and information dissemination, especially on coral reef and mangrove protection, should be accorded top priority. This will create awareness of the importance of returning the productivity aspects.
2. An implementable and effective authority that will unequivocally look after the coral reefs, mangrove and seagrass ecosystem productivity should be created. To be more effective, this authority should include regional, provincial, municipal and barangay levels. It is also essential to encourage, promote and sustain the support of non-government organizations in the management of the authority for greater marine ecosystem productivity and usefulness.
3. There is a need to encourage an integrated study or research on seagrass-mangrove-coral reef ecosystem to maximize the benefits with socio-economic implications.
4. It appears there is again a need to reiterate the problem on population pressure as the most important factor in the destructive coral reef fishing and utilization. Hence, promoting an urgent, acceptable, viable and effective family planning program is strongly emphasized.

PLENARY SESSION II

Plenary Paper:	**The Conservation and Management of our Freshwater Ecosystems**
Chairman:	Magdalena C. Cantoria
Rapporteur:	Clare R. Baltazar
Discussants:	Carlito R. Barril
	Macrina T. Zafaralla
	Jose R. Velasco

I. SUMMARY/HIGHLIGHTS OF DISCUSSIONS

The discussants focused on three inland resources, namely, Laguna de Bay, Lake Taal and Lake Buhi. Interrelationships of human activities and the changes taking place in each aquatic resource were presented. All agreed that each inland resource must be managed individually using appropriate management strategies because each lake had its own unique set of problems depending on the particular set of environmental conditions surrounding it.

1. Laguna de Bay is the largest and most important lake in the Philippines with about 76,000 families directly dependent on the lake for their livelihood. But the lake is considered by many as dying and according to Dr. Carlito Barril, "Laguna de Bay could be on the threshold of an ecocatastrophe with very serious implications, including social unrest and political upheaval." He focused on water quality as a key environmental indicator needed for sound management and sustainable development of the lake.

Dr. Macrina Zafaralla, on the other hand, identified three factors responsible for stunted fish growth in Laguna de Bay: 1. heavy siltation; 2. pollution; and 3. salinity. Allowing polluted Pasig river water to flow into the lake is considered a risk and a hazard to people's health and well being. Further, she concluded that the complex problems in Laguna de Bay were primarily rooted in the lake's multiple uses and destruction of the watershed.

How can Laguna de Bay be saved? Dr. Barril proposed seven urgent measures and two ultimate measures as water quality management strategies to save Laguna de Bay.

2. Taal Lake has its own set of management problems but it is ecologically better situated compared with Laguna de Bay, Dr. Zafaralla explained. She considers sanctuary area dedication and gear regulation (particularly the "suro" or push net) as sound policies to apply to this lake. Four zones have been proposed for efficient management of Taal Lake -- the tourist zone, the aquaculture zone, the open fishery zone and the fish cage zone.

3. Lake Buhi is shallower than Taal Lake and its littoral zone slopes gently so that it favors the establishment of fish cages and fish pens. Dr. Zafaralla identified three problems in this lake.

1. When the lake was used in 1983 to irrigate five adjoining towns, the operation caused the lake water to drop below the critical level of 0.8 m, thus exposing the fish cage culture of tilapia which was teeming at that time.
2. The area allocated for the sanctuary was 86 ha but in 1987, about 400 fish cages were found within the sanctuary.
3. In 1983, the endemic sinarapan or the smallest commercial fish in the world was threatened with extinction when the predatory tilapia was released into the lake by BFAR.

Dr. Jose Velasco mentioned the dual functions of natural resources: productive and protective. He lamented the lack of funding or government support for research and development on production and on protection. We should cultivate both personal and the country's interest in the kind and direction of R & D we undertake. He reiterated the recommendations of Dr. Guerrero but with strong emphasis on discipline as individuals and as a society, even to the point of coercion. This is to minimize abuses and exploitation of our own natural resources.

II. RECOMMENDATIONS FOR CONSERVATION OF FRESHWATER ECOSYSTEM

1. More studies on endemic freshwater species and their ecology should be conducted.

   There are 43 indigenous freshwater fishes in addition to numerous aquatic invertebrates and plants found in our lakes, rivers and swamps. Studies to be pursued should be in the areas of biological assessment, population dynamics and fisheries management of natural freshwater ecosystems.

2. Anti-pollution laws and fishing regulations, particularly for rivers and lakes, should be enforced strictly.

   Little or no control has resulted in high morbidity of our rivers due to pollutants and depletion of fisheries resources brought about by overfishing. Existing laws and regulations for pollution control and fishing in rivers and lakes should be improved or revised and enforced.

3. There is a need to protect endangered species.

   The Liguasan marsh, Lakes Lanao, Taal and Buhi are recommended areas to be reserved for the protection of endangered freshwater fishes and mollusks.

4. There should be more stringent regulations on the introduction of exotic species.

Evaluation and impact studies should be done on all introduced species prior to their propagation and dispersal to ensure protection of both the endemic species and the environment.

5. Water in fishponds should be used more efficiently.

   Integrated farming and recycling should be considered.

6. There is a need for more small impoundments.

   In upland areas, where soil erosion is critical and poor living conditions exist, there should be more small impoundments to conserve water and protect the environment.

7. There is a need for multisectoral and wholistic approach to freshwater ecosystem management.

   Ecosystem management could best be accomplished through agroecological approach, proper watershed management and waste treatment by industries, and enforcement of fishing regulations considering socio-economic and political factors.

8. More public awareness and information on conservation of freshwater ecosystems should be generated.

   Information drives should be done at all levels through the educational system, churches, mass media and every possible venue.

PLENARY SESSION III

Plenary Paper:	**Conservation and Management of Philippine Terrestrial Ecosystem**
Chairman:	Ruben L. Villareal
Plenary Speaker:	Enrique P. Pacardo
Rapporteur:	Benito S. Vergara
Discussants:	Delfin M. Ganapin Jr.
	Domingo M. Ramirez

I. SUMMARY/HIGHLIGHTS OF DISCUSSIONS

The discussions centered on several important issues:

- Developing a rational land use plan adopted at all levels (national, regional, provincial, local) to ensure sustainable natural resource use and avoid conflicting use of resources;
- Protecting the remaining primary forest areas, especially in critical areas (national parks, forest, nature reserves, watersheds), as well as endangered flora and fauna, for maintaining biodiversity and ecological stability;
- Providing strong support for environment cum population related programs;
- Promoting and enhancing implementation of community-based, participatory and integrated approaches to natural resource management, taking off from successful examples;
- Providing adequate research and development support for long- term and priority concerns related to agricultural sustainability, management of logged over areas, upland, coastal and urban rehabilitation and management;
- Promoting environmental education and extension programs for all sectors of society;

- Establishing line agencies (DENR, DA, DAR), NGO and academe network for natural resource management at the national, regional, provincial and grass roots levels;
- Evolving a comprehensive national policy of environmental ethics and management, which would encompass promotion of environmental awareness; formulating and enforcing legislation, which would protect and enhance the environment; and giving support to NGOs to encourage their participation;
- Recognizing the scale and nature of current urbanization and assessing the extent of deterioration so that the resource base can be rehabilitated and preserved;
- Adopting low-waste and environmentally sound technologies utilizing recycling, rehabilitation and renewability;
- Adopting agriculturally sustainable technology and practices, such as organic agriculture, integrated farming and integrated pest management;
- Promoting economic incentive schemes for regulating use of natural resources and pollution control measures;
- Developing and adopting an environmental master plan for major urban centers and the 179 Community and Environment Natural Resource Offices throughout the country reflective of the Philippine Strategy for Sustainable Development (PSSD); and
- Developing strong and effective preventive and anticipatory environmental management mechanisms, such as Environmental Impact System (EIS) at all levels (national, regional and local) for hazardous biological and chemical handling and regulation, etc.

II . RECOMMENDATIONS

1. A national agricultural land use policy, which will resolve the conflict between agriculture and industry in terms of positioning for space, should be formulated.
2. Appropriate measures to protect prime agricultural lands from being converted to other agricultural uses should be carried out.

3. There is a need for a well-coordinated and well-funded national soil conservation program covering both lowlands and uplands.
4. There is a need for agricultural planners of the Department of Agriculture (DA) to acquire the necessary skills in agroenvironmental planning/program implementation. Likewise, there is a need for extension workers of the DA and the Department of Environment and Natural Resources (DENR) to acquire the knowledge and technology in upland soil conservation.
5. There is a need for the DA, DENR and the Department of Agrarian Reform (DAR) to colloboratively establish a coherent program for sustainable agroenvironmental development.
6. DAR and the DENR should provide security of tenure to the uplanders or those occupying areas classified as forestlands.
7. A program to monitor and control pesticide and fertilizer pollution should be implemented.
8. There is a need to pursue vigorously an integrated population and environmental resources development program.

PLENARY SESSION IV

Plenary Paper:	**Conservation and Management of Urban Ecosystem**
Chairman:	Solita F. Camara-Besa
Plenary Speaker:	Manuel M. Dayrit
Rapporteur:	Carmen Ll. Intengan
Discussants:	Gelia T. Castillo
	Orlando S. Mercado
	Antonio Abaya

I. SUMMARY/HIGHLIGHTS OF DISCUSSIONS

The following points were raised during the discussions:

- To upgrade living conditions in Metro Manila, the Asian Development Bank estimates that the amount involved is P16.6 billion. This will make the city livable but may attract more rural-urban migrants; also, there will be less money available for the rural areas.
- The government has no policy on air pollution, solid waste, sewage disposal. Sewer pipes cause water pollution in our waters.
- Squatters cannot just be ejected - this is human rights violation. In fairness to the evacuees, adequate facilities should be provided in the evacuation area. The ratio of rural to urban settlers used to be 70:30. It is now 47:53.
- There is no general plan for land use. If there is one, this is individually planned.

- We have a chronic shortage of things that we need:
  - flood control infrastructure;
  - water supply;
  - public transport facilities;
  - supply of electrical energy; and
  - health and hospital services.

- We have an overabundance of things we do not want or need:
  - air and water pollution;
  - uncollected or poorly disposed garbage;
  - unregulated and unmanaged population growth leading to the proliferation of squatter colonies; and
  - hazardous and toxic wastes.

II. RECOMMENDATIONS

1. In the coming 1992 elections, more accountable and environmentally friendly leaders should be elected.
2. There should be some 3500 natural family planning clinics all over the metropolis.
3. The government should come up with policies after consultation with various sectors of the population so that there can be mobilization and implementation by all sectors.
4. The network of urban barangays should be mobilized and given specific duties and responsibilities in the management of the urban ecosystem. To improve the status of a barangay with the active participation of its people, the process should be done one grade at a time to make development manageable.
5. There is a need to conceptualize and execute a massive program to build pre-fabricated, low-cost mass housing, especially for the poorest of the poor. Agrarian reform may benefit less than 10 % of the population while a housing reform can benefit all.

6. There is an urgent need for a network of elevated, rapid-transit LRTs that extend as far north as Malolos, as far east as Tanay and Los Baños and as far south as Cavite City. This would decongest the Metro area and provide commuters enough time to get to work, reach school or shop in the Metro area.

7. Metro Manila should be crisscrossed with non-stop road arteries where all intersections are marked by overpasses and interchanges. For example, a non-stop ring artery in EDSA would allow travel from Pasay to Kalookan in 15 minutes.

8. Manila's 4,000 tons of garbage daily should be compacted or incinerated in Tondo and used to reclaim land in Manila Bay.

9. Laguna de Bay can be made a source of drinking water by walling off the least polluted section of the lake and having this aerated, filtered and chemically treated.

PLENARY PAPERS

PLENARY I

Coral Reef Ecosystems and Resources of the Philippines

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INTRODUCTION

The educated layman may or may not have been introduced to corals and coral reefs in school. Today, however, he has probably been exposed to corals and coral reefs in real life or through the television screen. It is not uncommon to see the ornate skeleton of some corals in an aquarium, in a display window of jewelry and watches, or as part of a display of shellcraft in the home of a friend. What few of us may realize is that where such samples are seen, whether it be in the United States of America, in Europe, or elsewhere, chances are that the coral involved originated in the Philippines. As to the television screen, many have been exposed to documentaries involving marine life, including "The Coral Triangle" featuring our coral reefs (in which I served as the scientific adviser) and tourism or adventure clips which feature underwater scenes in the tropics.

But what is a coral? What has been referred to above generally applies to the stony corals, i.e., the coelenterates or cnidarians that secrete a calcareous, friable skeleton, usually white when separated from the living tissue. To a smaller number of us, coral may mean jewelry, usually pink or red and sometimes black. The sources of these precious corals are also coelenterates, but the animals are taxonomically distinct from the stony corals. Whether stony or precious, what we are often exposed to are the skeletons of colonial organisms long dead by the time we see their clean and sometimes polished remains.

Technically, most of the corals as the layman knows them belong the the Class Anthozoa or "flower animals" in the Phylum Cnidaria or Coelenterata. A few fall under the Class Hydrozoa, the best known being the fire coral of *Millepora*. The stony corals mostly belong to the largest order of anthozoans, the Scleractinia.

With few exceptions like the solitary mushroom coral whose polyp may reach a diameter of 25 cm, all the stony corals are colonial. Most of the scleractinians harbor symbiotic algae called zooxanthellae. Due to this mutualism, the stony corals are able to deposit significant quantitites of calcium carbonate as their housing or skeleton. The massive populations of the hundreds of species form geological structures that we term coral reefs, with contributions of other calcareous materials principally from algae, molluscs and echinoderms.

In terms of conservation, it is the coral reef as a unit that is of greater concern. A coral reef is a sizeable, living, calcium carbonate stucture in a shallow tropical sea supporting a diverse association of flora and fauna. Usually there is a complex sculpturing of high relief. It is to be stressed that a coral reef is a dynamic living structure, with both accretion and erosion of materials with a bias for the former, hence resulting in a net reef growth of a millimeter or so per year.

Of the associated organisms, the most obvious and the most important from an economic point of view are the fish. These are the objects of fisheries in addition to the edible invertebrates and the seaweeds that are found on reefs.

Besides organisms that are collected for food, there are those harvested for the live or dead ornamental trade, i.e., for aquarium or for decoration purposes. These include reef fish, corals and shells and other invertebrates for the live trade and corals and shells for the ornamental trade. But in addition to these, marine organisms are now being collected for studies in marine pharmacology. Novel compounds are being identified and extracted from various organisms which have medicinal potentials, including some of the conotoxins studied by Academician Lourdes Cruz with Dr. Baldomero Olivera and their colleagues.

One other use of corals has been to provide construction materials for roads, for churches of old and for new buildings. An excess of this activity could diminish one of the important functions of coral reefs which is shoreline protection. In some places, sandy beaches exist only because of the active growth of coral reefs offshore that serve as wavebreakers and that contribute sand particles to dynamic beaches. Without them, these beaches could erode and disappear.

Finally, more and more coral reefs are being appreciated for their aesthetic value. A pristine reef in a warm sea with myriads of colorful fish is truly a sight of unparalleled beauty. It is no wonder that more tourists from far and wide come to spend their holidays where good reefs are to be found.

RESEARCH

In the past two decades, coral reef research in the Philippines has taken virtually a quantum leap from what was almost purely taxonomic work to that whose breadth and quality are now almost at par with those in the most advanced scientific institution. The initial project in the mid-seventies (see following section) enabled local scientists to design and test various reef monitoring techniques. Contributions have been made to the international literature on suitable field techniques for detecting changes in coral reef structure (Gomez and Alcala 1984; Gomez 1984; and Yap 1984, 1986).

In the course of time and further imrpovement of community structure methods, particular methodologies have been standardized and adopted for use on a broader geographic scale (e.g., Southeast Asia). A new generation of studies using these techniques has shed much light on broad distributional patterns of reefs and their associated assemblages, such as fish, in the Philippines (Gomez et al. 1989; Hilomen and Gomez 1989; Licuanan and Gomez 1989).

Research into the dynamics of these ecosystems initially focused on the nature of their recovery from physical damage, the most notable being blast fishing (Alcala and Gomez 1979; Aliño et al. 1985; Yap and Gomez 1989). Emphasis was given to this aspect because of its important economic as well as ecological implications.

Crucial parallel activities were the studies on coral growth, both on natural as well as artificial substrates (Alcala and Gomez 1979; Alcala et al. 1989; Yap and Gomez 1981; Alcala et al. 1982; Alcala and Gomez 1982; Gomez et al. 1982; Yap and Gomez 1984, 1985a; Gomez et al. 1985; and Yap et al. 1990). Many of these studies involved the use of coral transplantation as a tool for studying physiological responses of the organisms. Another goal of coral transplantation is with respect to the possible rehabilitation of damaged habitats.

STATUS OF PHILIPPINE CORAL REEFS

Concern for the status of Philippine coral reefs in part due to the reports of illegal gathering of precious corals in the Batanes Islands by foreign ships was translated into action in the second half of the seventies. The Marine Science Institute, which was

then the fledgling Marine Sciences Center that was without proper building space, was requested to submit a proposal for the assessment of Philippine corals. With the help of a few colleagues, I drafted the proposal for the "Investigation of the Coral Resources of the Philippines". In the execution of the project which went into three phases and spanned about five years, two other units of the University of the Philippines, viz., U.P. College Cebu and the College of Business Administration, and the Silliman University were involved as collaborators. Many publications resulted from this project, among them: Alcala and Gomez 1979; Gomez 1979; Gomez and Alcala 1979; Gomez and Añonuevo 1979; Gomez 1980; Alcala et al. 1981; Yap and Gomez 1981; Alcala and Gomez 1982; Alcala et al. 1982; Gomez et al. 1982; Gomez 1983; Maragos et al. 1983; Gomez and Alcala 1984; Yap and Gomez 1984; Alcala and Gomez 1985; Gomez et al. 1985 and Yap and Gomez 1985a.

As to the status of coral reefs, two papers viz., Gomez and Alcala (1979) and Gomez et al. (1981) gave the broad picture of the situation. The tabular results may be compared (Tables 1 and 2). The second table was first updated for 1982 by Yap and Gomez (1985b) and appears here as Table 3.

Since the end of the benchmark project, there has been no formal and systematic survey of Philippine reefs. The results have been adopted by both governmental and non-governmental organizations in recent years without any recognition of the source. In a sense, this has been gratifying and a good sign because they have been accepted and indeed corroborated by other observers.

In more recent years two ASEAN marine science projects have gathered some additional coral survey data for limited areas. These are the ASEAN/US Coastal Resources Management Project which focused on the western side of Lingayen Gulf and the ASEAN/Australia Marine Science Project: Living Coastal Resources. A summary table was included as Table 2 in Gomez (1989). The most recent data from the two ASEAN projects are compared to the old data in Table 4. It should be stressed that the method used for these other projects is different from the original method. Nevertheless, the results are comparable since they used percent live coral cover. If these results are combined in the old format, the result is Table 5, the status of Philippine coral reefs in 1991, assuming that the general condition of the reefs surveyed earlier has not changed significantly.

PROBLEMS

Stresses or problems that beset coral reef ecosystems are of two categories: natural and man-induced. A number of publications dealing with coral reef problems throughout the world have appeard. One such publication on human induced stresses edited by Bernard Saliat, appeared in 1987. I contributed to two chapters, one on dynamite fishing (Alcala and Gomez 1987) and one on other fishing methods destructive to corals (Gomez et al. 1987).

In a situationer on coral reefs of Southeast Asia (Gomez 1980), I outlined the problems of reefs in the region. That report was used as the basis of a paper on the coral reef degradation in Southeast Asia (Yap and Gomez 1985b).

As to actual problems of Philippine coral reefs, one of the earliest publications was the proceedings of a forum on natural resources management (Gomez 1979). Hence, what I need to say here is a distillation of previous work. A broader treatment of Philippine corals was prepared subsequently by Alcala, Gomez and Yap (1987).

It is probably not very relevant to discuss natural stresses on corals except to say that in general, our reefs have developed through some eons of typhoons and volcanic activity, and they have shown that they have the resilience to survive these events. Areas that are unsuitable for reef growth simply have no reefs to speak of. For an idea of how typhoon-damaged reefs recover, see Alcala and Gomez (1990) and Alcala et al. (1986). The recent eruption of Mt. Pinatubo may provide an opportunity to study the effects of the volcanic ejecta on Zambales reefs.

In Yap and Gomez (1985b) we listed siltation, fisheries-related destruction, collection of building materials, tourism, collection of reef invertebrates and other pollutants as the major causes of coral reef degradation. Details may be seen from that paper or from Alcala et al. (1987). A broader review of marine environmental problems in the region is provided in Gomez (1988b).

Every once in a while, I take the opportunity to reflect on these problems. Coral reef science and concerns for conservation in the Philippines have now broadened a great deal in contrast to those two decades ago. There are now dozens of investigators and activists taking up the cause of coral reefs. I am therefore now somewhat away from the frontlines, so to speak. This has allowed me to have a broader perspective.

If asked what the major problem of the coral reef is, I would probably say it is the pressure of human populations. A visit to any fishing village situated near a reef will demonstrate the situation very well. There are, in the first instance, just too many fishermen. They overfish the reefs and even if they use non-destructive fishing gear, the coral reef ecosystem in question is stressed. This stress is exercised from the bottom to the top of the reef. The pressure is especially heavy on the reef flats or tops of reefs. Here, at practically every low tide, reef gleaners comb the area for anything edible and marketable. Hence, accessible reef flats become less diverse and less productive from overharvesting and from trampling.

If you superimpose on the pressure of sheer numbers the destructive practices of some fishermen, then the reefs really regress. Blast fishing, muro-ami and the use of poisons all have more lasting negative impacts on the reefs.

With the possible exception of man-induced siltation, there is no greater stress on our reefs than population pressure.

MANAGEMENT

In view of all the problems alluded to above, what management measures might be recommended to conserve coral reefs? The most obvious is for this government and for each citizen to do something about our population problem. The details of how that might be done are beyond the scope of this paper.

Since the above is a broader and longer term approach, I should like to mention a management measure that could be adopted with great effort. From my perspective, the only way to save our reefs and restore their productivity is by limiting access to them. It is recognized that this will be no mean task. But if nations can agree to adopt the concept of the Exclusive Economic Zone among them, it should be possible to think in terms of exclusive economic zones among geographic or political units within the country.

A start is being made with the establishment of marine parks (cf. Gomez et al. 1984). Marine parks and marine reserves are beginning to be recognized in the country. We need many, such as the reefs of global significance (Gomez 1988a). However,

they can serve only as limited replenishment areas for neighboring habitats.

What I would like to see happen is for each reef to be under the management of an identifiable authority at the municipal or barangay level. Such an authority should be empowered to determine the number of fishermen allowed at any one time as well as the gear used. It would regulate the catch in terms of amount, size of individuals and kinds of species caught. Furthermore, it would exercise the authority to close a fishery or an area during certain times of the year.

Such an authority would obviously need much power and credibility. The power can come from the people themselves or from the government. The credibility would have to be cultivated and there must be inputs from scientists who would determine the biological basis for the regulations.

Table 1. Summary of Reef Survey Data ("Coral Cover" includes both soft and hard corals.)

Location	No. with Total No. of Stations	No. with Excellent (75-100%) Coral Cover	No. with Good (50-74.9%) Coral Cover	No. with Fair (25-49.9%) Coral Cover	No. with Poor (0-24.9%) Coral Cover
LUZON					
1. La Union	8	0	1	2	5
2. Zambales	4	0	0	1	3
3. Pangasinan	11	0	4	4	3
4. Bataan	6	0	3	2	1
5. Batangas	38	1	7	12	18
6. Mindoro Occidental	13	1	2	6	4
7. Mindoro Oriental	31	1	8	15	7
8. Palawan	5	0	3	2	0
Subtotal	116(100%)	3(2.5%)	28(24.2%)	44(37.95)	41(35.4%)
VISAYAS					
9. Bohol Island/Islets	22	0	8	7	7
10. Cebu Province	57	3	15	18	21
11. Negros Island	100	4	24	37	35
12. Siquijor Island	31	0	9	9	13
13. Guimaras Island	32	8	8	11	5
Subtotal	242(100%)	15(6.2%)	64(26.4%)	82(33.9%)	81(33.5%)
MINDANAO					
14. Zamboanga del Norte	8	0	3	2	3
15. Aliquay Island	8	2	3	2	1
16. Selinog Island	7	0	0	1	6
Subtotal	23(100%)	2(8.7%)	6(26.1%)	5(21.7%)	10(43.5%)
TOTAL	381	20	98	131	132
Percentage	(100%)	(5.2%)	(25.7%)	(34.5%)	(34.6%)

Table 2. Status of Philippine coral reefs - 1981: 619 Stations in Four Categories of Living Coral Cover - Excellent (75-100%), Good (50-74.9%), Fair (25-49.9%) and Poor (0-24.9%)

LOCATION	No. of Stations	EXCELLENT		GOOD		FAIR		POOR	
		No.	%	No.	%	No.	%	No.	%
LUZON									
1. Albay	9	0	0	1	11.1	5	55.6	3	33.3
2. Bataan	10	0	0	0	0	0	0	10	100.0
3. Batangas	25	0	0	6	24.0	11	44.0	8	32.0
4. Cagayan	4	0	0	2	50.0	2	50.0	0	0
5. Camarines Norte	13	0	0	1	7.7	7	53.8	5	38.5
6. Camarines Sur	2	0	0	0	0	2	100.0	0	0
7. Cavite	9	0	0	0	0	6	66.7	3	33.3
8. Isabela	3	0	0	2	66.7	1	33.3	0	0
9. La Union	5	0	0	1	20.0	2	40.0	2	40.0
10. Marinduque	5	0	0	0	0	4	80.0	1	20.0
11. Mindoro Occidental	31	1	3.2	8	25.8	15	48.4	7	22.6
12. Mindoro Oriental	11	1	9.1	2	18.2	4	36.4	4	36.4
13. Palawan	49	6	12.2	17	34.7	20	40.8	6	12.2
14. Pangasinan	37	0	0	8	21.6	14	37.8	15	40.5

Table 2. Continued

LOCATION	No. of Stations	EXCELLENT No.	%	GOOD No.	%	FAIR No.	%	POOR No.	%
15. Quezon	4	0	0	2	50.0	2	50.0	0	0
16. Zambales	12	0	0	2	16.7	3	25.0	7	58.3
Subtotal	229	8	3.5	52	22.7	98	42.8	71	31.0
VISAYAS									
1. Antique	12	2	16.7	10	83.3	0	0	0	0
2. Bohol	22	0	0	8	36.4	8	36.4	6	27.2
3. Cebu	51	5	9.8	13	25.5	19	37.2	14	27.4
Hilutangan Island	4	0	0	1	25.0	0	0	3	75.0
Mactan Island	15	1	6.7	3	20.0	3	20.0	8	53.3
Olango Island	7	0	0	1	14.3	2	57.1	2	28.6
Sumilon Island	4	0	0	3	75.0	0	0	1	25.0
4. Iloilo	64	9	14.1	18	28.1	27	42.2	10	15.6
5. Leyte	12	0	0	0	0	6	50.0	6	50.0
6. Negros Occidental	18	1	5.6	2	11.1	5	27.8	10	55.6
Refugio Island	4	0	0	1	25.0	1	25.0	2	50.0
7. Negros Oriental	98	5	5.1	20	20.4	41	41.8	32	32.6

Table 2. Continued

LOCATION	No. of Stations	EXCELLENT No.	%	GOOD No.	%	FAIR No.	%	POOR No.	%
Apo Island	5	0	0	5	100	0	0	0	0
8. Siquijor	31	0	0	9	29.0	9	29.0	13	41.9
Subtotal	347	23	6.6	94	27.1	123	35.4	107	30.8
MINDANAO									
1. Misamis Occidental	9	0	0	0	0	4	44.4	5	55.6
2. Misamis Oriental	1	0	0	0	0	0	0	1	100
3. Zamboanga del Norte	18	1	5.6	3	16.7	6	33.3	8	44.4
Aliquay Island	8	2	25.0	3	37.5	2	25.0	1	12.5
Selinog Island	7	0	0	0	0	1	14.3	6	85.7
Subtotal	43	3	7.0	6	14.0	13	30.2	21	48.8
TOTAL	619	34	5.5	151	24.4	234	37.8	200	32.3

Table 3. **Status of Philippine coral reefs - 1982: 619 Stations in Four Categories of Living Coral Cover - Excellent (75-100%), Good (50-74.9%), Fair (25-49.9%) and Poor (0-24.9%)**

LOCATION	No. of Stations	EXCELLENT		GOOD		FAIR		POOR	
		No.	%	No.	%	No.	%	No.	%
LUZON									
1. Albay	9	0	0	1	11.1	5	55.6	3	33.3
2. Bataan	10	0	0	0	0	0	0	10	100.0
3. Batangas	25	0	0	6	24.0	11	44.0	8	32.0
4. Cagayan	4	0	0	2	50.0	2	50.0	0	0
5. Camarines Norte	13	0	0	1	7.7	7	53.8	5	38.5
6. Camarines Sur	2	0	0	0	0	2	100.0	0	0
7. Cavite	9	0	0	0	0	6	66.7	3	33.3
8. Isabela	3	0	0	2	66.7	1	33.3	0	0
9. La Union	5	0	0	1	20.0	2	40.0	2	40.0
0. Marinduque	5	0	0	0	0	4	80.0	1	20.0
1. Mindoro Occidental	31	1	3.2	8	25.8	15	48.4	7	22.6
2. Mindoro Oriental	11	1	9.1	2	18.2	4	36.4	4	36.4
3. Palawan	49	6	12.2	17	34.7	20	40.8	6	12.2
4. Pangasinan	37	0	0	8	21.6	14	37.8	15	40.5

Table 3. Continued

LOCATION	No. of Stations	EXCELLENT No.	%	GOOD No.	%	FAIR No.	%	POOR No.	%
15. Quezon	4	0	0	2	50.0	2	50.0	0	0
16. Zambales	12	0	0	2	16.7	3	25.0	7	58.3
Subtotal	229	8	3.5	52	22.7	98	42.8	71	31.0
VISAYAS									
1. Antique	12	2	16.7	10	83.3	0	0	0	0
2. Bohol	22	0	0	8	36.4	8	36.4	6	27.2
3. Cebu	64	6	9.4	14	21.9	27	42.2	17	26.6
Hilutangan Island	4	0	0	1	25.0	0	0	3	75.0
Mactan Island	15	1	6.7	3	20.0	3	20.0	8	53.3
Olango Island	7	0	0	1	14.3	2	57.1	2	28.6
Sumilon Island	4	0	0	3	75.0	0	0	1	25.0
4. Iloilo	64	9	14.1	18	28.1	27	42.2	10	15.6
5. Leyte	12	0	0	0	0	6	50.0	6	50.0
6. Negros Occidental	18	1	5.6	2	11.1	5	27.8	10	55.6
Refugio Island	4	0	0	1	25.0	1	25.0	2	50.0
7. Negros Oriental	98	5	5.1	20	20.4	41	41.8	32	32.6

Table 3. Continued

LOCATION	No. of Stations	EXCELLENT No.	%	GOOD No.	%	FAIR No.	%	POOR No.	%
Apo Island	5	0	0	5	100	0	0	0	0
8. Siquijor	31	0	0	9	29.0	9	29.0	13	41.9
Subtotal	360	24	6.6	95	26.4	131	36.4	110	30.6
MINDANAO									
1. Misamis Occidental	9	0	0	0	0	4	44.4	5	55.6
2. Misamis Oriental	1	0	0	0	0	0	0	1	100
3. Zamboanga del Norte	18	1	5.6	3	16.7	6	33.3	8	44.4
Aliquay Island	8	2	25.0	3	37.5	2	25.0	1	12.5
Selinog Island	7	0	0	0	0	1	14.3	6	85.7
Subtotal	43	3	7.0	6	14.0	13	30.2	21	48.8
TOTAL	632	35	5.5	153	24.2	242	38.3	202	32.0

Table 4. Status of Philippine coral reefs - based on surveys by three projects

SOURCE	No. of transects Stations	EXCELLENT (75 - 100%) No.	%	GOOD (50 - 74.9%) No.	%	FAIR (25 - 49.9%) No.	%	POOR (0 - 24.9%) No.	%
Yap and Gomez (1985b)	632	35	5.5	153	24.2	242	38.3	202	32.0
ASEAN - Australia MSP:LCR*	103	4	3.9	32	31.1	46	44.6	21	20.4
ASEAN - US CRMP	40	-	0	18	45	17	42.5	5	12.5

* Source: Unpublished data from the ASEAN-Australia Marine Science Project: Living Coastal Resources

Table 5. **Status of Philippine coral reefs - 1991**

LOCATION	No. of Stations	EXCELLENT		GOOD		FAIR		POOR	
		No.	%	No.	%	No.	%	No.	%
LUZON									
1. Albay	9	0	0	1	11.1	5	55.6	3	33.3
2. Bataan	10	0	0	0	0	0	0	10	100.0
3. Batangas	35	0	0	13	37.1	14	40.0	8	22.9
4. Cagayan	4	0	0	2	50.0	2	50.0	0	0
5. Camarines Norte	13	0	0	1	7.7	7	53.8	5	38.5
6. Camarines Sur	2	0	0	0	0	2	100.0	0	0
7. Cavite	9	0	0	0	0	6	66.7	3	33.3
8. Isabela	3	0	0	2	66.7	1	33.3	0	0
9. La Union	5	0	0	1	20.0	2	40.0	2	40.0
10. Marinduque	5	0	0	0	0	4	80.0	1	20.0
11. Mindoro Occidental	31	1	3.2	8	25.8	15	48.4	7	22.6
12. Mindoro Oriental	66	4	6.0	11	16.7	33	50.0	18	27.3
13. Palawan	71	7	9.9	23	32.4	29	40.8	12	16.9
14. Pangasinan	53	0	0	18	34.0	19	35.8	16	30.2

Table 5. Continued

LOCATION	No. of Stations	EXCELLENT No.	%	GOOD No.	%	FAIR No.	%	POOR No.	%
15. Quezon	4	0	0	2	50.0	2	50.0	0	0
16. Zambales	12	0	0	2	16.7	3	25.0	7	58.3
Subtotal	332	12	3.6	84	25.3	144	43.4	92	27.7
VISAYAS									
1. Antique	12	2	16.7	10	83.3	0	0	0	0
2. Bohol	22	0	0	8	36.4	8	36.4	6	27.2
3. Cebu	64	6	9.4	14	21.9	27	42.2	17	26.6
Hilutangan Island	4	0	0	1	25.0	0	0	3	75.0
Mactan Island	15	1	6.7	3	20.0	3	20.0	8	53.3
Olango Island	7	0	0	1	14.3	4	57.1	2	28.6
Sumilon Island	4	0	0	3	75.0	0	0	1	25.0
4. Iloilo	64	9	14.1	18	28.1	27	42.2	10	15.6
5. Leyte	12	0	0	0	0	6	50.0	6	50.0
6. Negros Occidental	18	1	5.6	2	11.1	5	27.8	10	55.6
Refugio Island	4	0	0	1	25.0	1	25.0	2	50.0
7. Negros Oriental	98	5	5.1	20	20.4	41	41.8	32	32.6

Table 5. Continued

LOCATION	No. of Stations	EXCELLENT No.	%	GOOD No.	%	FAIR No.	%	POOR No.	%
Apo Island	5	0	0	5	100	0	0	0	0
8. Siquijor	31	0	0	9	29.0	9	29.0	13	41.9
Subtotal	360	24	6.7	95	26.4	131	36.4	110	30.5
MINDANAO									
1. Misamis Occidental	9	0	0	0	0	4	44.4	5	55.6
2. Misamis Oriental	1	0	0	0	0	0	0	1	100
3. Zamboanga del Norte	18	1	5.6	3	16.7	6	33.3	8	44.4
Aliquay Island	8	2	25.0	3	37.5	2	25.0	1	12.5
Selinog Island	7	0	0	0	0	1	14.3	6	85.7
Subtotal	43	3	7.0	6	14.0	13	30.2	21	48.8
TOTAL	735	39	5.3	185	25.2	288	39.2	223	30.3

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The State of Seagrass Ecosystems and Resources in the Philippines

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INTRODUCTION

A major ecosystem threatened by the multiplicity of demands upon the coastal habitat in the tropical region is the seagrass ecosystem. In the ASEAN region, over 70% of its total population inhabit the coastal zone. Very recently, this area of the marine environment is characterized by a high degree of resource exploitation, raising serious doubts as to its capacity for biological sustainability and normal recovery within this generation.

The islandic nature of the Philippines partly dictates that many population centers be developed around sheltered bays and estuaries, the very places colonized by seagrasses. Because these plants thrive in relatively shallow waters, they are subject to increasing stresses caused by man and his diversified needs. It is not surprising, therefore, to see seagrass beds being filled, dredged and diked for conversion to other coastal uses. These activities of man need to be looked at from the conservation and management points of view.

This paper presents the status and importance of seagrass ecosystems in the Philippines, reviews the problems and issues associated with their conservation and protection and emphasizes their susceptibility and vulnerability in the face of our rapidly deteriorating marine environment. In addition, this paper introduces the potentials of seagrass in restoring degraded coastal habitats and proposes an agenda for action in order to manage the ecosystems and their resources.

Seagrasses: Definition, Systematics and General Distribution

Seagrasses are hydrophytic monocotyledonous plants that are completely adapted to the marine environment. They resemble

true terrestrial grasses in possessing features common in their life history, i.e., rhizomes, which are buried in soft, sandy or muddy bottom, and erect leafy shoots in the water. They, however, comprise only about 50 species falling into 2 families and 13 genera. Family Potamogetonaceae consists of 10 genera and 39 species; family Hydrocharitaceae consists of 3 genera and 11 species.

Sixteen taxa of seagrasses have been identified from the Philippines (Fortes 1986a), of which three are new to the local seagrass flora: (1) an undescribed species of *Halophila*; (2) a new variety of *H. minor* ; and (3) *Ruppia maritima* var. *rostrata. H. ovalis, Enhalus acoroides, Cymodocea serrulata, Halodule uninervis* and *H. pinifolia* exhibited intraspecific morphological and physiological plasticities that appeared to be generalized adaptive responses to local habitat conditions. *Halophila beccarii* and *R. maritima* var. *rostrata* were found only in brackishwaters, while *H. decipiens* and *Thalassodendron ciliatum* appeared confined to deeper waters of the western and southern parts of the archipelago. Three zones in local seagrass communities are recognizable: (1) Zone 1 (*Halodule uninervis* , HUN, Zone); (2) Zone 2 (*Halophila - H. uninervis*, HUW, Zone); and (3) 3 (*Thalassia -Cymodocea - Enhalus* Zone).

In relation to the Philippine seagrass flora, hierarchical (cluster) analysis partitioned the Indo-West Pacific seagrass ranges into seven discrete provinces (Fortes 1986a). A high probability exists that *Halophila*, due to its wide distribution from Tasmania to Japan, represents a major connection between most of these provinces, strongly influencing the clustering of the seagrass floras in the region. The phytogeographic affinities of the Philippine seagrass flora is primarily to the west with the Indian Ocean and Southeast Asian coasts.

Functions and Uses of Seagrasses

The small number of seagrasses is not proportional to their ecological and economic importance. Seagrasses, due largely to their enormous quantities, form dense beds which cover large areas in the coastal waters. They perform a wide spectrum of biological and physical functions in the marine environment. These functions, summarized by Wood et al . (1969) and den Hartog (1977), include substrate stabilization, sediment production, habitat and nursery for fish and many invertebrates, primary food source for fish, turtles, dugongs and invertebrates such as echinoderms, and an alternative feeding site for commercial and

forage organisms. As a discrete ecosystem, seagrass beds interact physically with coral reefs and mangroves in the reduction of water energy, sediment relationships and flow regulation (UNESCO 1983). Seagrass systems tend to "leak" or export nutrients to nearby ecosystem.

It was Petersen (1891, 1918) who first evaluated the contribution of seagrasses to the coastal ecosystem via its fisheries. McRoy and McMillan (1977) showed that seagrass productivity was as high as those of the world's best agricultural crops and this production was done without energy subsidies. Despite this high productivity, there are very few organisms which actually directly consume them.

The distribution of seagrasses within coastal areas dictates the kind of grazers that are associated with them. Vertebrates such as parrotfishes (Scaridae), surgeonfishes (Acanthuridae), green turtles and sirenia, are the main consumers in the tropics (McRoy and Helfferich 1980). Alcala (1986 personal communication) observed that rabbitfishes (Siganidae) were also important grazers in local seagrass beds. In the more temperate regions, geese and ducks are the main consumers. Among the invertebrates, sea urchins are the main grazers, although they reportedly possess no enzymes that could digest the plant materials efficiently. Hence, only a small amount of energy stored in seagrasses is transferred to the next upper level in the trophic hierarchy.

Seagrass beds form a community frame that controls the structure of communities. The well-known catastrophic effects of the "wasting disease" of *Zostera* beds along the coasts of the North Atlantic in the early 1930s attest to the fundamental ecological importance of the eelgrass community. Not only were the structure and composition of the associated flora and fauna altered, but regimes in salinity, temperature and the nutrient load in affected coastal waters were also changed drastically. Fishery production declined and an almost complete reorientation in fisheries strategies had to be effected. It was primarily this ecological catastrophe which triggered renewed and significant research activities on seagrasses in most parts of the world.

The role of seagrasses at the ecosystem level is matched by their importance at the component level. McRoy and Helfferich (1980) considered their use under two time categories: historical and contemporary. The first category includes their use as materials for woven baskets by North American Indians; as source of salt, soda and warmth; in coastal Denmark, as stuffing for mattresses and bed ticks and as substitute for animal straw, roof

thatch, upholstery material, as packing and stuffing material and as a component for fertilizer; in the U.S., as insulation for sound and temperature; and in Germany, as a substitute for cotton in the manufacture of nitrocellulose (Cottam 1934) or simply pressed into bales for shipment to manufacturers who derive products from them (Scagel 1961). Soviet workers considered the commercial potentials of seagrass fibers (Anonymous 1967). The Dutch built sturdy and durable dikes from eelgrass when bricks and concrete were not yet available. The Danes made cigars from eelgrass leaves. The Seri Indians used seagrasses in the making of children's toys.

The contemporary direct use of seagrasses is related to their ability to trap and bind sediments and organic material. Hence, they are used in the filtration of sewage (McConnaughey 1975) and stabilization of coastlines (Boone and Hoeppell 1976). They are potential sources of useful chemicals (Ovodova et al. 1968; Paimeeva 1973; Dudkin et al. 1975), raw materials in paper making (Leopold and Marton 1974), fertilizer (van Breedveld 1966) and fodder (Bauersfekd et al. 1969). Some seagrasses are used for direct human consumption that Ehrlich and Ehrlich (1970) postulated the utilization of seagrasses and the future development of "high yield strains of salt-tolerant terrestrial grasses" to solve the world food problems. There is a great potential for the seagrasses *Enhalus acoroides* and *Thalassia hemprichii* as sources of fodder and fertilizer. Crude protein levels from their leaves reach as high as 23% of dry weight (Fortes 1986b), comparatively higher than those of most terrestrial forage grasses. Low percentages of six-month-old seagrass leaf composts, when mixed with garden soil, appear helpful in the faster growth, better pod fecundity and higher leaf chlorophyll content in mungbeans (Bautista 1987).

THE NEED TO STUDY THE SEAGRASS ECOSYSTEMS IN THE PHILIPPINES

From the viewpoint of productivity, coastal production and their varied roles, the presence of extensive seagrass meadows is favorable and studies on our local seagrasses are most desirable. A greater portion of the Philippine population is living adjacent to coastal waters or to the shores of estuaries. It is almost certain that this percentage will increase in the coming years. As this increase continues, so will the multiplicity of

demands upon the marine environment mainly as producers of food, avenues of transportation, receptacles of wastes, living space and sources of recreational and aesthetic pleasure. Indeed it is no wonder that seagrass beds are being diked, filled, dredged and converted to other coastal uses. At this point, it is imperative that we first know the seagrass resources we have, their role and contribution to coastal stability and productivity. A synthesis of this knowledge is essential for a wise, predictive management of these resources. Only when this knowledge is available can we derive maximum benefits from these nearshore ecosystems.

REVIEW OF LOCAL SEAGRASS LITERATURE: 1819-1991

Despite the high diversity and abundance of seagrasses in Philippine waters, very few papers have been published on the local species. Available records resulted mainly from unsystematic taxonomic accounts done discontinuously from 1819 to 1965. The earliest records date back to the collection of *Cymodocea serrulata* and *Enhalus acoroides* made by Perrottet in 1819 (cited by den Hartog 1970). Blanco (1837) reported *Vallisneria sphaerocarpa* (= *Enhalus acoroides*) from Zambales and the same species was reported by Merrill (1918) from Pa- lawan. Ostenfeld (1909) recorded *Halophila ovata* (= *H. minor*) based on Merrill's collection from Manila Bay. Mendoza and del Rosario (1967) included some seagrasses in their vascular plant compendia.

More collections were made in the early and mid-1900s, but these were primarily incidental accounts on the seagrasses accompanying the seaweeds, which were the main objects of the collections. Pascasio and Santos (1930) did a critical morphological study of *Thalassia hemprichii*, and Domantay (1962) reported eight species from the Hundred Islands in Pangasinan. The "collection" type of study on Philippine seagrasses culminated in 1965 with that made by Doty and Alvarez from the Visayan region.

The subsequent records on local seagrasses started with the find of *Thalassia* from Sipalay, Negros Occidental (Fortes 1977). Calumpong (1979) reported some seagrasses from Negros Oriental. Cordero (1981) illustrated, but with the names erroneously interchanged, three common species of seagrasses. Fortes (1981) made the first local taxonomic and distributional study of algal epiphytes on the leaves of *E. acoroides* . In addition, he (1984) reported *Halophila decipiens* from Zamboanga del Sur. The paper of Menez et al. (1983) represents the most compre-

hensive taxonomic and distributional account of the country's seagrasses. However, it was primarily based on the monograph of den Hartog (1970) and old herbarium materials were used in the description of some of the species. In a more recent work, Calumpong et al. (1985) described the taxonomy and distribution of seagrasses from Davao Gulf.

With the exception of the distributional aspects of the local studies, no other works on Philippine seagrasses could be considered truly ecological. However, Fortes (1981) made the first account on the structure and productivity of the epiphytic community on *Enhalus* leaves at Calatagan, Batangas. He (1982) made a preliminary assessment of the organic matter contributions of dominant producer communities including *E. acoroides* at the same time. A joint Philippine-U.S. research project dealing with the distribution, biomass and taxonomy of Philippine seagrasses was concluded in 1985. In Bolinao, Pangasinan, the first ecological assessment and transplant study in the Philippines involving seagrasses has been conducted (Fortes 1984). This activity has been extended to rehabilitate degraded coastal areas in Puerto Galera, Marinduque, Bataan and Manila Bay. Two completed research projects looked into the identification and seasonality of some chemical constituents in *Thalassia hemprichii*, *Cymodocea rotundata* and *Enhalus acoroides*.

A significant contribution to our knowledge of Asian seagrasses was made by Fortes (1986a) who completed a doctoral dissertation on the taxonomy and ecology of Philippine species. In 1987, the first National Conference on Seagrass Management, Research and Development was held, mainly as an offshoot of a resurging interest and awareness on the importance and role of seagrasses in tropical coastal resource management.

The more recently published contributions to local seagrass literature include those of Estacion and Fortes (1988) on the growth rates and production of *Enhalus acoroides;* Fortes (1988a) on the Indo-West Pacific affinities of the local flora; and Fortes (1988b), on the stresses on seagrass ecosystems in the East Asian region. Fortes (1989) came up with a primer on the importance, status and management perspectives of seagrasses in the ASEAN region.

The latest update on Philippine seagrasses is composed of works that focus on their structural affinities (Rollon and Fortes 1991), associated fish fauna (Vergara and Fortes 1991) and on the management of the resources (Fortes 1990; 1991). Few other works now in press deal with the secondary productivity

and feeding energetics of seagrass-associated fish and invertebrates in Cape Bolinao.

BIOLOGY AND ECOLOGY OF PHILIPPINE SEAGRASSES

Although very little is known about the biology and ecology of Philippine seagrasses, there is a practical need to consider incorporating all available scientific findings into their management. Information on the period of flowering and fruiting, adaptations to different habitat conditions and factors controlling their distribution, abundance and production, are important considerations if one is to derive maximum economic benefits from these plant resources. The general favorability of Philippine coastal conditions for seagrass growth and development is reflected partly in the high diversity of the seagrass flora recorded in the country. This diversity results directly from the varied responses of the species to specific environmental conditions that prevail along its coasts. These biological and ecological responses are the key to their sustained abundance, which, from the point of view of a coastal dweller, is a requisite to their full economic potential as a renewable resource for coastal populations.

Flowering, Fruiting and Adaptations

Little is known about the phenology (i.e., timing of biological events, such as flowering, fruiting, seed dispersal, etc.) of seagrasses in the Philippines. In Puerto Galera, Oriental Mindoro, seed germination in *E. acoroides* generally commences in August when daylight hours are short and mean water temperature is low (Fortes 1986a). During this month, rainfall is high, and there is an average twofold increase in the amount of time the plants are exposed to air and sun. Rapid vegetative growth and increase in biomass immediately follow, with a peak in October, lasting till December, when temperature, tidal exposure and rainfall reach even higher levels.

Flowering in *E. acoroides* is generally initiated in late April and continues until late August. This process is directly related to progressions in daylength, temperature and rainfall. On the other hand, growth, biomass and production in the species are inversely related to such progressions. Fruiting occurs at the latter half of the flowering period, with a peak in July when daylength and rainfall have their highest values.

The general form of a seagrass is in itself the most remarkable features which adapts the plant to its environment. Grasslike

leaves and an extensive root and rhizome system enable it to withstand the impacts of waves, tides and shifting sediments in the shallow coastal habitat. However, certain species-specific features have evolved, which make each plant unique, adapting it to prevailing factors of its environment. These survival strategies, at least under Philippine conditions, are achieved through morphological, physiological and behavioral adaptations.

Morphological plasticity is evident in four species, namely, *Halophila ovalis, E. acoroides, C. serrulata, and Halodule uninervis. H. ovalis* shows five different foliar forms (ecomorphs) which vary markedly in size and shape of the leaves. It is known that smaller-leaved varieties of seagrass species are more abundant in areas frequently subjected to higher temperatures (McMillan 1984).

In *E. acoroides,* form adaptation is observable in two types of population: short, thin-leaved plants comprising sparser populations of shallow open reef; and long, thick-leaved plants comprising denser populations of deeper protected coves. This variability appears to be a nutrient effect brought about partly by topography.

In *C. serrulata*, the two morphological variants are differentiated by the presence or absence of the long, leaf-bearing branches. Johnstone (1982) suggested that stem length in the species was correlated with the degree of water movement. McMillan (1982), on the other hand, postulated that stem length was the result of a selective process in habitats differing in sediment type and/or depth of submergence, or an adaptation to low or high light conditions. These conditions appear as the most probable causal factors as far as data from northern Philippines are concerned. Poiner (personal communication 1986), from his observations on *C. serrulata* and *S. isoetifolium*, hypothesized that "abnormal" elongation of the stem might be a response to crowding and space competition. The three modifications in the leaf forms of *H. uninervis* (i.e., narrow-, wide-,and intermediate-leaf varieties) appear to be a specific response of the species to the nutrient and depth status of the local environment.

In each species, *E. acoroides, C. serrulata, H. pinifolia* and *Halophila minor*, two genotype variants are present, each with its own set of unique environmental tolerances: *stenobiontic*, or that variant exhibiting narrow tolerances along gradients in daylength, tides, rainfall and temperature; and *eurybiontic*, that exhibiting wide tolerances to gradients of these factors. It appears that the eurybiontic character, as well as the annual habit, reside in the

narrow, thin-leaved, sparser *E. acoroides* populations occupying the intertidal portions of open reefs. The stenobiontic character as well as the perennial habit, on the other hand, reside in the wide, thick-leaved, denser populations occupying subtidal habitats and protected embayments.

In many coastal parts of the country, anoxia (i.e., very low oxygen condition) often characterizes portions of shallow intertidal habitats as a result of tidal and wind conditions. Such features prevail during summer when water movement is almost negligible, elevating the daily ambient water temperature to abnormally high levels. Under such conditions, seagrasses are overgrown by thick mats of senescent and rapidly decomposing bluegreen or green algae. Consequently, the sediment becomes highly reducing and acidic, indicated by the smell of hydrogen sulfide gas when the plants are uprooted. Even under such conditions, however, *T. hemprichii, E. acoroides* and *C. rotundata* grow and develop optimally due to an apparent adaptive metabolic strategy, which enables them to colonize successfully such shallow-water marine habitats that have excluded most other plant groups. Interestingly, it is in these habitats where highest levels in crude protein from the seagrasses have been recorded (Fortes 1986b).

Seagrass Abundance, Biomass and Production

The seasonal abundance of local seagrasses is generally bimodal, with highest values in summer (March-May) and in the wet season (July-November) (Fortes 1986a). Biomass, on the other hand, is highest from June - November. Highest biomass value (61.7 gm organic matter per m2) was obtained from *E. acoroides.* Net production in the species was 1.4 gm carbon per day, while its growth rate average was 1.1 cm per day. The recorded mean turnover time in *E. acoroides* is 115 days, which means that the whole leaf biomass is produced after every 16 weeks, forming 2-4 leaf crops annually. For management purposes, these data suggest a year-round supply of organic matter by the seagrass. The maximum rate of 2.41 cm per day obtained in the species at Bolinao is the highest value so far recorded from the Indo-Pacific region.

Seagrass density is directly associated with water temperature conditions. In the Philippines, *T. hemprichii* has the widest range of temperature tolerance. In terms of biomass, however, daylength appears to be the most influential factor, while the number of lowest low tides during daytime plays a primary

negative effect on seagrass abundance, biomass, growth rate and production. This is related to the desiccation factor, which affects plant vigor and vegetative reproduction. Generally, salinity and rainfall are ineffective in directly controlling the above features in local seagrasses.

It is probable that daylength, maximum temperature and rainfall, interacting independently or in combination, make up the critical and primary environmental cues that control the reproductive periodicity, abundance and production of *E. acoroides* in the area. This information would be useful in solidifying the ecological basis of seagrass management in tropical coastal areas.

Seagrasses in Philippine Coastal Food Chains

In the Philippines, the trophic hierarchy involved in the processing and transport of organic detritus from seagrass ecosystems to consumers appears rather intricate. Actual observations, surveys and simple experiments indicate that detri-tivores, herbivores, carnivores and omnivores are all well repre- sented (Fig. 1). However, at this stage of our knowledge on seagrass ecology, our understanding of their relationships is based primarily on qualitative data. Many linkages within the trophic structure remain vague, unquantified or largely unknown.

Organic material in seagrass ecosystems primarily comes from production by the seagrasses themselves. However, contribution from the associated epiphytes and macrobenthic algae (Fortes 1981; 1986a), as well as organic matter from outside the system, i.e., phytoplankton and terrestrial plants, may sometimes be significant. The organic materials are utilized by the fauna either through grazing of the living plant tissues or consumption of the detritus. It is still not known which of these two pathways is more important under local conditions, since the initial linkages between plant production and animal consumption are based largely on direct observation of feeding behavior in the field and, to a very limited extent, under laboratory conditions.

SEAGRASS ECOSYSTEM COMPONENTS AND THEIR USES

A high diversity of plants and animals resides in Philippine seagrass beds. This is due in large measure, to the rich nutrient pool and the high diversity of physical structures that protect juveniles from predation. A large percentage of these biotic components are commercially important. At the component

level, fish and shrimp are probably the most important among these groups, although some coastal villages derive portions of their sustenance from other components of the grass beds. At the ecosystem level, a number of uses and potentials have been associated with the beds. In this paper, only the major components of seagrass beds contributing substantially to the coastal economy of the country, as well as those with important implications concerning conservation, will be mentioned, i. e., benthic seaweeds, epibenthic invertebrates, fish and reptiles and mammals. In addition, some of the ecosystem uses of the habitat will be presented as a strong justification for its total management and conservation.

Benthic seaweeds - Although few seaweeds grow in seagrass beds, they exhibit great abundance and are harvested either as food (e. g., *Enteromorpha* spp., *Ulva* spp., *Caulerpa* spp.) or as a rich source of chemicals for industries (e. g., *Gracilaria* spp, *Gelidiella acerosa, Sargassum* spp., and *Eucheuma* spp). It is of interest that the three largest seaweed-producing countries in the ASEAN region (Philippines, Thailand, Indonesia) produce at least 100,000 tons of dried raw seaweeds worth about US $30 million annually (Rabanal and Trono 1983). The primary components of the harvest are farmed *Eucheuma* and *Caulerpa*, and natural stocks of *Gelidiella* and *Sargassum*.

Epibenthic Invertebrates - This group is composed of shrimps, sea cucumbers, sea urchins, crabs, scallops, mussels, and snails. Shrimp production in the Philippines was 55,700 tons in 1982 (FAO Statistics 1983). This production is directly associated with seagrass beds since it is known that shrimps spend the early stages of their life-history in these areas. Vergara and Fortes (in press) found that of the 1,491 taxa trawled from seagrass beds, fish comprised 28.6% and shrimps, 71.4%.

In Bais Bay, Southern Philippines, the edible mollusks harvested by fishermen yield 69 kg wet weight per hectare of seagrass beds (Alcala and Alcazar 1984). In the same Bay, Alcala (1979) reported that about 1,000 - 2,000 kg of the eggs of the sea hare, *Dolabella auricularia*, valued at US $228-456, were gathered annually.

Coastal inhabitants of the Philippines gather sea urchins, sea cucumbers and other echinoderms from seagrass beds, mainly as supplement to their daily nutrition and income. Gonads from the urchins are usually eaten raw with vinegar, serving as the "caviar" of the coastal Third World. Marketable crabs (*Portunus pelagicus*

and *Scylla serrata*) are frequently caught by trawls in seagrass beds. Scallops and mussels are more common in muddy protected coves where seagrasses and mangroves abound.

Fish - The economic usefulness of a seagrass bed rests primarily on the fisheries it supports. In developing countries, coral reefs with their associated seagrasses potentially could supply more than 20% of the fish catch (McManus 1988). A total of 1,384 individuals and 55 species from 25 fish families have been identified from 5 seagrass sites in the Philippines (Vergara and Fortes, in press). All members of these families have economic values mostly as food and aquarium specimens. Estacion and Alcala (1986) reported adults of about 52 fish species from 31 families from seagrass beds in Central Philippines. Carangids, clupeids, lutjanids and scarids, all amply represented in the catch, are popular food fishes. At least 123 fish species representing 51 families have so far been reported from both natural and artificial seagrass beds in the Philippines (Fortes, unpublished report). All the species have known economic uses.

Reptiles and Mammals - Some endangered species of reptiles and mammals have been reported in Philippine seagrass areas. Among these species, the green sea turtle (*Chelonia mydas*), the olive ridley (*Lepidochelys olivacea*), the loggerhead (*Caretta caretta*) and the flatback (*Chelonia* depressa), together with the wart snake (*Acrochordus* granulatus) are frequently found in dense seagrass meadows (IUCN/UNEP 1985). Sea turtles at the Turtle Islands off Sulu Sea include seagrasses in their daily diets (Estacion and Alcala 1986). The sea cow (*Dugong dugon*), a mammal considered an endangered species throughout its range in the region, feeds directly on seagrasses, especially *Cymodocea* and *Thalassia*.

At the ecosystem level, seagrass beds have the potential to filter sewage, thereby reducing the threat from pollution which would otherwise affect the components of both coral reefs and mangroves. Seagrasses are biotic heavy metal reservoirs or sinks in the marine environment (Wahbeh 1984). But unlike the abiotic sediments, which also act as heavy metal sinks, the seagrass bed may remobilize and transport these elements to higher trophic levels in the food chain (Burrell and Schubel 1977). The habitat is also known to stabilize the coast mainly due to its ability to trap sediments via the extensive mats of root and rhizome systems.

Actual observations and simple experiments indicate that detritivores, herbivores, carnivores and omnivores are all well represented in the trophic hierarchy involved in the processing and transport of organic detritus and nutrients from the ecosys-

tem to consumers even in other nearby ecosystems. It is now indisputable that some seagrass beds in the Philippines are nursery grounds for a number of commercially important fishes and invertebrates. In the Pacific Northwest, there has beer. a drastic 90% - 99% decline in black brant geese population since 1981, due to the disturbance and noise from boats near the large eelgrass meadows where the geese thrive (Reiger 1982).

SUSTAINABILITY AND VULNERABILITY OF THE SEAGRASS ECOSYSTEM

Primarily because of the shallow existence of seagrass beds, they are susceptible and vulnerable to natural stresses, as well as to acute and sometimes chronic perturbation from man and his needs. In coastal Southeast Asia, cyclones, typhoons, tidal waves, volcanic activity , pests and diseases, population and community interactions (grazing and competition) constitute the natural stressors to this ecosystem (Fortes 1988b). On the other hand, seagrass beds have been the objects of relentless pressure associated with man's basic needs for food production, transportation, waste disposal, living space, recreation and aesthetic pleasure. Unfortunately, not all of these uses are compatible and in many cases, they are mutually exclusive (Fergusson et al. 1980). From the point of view of management and conservation, environmental damage and degradation brought about by natural causes may not be as directly related to man's actions, attitudes and inadequacies. Hence, they will not be given emphasis in this part of the paper. What will be emphasized are the stresses under man's control. It is unfortunate that in Southeast Asia, the effects of the latter are largely unquantified and unmitigated.

In the Philippines some mesoscale changes are becoming easily observable in seagrass areas. Hence, we see sudden changes in their species composition, stunting of growth, phenological alterations in relation to seasons and movement of ecosystem boundaries. These changes are symptoms or large-scale cumulative effects of subtle, unobservable alterations in the biological make-up of the units that compose the four different levels of organization in the biotic environment. These levels are: (1) biochemical and cellular; (2) organismal; (3) population; and (4) community. The responses of organisms to degradative

perturbations, categorized under these levels, are given below (modified from Capuzzo 1981):

(1) CELLULAR
- gametogenic cycle
- nutrient storage
- deformity
- neoplasms and tumors

(2) ORGANISMAL
- feeding
- excretion
- growth
- fecundity
- reproductive effort
- larval viability
- swimming

(3) POPULATION
- biomass
- productivity
- age/size structure
- recruitment
- mortality

(4) COMMUNITY
- biomass
- species abundance
- species distribution
- species diversity
- trophic interactions
- energy flow
- spatial variability
- temporal variability

Signs of susceptibility and vulnerability on the part of the components of seagrass beds in relation to environmental impact can be manifested at each of the levels of organization before disturbances are seen at the population or community levels. Hence, impairment of feeding, growth, recruitment, development and energetics resulting from disruptive activities of coastal inhabitants may result in alterations both in reproductive and developmental successes and changes in community structure and dynamics. This in turn would manifest itself as the observable advance or retreat of the physical boundaries of the ecosystem. In addition, remarkable changes in the structure and species composition and spatial or temporal distribution occur.

Disruptions in the responses at a lower level of organization do not necessarily result in degeneration at the next higher level. Only when the compensatory or adaptive mechanisms at one level begin to fail do deleterious effects become apparent at the next level (Capuzzo and Kester 1987). The "wasting" disease that almost completely decimated the eelgrass (*Zostera*) beds of the North Atlantic in the early 1900s attests to the weakness of such compensatory mechanism in a seagrass ecosystem. While the exact cause is not yet fully understood, the death of the beds appears to be related to a major and abrupt change in the climatic

(temperature) regime (Rasmussen 1977) or to the amount of increasing pollution (silt, toxic materials, e. g., heavy metals, pesticides, PCB, detergents) carried by the river Rhine, which empties into the Waddenzee (Phillips and Menez 1988). There are at present indications that a similar catastrophe might occur in Southeastern United States (Fonseca, personal communication). Lewis et al. (1985) documented the loss of 80% of the seagrass stands in Tampa Bay, Florida, from 1880 -1980, which was due to the decline in water quality (increasing turbidity, toxicity) coincident with the influx of people into the area. On the other hand, the "Amoco Cadiz" oil spill in the coast of Roscoff, France, in 1978, demonstrated that the seagrass ecosystem was a resistant unit, the oil pollution causing only a short-term change in diversity without really affecting the stability of the ecosystem (Jacobs 1982).

Each species or small association of seagrasses grows between specific tidal levels. The long-term variations in sea level should lead to a migration of these plants to maintain their position relative to these levels. The primary cause of the decline of seagrasses in Moreton Bay, Queensland, was sand movement towards seagrass beds, physically smothering and displacing them to greater depths beyond their ability to survive (Kirkman 1978).

PERSPECTIVES AND ADVANCEMENTS IN SEAGRASS ECOSYSTEM MANAGEMENT

Institutional Linkages

The last two decades saw a rapid increase in pressure on the shallow coastal resources of the Philippines. This resulted largely from the increasing industrial and commercial development of the country's shorelines, coupled with the multifaceted demands of the population for food, transportation, waste disposal and living space. Fortes (1988b) mentioned the natural and man-induced stresses on seagrass habitats in East Asia, implicating the need for intensive and sustained investigations of these resources. Indeed, if one considers the fundamental functions of seagrass ecosystems in mitigating, if not completely solving, the coastal environmental and socio-economic problems of the ASEAN region, it would be worthwhile, even imperative, to manage the resource. The marked dependence of ASEAN countries upon their marine resources makes the improvement of marine environmental quality a policy objective common to the countries of the region.

Management policies and conservation projects specific on seagrass ecosystems are yet non-existent in the Philippines. However, in 1986, UNDP/FAO, in association with UNEP, has formulated for the region a project on coastal fisheries rehabilitation through seagrass transplantation. A regional study under the ASEAN-Australia Coastal Living Resources Project is currently investigating the structural and functional aspects of local seagrass resource. The Environmental Management Bureau of the Department of Environment and Natural Resources has embarked on projects which, together with that initiated with MARCOPPER to rehabilitate the mine tailings causeway at Calancan Bay, would serve as a framework for coastal protection with seagrasses playing a major role.

Among the ASEAN countries, only the Philippines, through inter-agency cooperation, has formulated a National Seagrass Management Program and proposed the creation of a Philippine National Seagrass Committee. These steps are aimed at optimizing the use and conservation of seagrass systems. The Program is envisioned to consist of five parts, namely: resource mapping and survey; research and development; information dissemination, education, training and publication; environmental management; and policy and legislation.

Mapping of Local Seagrass Resources

Mapping of seagrass areas for coastal management purposes has been undertaken successfully in some parts of the Philippines. More importantly, the identification of the centers of distribution of seagrasses in Bolinao Bay and in other study sites would indicate the greater probability that these areas are nurseries for juveniles of some economically important species of vertebrates and invertebrates. In addition, they could be rich collecting grounds of these species. For practical purposes, the data would facilitate the classification of seagrass beds for coastal zoning and conservation purposes. Their availability makes ground truth surveys more economical and efficient.

For selective protection and use, the seagrass beds of the Philippines were classified (Fortes 1986c) into categories on the basis of the degree and nature of alteration to which they were subjected and their general community response to specific habitat conditions. Hence, seagrass areas may be *pristine, disturbed, altered* or *emergent*.

Pristine seagrass meadows are those with high or low diversity of species, bordering land masses far removed from

human habitations, disturbed only by the normal intensity of natural elements. These meadows form climax assemblages in shallow waters, usually dominated by *E. acoroides, T. hemprichii* and *C. rotundata* . This type of habitat should be preserved and protected from any form of alteration, to be made available only for scientific and educational purposes.

Disturbed seagrass meadows are highly or lowly diverse beds occupying bays and coves, adjacent to human habitation. These are the constant objects of man's activities and impacted by domestic and industrial effluents. These meadows may yield the highest biomass, protein levels and production rates and should be the subject of effective mitigation measures.

Altered seagrass meadows are low species diversity areas, permanently and completely changed or converted to other coastal uses, like salinas and fish or shrimp ponds. They can be reconverted into seagrass areas through massive transplantation and rehabilitation. This type of seagrass habitat should be the subject of proper multiple use programs.

In the "emergent" category, seagrass community diversity is low, controlled in large part by extreme physico-chemical conditions. *Ruppia maritima* and *Halophila beccarii*, which form extensive growths in almost freshwater or in brackishwater, and terrestrial macrophytes and herbs may co-exist with the seagrasses.

At six sites in the country, seagrass surveys yielded a total area of 50.88 km^2 (Fortes 1989). The seagrass areas at the sites may be broken down as follows:

	Seagrass area, km^2			
Site	Total	Pristine/%	Disturbed/%	Altered/%
Bolinao Bay	37	0	37.00/100	
Pagbilao Bay	1.89	0	1.89/100	
Puerto Galera	1.14	0	0.87/ 76	0.27/24
Calancan Bay	0.12	0	0.07/ 58	0.05/42
Ulugan Bay	2.97	0.50/17	2.25/ 76	0.22/ 7
Banacon Is.	7.81	0.20/ 3	7.61/ 97	
TOTAL %	50.88	0.7/1.4	49.69/ 98	0.54/0.6

Although comprising a very small percentage of the total coastal area in the Philippines, the six study sites, among a few others, have been chosen to represent the wide range of habitat differences characterizing the marine environment of the archipelago. In addition, these sites are being subjected to the various impacts of both natural and man-made stresses. Hence, only Ulugan Bay (Palawan) and Banacon Is. (Bohol), areas relatively removed from human habitations, have semblances of pristine seagrasses. On the other hand, almost all of the seagrass areas surveyed are markedly disturbed.

Seagrass Bed-Coral Reef-Mangrove Ecosystems Interactions

While much has been done to understand coral reefs and mangroves (but much less, seagrass beds), information regarding interactions among these marine ecosystem "triumvirate" remains fragmentary and decidedly inadequate. However, there is a rapidly increasing consensus among marine scientists that in order to achieve any sustainable form of renewable resource development, more emphasis must be given to the interactions at the ecosystem level, rather than at the lower levels of organization of the marine environment. Burbridge (1988) agreed when he emphasized that such an objective was attainable if priority was given to the study of factors, such as the effects of changes in the hydrology of rivers, conversion of swamp forests to agriculture, the reclamation of mangroves for fishponds and their impact on marine fisheries and other activities. The prevailing sectoral approach to marine resource management and conservation is based upon a single exclusive purpose, disregarding the fundamental concept of biological continuum, which is the key to the integrity of the marine environment as an integrated macroecosystem. Indeed it is increasingly being realized by the scientific circles that one of the causes of failures in marine resource management has been the failure of leaders and investigators to view the marine habitats as one interacting macroecosystem.

An integrated study of seagrass-mangrove-coral reef ecosystems is the most practical approach towards the sustainable development and conservation of these ecosystems. It treats the resource-base as a dynamic system where biophysical, socio-cultural and economic factors continuously change and interact. From the discussions presented, it is clear that many species of fish, invertebrates, birds and mammals move between mangrove

forests and seagrass beds, exchanging energy and energy sources. Tidal flushing and faunal feeding and movement extend the sphere of influence of seagrass beds and mangroves well beyond their physical boundaries. To save the mangrove trees would do the gray snapper little good if the associated seagrass beds were destroyed. Pink shrimp populations would be enhanced by the preservation of seagrass beds and mangrove- lined waters. Indeed, a most plausible (but for a poor maritime country like the Philippines, a most impractical) conclusion is that the best management practice is to preserve Philippine seagrass and mangrove ecosystems. Central to this concept, however, is the preservation of adjacent ecosystems that are linked significantly by functional processes. In Southwest Florida, the continued and enhanced role of the mangrove forests is highly dependent on the continual and effective preservation of the Everglades and Big Cypress Swamp.

Seagrass Role in Environmental Impact Mitigation and Habitat Restoration

In developing countries, resource management decisions are normally compromises, which place higher premium on economic imperatives at the expense of the environment. Hence, destruction of portions of seagrass areas are sanctioned with some specified precondition to at least mitigate the impact in order to compensate for the unavoidable loss of important ecosystem components. At this point, two considerations must be made. First, if any biologically productive habitat is destroyed, it is improbable that its original productivity can be returned. This is because the biological and physico-chemical interactions, which took hundreds of years to evolve and be developed among its components, are permanently disrupted. This is true even if a new habitat is established elsewhere within the system (Thayer et al. 1984). Secondly, mitigation rarely creates a new and similar habitat where the original components would normally thrive. What current mitigation achieves is often a transitory enhancement, since no long-term additions to the system are realized. Only the creation of an entirely new, previously biologically desolate aquatic habitat (that has not been created at the expense of another biologically productive habitat) can be considered and allowed as true mitigation.

Transplantation

Seagrass transplantation has been developed as a viable means to initiate the recovery or restoration of degraded or destroyed coastal habitats. Restoration techniques using seeds, seedlings and vegetative materials offer a great potential for investigating basic biological problems, which may prove useful in understanding biotic responses to degradative impacts. With this information, techniques could be developed to recolonize areas, which have lost their plant cover or to create new areas, which would restore and create associated plant and animal communities that lead to food resources.

Phillips (1980), applying most known techniques in seagrass transplantation, found differential success according to the species and methods used. He concluded that the sod method provided the greatest potential in the establishment and growth in all species tested. In the Philippines, Fortes (1984) initiated the first local transplantation of seagrasses as a means to rehabilitate highly silted areas in Bolinao Bay, Pangasinan. In 1986, FAO-NEPC tested the transplant and rehabilitation potentials of some selected seagrass species against pollution from phosphate and copper mine tailings, and domestic industrial effluents. However, these two undertakings were basically experimental so that the results could not provide conclusive data as to the true restoration potentials of the seagrasses. In Calancan Bay, Marinduque, existing eight-month-old transplants (using plugs of *Halodule uninervis,* sprigs of *Cymodocea rotundata,* and seedlings of *Enhalus acoroides*) are showing highly positive indications (in terms of spread, mortality and growth rates) that the methods are potentially useful in rehabilitating a portion of the tailings causeway.

Artificial Seagrass Systems

A new approach that is gaining interest and acceptance in coastal restoration ecology utilizes artificial seagrass systems (ASS). Indirectly, it has been used to enhance the biological productivity of coasts (Barber et al. 1979) and sediment stability, especially of degraded fishery habitats. Apparently, the enhancement effect of the system is achieved via the creation of more ecological niches available for occupancy mostly by juveniles of fish and invertebrate fauna. In addition the epibiota that grow on

the system are direct food sources, while the system itself serves as a refuge and spawning ground, especially for fauna of economic value. It could also be surmised that the physical components of the ASS act as effective and durable structures in stabilizing the sediments.

Bell et al. (1985) have shown that ASS attract vagile macrofauna typical of real seagrass beds. Hill (1988, personal communication) has demonstrated that strips of solar mat (plastic material used in heating swimming pools) are effective as ASS. Using a slightly modified ASS established at 5 m depth in a highly silted area, Fortes and Espinosa (unpublished manuscript) found 986 fish individuals representing 70 species and 27 families in Bolinao Bay (Pangasinan) from June 1988 -November 1989. Interestingly, these figures are significantly higher when compared to those they found from four natural seagrass sites in the bay for the period (229 individuals, 29 species from 24 families). These results indicate that the artificial structures indeed have great potential to attract fish. Of further interest are the percentages in species overlap between the two systems (in terms of fish families = 47.1%; species = 20.2%), which suggest that ASS support a relatively distinct ichthyofaunal composition. However, results from morphometric measurements also suggest that mostly juveniles to medium adults comprise the fish fauna. This finding confirms the nursery role of both systems.

As an important component of the Calancan Bay Rehabilitation Project in Marinduque Island, the use of ASS has been intensified primarily to attract fauna and improve the biodiversity of the lower western half of the mine tailings causeway at the bay. The system makes use of cheap plastic and PVC materials, constructed to simulate stands of *Thalassia hemprichii, Halodule uninervis* (wide-leaf variety) and clumps of *Enhalus acoroides*. Monitoring of the relative success of the system in terms of fish and invertebrate recruitment, yield and epibiotic composition has recently been initiated. A cost-benefit analysis is being made to evaluate the economic feasibility of the system.

MANAGEMENT GOALS AND NEEDS

It is unfortunate that there have been practically no significant positive results that lead to the conclusion of coastal resources'

successful management and conservation. Such is the case although schemes to manage the coastal resources have been initiated and implemented for some time in the Philippines. With seagrass ecosystem in particular, similar efforts are as yet at the very initial stages; but there are indications that these systems will not be prone to the fate, which has befallen mangroves and coral reefs. More importantly, the sets of activities proposed by Fortes (1987) for the research and development (R and D) of seagrass beds are largely applicable to that of mangroves, making management goals and needs closely similar for these two ecosystems. Indeed this striking similarity between these two closely evolved systems directly implies similarity in approach to understanding their responses as an integrated system to environmental impacts.

Management Goals

In retrospect, management and conservation of Philippine seagrass beds as an ecosystem would tend to be difficult. This is in view of the current state of knowledge people have on the fundamental interactions that connect seagrass, mangroves and coral reef ecosystems. In addition, people in a developing country like the Philippines tend to place a higher premium on economic gains, no matter how short-lived these are, at the expense of marine environmental imperatives and without regard to the costs of ecosystem rehabilitation and restoration. The need arises for management and conservation of these resources along major relevant goals. These goals include:

1. To preserve the natural interconnections known of the ecosystems;
2. To protect their ecologically valuable and economically harvestable fisheries;
3. To protect the coastlines from erosion, siltation and pollution;
4. To establish seagrass, mangrove, coral reef reserves for research and educational purposes; and
5. To preserve the aesthetic and recreational qualities of the natural shorelines.

Specifically, the key areas of research in the management and conservation of seagrass beds include:

1. Acquisition of baseline data on their inter-connections and the quantification of these interactions with coral reefs and mangroves;
2. Time-series study on the stability dynamics of the ecosystem anchored on a thorough understanding of their biophysical and hydrological conditions;
3. Mapping of seagrass beds using remote sensing technology and ground truth surveys in order to pinpoint areas suitable for restoration;
4. Studies on the ecosystem-level functions in restored or rehabilitated seagrass areas;
5. Studies on the vulnerability of the ecosystem and their components to and their recovery from human impacts; and
6. Coordination of all technical information on the ecosystems and their resources.

Socio-Political Needs - The environmental crisis in our coastal zone is largely rooted in an improper system of Philippine socio-cultural values. It arises directly from the tendency of policy-makers to deal with environmental problems mostly with curative rather than preventive measures embodied in a framework of inconsistent policies. In order to obviate this problem, certain pointers, some of which are not uncommon in marine resource management circles, need to be emphasized:

1. To ensure that local decisions and actions on ecosystems management are based on local perspectives;
2. To make the public aware and appreciative of the intrinsic value of the ecosystems in relation to the total environment; to make the public initiate positive action for the cause of the few who make extra effort to properly manage and conserve these ecosystems; to make the public sympathetic to such cause;
3. To formulate sound legislation that would prohibit specific activities disrupting the ecosystem connections and degrading their resources;

Management Needs

To effectively address the above management goals, certain specific needs arise. These necessary factors or attributes are based on the dictum: SOUND ECOLOGY IS GOOD ECONOMICS. Primarily they can be divided into technical or scientific needs and administrative or socio-political needs.

Technical Needs - Management and conservation within the physical boundaries of seagrass beds, mangrove forests and coral reef cannot succeed outside a sound ecological framework. Nor is the "lifting" of oceanographic principles, largely developed to manage and conserve temperate coastal resources, an adequate basis for the management of these resources. Management planning requires adequate field data for each specific ecosystem for the identification of those interconnections, dynamic qualities and populations which need protection, as well as the explanation of how these may be vulnerable to disruptive human influences. Persons primarily involved in the management process must base decisions on solid scientific grounds, not solely on the laws of short-term economics. Otherwise, remedial expense or irreversible losses will be encountered.

Technically, what is required is to identify and initiate studies in representative natural seagrass, mangrove and coral reef ecosystems in regional and local areas, so that we may know what constitutes harmful activity or even change in the ecosystems. Without baseline data obtained from the few remaining natural and unspoiled systems, we will never have a measure of human impact on these systems and will never attain wise management and stewardship of their resources.

In developing countries, it is not a sound policy to advocate the physical separation of the users from their source of nutrition and livelihood. However, the continued availability to these people of the resources depends on the maintenance of natural genetic and species diversity in these systems. Biological productivity depends largely on the availability of nutrients, light and temperature. Hence, the stability of the systems becomes a function of the stability of these factors and the genetic and species diversity present. Human interventions, such as farming of the desired species, may increase the short-term productivity of ecosystems, but they may also lead to an extinction of genotypes and species or to the spread of less desirable ones.

4. To encourage and facilitate the coordination of all information with major coastal projects;

5. To incorporate a holistic approach in planning for both scientific research and decisions that are related to the marine ecosystem;

6. To encourage and facilitate the cooperation among institutions and social groups on marine environmental issues;

7. To choose strategies that do not equate management with control; and

8. For the educational system to inculcate environmentalism at all levels, as well as the practice of environmental ethics.

To effectively manage and conserve the seagrass ecosystems, it is necessary to relate them with management problems of associated nearshore habitats and adjoining tidal lands. It is the natural movement of water that provides the fundamental connections between the two ecosystems. Hence, in the planning process, it should be recognized that some activities in these associated areas can have far-reaching effects through their influence on water quality.

Seagrass beds must be managed on the basis of a philosophy of conservation. Most importantly, it should begin by preventing further degradation and loss of existing ecosystems while accommodating traditional and contemporary needs, with adequate provision of reserves suitable for protection of the biodiversity within them. These unexploited areas may serve as a refuge for fauna and flora and as sources of materials for restoring areas in which management strategies failed.

As renewable resources, seagrass ecosystems must be managed on a sustainable use basis. This concept places economic benefits at par with the maintenance of the ecosystems as close to their original state as possible. However, depending on established priorities, a compromise allowing sustainable yield and reasonable resemblance to an undisturbed system may be reached. During the First National Conference on Seagrass Management Research and Development in December 1986, Fortes (1987) proposed an R and D program for seagrass ecosystems and their resources. The central issue in the proposal was multidisciplinarity in approach because resource manage-

ment "...begins with the ecologist's discussion of the productivity of the ecosystem, continues with the lawyer's discussion of the legal nature of resource utilization, includes the economist's concern for efficient use of equipment and facilities and the politician's concern for proper allocation of the shore among the users."

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Managing the Philippine Mangal for Long-Term Human Survival

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INTRODUCTION

The mangal is one of several critically significant ecosystems that characterize the country's environment. It is considered a minor type, compared for instance to the mixed lowland rainforest ecosystem type, in that it occupies only a very small proportion of the total forest cover of the country. However, it is also of paramount importance to Filipinos in innumerable ways, especially to those who live in the coastal areas.

Economically, the mangal has been, still is and will be directly beneficial (Brown and Fisher 1920; Aguilar 1949; Francia et al. 1971; Bumarlong and de la Cruz 1976; Robillos 1976; Bagawan and Francia 1979; Amio et al. 1979; Gonzales 1980; Arroyo 1982; Lorica and Fortes 1982; Manas 1982; Redoloza 1982; Tesoro 1982; Zamora 1982, 1984, 1986, 1987, 1988, 1989, 1990; Escolano 1987). Specifically, the mangal is an important source of the following forest products: (1) poles for house construction (traditional use); (2) firewood (fuelwood) for cooking (traditional use); (3) charcoal as domestic energy source and as source of income; (4) tanbark for coloring tuba (fermented nipa juice); (5) nipa sap for various uses (i.e., tuba, vinegar, alcohol, sugar), both for domestic use and as source of income; and (6) nipa

shingles (for roofing and walling) for domestic use and source of income, source of fishery products (mangrove-dependent species), such as adult forms (finfishes, shellfishes, shrimps, mangrove crabs) for domestic consumption as well as a source of income and juvenile forms (fry) for food (domestic use) and for stocking brackishwater fishponds (as source of income). Mangal areas are also sites for human activities other than forest product gathering, fishing or gleaning industrial waste disposal, human occupancy, farming, commercial and industrial establishments and fishpondification. [Fishpondification was introduced into the literature by Zamora (1988) to refer to the massive conversion of mangal into brackishwater ponds for rearing marine animals for food production.]

The mangal is likewise beneficial indirectly to the coastal dwellers (Ronquillo and Llana 1976; Anonymous 1977; Gabriel 1977; Atchue 1978; Fortes 1982; Fortes and Jara 1987; Zamora 1979, 1982, 1984, 1985, 1986, 1987, 1988, 1989, 1990; Arroyo 1984; NMC Technical Staff 1986; Serrano and Fortes 1987; Paw and Chua 1988; Florido and Miclat 1988). For example, there are strong indications that the mangal: (a) exports detritus and nutrients, which form the food base of a complex of marine organism [This in turn supports valuable estuarine and nearshore fisheries, such as finfishes, shellfishes and crustaceans (Gomez 1983; Reyes 1983; Zamora 1987, 1990)]; (b) serves as feeding, nursery or spawning grounds for economically important marine fishes (Motoh and Solis 1978; NATMANCOM 1982; Pinto 1985); (c) serves to protect valuable properties by dampening storm surges and high winds associated with tropical typhoons (About 20 typhoons visit the Philippines each year. While the mangal coastal barriers may be battered and damaged in severe storms, they will grow naturally without cost to man.); (d) performs a flood reduction function (where it occupies estuarine floodplains; This function may be lost if the mangal is filled up and converted into other uses.); (e) helps prevent erosion of banks of rivers, thus, protects properties adjacent to the mangal; (f) serves as habitat for wildlife and provides

valuable opportunities for education and scientific study (While putting monetary value on these wildlife-based activities is difficult, they are nonetheless significant uses which add to the importance of mangal.); and (g) improves water quality by taking up suspended solid matter and floating materials (Zamora 1990).

STATUS

Areal Extent. The National Mapping and Resource Information Authority (NAMRIA) updated the Philippine mangrove area statistics by manual (visual) reinterpretation. The 1987 SPOT satellite data using the scale 1:100,000 was enlarged to the scale of 1:50,000.As a result of this activity, NAMRIA reported that the mangrove area for the Philippines came to 139,725 hectares, as may be gleaned from Table 1.

Geographical Distribution. The rough geographic distribution of mangroves in the Philippines is shown in Figure 1. Table 2, on the other hand, shows a summary of the mangrove areas in the four major islands for 1972, 1982 and 1988. According to the most recent statistics (1988), mangrove distribution in the major island groups and their percentage equivalents are as follows:

Islands	Hectares	Percent
Luzon	22,700	16
Visayas	37,325	27
Mindanao	37,400	27
Palawan	42,300	30
Philippines	139,725	100

Of the estimated total mangrove forest as of 1 December 1984, approximately 49% is reproductive brush, 49% is young growth and 2% is old growth. Old growth stands remain only in Mindanao (4,582 hectares) and Palawan (5,317 hectares).

Economically Important Species of Flora and Vegetation. The known vascular flora of Philippine mangroves are categorized into

four groups, following Tomlinson's (1986) criteria: (1) major mangal elements (19 species, Table 3); (2) minor mangal elements (12 species, Table 4); (3) mangal associates (40 species, Table 5); and (4) specialized elements (24 species, Table 6). There are 95 species altogether of vascular plants in Philippine mangroves.

Zonation. Several zonation patterns of the Philippine mangroves have been described. A compilation of this information is shown in Table 7. The distribution of species within zones is probably a function of substrate type and salinity. However, there has been severe disturbance through most of the country and much scrub mangrove may not have recovered to the original species. Those species, which are active primary colonizers of treeless sites, are sometimes referred to as "frontal" species. In particular, *Rhizophora stylosa* colonizes coral rubble and sandy sites; *Avicennia marina* and *Sonneratia* spp, sandy loam to loamy sites; *R. mucronata, R. apiculata,* silty and clay loam river sites.

Wood Products. The most economically important species belong to the first group (major mangal elements). These species are sources of construction or building materials, fuelwood (firewood) and charcoal.

Non-wood Products. Some of the major mangal elements are sources of non-timber products like tanbark, nipa sap and thatching materials.

With regard to the volume of standing timber in the existing mangrove forest, the Forest Management Bureau (FMB) reported the following statistics in 1982: (1) Luzon, 308,000 cu m; (2) Visayas, 1,375,000 cu m; and (3) Mindanao, 2,626,000 cu m.

Economically Important Species of Mangrove Fauna. Mangrove- dependent species of economic value include: (1) finfishes (milkfish, mullets, gobies, others); (2) crustaceans (shrimps, mangrove crabs); and (3) molluscs (oysters, mussels, clams). There are five types of natural fisheries-dependent fishermen in the country: (1) shell gatherers; (2) fishermen; (3) crabbers; (4) nipa sap gatherers; and (5) baroy gatherers. Production statistics on mangrove fisheries may be gleaned from Camacho and Malig (1988).

Ecological Values of Philippine Mangal. An important role of the mangal is that it contributes immensely to the provision of

nutrients needed by fishes, shellfishes and crustaceans. Mangal achieves high productivity due largely to its ability to grow in areas with high solar radiation and its ability to take up freshwater from salt. However, local studies on this important aspect of mangrove ecology are wanting. Data on litter production and decomposition and nutrient transport are available through the studies of Fortes and his students, which were done in Calatagan (Batangas) and Pagbilao (Quezon) (Tables 8, 9). Fortes (1982) found that the major communities in a mangrove-reef flat in Calatagan contributed about 2.49 gm C/sq m/day to the food chain. Of the producer communities, the mangroves (leaves of *Rhizophora apiculata* and *Avicennia marina*) contributed the highest organic carbon to the nutrient pool, i.e. 1.38 gm C/sq m/day or 58% of the total output. In Pagbilao, the four dominant mangrove species were shown to have high annual organic matter yield (gm/sq m), thus:

Species	Organic Matter Production gm/sq m/yr
Avicennia officinalis (Api-api)	522.24
Ceriops decandra (Malatangal)	428.57
Scyphiphora hydrophyllacea (Nilad)	553.63
Osbornia octodonta (Taualis)	422.52

These values are fairly high, considering that mangrove trees grow naturally without energy subsidies, like fertilizers, and that they are not subjected to selective weeding and other means of yield improvement.

CONVERSION USES OF PHILIPPINE MANGAL AND THEIR PROBABLE IMPACT

In 1920, Brown and Fischer placed the area of the mangrove swamp forest of the country between 400,000 and 500,000 hectares (average of 450,000 hectares). In 1988, Bina placed the total mangrove area at 139,725 hectares. If we subtract the 1988 estimate (139,725 hectares) from the 1920 estimate (450,000 hectares), the difference comes to 310,275 hectares representing the denuded mangrove area over the past 71 years (1920-1991). In other words, nearly 70% of the total mangrove forest is

denuded, while nearly 30% remains covered by mangrove vegetation. Thus, based on the foregoing fact, the denudation rate of the mangrove forest cover of the country comes to 4,325 hectares per year. As may be gleaned from Figure 2 (Zamora 1990), a large portion of the denuded areas (which comes to more than 210,457 hectares out of the 310,275 hectares or nearly 68% of the total denuded mangrove areas) is now devoted to brackishwater fishponds (BFAR 1987). It is for this reason that the conversion of mangrove swamps into capital-intensive brackishwater fishponds is considered the more controversial issue in mangal development planning and management.

The increase in areal extent of fishponds and the concomitant decrease in areal extent of mangroves have caused a growing concern among environmentalists that further fishpondification may impose significant negative ecologic, social and cultural impact on the nearshore and nearby oceanic systems of the country. National and international conferences during the last decade (1977-1988) have arrived at the consensus that fishpondification has contributed to the decline in the yield of other mangrove products. Camacho and Bagarinao (1986), analyzing 1976-1982 data of the Bureau of Fisheries and Aquatic Resources (BFAR), found that municipal marine fish production was positively correlated with the area of existing mangrove swamps (see also Paw and Chua 1988; Mastaller 1989). They concluded that the levelling off of marine fish production during the past 10 years was in part due to the drastic alteration or destruction of the mangrove environment around the ocuntry (Zamora 1990).

As written earlier, the mangrove ecosystem has many functions, among which are nutrient export, breeding or nursery ground function and erosion control. All these are important to the maintenance of ecological stability of the ecosystem itself, as well as the surrounding nearshore ecosystem and nearby oceanic system (Zamora 1990).

For fishponds to function, the developer must change the mangal from a natural state to an artificial state. Once an area is converted into a fishpond, it can no longer function as a natural system. Thus, as a hectare of fishpond is improved, that same hectare can no longer be counted on to contribute to the productivity of the nearshore ecosystem. Thus, continued reduction of mangrove forest area is predicted to have the following

harmful effects: (1) decrease in nutrient export, which also affects the food chain of man; (2) decrease in area available for protection and nursery grounds for seed fish (fry) needed for stocking fishponds; and (3) decrease in the catch of the other fishery sectors (Fig. 3, Zamora, 1990). Consequently, the livelihood of a significant segment of the coastal population will be affected. These include: (1) 522,418 sustenance or municipal fishermen; (2) 42,947 commercial fishermen; and (3) 170,000 fry gatherers. Even the 12,660 fishpond operators will be affected (Fig. 4, Zamora 1990) for both the "adult pool" (mature forms) and "replacement pool" (fry for stocking fishponds) . The effect may lead to "crashes" of the populations and ultimately when large numbers of populations are affected, the mangal itself will be affected. The continued proliferation of fishponds along the coastal areas means the clearance of more vegetated areas and subsequent diking and fertilization of the denuded areas. The disturbance of the mangal is said to be maximal because: (1) all standing biomass is completely removed; (2) soil profile is totally disrupted or covered up; and (3) subsequent natural regeneration cannot take place (Zamora 1990). Yet the effects of this artificial fish production technology on natural fisheries are not clearly understood.

If it is assumed that certain stages of the life cycle of some commercially important species of fish and shellfishes require the protection of the mangal, then it follows that fishpondification will affect natural fisheries. While quick and lucrative economic returns and benefits form fishpondification contribute to the escalation of protein production, fishponds still need fish and shrimp fry. The shallow littoral areas near mangrove areas are still the principal natural sources of seed fish for stocking fishponds (Zamora 1990).

Furthermore, if it is assumed that four hectares of mangrove forest are required (Hamilton and Snedaker 1984) to support one hectare of intensive oyster culture, i.e., ratio of 4 mangrove forest: fishpond, then it follows that further clearing of mangrove forest for fishpondification purposes will affect mariculture. At present, the country has only 139,725 hectares of mangrove forest, and 310,275 hectares of denuded mangrove area or close to a ratio of 1:4. Said ratio is the reverse of what is claimed to be needed to support intensive oyster culture (Zamora 1990).

MANAGEMENT STRATEGIES

In view of available data and information on the present state of the mangrove forest, the government became greatly concerned and acted accordingly by formulating and implementing measures directed toward the: (1) protection and conservation of the remaining mangrove forest areas; and (2) rehabilitaiton of the critically denuded mangrove areas.

Most of these measures are now embodied in DENR Administrative Order Number 15 Series of 1990, which is a set of regulations governing the utilization, development and management of mangrove resources. This was formulated by the Coastal Resources Management Committee created by DENR Special Order Number 982 dated 25 October 1989; this committee replaced the former National Mangrove Committee (Appendix 1). In conclusion, it can be said here that this set of regulations is meant to promote a mutually productive and long-term coexistence of artificial fisheries (aquaculture) and natural fisheries (municipal fisheries, including fry fisheries and commercial fisheries), as well as to minimize environmental degradation of the mangal and nearshore ecosystem (Zamora 1990).

Table 1. Mangrove Areas (in Hectares) of the Philippines for 1988 (by Region and Province)

REGIONS	PROVINCES	TOTAL
I	Pangasinan (200)	200
II	Cagayan (3,000), Isabela (400)	3,400
III	Pampanga (300), Zambales (200)	500
IV	Aurora (300), Marinduque (1,100), Occidental Mindoro (900), Oriental Mindoro (1,500), Palawan (42,300), Quezon (4,000), Romblon (700)	51,000
V	Albay (400), Camarines Norte (2,500), Camarines Sur (2,500), Catanduanes (1,200), Masbate (1,500), Sorsogon (1,800)	9,900
VI	Aklan (0), Antique (100), Capiz (1,700), Iloilo (300), Negros Occidental (725)	2,825
VII	Cebu (400), Bohol (8,700), Negros Oriental (550)	9,650
VIII	Eastern Samar (6,000), Northern Samar (5,500), Western Samar (10,450), Leyte (2,900)	24,850
IX	Basilan (3,600), Sulu (*), Tawi-Tawi (*), Zamboanga del Norte (300), Zamboanga del Sur (15,400)	19,300
X	Agusan del Norte (1,100), Agusan del Sur (-), Misamis Occidental (1,200), Misamis Oriental (-), Surigao del Norte (6,300)	8,600
XI	Davao del Norte (0), Davao del Sur (0), Davao Oriental (800), South Cotabato (0), Surigao del Sur (6,300)	7,100
XII	Lanao del Norte (1,300), Maguindanao (300), Sultan Kudarat (800)	2,400
TOTAL (**)	48	139,725

* Data on these two provinces were not included.

** Based on the manual interpretation of SPOT multispectral satellite images 1987-1988 by NAMRIA (National Mapping and Resource Information Authority), Department of Environment and Natural Resources

(Source: Bina R. T. 1988 **"Updating mangrove forest area statistics in the Philippines"**, presented tables in transparencies. Text read during the NATMANCOM (National Mangrove Committee) Symposium- Workshop on Mangrove Research, Environment, Policy and Information on 28-30 November 1988 at the Sulo Hotel, Quezon City, Philippines).

Table 2.. Summary: Mangrove Areas of the Philippines (in Hectares) for 1972, 1982 and 1988 (in the four major islands)

Major Islands	1972[a]	1982[b]	1988[c]
Luzon	34,409.9	54,117	22,700
Visayas	38,467.6	46,146	37,325
Mindanao	120,066.3[d]	92,513	37,400[e]
Palawan	35,004.1	40,855	42,300
Total	227,947.9	233,631	139,725

a Based on digital analysis of 1972 LANDSAT I Data (Source: NRMC Report 1979. Mangrove Inventory of the Philippines Using LANDSAT Data)

b Based on high altitude aerial photography, LANDSAT and aerial reconnaissance survey (Source: BFD NRMC-UPCF Forestry Statistics 1984)

c Based on the manual interpretation of SPOT satellite data by NAMRIA (Report to be finalized)

d Does not include Tawi-Tawi

e Does not include Sulu and Tawi-Tawi

Note:

Luzon [Regions I, II, III, IV (including Palawan) and V]

Visayas (Regions VI, VII and VIII)

Mindanao (Regions IX, X, XI and XII).

Source: Bina, R.T. 1988. **Updating Mangrove Forest Area Statistics in the Philippines.** Paper read during the NATMANCOM Symposium- Workshop on Mangrove Research, Environment, Policy and Information held on 28-30 November 1988 at the Sulo Hotel, Quezon City, Philippines.

Table 3. Major Elements of Mangal in the Philippines

1. *Avicennia alba*
2. *Avicennia eucalyptifolia*
3. *Avicennia marina*
4. *Avicennia marina var rumphiana*
5. *Avicennia officinalis*
6. *Bruguiera cylindrica*
7. *Bruguiera gymnorrhiza*
8. *Bruguiera parviflora*
9. *Bruguiera sexangula*
10. *Ceriops decandra*
11. *Ceriops tagal*
12. *Lumnitzera littorea*
13. *Lumnitzera racemosa*
14. *Nypa fruticans*
15. *Rhizophora apiculata*
16. *Rhizophora mucronata*
17. *Rhizophora stylosa*
18. *Sonneratia alba*
19. *Sonneratia caseolaris*

Table 4. Minor Elements of Mangal in the Philippines

1. *Acrostichum aureum*
2. *Acrostichum speciosum*
3. *Aegiceras corniculatum*
4. *Aegiceras floridum*
5. *Camptostemon philippinense*
6. *Excoecaria agallocha*
7. *Heritiera littoralis*
8. *Osbornia octodonta*
9. *Pemphis acidula*
10. *Scyphiphora hydrophyllacea*
11. *Xylocarpus granatum*
12. *Xylocarpus moluccensis*

Table 5. Mangrove Associates in the Philippines

1. *Acanthus ebracteatus*
2. *Acanthus ilicifolius*
3. *Acacia farnesiana*
4. *Albizia saponaria*
5. *Alstonia macrophylla*
6. *Ardisia elliptica*
7. *Barringtonia asiatica*
8. *Barringtonia racemosa*
9. *Brownlowia tersa*
10. *Cassine viburnifolia*
11. *Cerbera manghas*
12. *Chloris barbata*
13. *Corypha elata*
14. *Crinum asiaticum*
15. *Cynometra ramiflora*
16. *Cyperus malaccensis*
17. *Derris indica*
18. *Desmodium umbellatum*
19. *Dolicahndrone spathacea*
20. *Ervatamia pandacaqui*
21. *Fimbristylis ferruginea*
22. *Flagellaria indica*
23. *Glochidion littorale*
24. *Glochidion mindorense*
25. *Hibiscus tiliaceus*
26. *Intsia bijuga (I. retusa)*
27. *Ipomoea pes-caprae*
28. *Kleinhovia hospita*
29. *Mallotus papillaris*
30. *Morinda bracteata*
31. *Oncosperma tigillarium*
32. *Pluchea indica*
33. *Pongamia pinnata*

34. *Premna integrifolia*
35. *Pseuderanthemumpulchellum*
36. *Schefflera odorata*
37. *Sesuvium portulacastrum*
38. *Strophanthus cumingii*
39. *Thespesia populnea*
40. *Thespesia populneoides*

Table 6. Specialized Groups in the Mangal of the Philippines

Climbers

1. *Bauhinia binata*
2. *Caesalpinia crista (C. nuga)*
3. *Columella trifolia*
4. *Dalbergia candenatensis*
5. *Derris heptaphylla*
6. *Derris lianoides*
7. *Derris trifoliata*
8. *Finlaysonia obovata*
9. *Tristellateia australasiae*

Epiphytes

1. *Asplenium nidus*
2. *Cymbidium finlaysonianum*
3. *Dischidia saccata*
4. *Dendrobium crumenatum*
5. *Dendrobium distichum*
6. *Dendrobium luzonense*
7. *Drynaria quercifolia*
8. *Hoya merrillii*
9. *Hoya reticulata*
10. *Hydnophytum membranaceum*
11. *Hydnophytum philippinense*
12. *Lecanopteris sinuosum*
13. *Myrmecodia echinata*
14. *Pyrrosia adnascens*
15. *Sarcochilus pallidus*

Table 7. Zonation of Philippine Mangroves

ZONE	TIDAL INUNDATION REGIME	SOIL TYPES	COMMON MANGROVE SPECIES
Seaward	Daily, including	Coral rubble, sandy,	*Avicennia marina, Sonneratia*
	sandy loam/alba, *S. caseolaris,*	neap tides	
	Rhizophora stylosa, R. apiculata		
Middle	Daily, except	Silty to silty clay	*Avicennia alba, A. eucalyptifolia,*
during neap	*A. officinalis, Rhizophora apiculata,*	tides	
	R. mucronata, Aegiceras floridum,		
	A. corniculatum, Bruguiera cylin-		
	drica, B. gymnorrhiza, B. parviflora,		
	B. sexangula, Ceriops tagal, C. de		
	candra, Excoecaria agallocha,		
	Lumnitzera racemosa, Xylocarpus		
	moluccensis		
Landward	Inundated only	Silty to silty-clay	*Avicennia alba, Bruguiera sexangula,*
to clay	*Excoecaria agallocha, Heritiera*		
	littoralis, Scyphiphora hydrophyllacea,		
	Xylocarpus granatum, X. moluccensis,		
	Nypa fruticans		
Riverine subdivided into river mouth and upstream foreband and backband	Variable inunda- tion, brackish/ freshwater	Sandy to silty to clay	Rivermouth: *Avicennia marina, A. officinalis, Aegiceras floridum, A. corniculatum, Camptostemon philippinense, Rhizophora mucro- nata, R. apiculata, R. stylosa*
			Upstream: *Avicennia alba, A. offici- nalis, Aegiceras floridum, A. corni- culatum, Bruguiera cylindrica, B. gymnorrhiza, B. parviflora, Camp- tostemon philippinense, Excoe- caria agallocha, Heritiera littoralis, Nypa fruticans, Rhizophora mucro- nata, R. apiculata, Xylocarpus granatum, X. moluccensis*

Source: Final Draft Report of the ADB/DENR Mangrove TA Develop- ment Project Feasibility Study 13 August 1990 (prepared by Crown Agents for Overseas Governments and Administrations)

Table 8. **Annual Organic Matter Production of Four Dominant Mangrove Species in Pagbilao, Quezon Province, Luzon (Reyes 1983)**

Species	Local Name	Category	Leaf[a]	Total[a]
Avicennia officinalis	(Api-api)	Major	394	522
Ceriops decandra	(Malatangal)	Major	325	429
Scyphiphora hydrophyllacea	(Nilad)	Minor		554
Osbornia octodonta	(Taualis)	Minor		423

[a] Grams/square meter/year

Reyes M.R.C. 1983. **Litter Production and Leaf Litter Decomposition Rates of Dominant Species in Two Sites Within the Mangrove Forest at Pagbilao, Quezon.** M S Thesis, University of the Philippines, Diliman, Quezon City.

Table 9. **Productivity of Two Mangrove Species in Calatagan, Batangas, Luzon (Fortes 1982)**

Species	Local Name	Category	Productivity[a]
Rhizophora apiculata	Bakauan-lalake	Major	0.74
Avicennia marina	Bungalon	Major	0.64

[a] Grams carbon per square meter per day

Fortes, M.D. 1982. **Productivity studies on mangrove, seagrasses and algae at Calatagan, Batangas (Philippines).** Mangrove Forest Ecosystem Productivity in Southeast Asia. BIOTROP Spec. Publ. 17:17-24.

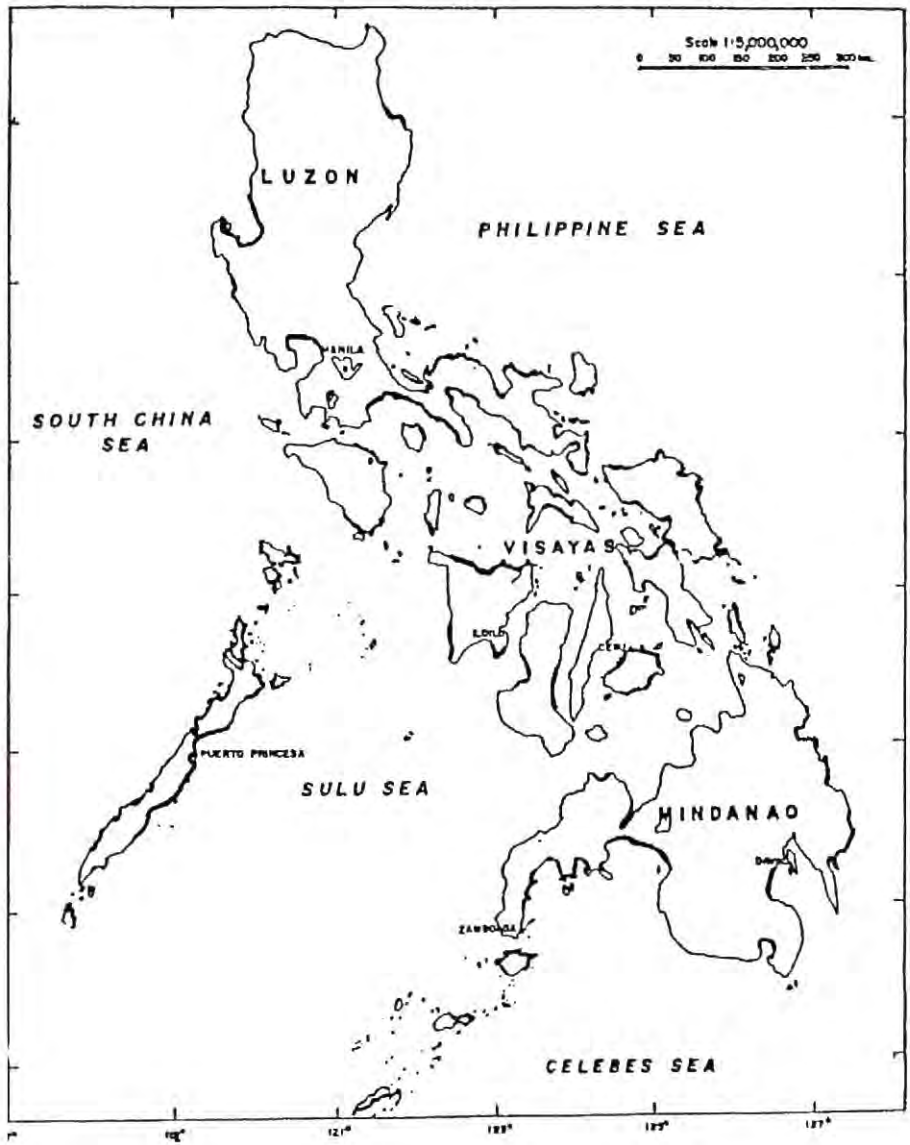

Figure 1. Geographical Distribution of Mangroves in the Philippines.

(Zamora, P.M. 1987. Mangrove resources in the Philippines. Proceedings of First National Conference on Seagrass Management Research and Development. Metro Manila 1986: 16-42)

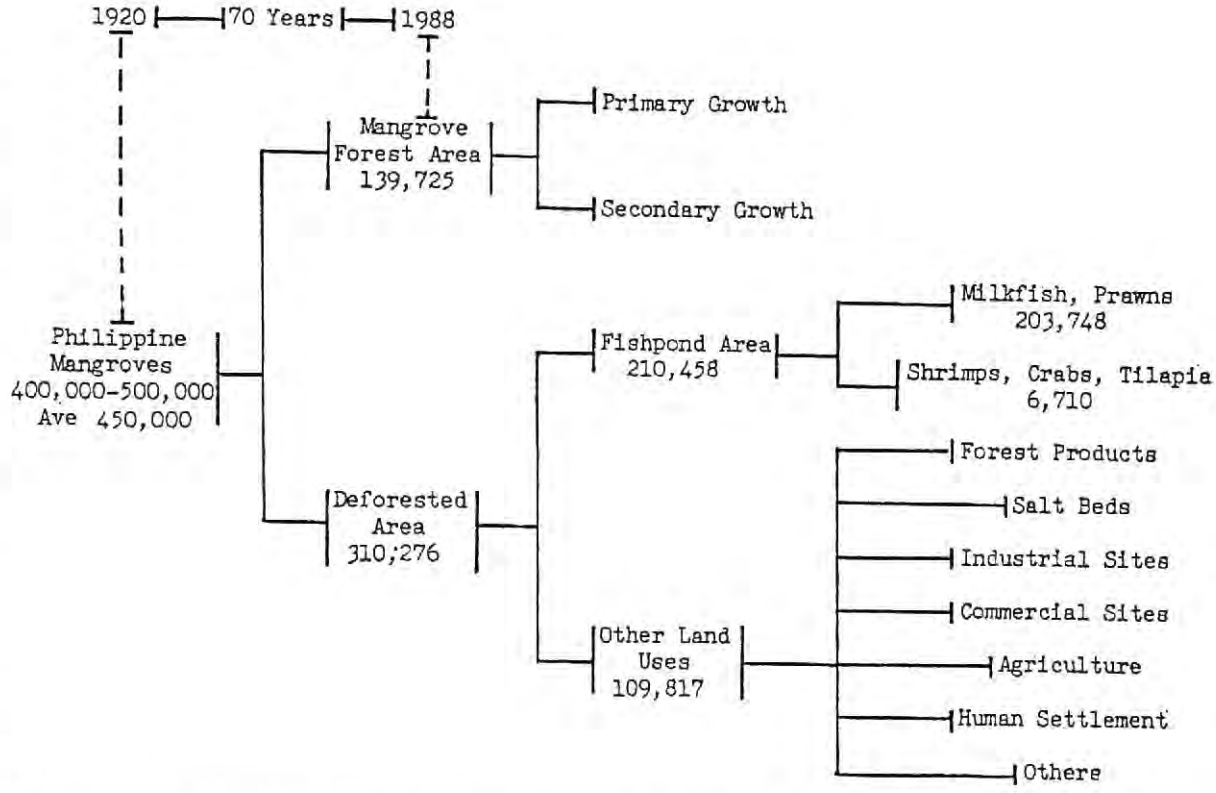

Figure 2. Estimated Area in Hectares of Existing Mangrove Forest and Mangrove Area Uses in the Philippines

(Updated from Zamora 1981, unpublished. For 1988, Annual Rate of Denudation was placed at 4,432.5 hectares.)

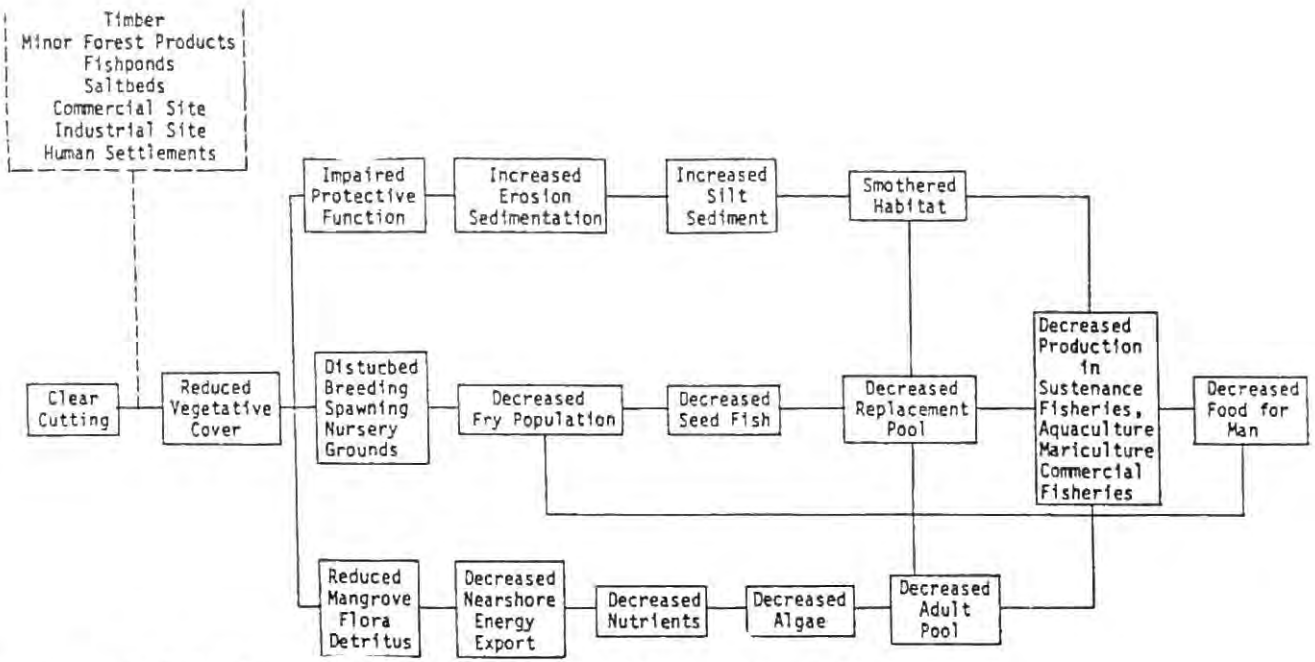

Figure 3. Probable Chain of Effects of Clear-Cutting of Mangrove Vegetation Cover

Source: Zamora, P.M. 1985. Mangrove management strategies: Philippine perspective. Lecture prepared for the UNDP/UNESCO Regional Mangrove Project RAS/79/002 Training Course on Life History of Selected Species of Flora and Fauna in Mangrove Ecosystems held on 2-16 October 1985 at Bangkok, Thailand.

(After Zamora, P.M. 1990. Wallaceana, Kuala Lumpur, Malaysia 58 (1989): 1-5).

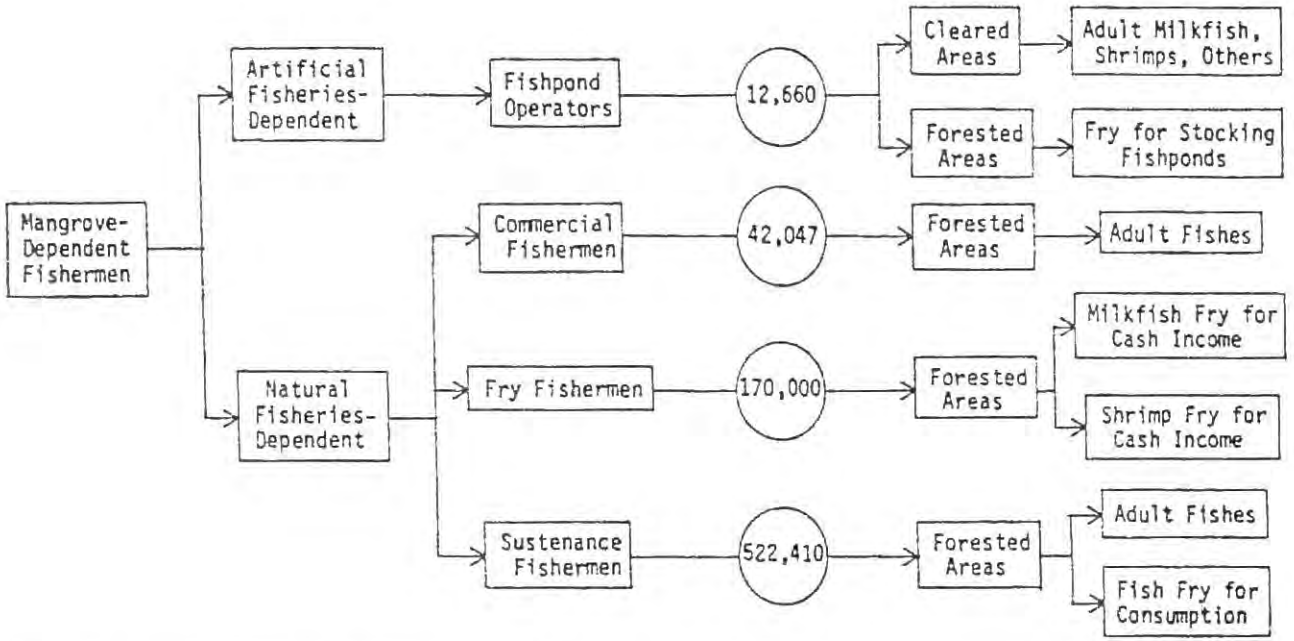

Figure 4. Mangrove-Dependent Fishermen in the Philippines

Source: Zamora, P.M. 1985. Mangrove management strategies: Philippine perspective. Lecture prepared for the UNDP/UNESCO Regional Mangrove Project RAS/79/002 Training Course on Life History of Selected Species of Flora and Fauna in Mangrove Ecosystems held on 2-16 October 1985 at Bangkok, Thailand.

(After Zamora, P.M. 1990. Wallaceana, Kuala Lumpur, Malaysia 58 (1989): 1-5).

APPENDIX 1

1 February 1990

DENR ADMINISTRATIVE ORDER
Number 15
Series of 1990

SUBJECT: REGULATIONS GOVERNING THE UTILIZATION, DEVELOPMENT AND MANAGEMENT OF MANGROVE RESOURCES

In accordance with the provision of PD 705, as amended, otherwise known as the Revised Forestry Code of the Philippines, the following rules and regulations governing the utilization, development and management of mangrove resources are hereby promulgated for the information and guidance of all concerned:

SECTION 1. Policy and Objectives. Mangroves have multi-uses. As such, the utilization, development and management of mangrove resources shall involve as many uses as possible for the benefit of the greater number of users. To sustain optimum productivity, it shall be the policy of the government to conserve, protect, rehabilitate and develop the remaing mangrove resources of the country; give preference to organizations, associations or cooperatives over individual users in the utilization and development of the mangrove resources; stop the wanton exploitation of the mangrove resources; and enhance the replenishment of the denuded areas through natural or artificial means.

SECTION 2. Definition of Terms. For the purpose of this Order, the following terms are defined:

a. **Alienable and Disposable Lands** refer to those lands of the public domain which have been the subject of the present system of classification and certified as not needed for forestry purposes.

b. **Communal Mangrove Forest** refers to a tract of public forest set aside by the Secretary of the Department of Environment and Natural Resources upon the recommen-

dation of the Director of the Forest Management Bureau for the exclusive use of the residents of the municipality from which said residents may cut, collect or remove mangrove forest products, such as firewood and mangrove timber for charcoal production for home consumption in accordance with existing laws and forest rules and regulations.

c. **Denuded Areas** refer to mangrove areas which have been devoid of mangrove trees, shrubs and/or nipa palms. Treeless areas covered with weeds and vines fall under this definition.

d. **Fishpond Lease Agreement** is a privilege granted by the State to a person or group of persons to occupy and possess in consideration of specified rental any public lands for the raising of fish and other aquatic products.

e. **Forest Lands** include the public forest, the permanent forest or forest reserves, and forest reservations.

f. **License** is a privilege granted by the State to a person to utilize forest resources within any forest land, without any right of occupation and possession over the same, to the exclusion of others, or establish and operate a wood processing plant, or conduct any activity involving the utilization of any mangrove forest resources.

g. **Mangrove Area** refers to the area found along the seacoast and estuaries whether sparsely or thickly vegetated with true and/or associated mangrove species, or open swampy areas, including brackish fishponds, extending along stream where the water is brackish.

h. **Mangrove Buffer Zones** are strips of land at least 50 meters in width fronting seas, oceans and other bodies of water and 20 meters on both sides of river channels/banks maintained and developed to enhance the protective capability of the mangroves against strong currents, winds and high waves except in areas covered by Ministry Administrative Order No. 42, Series of 1986.

i. **Mangrove Forest** refers to forest stand found in the mangrove areas and composed primarily of mangrove and associated species.

j. **Mangrove Plantation** refers to a stand of mangrove trees and/or palms of true or associated species planted in the mangrove area.

k. **Mangrove Resources** refer to all terrestrial and aquatic flora and fauna in the mangroves including land and minerals which could bestow any form of services, influences and amenities to man and the environment.

l. **Mangrove Swamp Forest Reserves** are mangrove areas of the public domain which are declared as such under Presidential Proclamation 2152 and are determined to be needed for conservation and protection purposes.

m. **Permit** is a short-term privilege or authority granted by the State to a person or group of persons to utilize any limited forest resources or undertake a limited activity within any forest land without any right of occupation and possession therein.

n. **Protected Areas** refer to mangrove areas declared as such under the Integrated Protected Areas System to be instituted by the DENR.

o. **Timber** refers to any piece of wood more than 1.5 meters long and having an average diameter of more than 15 centimeters.

p. **Wilderness Areas** refer to the mangrove areas which have been declared as such by the President of the Philippines under Presidrential Proclamations for the preservation of the floral and faunal species found therein to prevent their extinction and to serve as gene pool for the proliferation of said species.

SECTION 3. Prohibition in the Issuance of License and Permit. Upon the effectivity of this Order, the granting and/or renewal of mangrove timber license and/or permit of any kind that authorizes the cutting and/or debarking of the trees for commercial purposes in areas outside the coverage of Fishpond Lease Agreements and mangrove plantations shall no longer be allowed.

SECTION 4. Conversion of Mangrove Areas into Fishponds. Conversion of thickly vegetated mangrove areas into fishponds shall no longer be allowed. All mangrove swamps released to the Bureau of Fisheries and Aquatic Resources which are not utilized, or which have been abandoned for five years from the date of such release shall revert to the category of forest land in accordance with existing laws and regulations.

SECTION 5. Fishponds in Mangrove Forest Reserves and Wilderness Areas. In accordance with the national policy fishponds will not be allowed within mangrove forest reserves and wilderness areas. However, in cases where legally acquired productive fishponds are found within such areas, and the government opts to revert them to the category of forest lands and if public interest so dictates, the operator would be justly compensated.

SECTION 6. Issuance of Certificate of Stewardship Contract. A Certificate of Stewardship Contract may be issued covering mangrove areas to individuals, communities, associations or cooperatives, except in wilderness areas, provided that the acitivities shall be limited to sustainable activities as indicated in the approved Management Plan for such areas. Conversion of mangroves for, but not limited to, fishpond development, saltworks and paddy cultivation shall not be allowed under the Certificate of Stewardship Contract.

SECTION 7. Cutting of Trees within FLA Areas. No cutting of trees within existing Fishpond Lease Agreement (FLA) areas shall be allowed without the benefit of a permit from the Department of Environment and Natural Resources. The trees cut in FLA areas through a permit shall be turned over to the DENR for disposition through public bidding. FLA holders are given the right to equal the highest bidder, in which case the bid is automatically awarded to him.

SECTION 8. Establishemt, Development and Management of Communal Mangrove Forest. Communal mangrove forests may be established in mangrove-endowed municipalities/cities in accordance with the policy guidelines as enunciated in Ministry Administrative Order No. 48, Series of 1982, as amended. The development and management of the communal mangrove forest shall be the responsibility of the community people concerned under the concept of community-based forest management and in accordance with an approved Management Plan to be monitored closely by the Regional Offices of the DENR. However, the DENR may disestablish a mangrove area as communal mangrove forest if the allowable activities thereat are found to be non-sustainable to the resource.

The DENR through its field offices shall conduct a sustained information dissemination campaign on the environmental aspect of mangrove management. Local immersion should also be used as a tool to train the people on the technical aspect of mangrove management. The substance of the training should be attuned to the policy as enunciated in this Order.

SECTION 9. Fishpond Development. Fishpond development shall only be allowed in denuded areas which have been zonified as suited for such activity. Estuarine mangroves which are predominantly, if not totally, vegetated with shrubs shall not be disposed for fishpond development as such areas still contribute to the productivity of the nearby marine ecosystem, hence should also be extensively rehabilitated. Applications for fishpond development covering the estuarine areas shall be returned to the applicants immediately with a corresponding responsibility on the part of the Department of Environment and Natural Resources to assist the applicants in locating suitable areas as an alternative area for fishpond development in accordance with the provisions of this Order.

SECTION 10. Responsibility and Authority on the Protection, Development and Management of Mangrove Areas. The protection, development and management of mangrove areas shall be the responsibilty of the concerned Regional Offices of the Department of Environment and Natural Resources in coordination with the Department of Agriculture.

SECTION 11. Continuing Assessment of Mangrove Resources. There shall be a periodic assessment of the mangrove resources throughout the country. The National Mapping and Resource Information Authority (NAMRIA) shall be responsible in the interpretation of aerial photographs, Land Satellite (LANDSAT) and other remote sensing data while the Regional Land Evaluation Teams will do the ground verification activities. The involvement of interested non-government organizations shall also be solicited in the conduct of the assessment.

SECTION 12. Establishment of Mangrove Plantations. Mangrove plantations are allowed to be established in denuded or sparsely- vegetated mangrove forest lands and A and D areas through an approved permit in accordance with the relevant provisions of Forestry Administrative Order No. 8-3, Series of 1941, prescribing the revised guidelines governing the special uses of

forest lands, as amended, and other related laws, rules and regulations. The initial maximum area allowed for mangrove plantation establishment shall be fixed at 50 hectares for corporations, cooperatives and associations and 10 hectares for individuals. However, additional areas may be subsequently granted to existing developers after thorough evaluation of accomplishment provided that the accumulated area does not exceed 200 hectares for corporations, cooperatives and associations and 50 hectares for individuals.

SECTION 13. Cutting of Trees in Mangrove Plantations. Mangrove plantation developers shall be allowed to cut the planted trees found within their respective plantations through clear cutting by strips system, whether such is intended for personal or commercial purposes; Provided, That they secure a permit from the immediate office of the DENR.

SECTION 14. Silviculture. Silvicultural practice allowed in naturally grown mangrove forest shall be a combination of seed-tree method and planting. In the course of harvesting, at least 40 healthy trees per hectare, spaced regularly over the area, and representative of the species in the area, shall be retained to provide the seeds necessary for regeneration purposes.

SECTION 15. Penal Provision. Violations of any of the provisions of this Order shall be penalized in accordance with existing laws and regulations.

SECTION 16. Repealing Clause. This Order supercedes radiogram message dated June 13, 1986; BFD Circular No 13, Series of 1986; and all previous administrative orders, regulations, circulars, memorandum orders or instructions involving disposition of mangrove resources inconsistent herewith.

SECTION 17. Separability Clause. Should any of the provisions of this Order be subsequently or otherwise revised, modified or repealed accordingly, the same shall not affect the validity or legality of the other provisions so far as they could stand independently of the provisions so revised, modified or repealed.

SECTION 18. Effectivity. This Order shall take effect 15 days after its publication in a newspaper of general circulation.

FULGENCIO S. FACTORAN JR.
Secretary

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Why Conserve and Manage Coral Reefs and Seagrass Beds?

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This session focuses on the conservation and management of Philippine marine ecosystems. It may be useful at the outset to have an idea of the types of marine ecosystems found in the littoral and the sublittoral or continental shelf zones from a depth of 0 to 200 meters (Smith and Heemstra 1986). These are the mangroves, estuaries, seagrass, coral reefs, soft-bottom ecosystems and pelagic or open water ecosystems. Two of these ecosystems, seagrasses and coral reefs, have been discussed. My assignment is to comment on the paper on coral reefs by Dr. Edgardo D. Gomez and on the paper on seagrasses by Dr. Miguel D. Fortes, with primary emphasis on the natural and human impacts on these two ecosystems. Since the theme of the 13th NAST Annual Scientific Meeting is "Managing Ecosystems for Long-Term Human Survival," my discussion will stress the consequences of the impacts on sustainable productivity of these marine ecosystems and on the conservation of biodiversity.

CORAL REEFS

Dr. Gomez has discussed the problems and issues in coral reef conservation and management in his brief but comprehensive paper. He has identified the major problem as the destruction of coral reefs through the relentless exploitation of coral reef resources by an ever-increasing number of people. A substantial number of papers published since the late 1970s, including those by Dr. Gomez and myself, have documented the status and the specific natural and human-induced stresses on coral reefs. There is no question that the coral reef is the most heavily stressed marine ecosystem in the Philippines with the possible exception of the mangroves. All Philippine coral reefs, irrespective of location,

are undergoing degradation. This includes even those far from centers of human population, such as those in the middle of the Sulu Sea.

The stresses on coral reefs are of course man-induced and caused by natural occurrences. Natural occurrences, which include typhoon and volcanic eruptions, have affected us for eons, and coral reefs have adapted to these occurrences. Dr. Gomez has commented on our work dealing with the recovery of coral reefs in the Central Visayas (Alcala and Gomez 1990), which is fairly fast provided no human interference occurs. From the scientific point of view, natural occurrences may be useful in providing natural experiments against which the effects of human disturbance may be measured or compared. The Pinatubo eruptions, which have affected the coral reefs of Zambales, will surely give us a better understanding of coral reefs in the same way that typhoons have been useful in studies of natural recovery. Human-induced stresses, however, belong to a different category, as they are geometrically increasing in severity without let-up, giving no time for the reefs to recover.

This brings us to two major points I would like to comment on. These are: (1) the long-term effects of coral reef destruction on the fisheries component; and (2) the issue of coral reef management.

Dr. Gomez has pointed out that coral reefs are a source of a number of materials that are harvested for food and for commerce. Research has also shown that a number of reef organisms produce a variety of chemicals useful to man. I would like to focus on the fisheries aspect. Coral reefs have been a source of fish for our people. There was a time when our coral reefs were teeming with fish and it took only several minutes perhaps to gather a kilogram. Now, our studies of a protected island in the Central Visayas show that the average catch per two-to three-hour trip ranged from 1.6 - 2.0 kg (Alcala 1988). In healthier reefs in southwest Philippines, the catch is a little better -- 2 to 3 times this much. What I am trying to say is that the fishery production of coral reefs has definitely been decreasing.

In terms of the national significance of coral reefs as a source of food fish, some workers have estimated that they supply 15% of the marine fishery production. This may be a low estimate. If all reefs of the Philippines were of the good to excellent quality, the potential fish production of 27,000 km^2 would be about 540,000 tons/yr, one km^2 producing 20 tons/yr (Alcala 1981,

1988). And this would be a sustainable yield. But the fact is that the majority of Philippine reefs, as Dr. Gomez has pointed out, are of the poor to fair condition, producing only 4-5 tons/km^2/yr (Luchavez et al. 1984). It is thus easy to see that the continued degradation of coral reefs has already decreased the supply of coral reef fish used for food. Evidence has been accumulating that some species useful in the aquarium trade are present in much diminished numbers if not totally absent in certain collecting sites (Pajaro nd).

On the issue of coral reef management, Dr. Gomez has recommended the strategy of limited access to allow recovery of coral reefs and the restoration of their normal productivity levels. If combined with another strategy, the establishment of protected areas (parks, reserves), degraded coral reefs will most likely return to their normal conditions within a fairly short period of 5-10 years. This has been demonstrated in this country by our experiments at Sumilon Island (Alcala 1981, 1988; Alcala and Russ 1989; Alcala and Russ 1990). However, it is necessary to have a strong enforcement mechanism to sustain the positive effects of protection. It has been found that, for small fringing reefs near centers of population, organized communities can provide a sustained management mechanism (Savina and White 1986). Its drawback would seem to be the large expense and the relatively long period of time needed to organize communities before they become effective managers. Obviously, different management mechanisms are needed for reef systems away from human population centers. An example of such reefs is the Tubbataha National Marine Park, which lies in the Sulu Sea, about 12 hours away (by boat) from land. Here, enforcement measures involving a strong political will on the part of the government and non-government agencies charged with its protection are required. This is the only way to stop the violations which as of May 1991 were observed to be rampant in the park (Silliman Marine Laboratory Report to PCAMRD; observations by G. Hutchinson).

The establishment of many protected marine areas (parks, reserves and sanctuaries) is important for at least two reasons. First, they export adult fish biomass to areas being fished by fisherfolk (Alcala 1988; Alcala and Russ 1990). Second, they serve as replenishment areas or recruitment sources for larvae and fry of marine organisms, including fish, thus maintaining

biodiversity. Protected areas serve as replenishment areas for artificial habitats now being constructed. Without natural reefs, artificial reefs would probably not be productive, as only coral reef fish seem to colonize artificial reefs (Alcala nd).

As I see it, in this country we no longer have the luxury of choosing large areas for such protected areas; we have to be content with small ones. The many such small protected areas, the greater the probability of fishing areas receiving recruits to sustain the fishery. It is known that coral reef fishes produce long-lived larvae that could be carried by water currents through long distances (Williams et al. 1984; Frith et al. 1986).

Because most of our fringing reefs have been destroyed, we are left with no alternative but to protect relatively inaccessible but good to excellent reefs like the Tubbataha reefs and the adjacent ones on the Cagayan Ridge. It should be pointed out that these reefs, which are about 150 kilometers from Puerto Princesa, Palawan very likely export fish larvae and fry to the island of Palawan. Some evidence based on fish larvae and the favorable currents moving westward favor this hypothesis. What is needed is more oceanographic research to provide the needed critical evidence. If this hypothesis is confirmed, the role of Tubbataha reefs as a fishery replenishment area will become evident and should provide a relevant reason for their total protection.

SEAGRASS BEDS

Seagrass meadows are a distinct ecosystem dominated by 15-16 species of marine flowering plants collectively called seagrasses. Dr. Miguel D. Fortes has exhaustively discussed the ecology, conservation and management of the seagrass ecosystem. I shall focus my brief remarks on the conservation and management of this ecosystem.

Seagrasses, which generally lie adjacent to coral reefs, share with coral reefs a number of marine organisms. The two ecosystems also interact in terms of not only living organisms but nutrients as well.

As Dr. Fortes points out, there is an immediate need to conserve and manage seagrass meadows. But first of all there is a need to map the seagrass beds of the country as there are no estimates of the area of this ecosystem.

Why should seagrass beds be conserved and managed? One of the reasons is that seagrass beds serve as nursery areas for a number of important marine species.

The second reason is that seagrass beds have potentials for fishery production (Alcala 1990). Their relative accessibility to fisherfolk (they are found in intertidal flats and in shallow water) is an advantage. Sea ranching of such organisms as fish, clams, sea urchins and abalones are feasible. PCAMRD (1990) has documented the successful use of seagrass beds as sea ranching sites for finfish, including the highly-prized groupers, using a simple technology termed "rock mound." The technology has been adopted by fishing villages of an island off northern Negros.

The third reason is that seagrass beds produce organic matter and may, like mangroves, contribute substantial amounts of carbon to the shallow benthos and are thus important in food chains. Our studies in the ASEAN-Australia Living Coastal Resources Project should in time provide us good information on this function of seagrasses.

The fourth reason is that seagrass meadows which grow in intertidal areas located along the flyways of migratory birds serve as feeding areas for these birds. Example are the seagrass meadows between Cebu and Bohol. The excreta of these birds fertilize the sea bed. The bird-seagrass interaction is an interesting research area.

With the foregoing discussion, at least we know what we stand to lose if we do not conserve and manage seagrass beds.

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The Conservation and Management of the Philippine Marine Ecosystem

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Dr. Velasquez, distinguished scientists, ladies and gentlemen, good morning.

When Undersecretary Umali asked me last Thursday afternoon to take his place as one of the discussants on the subject of "Conservation and Management of the Philippine Marine Ecosystem", I thought that I would review only one paper. I was surprised later to receive two papers - one on "Coral Reef Ecosystems and Resources of the Philippines" authored by Dr. Edgardo D. Gomez and another on "The State of Seagrass Ecosystems and Resources in the Philippines" authored by Dr. Miguel D. Fortes - with a warning that a third paper on Mangroves was forthcoming. As of yesterday afternoon, however, the threat had not materialized, fortunately. I finally received the third paper entitled "Managing the Philippine Mangal for Long-Term Human Survival" by Dr. Prescillano M. Zamora only this morning. So instead of a paper on "Conservation and Management of the Philippine Marine Ecosystem" with three co-authors, we have three papers authored individually by separate experts. This situation probably highlights the great complexity of the subject matter - the Marine Ecosystem.

1. The State of Seagrass Ecosystem and Resources in the Philippines

Let me now take up the paper on Seagrass Ecosystem.

First of all, I would like to congratulate Dr. Miguel D. Fortes for coming up with a very comprehensive paper on the state of the seagrass ecosystems and resources in the Philippines as well

as the problems and issues associated with their conservation. Reading through the paper and listening to the presentation was a very interesting and educational experience, especially for one whose professional exposure has been entirely on forests including grasses that thrive on *terra firma*. The sources of information are quite exhaustive and, combined with Dr. Fortes' personal expertise on the subject, assure us that the discussions cover the "state of the art" as far as available knowledge on seagrass ecosystems and resources in the country is concerned. The paper gives us, among other vital information, a clear picture of the nature and variety of the seagrass ecosystem components.

Considering their multifarious functions and uses, there is really an urgent need to ensure that the seagrass ecosystems are sustainably managed and effectively protected. We are cognizant of the ever-increasing pressures on the coastal areas by the rapidly expanding population and industrial activities, especially mining. The paper notes that information on the extent of damage and the costs of restoring the seagrass beds are still wanting. A few studies which provide some benchmark information on the subject have already been made in some parts of the country. Further studies, however, should be conducted in other parts of the country to show a national perspective.

It is interesting to note that a high diversity of flora and fauna reside in seagrass beds and that a great percentage of the biotic components are commercially important; a fact which the layman, like myself, just takes for granted due to ignorance of their inter-relationships. Furthermore, it appears that two important endangered species of marine animals - the green sea turtle (pawikan) and dugong feed directly on seagrasses. Survival of these species would, therefore, largely depend on how effectively these feeding grounds are protected.

The role of seagrasses in the stabilization of coastal areas, their ability to filter sewage and their vulnerability to natural stresses as well as human pressures, as a result of his basic needs and industrial pursuits, should awaken our policymakers to the need to conserve these resources.

Regarding the prospects and developments in the seagrass ecosystems management, we quite agree that management policies and conservation projects specifically on seagrass ecosystems are yet non-existent in the Philippines. However, it was

also represented that among the ASEAN countries, only the Philippines, through an inter-agency cooperation, has formulated a National Seagrass Management Program including the proposed creation of a Philippine National Seagrass Committee. Furthermore, the author pointed out that in 1986. UNDP/FAO in association with UNEP, formulated a regional project on coastal fisheries rehabilitation through seagrass transplantation and that the ASEAN-Australian Coastal Living Resources Project is investigating the structural and functional aspects of local seagrass resource at present. But as the author correctly noted, the most practical approach toward sustainable development and conservation of the marine ecosystems is to have an integrated study of seagrass-mangrove-coral reef ecosystems as they are intimately associated or inter-related with one another. We hope that this integrated study would soon be implemented.

The paper pointed out that the Philippines has formulated a National Seagrass Management Program which consists of five parts namely: resource mapping and survey; research and development; information dissemination; education, training and publication; environmental management; and policy and legislation. The paper did not indicate if the program has been implemented and, if so, its current status. In any case, I believe that the policy and legislative component should not be neglected. I mentioned this because a look at the list of bills on natural resources filed in the House of Representatives and the Senate revealed that there are no bills addressing the concerns of these ecosystems except a few which mandate the reforestation of mangrove areas.

Likewise, the component on education and information dissemination should be accorded priority. It appears that much of the work done, especially the papers on studies so far undertaken, are mainly for the scientific community. Hence, there appears to be an utter lack of information about and appreciation of these vital ecosystems among policymakers, especially the legislators and the communities who are directly interacting or who make their living by harvesting the products of these marine ecosystems and resources. I think everybody will agree that if we are to effectively manage and conserve our seagrass and associated resources, the people who directly relate to these resources should be properly educated and informed on the need to protect and conserve them.

2. Coral Reef Ecosystems and Resources of the Philippines

The paper gives us a concise account of the Coral Reef Ecosystem and the valuable resources intimately associated with it. In contrast to research on the seagrass ecosystem, coral reef research has received considerable attention and has progressed so much since the 1970s. This is perhaps because corals, aside from providing habitat to various marine life, especially fish, are extremely valuable in commerce. Judging from the list of references at the end of the paper, however, there appears to be only very few Filipino scientists involved in research on this ecosystem.

Among the problems related to the conservation of the coral reef ecosystem, the author pointed out that pressure of human population is one of the major ones. This indicates that coral reef ecosystems and resources have at least one thing in common with the forest ecosystem, that is, both suffer from population pressure.

To address this problem, the author proposes that for the long term, something must be done about the population problem. For the immediate future, the author indicated that access to them should be restricted or controlled which is quite obvious, but is easier said than done. The approach through some local authority, while in consonance with the current thrust for local autonomy, may be quixotic considering the deeply ingrained Filipino value of **pakikisama**. I must admit, though, that we in the DENR are also proposing to adopt such a scheme when the proposed Local Government Code becomes a law.

3. Managing the Philippines for Long-Term Human Survival

Finally, I shall say a few words about the paper of Dr. Zamora, which as I said earlier, I received only this morning. The paper discussed the present status of the Philippine mangrove ecosystem and indicated that out of an estimated area of 450,000 hectares in 1920, it is now down to 140,000, an average annual decrease of over 4,400 hectares over the last seven decades. A large portion of the mangrove forests has been converted to fishponds and industrial uses.

In this connection, I would like to mention an incident which is very relevant to the subject. In 1967, President Marcos

instructed the Director of the Bureau of Forestry to release 500,000 hectares of mangrove swamps for fishpond development. The directive was supported by supposed studies of the Presidential Economic Staff, the forerunner of the NEDA, which showed that the value of mangrove areas would be more if converted to fishponds. We, however, knew that the President was being misled by an influential group of war veterans who were then engaged in the lucrative export of mangrove timber to Taiwan. In other words, the proposed conversion of mangrove forests to fishponds was just a cover or justification for wholesale clear cutting of the mangrove forests. Recognizing this ruse, we prepared a position paper and requested the President to reconsider his directive. Fortunately, the President heeded the Bureau's advice and did not pursue his directive. Otherwise, we probably would not have a single hectare of mangrove swamp left at present.

Like the other ecosystems, the mangrove ecosystem is being damaged and depleted. However, at present, we have regulations that prohibit the conversion of forested mangrove swamps to fishponds. Even areas covered by fishpond leases which are still forested with mangrove species are required to be left in their natural state. The titling of fishpond areas,which was allowed before, has been stopped. Furthermore, areas zonified for fishpond purposes which are not utilized within five years after their certification are reverted to the category of forestland.

Finally, let me congratulate the authors of the papers on Marine Ecosystems and Resources for their excellent exposition of the present status and the issues and problems affecting them.

Mangrove Reforestation in Bohol: A Success Story

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ABSTRACT

Mangrove reforestation in Bohol started in Banacon Island in 1957. The need for life's essentials, such as firewood and poles for hut and fish fence construction, as well as the love for planting trees have been the driving forces in an ordinary man's establishment of his first bakauan plantation. The plantation, unexpectedly, had inspired and shaped the lives of the rest of the people in the island and the neighboring towns. At present, Boholanos, particularly in Banacon Island, are realizing bounties from their bakauan plantations aside from firewood and poles.

INTRODUCTION

Mangrove reforestation in Bohol first started in 1957 in Banacon, a small island north of Jetafe, Bohol. It has an approximate area of 425 ha, only 15 of which was dry land. The rest (410 ha) was mangrove plantation (tidal flat) area.

Banacon Island got its name from a species of fish called "banak" or mullet (*Mugil caphelus*), which was very abundant in the area before the proliferation of destructive fishing methods.

[1] Pioneer bakauan planter of Banacon Isalnd, Jetafe, Bohol and 1989 DENR Likas Yaman Awardee

Banacon is now inhabited by about 550 families dependent on the mangrove plantations and foreshore marine resources, such as fin fishes, shells, crabs and other mollusks and crustaceans. The most important commodities of the island today are the Bakauan (*Rhizophora stylosa*) plantation, the crabs and the shrimps that are directly dependent on the established mangrove forest.

How Bakauan Planting Started

At about the end of 1950, there were almost no mangroves left in Banacon Island. This was because of excessive cutting done by a certain Pilot Camacho, who professed to be the holder of a mangrove concession permit which included Banacon. Moreover, the people of the island were entirely dependent on their mangroves for firewood and poles for the construction of their dwellings and fish fence due to very limited dryland area. It was this great need for firewood and poles that gave me the idea to establish my own bakauan plantation. My observation of the propagules that fell, stuck in the mud and grew into young plants gave me the idea that bakauan could be direct planted/seeded. Hence, in 1957, I established my first bakauan plantation by direct seeding propagules.

At first, folks in the island called me "crazy." However, when I started harvesting and selling firewood and pole out of the bakauans I planted, that was in 1966, people in the island were encouraged to establish their own bakauan stand by following what they saw me doing. From then on, planting became extensive and every family wanted to establish its own bakauan plantation.

Hence, to facilitate passage, I, together with the other folks in the island, agreed to establish a 10-meter-wide highway at the center of the plantation (for motor boat passage) and foot trails between individual plantations (for human passage.) We are proud to claim that such beautiful experience we had in the island was appreciated and copied by residents of Pangangan Island, Calape, Talibon, Bien Unido, Ubay, Candijay and other neighboring islands and municipalities of Bohol. That is how planting of bakauan or "mangrove reforestation" became popular and successful in Bohol.

Tips/Technology To Share

1. **Selection of Planting Site.** The site best for planting bakauan-bangkau should have soft, stable and deep

sandy to sandy loam soil. Water must be shallow enough that during neap tides (when high and low tidal fluctuations are minimal), planted bakauans should be above or without water. The shallower the water that inundates the tidal flat, the higher the chance for bakauans to survive and get established. Moreover, the area should be sheltered from the general direction or yearly route of strong winds and typhoons.

2. **Selection of Propagules.** Bakauan propagules for planting should be mature, not defective and free from oviposition holes of beetles. Indicators of mature propagules are as follows: (a) they have attained the desired length of 40 - 60 cm for bakauan- bangkau; (b) extended appendages have appeared at the base of the pericarp; (c) pericarps are easy to remove; and (d) hypocotyls are sturdy, robust and with distinct lenticels, which appear as numerous black dots at the basal end.

3. **Planting.** Plant bakauans by direct seeding the mature propagules. Simply shove the pointed basal end of the propagules into the ground at very close spacing of around 25 - 30 cm without proper alignment (old method). Nowadays, we are planting at regular spacing of either 0.5m - 1.0m and observing uniform alignment of plants. Technicians of the Department of Environment and Natural Resources (DENR) have taught us the importance of proper alignment and spacing.

Benefits/Rewards Realized From Planting Bakauan

1. **Monetary.** In 1966, I started to harvest/cut my bakauans and sell them for firewood and poles. I was at that time selling my poles at ₱25 - ₱50 per hundred depending upon the size. From the income I got from my bakauan plantations, I was able to build a house and a concrete water tank for the collection of rain water. The collected rain water also gave me a little additional income because rain water in the island was sold at ₱0.20 per container (four-gallon capacity). People in the island realized that there was money in planting bakauans. Nowadays, bakauan poles aged 25 - 30 years, which are used for house posts, are sold at ₱17 per pole. The 20-year-old poles for beams, rafters and other skeletal parts of the

house are sold at ₱10 each; the 15-year-old poles at ₱5 each; and the 10-year old poles at ₱3 each.

2. **Mangrove-Related Products**

   a. **Firewood and Poles.** The Banacon populace are largely dependent on their bakauan plantations for firewood and poles. Having a free source of firewood, as well as poles for house construction/repair and for fish fence is of great help to us. If a fisherman has to buy his poles for a fish fence, he has to spend ₱3,000 - ₱5,000 every seven months for poles alone.

   b. **Shells, Shrimps, Crabs and Fin Fishes.** These are important mangrove-related commodities that largely comrpise the daily diet and contribute to the daily income of the people in the island. "Fat and Fleshy" crabs from Banacon Island are preferred by buyers because these are reportedly of superior quality compared to those of the neighboring islands. We attributed this to our extensive bakauan plantations which are not found in the neighboring islands. These extensive plantations provide rich foraging grounds for the shrimps and crabs.

3. **Protection.** Our extensive bakauan plantations have afforded us protective cover during typhoons. When there is a typhoon warning, the fishermen would keep their boats inside the plantations along the established highway for cover.

4. **Expansion of Land Area.** There is an observed rise of the land occupied by the old plantations, making the water shallow. The established plantations have joined Banacon to its neighboring island, Jagoliao. During low tide, people from Banacon could walk to and from Jagoliao thru the highway provided at the center of the plantation.

5. **Other Livelihood Opportunities.** Our established bakauan plantations have afforded us opportunities for additional income. One such opportunity was the seasonal income we realized by collecting and selling bakauan propagules during fruiting season. The propagules were sold in the island at ₱0.05 - ₱0.10 each. "Amatong", a type of artificial reef established along the highways and bounda-

ries of established plantations, provided another livelihood opportunity. Amatong was established by digging a hole (1 m deep and 1 m wide) and filling it with coral stones and tree branches. The hole retains water even during low tide hence affording sancturies to fishes. We are harvesting 3 - 4 kilos of finfishes from our amatong every three months.

Problems

1. **Tenurial problem.** The Island of Banacon was declared "Wilderness Area" by virtue of P.D. 2151. Hence, bakauan planters could not be issued Certificates of Stewardship Contract over the plantations they have established.
2. Due to instant need for cash, some of the planters had sold their young crops to people who were either residents or non-residents of the island. Some of the stands are now overmature, yet the buyers have not harvested the crops. Hence, the system deprives the planters of the opportunity to reuse the area. Some buyers are even presuming that they have bought the crops including the right for the land.

PLENARY II

The Conservation and Management of Our Freshwater Ecosystems

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ABSTRACT

The Philippines has various freshwater ecosystems consisting of lakes, swamps, rivers, reservoirs and ponds with an aggregate area of more than 370,000 hectares. These resources are valuable to the economy and to biodiversity.

Reservoirs provide over 40% of the country's power requirements in the form of hydroelectricity, which supplies water for irrigation, industries and domestic needs. There are 43 known indigenous freshwater fishes in the country in addition to numerous aquatic invertebrates and plants present in our lakes, swamps and rivers.

The major problems affecting the conservation of our freshwater ecosystems are pollution, siltation and overfishing. Enforcement of the existing anti-pollution and fishing laws and regulations is weak in regions outside Metro Manila. Heavy erosion of watershed areas and unabated release of mine tailings and other wastes into rivers have contributed much to their deterioration in many areas of the country, particularly in Luzon. Use of illegal fishing gear has resulted in the depletion of many endemic fishes. Accidental stocking and indiscriminate introduction of exotic species have also threatened the existence of our endemic species.

Following is an eight-point agenda for action on conservation of our freshwater ecosystems.

INTRODUCTION

Water is essential for life. The development of civilization has been dependent on the availability of freshwater for domestic and industrial uses. Although over 70% of the world's surface is covered with water, only less than 1% is available as freshwater for human life. The conservation of our freshwater resources is thus vital for our survival.

The Philippines has various freshwater ecosystems consisting of lakes, swamps, reservoirs, rivers, ponds and small impoundments with an aggregate area of over 370,000 ha (Table 1).

Our freshwater ecosystems are valuable resources from the standpoint of the economy, as well as that of biodiversity. Lakes, swamps and rivers are sources of fisheries products and livelihood to thousands of fisherfolk. Reservoirs provide more than 40% of the power requirement of the country through hydroelectric plants, which supply irrigation to about 1.5 million ha of agricultural land. The domestic water supply of Metro Manila mainly comes from dams. The freshwater fisheries from our inland waters contributed 222,257 metric tons or 11% of the total fisheries production of the country in 1989 (BFAR 1990). There are 43 indigenous freshwater fishes recorded in the Philippine taxa (De la Paz 1988) in addition to numerous aquatic invertebrates and plants.

This paper discusses the status and management of the freshwater ecosystems in the Philippines. An agenda for action on the conservation of these resources is also presented for consideration by policymakers and implementing agencies.

STATUS AND MANAGEMENT OF OUR FRESHWATER ECOSYSTEMS

A. Lakes

Lakes are natural water impoundments formed by processes like volcanic or seismic activity. There are six major and 52 minor lakes in the country with an aggragate area of 200,000 ha (PCARRD 1981). Our six major lakes are Laguna de Bay (90,000 ha), Lake Lanao (34,000 ha), Lake Taal (33,432 ha), Lake Naujan

(8,000 ha), Lake Mainit (7,340 ha) and Lake Buluan (6,500 ha). Except for Laguna de Bay, there is a paucity of information available on the status of lakes in the Philippines. Only Laguna de Bay and Lakes Lanao, Taal, Naujan and Buhi are discussed here.

Laguna de Bay is a eutrophic lake with an average depth of 2.5 m. It has multiple uses, which are for fisheries, irrigation, power generation and industry (Davies 1988). There are eight endemic fish species in the lake in addition to six migratory species and six other introduced ones. Only one endemic species, the kanduli (*Arius manilensis*), has been extensively studied.

There are about 10,000 fishermen dependent on the lake for their livelihood. The fish catch consists of the **ayungin** (*Therapon plumbeus*) and the **puting bia** (*Glossogobius giurus*), which represent about 93 percent of the open-water fish harvests. Low-value snails and shrimps utilized largely for duck feed constitute the bulk of the capture fisheries of the lake. The total fisheries catch of the lake decreased by 75% from 82,882 mt in 1961-63 to 20,723 mt in 1979 because of overfishing with the use of efficient fishing gear like the motorized push net (Mercene 1990).

The fishpen culture of milkfish was introduced in the lake in the early '70s to utilize its primary production and lessen fishing intensity by encouraging fishermen to engage in fishfarming (Delmendo 1987). In the middle '80s the fishpen hectarage in the lake had reached 35,000 ha, far exceeding the 20,000 ha authorized by the Laguna Lake Development Authority (LLDA) which set a fishpen belt zone to regulate the industry. While increasing the fisheries production of the lake through aquaculture, the fishpens reduced the fishing area for fishermen and brought about not only a decrease in their fish catch but also social conflict. To protect the open-water fisheries, a 5,000-ha fish sanctuary was established in the lake by the LLDA.

Domestic sewage and agricultural run-off made Laguna de Bay highly eutrophic and vulnerable to fishkills in the '60s and the '70s. Soil erosion from the watershed of the lake has resulted in its rapid sedimentation. To prevent the backflow of the polluted waters of the Pasig River into the lake during the months of April to July and to protect Metro Manila from floods in the rainy season, a hydraulic control structure was constructed in the Napindan Channel in 1983.

Pollution and siltation are the two major problems adversely affecting the water quality and fisheries of Laguna de Bay at present. There are more than 1,100 industrial and agricultural establishments operating in the lake basin that contribute to its pollution (Cuvin-Aralan 1989). Heavy siltation of the lake has resulted in high turbidity of its waters, which limits primarily productivity and consequently production (Santiago 1991).

Lake Lanao is an oligotrophic lake with an average depth of 60.3 m. It has been declared a national park and reserve, and is under the jurisdiction of the Bureau of Forest Development. The lake and Agus River are the sources of hydroelectricity supplying 70% of Mindanao's power requirements.

The lake is important in terms of biodiversity because of its 20 endemic species of cyprinid fishes (IUCN 1990). Accidental introduction of the white goby (*G. giurus*) has threatened the cyprinid population of the lake (Juliano et al. 1989). No fisheries conservation program for the lake is being done.

Lake Taal is an oligotrophic lake with a maximum depth of 200 m. The lake is famous for its volcano island, which is a national park, and its **tawilis** (*Harengula tawilis*) fishery. The tawilis is one of the few freshwater clupeid fishes in the world. The fishery provides livelihood to 80 percent of the fishermen of the lake. In 1989, the total landed catch was 10,650 mt or 4.7% of the total catch from the country's inland waters (Castillo undated).

The tawilis was at one time threatened with overfishing by ring- net fishermen using lights to attract the shoaling fish. As a conservation measure, the Department of Agriculture, through the Bureau of Fisheries and Aquatic Resources, promulgated Fisheries Administrative Order 82 banning the use of strong lights and limiting the operation of ring nets only from mid-August to mid- October. Fishing for the species is also prohibited during its spawning season from November to January. A fish sanctuary to protect the tawilis has also been established in the lake (Zafaralla 1989).

Lake Naujan was declared a national park in 1956. It has a rich fish fauna, which supports the fisheries of demersal and pelagic species. Fishing in the lake is intensive and there is conflict between conservation of the resource and commercial fishing activities. Among the fishing regulations imposed are the

issuance of fishing permits, prohibition of fishing between 4 a.m. and 5 p.m. and the maintenance of a fish sanctuary. Enforcement of the regulations, however, is poor (IUCN 1990).

Lake Buhi, although a minor lake in terms of area (1,707 ha), is significant for having the **sinarapan** (*Mistichtys luzonensis*), considered the world's smallest commercially important fish. The population of the species has been declining since 1979 as a result of overfishing, introduction of exotic species and siltation (Depthnews 1990).

The operation of motorized push nets, which destroys the shelters of the sinarapan, has been banned by the Bureau of Fisheries and Aquatic Resources (BFAR). Introduction into the lake of the Mozambique tilapia in the '50s and the freshwater shrimp (*Macrobrachium* sp.) in the '70s is believed to have contributed to the depletion of the fishery (Gindelberger 1981). The BFAR has taken steps toward the protection of the species by prohibiting sinarapan fishing in nearby lakelets where it still abounds.

B. Swamps

Swamps or marshes are water-logged areas with grassy vegetation. The major freshwater swamps of the Philippines are the Liguasan Marsh (220,000 ha) and the Agusan Marsh (89,359 ha) in Mindanao and the Candaba Swamp (32,000 ha) in Central Luzon (IUCN 1990).

Liguasan Marsh forms the basin of the Mindanao River in North and South Cotabato and consists of a vast complex of river channels, small freshwater lakes and ponds. A portion of the marsh (30,000 ha) was declared a Game Refuge and Bird Sanctuary in 1979. There are about 20 species of fishes described in the swamp. Access to the area is limited because it is the stronghold of the MNLF. This freshwater ecosystem is probably the best protected in the country today.

Agusan Marsh consists of freshwater marshes and water courses with numerous small shallow lakes and ponds in the upper basin of the Agusan river and its tributaries. The area has been declared a reserve and crocodile sanctuary.

Candaba Swamp is a complex of freshwater ponds and grasslands on a vast floodplain. The area is flooded in the wet

season but it becomes arable in the dry season. Among the conservation measures proposed are the establishment of a wildlife sanctuary and a reservoir for fisheries and irrigation.

The other swamps of importance in the country are the Leyte Sab-a Basin (90,000 ha) and the Buguey Wetlands (14,400 ha) in Cagayan. Little is known about the fisheries of these swamps.

C. Reservoirs

Reservoirs are man-made impoundments constructed primarily for irrigation and power generation. They are also important for fisheries because of the endemic species in the rivers that supply them, fish introductions and aquaculture. There are more than 1,000 impounding dams and reservoirs in the country with a dam height of over 3 m (PCARRD 1981). The common fishes caught in our reservoirs are tilapias, the common carp and the white goby. The management of only two of our largest reservoirs is discussed in this section.

Pantabangan Reservoir in Nueva Ecija, built in 1974, is the largest in the country with an area of 8,900 ha. It is managed by the National Irrigation Administration (NIA). Introductions of tilapias, carps and milkfish into the reservoir by the BFAR have contributed to a limited extent to the livelihood of the fishing community in its watershed. Heavy erosion of the watershed has resulted in the reservoir's rapid sedimentation and relatively low fisheries productivity. Fishing rules and regulations have been formulated by the NIA but these are poorly enforced (Johnson 1975).

Magat Reservoir in Ramon,Isabela is the second largest in the country with an area of 4,460 ha and a maximum depth of 80.5 m. The reservoir sits at the foothills of the Cordillera Mountain Range. It became operational for generating hydroelectricity, as well as irrigation and fisheries purposes in 1982.

The total fish landings in 1987 and 1988 by about 700 fishermen using gill nets, hooks and lines, traps and case nets were 1,785 mt and 1,713 mt, respectively. Nile tilapia (*Oreochromis niloticus*) comprised 31.8% of the total catch, followed by the ayungin (*Therapon plumbeus*), catfish (*Arius magatensis*), carps, goby, mudfish, eel and shrimps. In addition to the open-water fisheries, Nile tilapia is also extensively cultured in floating

fish cages in the reservoir. There were 785 cage units with a total area of 56.3 ha operated by 212 fishfarmers in 1986 (De los Trinos personal communication). NIA strictly enforces fishing regulations.

The fisheries of the other major reservoirs, such as those of Ambuklao, Binga, Angat and Caliraya, have not been studied well. These reservoirs are being managed by the National Power Corporation. Late report has it that the Ambuklao and Binga Reservoirs in Benguet are heavily silted due to serious watershed erosion and are no longer suitable for fish production (Angulan personal communication).

D. Rivers

There are 421 major rivers in the Philippines, with drainage areas or basins ranging from 40 - 25,000 sq km. With an annual precipitation of 2,269 mm, the country has an estimated average annual run-off of about 256,980 million cubic meters (EMB 1990). Because of their proximity and accessibility to urban and industrial centers, our river systems are the most vulnerable to pollution among our freshwater ecosystems.

A recent item in a national newspaper had the lead: "Four of 17 Luzon Rivers Found 'Dead'." The biologically dead rivers identified were the Maasin-Potiero-Gumain River System, Caulamang-Marella River System, Pamatawan River and Rio Chico in Bulacan and Pampanga. The San Fernando River in Pampanga was also reported to be the most heavily polluted (Arias 1991). In another news item, it was revealed that only 12% of the Metro Manila population had access to sewers and that while 40% of all rivers in the country are now "dead", all the rivers in Metro Manila are "dead" (Giron 1991).

Four major rivers in the Ilocos Region -- the Abra, Agno, Amburayan and Bued Rivers -- have been found to be heavily polluted with cyanide from tailings of mining operations. Such pollution and sedimentation have resulted in the decline of productivity of agricultural lands irrigated by these rivers, as well as losses to fishermen. Over 200 mines and quarries in the country discharge an estimated 190,896 mt of tailings and 371,644 t of mine wastes daily (Zafaralla 1982).

The pollution of the Pasig River in Metro Manila is a classic example of how a freshwater ecosystem has been decimated. Domestic and industrial wastes are dumped at the rate of 3,600 t and 30,137 gal, respectively, into the river and its tributaries each day. There are 313 industrial firms along the banks of the river in addition to the squatter families that discharge liquid and solid wastes (NEPC 1983)

Rivers in the other regions of the country in the Visayas and Mindanao have not been spared of the ravages of pollutive industries. In the Visayas, sugar mills and alcohol distilleries are responsible for the deterioration of rivers. The Hijo and Liboganon Rivers in Tagum, Davao del Norte were found to have high mercury levels with the tailings from gold mining operations (EMB 1990).

Aside from pollution and siltation, overfishing has also contributed to the decline of fisheries in our rivers inhabited by at least 234 Catadromous fishes (De la Paz 1988). As early as in the '30s, conservation of the **ipon** fisheries in Northern Luzon was already stressed (Montilla 1931). In the Cagayan River of Mindanao, the **dulong** (*Sicyopterus extraneus*) has been threatened not only by siltation of the river but also by too much fishing pressure (Manacop 1953).

Regulations for pollution control are enforced by the Department of Environment and Natural Resources through its Environmental Management Bureau, which is also tasked with monitoring water quality in our rivers. The DENR launched the Rivers Revival Program in 1987, initially with the **Ilog ko, Irog ko** Project, a multi-agency effort with the objective of lessening the pollution load of the Navotas-Malabon-Tenejeros-Tullahan River System of Metro Manila by 50% in 1992 (EMB 1990).

E. Fishponds and Small Impoundments

Fishponds are water-holding structures with dikes made of soil or concrete for growing fish and other aquatic species. Water supply for fishponds may come from rivers, irrigation or groundwater. The total area for freshwater fishponds in the country was estimated to be 13,847 ha in 1989 (BFAR 1990).

The largest concentrations of fishponds are found in Central Luzon and the Ilocos Region, with 9,114 ha and 1,342 ha,

respectively. Most of the fishponds are privately owned and have an average annual production of 2.47 mt/ha. The Nile tilapia is the main species of fish cultured, along with the common carp, shrimps and snails.

Two environment-related constraints that affect the freshwater pond industry are pollution of rivers and other waterways, and the introduction of exotic species. Fish kills in ponds occur when pollutants, such as agricultural pesticides and industrial wastes, contaminate fishponds through the water supply system. Polluted water has also been identified as one of the factors contributing to fish disease (De los Reyes 1986).

With the unsuitability of our freshwater endemic fishes for culture, there have been numerous fish introductions made in the country since 1905. A total of 34 exotic fish species have been introduced to date (Juliano et al. 1989). Most of these introductions have not had adverse impacts on the environmet. At least one introduced fish, the Nile tilapia, is now the second most important cultured fish in the Philippines.

On the negative side, however, our native freshwater catfish (*Clarias macrocephalus*) has disappeared in areas where its foreign counterpart (*C. batrachus* from Thailand) was released in the '70s. Similarly, the introduction of the freshwater snail, *Pomacea* sp., from South America in the early '80s, has resulted in the ecological displacement of our *Ampullaria luzonica*, a popular food item of the Ilocanos and Tagalogs. The "golden kuhol", as the foreign species is popularly called, has also become a major pest of irrigated ricelands in the country.

Small impoundments are dugout ponds or earthen dams with a height of 3 m or less. They are primarily built for rainwater storage and for flood and erosion control in the uplands. Water from these impoundments is used for agriculture, fisheries, power generation and recreation (PCARRD 1986).

A modified version of the upland small impoundment in the lowland is the small farm reservoir (SFR) for rainfed areas. The typical SFR has an average depth of 1 m, open water area of 0.2 ha and a water storage capacity of 2,000 cu m. Through such impoundments, rice farmers can earn additional income from a second crop of rice in addition to the fish production (Watson et al. 1988).

AN AGENDA FOR THE CONSERVATION OF OUR FRESHWATER ECOSYSTEMS

Our freshwater ecosystems provide us with many of our basic needs for survival and progress as a nation. It is imperative that we wisely manage these resources on a sustainable basis for the benefit of present and future generations of Filipinos.

The following eight-point agenda for conservation of our freshwater ecosystems is proposed.

1. Need for more studies on our endemic freshwater species and their ecology

We know very little about the biology and ecology of our endemic freshwater species. Their conservation and management can only be possible if we understand how they live and what they need to survive.

Our scientific literature contains mostly surveys and taxonomic information. Not much has been done in the areas of biological assessment, population dynamics and fisheries management of our natural freshwater ecosystems, particularly lakes, swamps and rivers. There is also a need for more researchers in the regions where such resources are found.

2. Need for strict enforcement of anti-pollution laws and fishing regulations, particularly for rivers and lakes

The existing laws and regulations for pollution control and fishing in our rivers and lakes need only to be enforced and revised, if necessary. This is crucial for us to be able to deal with the alarming trend in the high morbidity of our rivers and depletion of fisheries resources due to overfishing. Action is urgently needed in the provinces where little or no control is being done. Public support and political will for the conservation of the fisheries of our lakes and rivers are important.

3. Need for protection of endangered species

The endangered freshwater fishes and mollusks in the country should be propagated in protected and reserve areas to ensure their perpetuity and conservation for biodiversity. The Liguasan Marsh, and Lakes Lanao, Taal and Buhi are recommended areas for protection.

4. Need for greater public awareness and more information on conservation of freshwater ecosystems

Since people are the objects of management, people should be made aware of the urgent need for conservation and the consequences of unabated human stresses on the environment. Information drives should be done at all levels -- from kindergarten schools to the halls of Congress -- through the educational system and the mass media.

5. Need for more stringent regulations on the introduction of exotic species

To avoid further negative impact on our freshwater ecosystems, more stringent quarantine and import regulations for exotic species of aquatic organisms should be enforced. Qualified personnel and institutions should undertake evaluation and impact studies under controlled conditions on all introduced species before these are propagated and dispersed to ensure protection of our endemic species and the environment.

6. Need for more efficient water use in fishponds

With the increasing hectarage of freshwater ponds in the country, more efficient water use systems, such as integrated farming and recycling, should be considered.

7. Need for more small impoundments

One of the most effective and economical ways of conserving water and protecting the environment is to have more small impoundments in the country, particularly in upland areas where soil erosion is critical and living conditions of inhabitants are depressed. Aside from the conservation of soil and water, productivity of the land will be enhanced and nutrition of the uplanders will be improved.

8. Need for multisectoral and wholistic approach to freshwater ecosystems management

Management of freshwater ecosystems is a complex task that can best be done through the multisectoral and wholistic agroecological approach. The problems of siltation and pollution of the ecosystems can only be minimized with proper watershed management and waste treatment by industries. Overfishing of

the fisheries resources, on the other hand, cannot simply be solved by enforcing fishing regulations without considering socio-economic political factors.

Table 1. Freshwater Ecosystems of the Philippines

Ecosystems	Estimated Area (ha)
Lakes	200,000
Swamps	106,328
Rivers	31,000
Reservoirs	19,000
Ponds	13,874
Total	370,202

Source: Bureau of Fisheries and Aquatic Resources (1990)

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Water Quality Management: Key to the Sustainable Development of Laguna de Bay

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INTRODUCTION

Dr. Guerrero gave us a good overview of the major freshwater aquatic ecosystems in the Philippines including a set of recommendations on how they may be properly conserved and managed. My reaction is to focus on one of these lakes, which is Laguna de Bay, in terms of the contemporary issues confronting the management and development of this lake. In particular, I shall focus on water quality as a key environmental indicator needed for sound management and sustainable development of the lake.

Laguna de Bay is the largest and certainly most important freshwater lake in the Philippines. It has an area of 90,000 hectares; it is situated between the provinces of Rizal and Laguna, including a good part of Metro Manila. Its watershed covers about 300,000 hectares and it had an estimated population of about 3.5 million people in 1988. In 1976, its average depth was estimated at 2.8 meters at the mean annual low lake level of 10.5 m which is equal to the mean sea level in Manila Bay. The lake is a tropical one with seasonal water temperature ranging from 25° to 32°C and hardly exhibits thermal stratification between the surface and bottom temperatures. The large surface-to-volume ratio, high nutrient level and high water temperature of the lake categorize it as a highly eutrophic lake.

The main uses of the lake include: (a) as a fishery for both open fishing and aquaculture; (b) for power generation and indus-

trial use; (c) agricultural uses for irrigation and livestock production; (d) for navigation and transport of goods and people; (e) for recreation and aesthetic use; (f) as a convenient dump for domestic, industrial and agricultural and other wastes; (g) for flood control; and (h) as source of public water supply.

These extractive demands coupled with the negative impacts of the wasteloads from natural and anthropogenic sources have resulted in the accelerated depletion of its resources and continued decline in its water quality thereby threatening the continued productivity and viability of the lake. The need for the rational utilization and holistic approach to the management of Laguna de Bay based on a sustainable development concept is imperative. The ecological monitoring of its water quality and institution of control measures to maintain desired water quality standards are prime requisites for the conservation and management of Laguna de Bay.

CONCEPTUAL FRAMEWORK FOR WATER QUALITY MANAGEMENT OF LAGUNA DE BAY

The conceptual framework for the assessment and management of water quality of Laguna de Bay (or any lake) is shown in Figure 1. The prevailing water quality of a lake is determined by the different factors obtaining in the ecosystem and its watershed. These factors include both controllable (e.g. point sources of pollutants, siltation rate, water flow rates, etc.) and noncontrollable (e.g. rainfall, solar radiation, weather, etc.) ones. The impact of all these factors may or may not be apparent in the short term because of the inherent resilience of balance natural ecosystems. Monitoring of the water quality which could detect subtle changes in the physical and chemical properties of the water provides a convenient way of assessing the impact of environmental stressess. Thus, water resources are classified according to their best uses based on prescribed water quality criteria and standards.

The conceptual model shows how the ambient water quality of the lake, as revealed by monitoring, is operationally assessed and compared with the water quality standards prescribed for the best use of the lake. In principle, water quality standards are prescribed based on water quality criteria which are established as a

result of research studies on ecotoxicological testing, bioassay and impact assessment. The setting up of water quality standards and criteria as well as the implementation of mitigating or control measures are subject to government intervention or policy which in turn are influenced by social, political and economic considerations. These factors certainly complicate further the management of water quality as affected by the different external variables or environmental factors. The extent to which this conceptual model operates in reality in the case of Laguna de Bay remains to be seen.

WHAT IS THE PRESENT WATER QUALITY OF LAGUNA DE BAY?

Laguna de Bay has been classified as Class C waters, i.e. good for fishery production. However, results of water quality monitoring studies have shown that many water quality parameters have exceeded the standards prescribed for class C waters indicating the worsening condition of the lake. Among these are temperature, turbidity, dissolved oxygen, ammonia-N, nutrients, coliform bacteria and heavy metals (See Table 1). This continued deterioration in water quality must be stopped by identifying and controlling the factors causing it.

ENVIRONMENTAL PROBLEMS AND FACTORS AFFECTING WATER QUALITY OF LAGUNA DE BAY

The major environmental problems confronting Laguna de Bay and causing the deterioration of its water quality, general loss in its beneficial uses and aesthetic attributes, as well as posing health hazards to humans include the following:

a) Rapid siltation as a result of erosion of the watershed from forest denudation, infrastructure development and process of urbanization, etc. The change in forest cover in the watershed has been reduced from 93,000 ha in 1963 to less than 18,000 ha by 1988 which shows an average rate of decrease of 6.56% annually. On the other hand, as of April 1989, there are some 1,898 subdivisions in the watershed covering a total

area of 8,363 ha which contribute to land disturbance and erosion. The sedimentation rate in the lake has been estimated to be about 1.5 MCM/year.

b) Cultural eutrophication as a result of increasing wasteloads of nutrients from domestic households and expanded agricultural and livestock production and intensive fishpen operations.

c) Increasing wasteloads of toxic and hazardous substances from industrial activities including the operation of power plants. From 117 firms in 1963 the number of industrial establishments has reached about 1200 in 1988 (for an annual growth rate of 9.88%) with about 65% of them considered pollutive industries (Valerio 1990). Only about 50% of these pollutive industries have wastewater treatment facilities.

d) Increasing wasteloads of organic wastes and pathogenic organisms from the animal and livestock industry. The populations of poultry, hogs, cows, carabaos and ducks have escalated along with human population in the area.

e) Rapid population growth in the basin which exerts its pressure on human settlements both on the lowlands and uplands. Increase in human population in the Laguna de Bay Basin from about 0.85 million in 1960 to about 3.50 million in 1989 registered an annual growth rate of 4.9%

The complexity of factors causing water pollution and deterioration in the water quality of Laguna de Bay, as well as their impact on the lake ecosystem, is shown in Figure 2. The pollutants include chemicals (or toxic and hazardous substances), organic wastes, nutrients, pathogenic organisms, silt, oil, thermal, etc. They came from different sources, point and non- point, natural and anthropogenic. For the past 30 years or so, due mainly to man's activities in the watershed, the inputs of these pollutants have escalated in line with the exponential population increase in the area (see Table 2). The impacts of such unmitigated and continuous inputs of pollutants and environmentally disturbing factors are bound to destabilize or destroy any natural ecosystem, particularly where no concrete or effective steps are taken to

control pollution, as in the case of LDB. As an analogy, what can you say of an ecosystem like an aquarium if pollutants of all kinds were dumped into it until its water turned turbid, dirty and smelly, and all the rooted plants were gone, smothered by sediments. Certainly it is not balanced anymore. Yet such is the condition of LDB now, only on a much larger scale - with turbid, dirty and smelly waters with its rooted bottom aquatic plants entirely gone, covered and smothered by three meters of sediment. No wonder, people say, it is a dying lake, or worse, it is already biologically dead. This verdict is not without reason for among the negative impacts of pollution and water quality degradation are the following:

a. Decreasing productivity of the lake as reflected in decreasing harvests of fish, shrimps, snails, etc. as noted above. Capture fishery production decreased by 556% between 1963 and 1988 while yield of shrimps and snails decreased by 192% and 93%, respectively, for the same period. Fishpen productivity also decreased. With an average yield of 4,311 kg/ha in 1973 and a peak yield of 10,038 kg/ha in 1977, the yield decreased to 1,818 kg/ha by 1983 which means that there was more than five times decrease in productivity (LLDA 1988; BFAR 1988).

b. Fish Kills. As early as the seventies, fish kills had been reported in Laguna de Bay primarily due to its highly eutrophic nature.

c. Habitat destruction and loss of endemic fish species. The present state of Laguna de Bay puts it in the category of a hypereutrophic lake, one fast approaching its death. Ecologically, it is a tragedy. Even before we could identify and classify all its indigenous fauna and flora, water pollution has decimated their numbers and changed their composition. Of the 23 or so species of fish and mollusks originally present, very few species remain. Freshwater fishes that thrive in clear waters like the therapon or ayungin, white goby or bia and shrimps which used to dominate the lake are now almost gone, replaced by new and hardy species like tilapia and bighead carp which could thrive even in muddy and dirty waters. Gone also are the rooted

aquatic plants that help to cleanse the water of pollutants and serve as fish habitats. They were overwhelmed and smothered by heavy siltation and toxic wastes.

d. Social unrest and plight of the small fishermen and the lakeshore inhabitants in general whose primary means of livelihood is threatened both from the increased encroachment of fishpen operators of the open fishing area and the decreasing productivity of the lake due to the deterioration of its water quality. There are 76,000 families which are directly dependent on the lake for their livelihood.

Since ecological impact increases more rapidly than any of the factors causing it, it is expected that as the magnitude of the different environmental factors involved increases, the ecological effects would escalate further. **Laguna de Bay could be on the threshold of an ecocatastrophe with very serious implications including social unrest and political upheaval.**

How then do we approach the management and conservation of this lake? Or more appropriately, how can we save Laguna de Bay?

WATER QUALITY MANAGEMENT STRATEGIES TO SAVE LAGUNA DE BAY

Considering the complexity of the environmental problems and their impacts upon the water quality and beneficial uses of Laguna de Bay, it seems that the only way toward its sustainable development and management is to adopt the basin or integrated system approach (in the context shown in Figure 1). This considers all the factors obtaining in the watershed which have direct and indirect impact upon the lake ecosystem. However, this approach is much easier to conceptualize than to implement particularly in an ecosystem like Laguna de Bay where various government agencies and other groups have overlapping operations and interests. It will certainly be a Herculean task to coordinate and harmonize the activities of these interest groups toward a common goal of sustainable development of the ecosystem. Yet it is precisely for this reason that the process

should and must be pursued with more vigor and determination. There is an urgent need to act swiftly and decisively if only to forestall the accelerating rate of degradation of the lake. No less than a strong political will is needed.

For my part, I wish to reiterate the following water quality management strategies or measures needed to save the lake: the first set I call *Urgent Measures* can not be postponed without aggravating further the sorry condition of the lake; the second set or long term *Ultimate Measures* which is bound to affect the physiographical structure of the lake, must be subjected to a critical and thorough environmental impact assessment before its implementation. Following are the proposed water quality management strategies for Laguna de Bay.

A. Urgent Measures

1. Zoning and limitation of fishpen areas corresponding to sustainable productivity. This was estimated to be 18,000 hectares in 1984, but is dependent on primary productivity. Results of latest observations show decreasing primary productivity of the lake due to high water turbidity and other factors, such as control of illegal fishing methods like "pukot" (purse seine) and "suro" (push net).

2. Control of agro-industrial waste input into the lake. Industrial and other waste sources must be identified and required to control and treat their wastes in order to minimize waste discharges into the lake. The concept of the industrial waste interceptor system, as well as the CALABARZON PROJECT must be reviewed and subjected to environmental impact assessment.

3. Strict implementation of land use policies and regulations particularly on forestry activities, and agricultural land management, infrastructure development, housing programs, etc. including a vigorous reforestation program in the watershed

4. Domestic waste treatment and control to minimize the discharge of nutrients (nitrogen and phosphorus) and

pathogenic organisms into the lake. The government should install community toilets and garbage collection system in the foreshore communities. An ecological solid waste recycling and disposal system must be adopted. In this system, wastes are segregated at source for recycling of paper, plastics, metal, and glass, while organic wastes are made into compost.

5. Clean-up and control of pollution in the different tributaries. Most of the major rivers and tributaries of the lake are highly polluted and they contribute a lot of wasteloads. They must be cleaned and dredged and all polluters must be made to treat their wastes.

6. Promotion of sustainable development concept through intensive educational campaigns on ecological awareness and resource conservation at all levels using the power of the media, schools, churches and every possible venue

7. Consideration of the plight of the fisherfolks including the provision of alternative means of livelihood to them. They must be consulted and involved in the development and management plan of Laguna de Bay.

B. Ultimate Measures

1. **Dredging and deepening of the lake to a depth that would promote sustainable development.** This is necessary to remove the sediments that have accumulated which are the main causes of the turbidity and decreased productivity of the lake. The heavy siltation is also the cause of the disappearance of the bottom aquatic plants which serve as nursery and breeding places for fish. Land that will be reclaimed can be used for residential, agricultural, industrial or tourist purposes. This will compensate for the cost of dredging. The lake will be cleaner and clearer which will be ideal for its multipurpose uses including recreational swimming, fishing and boating for tourists especially if the coastal highway suggested below will be built. This project on dredging must however, be subjected to critical EIA.

2. **Construction of Coastal Super Highway (CSH) on Reclaimed Area**

   a. *Phase I. Calamba-Los Baños-Bay CSH*: To connect present South Super Highway at Real, Calamba to the highway at Maitim, Bay, Laguna. This is the first phase which is urgent in view of the fact that at present the Calamba-Los Baños stretch is a very congested bottleneck. This is particularly true during weekends when Metro Manilans flock to the hotspring resorts in the area.

   b. *Phase II. Bicutan/Taguig-Pateros-Angono CSH.* To decongest the South Superhighway and part of EDSA and Ortigas Ave. and provide rapid access to Marikina Valley.

   c. *Phase III. Peripheral CSH Along Western and Southern Bay starting at Taguig and ending at Sta. Cruz, Laguna.* This tollway is the final phase which will promote the multipurpose uses of the lake especially the flow of tourists to the tourist spots in Laguna and Quezon.

Of course, the fishermen within the area covered by the CSH should be given access to the lake at strategic points through a system of underpasses, quays and landing areas.

CONCLUSION

To recapitulate, I have discussed the conceptual framework for the water quality management of Laguna de Bay as a key to its sustainable development and management. To what extent this conceptual model operates in reality amid the complex environmental problems and factors affecting the said ecosystem will determine the future viability and productivity of the lake. A lot depends upon the political will and the setting up of the mechanism for coordination and harmonizing of the different activities and interests of the different agencies and vested groups involved in the use and conservation of the lake. The tasks are complex and very difficult. But they must be tackled if we want Laguna de Bay to survive.

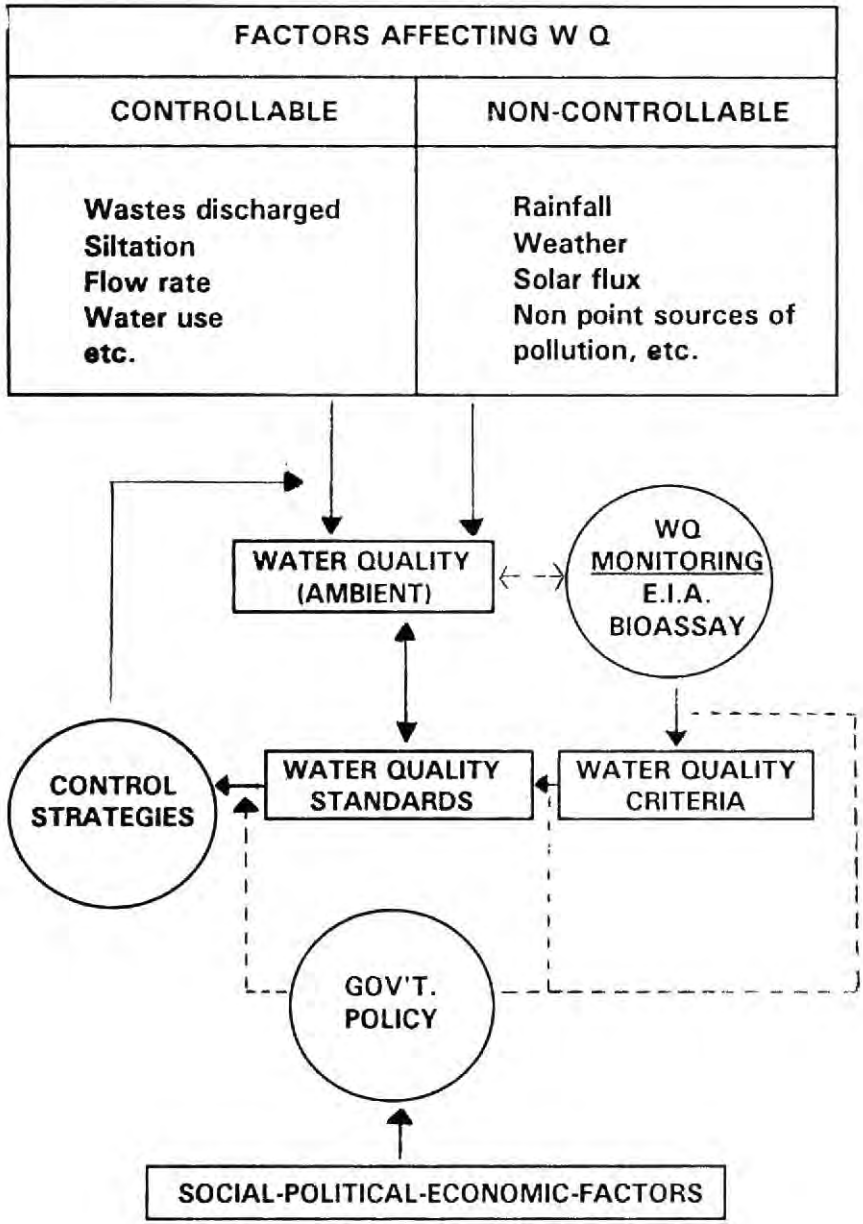

Fig. 1. Conceptual Framework for Water Quality Assessment and Management of Laguna de Bay

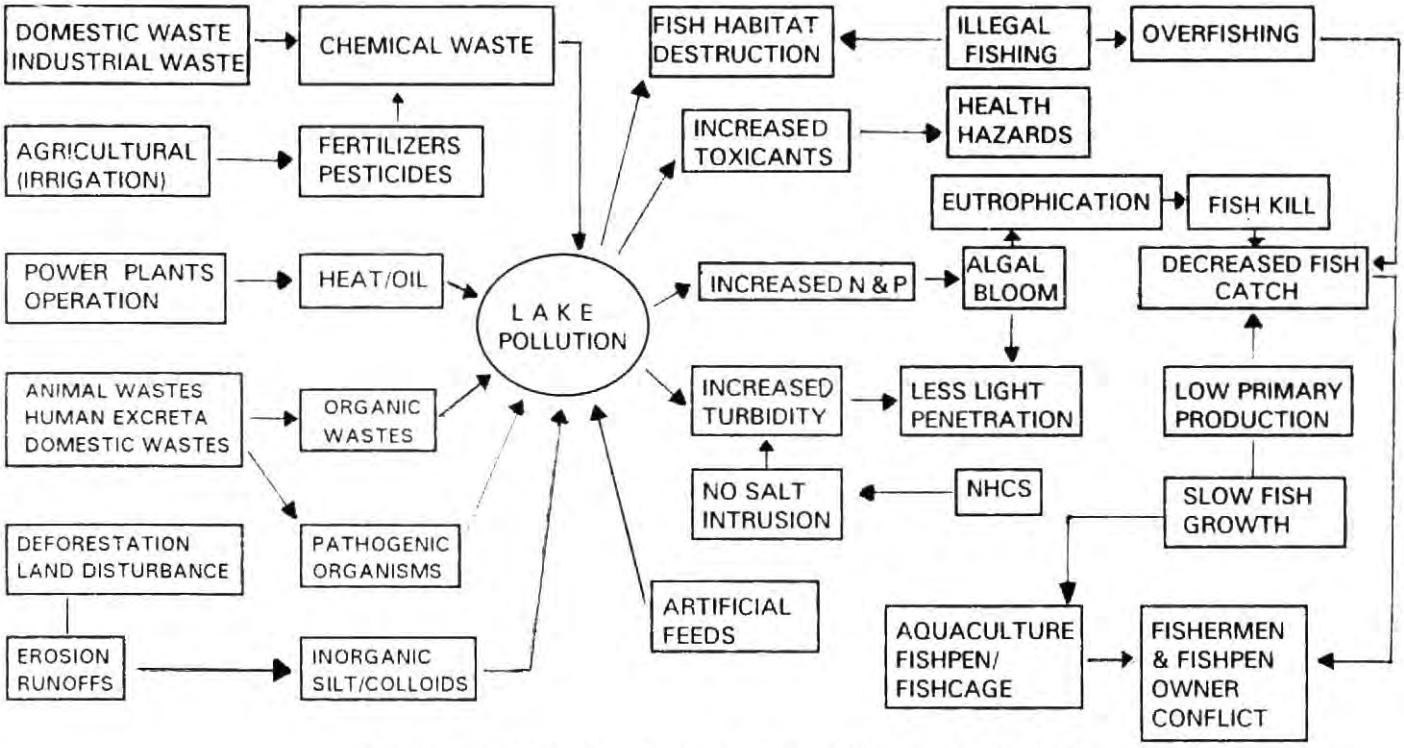

FIGURE 2. KINDS OF POLLUTION AND THEIR IMPACTS IN LAGUNA DE BAY

TABLE 1. Summary of Results of Water Quality Monitoring Studies in Laguna de Bay for the Period 1986-1989

Parameter	Average of Annual Means	Standard for Class C
Temperature (°C)		Rise not more
East Bay	Surface - 29.2 (25.0-32.2)	than 3°C of min. or
	Bottom - 28.3 (24.0-31.0)	max. value
West Bay	Surface - 29.9 (24.9-33.0)	
	Bottom - 28.8 (24.7-31.7)	
Transparency (cm)		no less than 100 cm
East Bay	1986 - 45 cm (15-75)	
	1988 - 41 cm (20-90)	
	1989 - 20 cm (5-40)	
West Bay	1986 - 59 cm (10-140)	
	1988 - 43 cm (10-75)	
	1989 - 19 cm (5-50)	
Ammonia-N (mg/L)		
East Bay	0.11 mg/L (nil - 0.369)	0.02 mg/L
West Bay	0.13 mg/L (nil - 0.348)	
Nitrate-N (mg/L)		
East Bay	0.124 mg/L (0.045 - 0.205)	0.30 mg/L
West Bay	0.146 mg/L (0.040 - 0.264)	
Ortho-P (mg/L)		
East Bay	0.080 mg/L	0.025 mg/L
West Bay	0.14 mg/L	
Total P (mg/L)		
East Bay	0.14 mg/L	
West Bay	0.19 mg/L	
Coliform Bacteria (counts/ml)		500 MPN/100 ml
Entire Lake	1984 - 3,290/ml (2,160-4750)	
	1985 - 12,580/ml (8,200-20,500)	

Table 1. Continued

Heavy Metals

Hg water - 0.23 mg/L (0.02 - 2.08 mg/L)
sediment - 0.64 mg/Kg(0.07 - 2.86 mg/Kg) (Reyes 1988)

sediment - 1.0 mg/Kg (LLDA 1977) 0.4 mg/Kg

Levels of Cu, Zn ad Pb are relatively higher than normal.

TABLE 2. Growth Rates of Population, Industry and Deforestation in Laguna de Bay Watershed. (Valerio 1990)

Item	*Annual Growth rate (period; number)*
Population	4.9% (1960-1989; 0.85M - 3.5M)
Deforestation	6.56% (1963-1988; 93,000 ha - 18,000 ha)
Industrial Firms	9.88% (1963-1988; 117 - 1200)

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Discussion Paper on: "Conservation and Management of Freshwater Resources"

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First, I would like to thank the NAST for the opportunity given me today to discuss the paper entitled "Conservation and Management of Freshwater Resources" by Dr. Rafael Guerrero. I am extremely interested in this topic which I consider very relevant and timely. The exchange of ideas on it should help put into correct perspective the importance of inland water resources to the country's economic stability. I think it is of utmost importance that inland water resources be underscored among our development priorities because of the unjustifiable low priority these resources receive at present.

There are a number of reasons why management should take a second look at the state of freshwater resources of this country. The ones which I consider important reasons are:

1) these resources have not been performing to the level of our expectations,
2) our inland water resources today are highly threatened by misuse and abuse; and
3) there is much to be desired in terms of the equitability of the distribution of the benefits derivable from their use.

For us here gathered today, it is therefore relevant that we ask ourselves why these observations hold.

Dr. Guerrero has given us a good vista on the state of the most significant of our inland water resources, namely, lakes,

swamps, reservoirs, rivers, ponds and small impoundments. We submit that these resources are significant based on areal coverage alone. Alternately stated, area-wise they have a potentially high production. But let me focus your attention on the distinction between secondary (fish) production and secondary (fish) productivity and in the process put emphasis on which of the two tells us more about the ecological status of an aquatic resource.

Production or fish production to be specific, refers to the quantity of fish produced within an area or within a year. Table 1 shows the production of some well known lakes and reservoirs.

Productivity, on the other hand, refers to the rate of fish production per unit area or volume of water per unit time. (Note how this differs from primary productivity which refers to plants). The same table above shows the differences in the productivity of the same lakes.

As you can see from the table, productivity can change the whole picture of performance. Productivity gives us an idea of how each resource is performing in comparison to another. From the tables presented you can very well see that production values cannot be taken at their face value without first having an idea about productivity.

Productivity therefore is an expression of the performance of a resource. Because it reflects the rate of performance, it gives a better basis for the comparison of different lakes. Productivity depends on such natural factors as land use and water use. Fig. 1 shows how these two elements impinge upon productivity by way of their effects on light, temperature and nutrients. Fig. 2 shows the larger context in which Figure 1 would fall. Within both contextual frameworks one can readily understand that little disturbed watersheds lead to little disturbed lake ecosystems (as shown by the nutrient fluxes that take place there). In contrast, disturbed watersheds which result from various activities including the conversion of forests into agricultural lands, industrial estates and settlement areas can cause a disturbed lake. Disturbance can be seen in the highly variable nutrient levels, the excessive loosening of the soil which in turn results in higher erosion rates. The latter aggravate siltation problems in impounded waters.

Having presented the interrelationships of human activities with the changes taking place in an aquatic resource, I shall now dwell on my views on what should be the concerns of manage-

ment in three inland resources, namely, Laguna de Bay, Taal lake and Lake Buhi. In my short discussion I shall expound on the reasons why I believe that some of our inland aquatic resources must be managed using unique and appropriate management strategies.

With Laguna de Bay, I tend to think that the problem of declining fish yield is more complex than we care to imagine. At least three factors interplay in a yet not so well understood way and bring about the stunted fish growth. These factors are: (1) heavy siltation; (2) pollution; and (3) salinity. Dr. Andy Santiago and Dr. Nielsen correctly predicted in the early eighties that siltation will become the problem to contend with where primary production in Laguna de Bay is concerned. And yet today, even lake parts that are not heavily silted also experience fish growth stunting. This focuses our attention on another lake problem - that of pollution. But pollution itself has a hidden facet that is associated with salinity. Such an association is given importance by both fishermen and fishpen operators.

Relevant to the discussion of the problem of siltation is the issue on the Hydraulic Control Structure. The HCS is the monumental realization of the technocratic minds that foresaw the need to prepare the lake for: (1) tapping as a source of domestic water supply by the year 2000; (2) water quality improvement for the purpose of meeting the irrigation needs of agricultural areas surrounding the lake; and (3) controlling the infusion of pollutants collected and dumped into it by the inflowing Pasig River.

The changes in lake behaviour noted after the start of operation of the HCS are: (1) the lowering of the slinity level particularly in areas directly surrounding the mouth of th Pasig River; (2) the proliferation of water hyacinths in various lake areas; (3) the alteration of the priod of development of clear and turbid waters; and (4) the continue decline in fish production. Of these reported changes, that involving slinity decrease was most expected since the salinity of the Pasig River itself can go up to 20 ppt. Mixing with lake's own water, this saltwater intrusion can very well make the latter brackish. Water hyacinth proliferation following operation of the HCS may therefore have arisen from the altered osmotic conditions within the plant system as a result of the fall in lake salinity. Parenthetically, it must also be pointed out that the river aides who were in charge of the removal of this plant from the water stopped doing their job even before then president Ferdinand E. Marcos left the country.

The occurrence of lake clearing is a phenomenon that highly rests on the slinity of the water. Saline water intrusion from Manila Bay via the Pasig River is an important factor that causes the autoflocculation of suspended organics in Laguna de Bay. Clearing takes place from March to April of each year for the Pasig River generally bacflows prior to this when the lake loses depth in relation to the Manila Bay. Fishpen operators and fishermen of Laguna de Bay claim that saline water entry is vital to a good harvest. Thus, the opening of the HCS' gates in 1990 caused a tremendous boom in fishpen production, so the fishpen operators claim.

Given the above observation of people who have practically been living their lives out in the lake's waters, it is logical to consider the notion that saline water entry into the lake is not only physically rejuvinating to the lake but is also ameliorating. It could chemically bind the suspended and dissolved pollutants which in one way or antoher contribute to the fish's stunted growth. It could also remove the suspended materials that reduce light penetration and therefore lead to lower photosynthetic production in the plankton. Limited phytoplankton photosynthesis or production also reduces the amount of plant-derived nourishment for the fish. But allowing polluted Pasig River water to flow in is also a way of exposing peoples health and well being to risks and hazards of unknown nature and dimension. For Laguna de Bay, the complex problems that now beset it are primarily rooted in the lake's multiple uses coupled with the destruction of the watershed.

Taal Lake is another lake with its own set of management problems. Compared with Laguna de Bay, however, it can be considered as ecologically better situated. Free form the influences of industries, Taal Lake teems with tawilis (*Harengula tawilis*) whose population fluctuates with the alternating dry and wet seasons. The fish, whose biology needs further elucidation, seems to survive the growing pressure of catch fishing. The fishcage practice of raising tilapia does this fishery a lot of good by making the water more fertile. The lake, as you will know, is 198 meters deep and is oligotrophic.

With the sustained high demand for *tuyo* that the tawilis processed into, it beocmes readily appreciable why pressure on this fishery would continually rise. The impending threat of overfishing ha sjustified the establishment of a fish sanctuary in the lake extending from the municipalities of Agoncillo to Laurel. This takes up roughly one-third of the entire lake surface area.

Sanctuary area dedication, on one hand and gear regulation, on the other, are sound policies to apply to this lake which is also a tourist spot although it is considered a high-risk area. But similar to what obtains in other lakes, the fishery regulations of Taal lake are not being implemented to the fullest. Forbidden areas for fishing remain as the haven of "suro"-using catch fishermen, the "suro" being a banned type of fishing gear.

Local fishermen claim that extreme measures like those of sanctuary establishment and gear regulation need not be implemented in the lake since the tawilis has its own self- preserving mechanism. According to the local fishermen, the fish migrates to great depths when its population is thinned to a critical level. During such time, there is no type of gear that could possibly be used for the economic advantage of the *tawilis*. In contrast, the times of fish abundance are a time of economic well being for the Taal Lake fishermen. The prolific tawilis could even force fishermen to turn off some of their lanterns at night so as to attract less fish. Where the tawilis is concerned, the need for management is to know more about the biology of the fish so that reduction in size of the sanctuary can be considered if this is warranted.

Efficient management of Taal Lake also calls for a zoning plan for this lake. We have proposed four zones for the lake, namely, the tourist zone, the aquaculture zone, the open fishery zone and the fish cage zone. The zones are necessary in order to: (a) regulate the fish cage prolifereation; (b) ensure the most economic routes for catch fishermen; (c) ensure fishery rehabilitation for a sustained high yield; (d) safeguard the lives of pleasure seekers inthis tourist spot; and (3) maximize the redound of benefits from resource use upon the poor that have settled around the lake.

Lake Buhi is yet another lake with another set of problems. Unlike Taal Lake, Lake Buhi si relatively shallower, its littoral zone gently sloping. The littoral being of such topography, Lake Buhi favors the establishment of fishcages and fish pens. In 1983, the National Irrigation Administration (NIA) found the lake ideal as water source for a hydraulic control structure that would meet the irrigation needs of at least five adjoining towns. Prior to the operation of the structure, however, there was a teeming fishcage culture of tilapia which was boosted by the KKK program of then first lady Imelda Marcos. The initial activities to put the HCS in full operation caused the lake water to drop below the critial level of 0.8 meters. This exposed the fishcages and caused the operators not to honor their debts with KKK.

A second problem in Lake Buhi had to do with the determination of the area to be allocated for the sanctuary. As it turned

out, the sanctuary was decided to have a size of 86 hectares. This implied prohibition of the establishemnt of fish cages within the sanctuary limits. This prohibition, however, never materialized. As of 1987, something like 400 fish cages have been established inside the sanctuary.

A third management problem relates to biodiversity. As everyone knows, Lake Buhi is the home of the smallest commercial fish in the world, *Mistichthys luzonensis* or sinarapan. As early as 1983, sinarapan faced the threat of extinction by the predator tilapia which was released into the lake by the BFAR.

The points I have raised indicate that our lakes are beset with their own unique set of problems dictated by the particular set of environmental conditions surrounding them. Each lake must, therefore, be dealt with on an individual basis. Nevertheless, it is of primary importance that the government set up a unifying policy to safeguard biodiversity, productivity and sustainability in these bodies of water.

Table 1. Fish production (tons of fish) of some lakes and reservoirs for 1986

Reservoir	Area (has.)	Fish production (tons/yr)	Productivity (tons/ha/yr)
Magat	4,460	6,726	1.5
Angat (Norzagaray, Bulacan)	2,300	87	0.04
Pulangui IV (Maramag, Bukidnon)	1,100	2.4	0.002
L. Buhi (1985-86)	1,707	1,547	0.91
L. Taal (1989)	26,368	10,650	0.4
L. de Bay*	90,000		0.167

* - 1988 Data

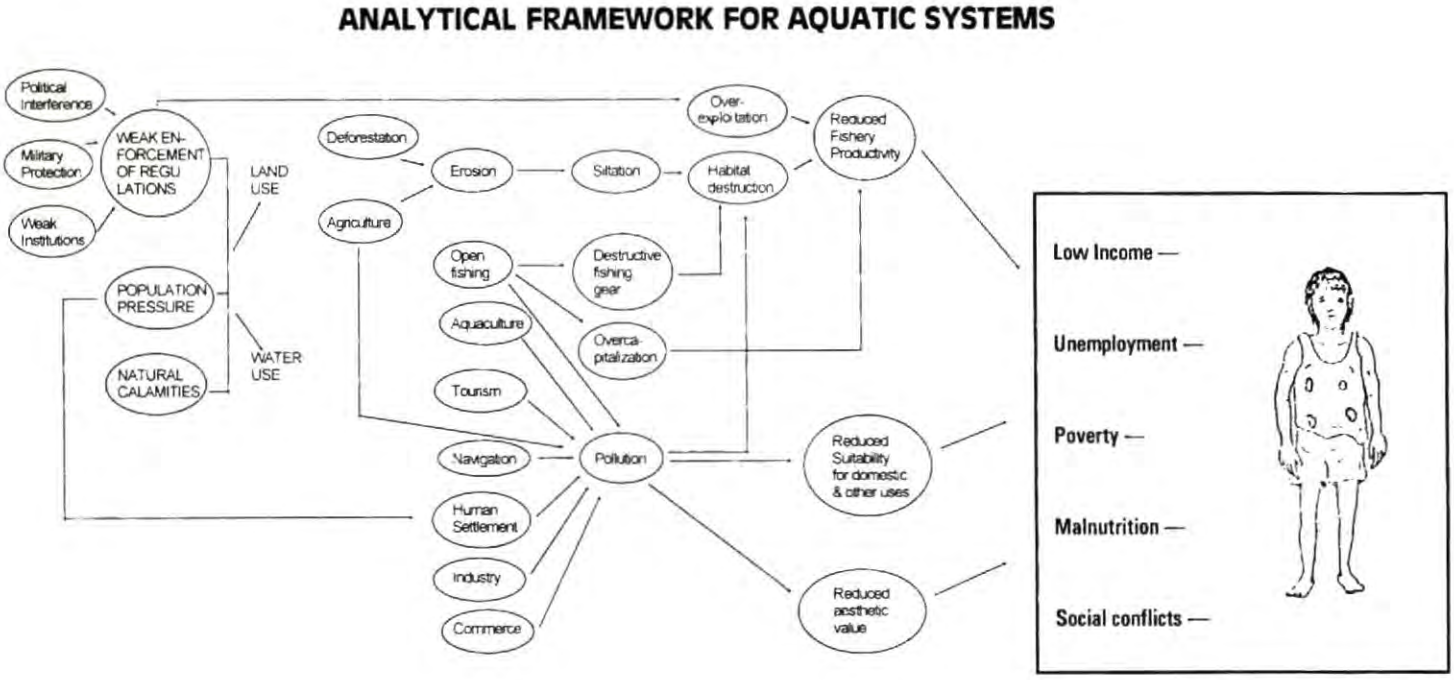

Figure 1. Analytical Framework for Aquatic Systems

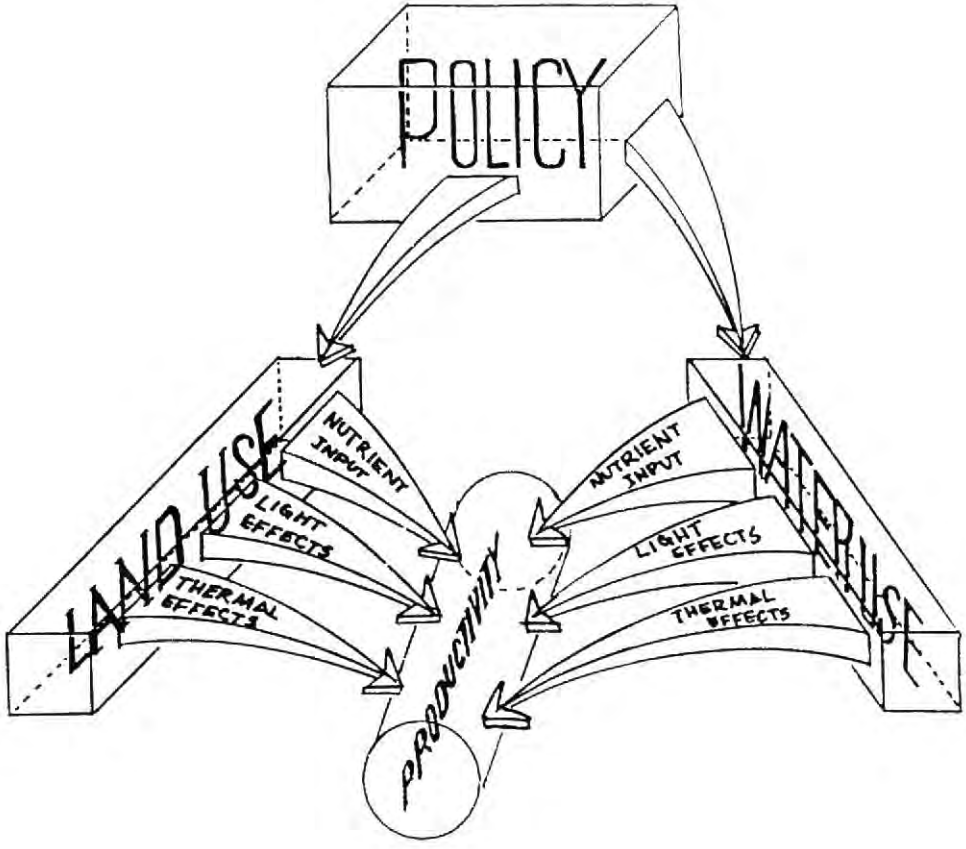

Figure 2. Context of the Analytical Framework for Aquatic Systems

PLENARY III

The Philippine Terrestrial Environment: Present Status, Problems and Prospects

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SUMMARY

The alarm bells now being sounded on the state of the Philippine terrestrial environment are for real.

The **Philippine forest,** which covered 60% of the archipelago 90 years ago, now covers only about 21%. It is expected to decline further at a rate of about 119,000 ha per year. All regions in the country, except the province of Palawan, have a forest cover way below the ideal level of 54%. However, the forest cover in this province is expected to suffer the same fate unless adequate measures are put in place soon enough.

The reforestation rate continues to lag behind deforestation by about 135,000 ha per year. At this present rate and efficiency, it would take about three generations or 177 years to regenerate enough forest cover for 54% of the Philippines.

The rapid rate of forest destruction in the country was, and continues to be, a result of many interlocking and complex factors: population pressure; weak enforcement of forest laws and policies; public apathy toward forest conservation; economic, military and political pressures on the conservation and protection efforts; unequal access to resources; and failure to address conservation and management concerns in development planning.

Forest destruction has brought about significant changes in the country's ecology: soil erosion; siltation; hydrologic impairment; climatic change; and extinction of species. Environmental degradation has continued to result in mass poverty and malnutrition.

On the other hand, the **marginal lands or grasslands**, which comprise about 41% of the total land area of the Philippines, are confronted by the following problems: soil erosion; low productivity; soil pollution and land degradation; loss of biodiversity; uncontrolled fires; utilization inefficiency; and social and economic displacement. These problems are due to the following: rapidly increasing population; insecurity of land tenure; slow rate of reforestation; poverty; and people's lack of knowledge on appropriate and sustainable soil and resource management technology. These factors also aggravate the problems.

The country's **croplands,** which comprise one-third (33%) of the country's total land area, are similarly under environmental stress. Prime agricultural lowlands and alluvial plains are rapidly shrinking from about 8,000,000 ha to less than 5,000,000 ha. Soil erosion is severe in 36 of the 81 provinces; farm lands are threatened by chemical pollution; and urban encroachment into prime and productive farm lands has reduced the country's agricultural capacity. Furthermore, the decreasing man-land ratio has led the landless to occupy and cultivate ecologically unstable croplands. Because of this, food shortages may soon occur and the country may resort to heavy food importation of rice and corn because the efficiency of production of these important grain crops has been going down since 1985.

The present state of the terrestrial environment poses a great challenge to all. The terrestrial resources have diminished not only in size but also in quality. More alarmingly, the factors that have brought about this degradation are not expected to disappear and a herculean task is necessary to even alleviate the present problems. The environmental problems during the next two decades will be exacerbated by:

a. High foreign debt burden;
b. Poverty;
c. Rapid population growth;
d. Inequity;
e. Weak institutional capacity;
f. Non-participation of local communities; and
g. Possibly, a stagnant economy and non-responsive political set-up.

There are successful examples of locally-evolved and implemented natural resource management projects from which lessons can be drawn. These include the Bontoc system, Ifugao, Mangyan, Kalahan, SALT, Jose Panganiban, RRDP and many more.

This is the background which must be considered in drawing up an environmental agenda for the next decades.

THE ENVIRONMENTAL AGENDA

* Developing a rational land use plan adopted at all levels (national, regional, provincial, local) to ensure sustainable natural resource use and avoid conflicting use of resources

* Protecting the remaining primary forest areas, especially in critical areas (national parks, protection forest, nature reserves, watersheds), and endangered flora and fauna for maintaining biodiversity and ecological stability

* Generating strong support for environment-cum-population- related programs

* Promoting and enhancing implementation of community-based, participatory and integrated approaches to natural resource management, taking off from successful examples

* Providing adequate research and development support for long term and priority concerns related to agricultural sustainability, management of logged over areas, upland, coastal and urban rehabilitation and management

* Promoting environmental education and extension programs for all sectors of society

* Establishing a network involving line agencies (DENR, DA, DAR), NGOs and the academe for natural resource management at the national, regional, provincial and grassroots levels

* Evolving a comprehensive national policy of environmental ethics and management, which would encompass

promotion of environmental awareness, formulation and enforcement of legislation to protect and enhance the environment and giving support to NGOs to encourage their participation

* Recognizing the scale and nature of current urbanization and assessing the extent of deterioration so that the resource base can be rehabilitated and preserved

* Adopting low-waste and environmentally sound technologies and efficient utilization, recycling and rehabilitation of nonrenewable and renewable resources

* Adopting agriculturally sustainable technology and practices, such as organic agriculture, integrated farming and integrated pest management

* Promoting economic incentive schemes for regulating use of natural resources and pollution control measures

* Developing and adopting an environmental master plan for major urban centers and the 179 CENROs throughout the country reflective of the Philippine Strategy for Sustainable Development (PSSD)

* Developing strong and effective preventive and anticipatory environmental management mechanisms, such as an Environmental Impact System (EIS), at all levels (national, regional and local), those concerning hazardous biological and chemical handling and regulation, etc.

These agenda must be pursued vigorously through policy reforms, legislation and programs if we hope to pursue a direction of sustainable development instead of short term growth and even probably national suicide by installment. The time to act is now.

THE SCOPE OF THE TERRESTRIAL ENVIRONMENT

Environment is so broad that it encompasses almost everything. It is defined as:

the circumstances, objects or condition by which one is surrounded,

or

the complex of physical, chemical and biotic factors that act upon an organism or an ecological community that ultimately determine its form and survival.

The scope of concern of the Philippine terrestrial environment is broad. Three ecosystems are involved and interacting with one another: forest, marginal lands and croplands. The capacity of these ecosystems to support the needs of the people on a sustainable basis and the impact of human acitivities on them should be considered in defining this scope.

THE TERRESTRIAL ENVIRONMENT AND SUSTAINABLE DEVELOPMENT

The Philippine dipterocarp forests are evergreen closed canopy forests, which are generally of the perhumid tropical zone. They constitute a highly diverse, multistory, productive and stable system, the woody flora of which are dominated by the family dipterocarpaceae. Philippine forests support one of the world's richest plant and animal lives. They have continued to be the life blood of the nation, supplying its ecological and economic requirements.

The Philippine marginal lands constitute a very important ecosystem type, both in terms of extent and the variety of its ecologic and economic importance. For instance, the reforestation efforts of the government and the private sector depend vitally on the type, diversity and successful succession of species within marginal habitats.

The Philippines has a total land area of 30,000,000 ha, about 9,900,000 ha of which or 33% are considered croplands (SPOT 1987). Per capita agricultural land in the country, which is about 0.50 ha, is lower than the world average of about 1 ha.

Sustainable development of these three terrestrial resources would require that the four sustainability components - - **resources, technologies, institutions** and **investments** - - be in harmony with one another and enhance both present and future satisfaction of human needs and aspirations (Fig. 1).

Two relevant issues emerge with the operationalization of sustainable development. First is the time frame involved between present and future satisfaction of needs and aspirations. Forests, marginal lands and croplands are all renewable resources but we still lack data to guide us in using these resources optimally and in a sustainable way. Moreover, is it right to forego present consumption of a resource in order to preserve it for generations not yet born, especially in the light of present economic uncertainties? While the interdependence between economic development and environmental soundness has long been recognized, large population increases, poverty, immediacy of needs, as well as corruption and equity issues, force environmental concern to take a backseat.

The time frame in sustainable development may ideally be very long (one generation of 60 years at least) in marginalized and resource poor areas. But in these areas where total dependency on natural resources determines survival, concern for intragenerational allocation of resources may not even be ethical in consideration of those living at present.

The second relevant question concerns hierarchical level of achieving sustainable development. What is the relevant level for its achievement? Project level? Provincial level? Regional? National? This question is important in resource systems that cut across different boundaries and have varied or multiple but conflicting uses. Lake Buhi is one very good example.

There are a lot of other questions that may need to be resolved in order to operationalize sustainable development. One thing, however, is sure. Sustainable development of the terrestrial environment cannot be achieved without addressing the needs of 50 % of our people now living below the poverty line.

UPLAND FOREST ECOSYSTEM

Historical Trend

About 416 years ago, in 1575, the Philippine forest was estimated to cover almost 92% or 27,500,000 ha of the total land area of the country (Sajise and Tapay 1990). Under the Spanish regime forest conversion somewhat accelerated to meet the requirements for shipbuilding materials for the Galleon Trade.

At the beginning of American colonial rule toward the end of the 19th century, virgin dipterocarp forests still covered the islands of Mindanao, Luzon, Samar and Palawan (Porter with Ganapin 1988). While the "Spooner Amendment" initially delayed logging operations, the American logging firms, with help from Filipino legislators, later introduced modern logging techniques and equipment. Wanton logging thus became rampant.

Accelerated logging without concern for future sustainability of forest resource was similarly carried on during the time of the Commonwealth and the Japanese Occupation (Sulit 1958).

Logging activities were heightened in the years after the Second World War as the industry was looked at as a means of amassing capital for reconstruction. Widespread mechanization of the logging industry in 1947 resulted in dramatic increases in timber production and log exportation (Sulit 1958). Peak was in the late 1960s and 1970s due to the burgeoning Japanese demand for wood (Boado 1985, cited by Porter with Ganapin 1987).

The government, sensing the fast decline in forest cover, began introducing regulations in the logging business. In 1955, the policy of selective logging and sustained yield was introduced. But this was not successful (Porter with Ganapin 1988).

In 1981, timber concessionaires were required either to apply for industrial tree plantation lease or submit a seven-year-plan for reforestation. Again, this effort did not succeed because most concessionaires declared financial constraints.

Thus, annual exports from logs - - once the dominant export product of the country - - began to decline in 1980. Reduction in production and export resulted from the log export ban and the decrease in the number of licenses being issued.

Because of these new movements forest conversion began to slow down from 1980 to the present. This happened, however, when the country was almost devoid of forest areas.

The historical trend in the relationship among forest land use, population and agricultural land use from 1948 to 1988 is shown in Figure 2. Agriculture became the dominant land use system, finally surpassing forestry use beginning in the early 1980s. However, the fastest deforestation rates coincided with the logging boom, which cleared more forest areas than what was actually felled for logs.

Rapid deforestation resulted also when the availability of land per capita continued to decline with increase in population. In 1960, the per capita land was 1.11 ha; in 1970, 0.82; in 1980, 0.62; and in 1990, less than 0.50 ha.

The loss of forest cover has been a consequence also of government policies and incentive system. The Homestead Act, through which the government tried to broaden the agricultural base by opening up virgin forest lands, and the policy of "giving land to the landless" both contributed greatly to the massive conversion of forest lands for agricultural and non-forest use. Another factor was the government's policy in the post war years, which continued into the 1960s and 1970s, to utilize intensively its forest resources to increase export income essential for financing domestic capital formation and paying huge foreign debts.

Present Status

The forest cover of the country is only 23% or 7,200,000 ha (SPOT 1987) (Table 1) compared to 60% 90 years ago. Virgin forests cover only 8.06% of the total land area. Moreover, it would require 177 years at the present rate and efficiency of reforestation to regenerate enough forest cover to bring it back to the same level. By the end of 1990, forest areas may cover only 6,100,000 ha. The population by 1991 would have grown to 61,900,000 and the per capita forest would therefore only be about 0.099 ha.

The regions with the lowest forest cover are Regions 7 (3%), 5 and 6 (7%), 1 (14%) and Regions 3 and 4 (except Palawan) (16% and 19%, respectively) (Table 2). The fastest deforestation rates occur in Regions V, VI, VII and IV. These same regions have higher percentages of total cultivated areas, showing competition between forest and agricultural land uses.

Depletion in the forest cover of the country in the past 18 years was about 190,000 ha (EMB 1985). Estimates taken from LANDSAT, SPOT imagery and aerial photography interpretation by NAMRIA show that annual rate of deforestation in the last few years was about 119,000 ha (DENR 1989).

The Forest Management Bureau reported that as of 1987, the country maintained 59 national parks located in 441,331 ha of forest lands, 57 watershed reservations covering a total area of 747,984 ha and 7 game refuge and bird sanctuaries on an area

of 939,886 ha. However, only seven of the parks now meet international standards due to squatting, illegal logging, kaingin and conversion to subdivisions.

While reforestation, particularly in critical watershed areas, is an important means of maintaining forest cover, records show that there was a big gap between reforestation and deforestation rates (about 135,000 ha per year) over a 13-year period (1975-1988) (DENR 1989).

PROBLEMS AND PROBLEM ANALYSIS

The issues in Philippine forest ecosystem can be traced to interlocking problems. These are:

a. improper land use/land degrading cropping systems;
b. widespread degradation/depletion of the forest resources;
c. institutional weaknesses;
d. dismal performances in forest protection/reforestation/renewal;
e. people's lack of active support for forest development and conservation;
f. inefficient and destructive forest utilization and now, a forest-based industry in distress;
g. over-emphasis on short-term objectives;
h. peace and order problems in the uplands;
i. predominance of poverty in the countrysides;
j. evident inability of the socio-economic system to meet the basic needs of the people;
k. continuous incursion of agriculture into forest lands and forest reserves;
l. heavy depletion of forest cover in the face of heavy population pressure;
m. incapability to resist economic and political pressures on forest exploitation;
n. weaknesses of development planning in relation to ecological management and forest conservation and development.

The above problems are inextricably bound in and ultimately rooted in the population and poverty problems: forest destruction aside from commercial logging, being largely a result of cumulative effects of small-scale extractive activities of some 18,000,000 impoverished and expanding population in the uplands. Figure 3 illustrates that the process of degradation and depletion of the forest resource in the Philippines is closely linked up with the interwoven problems of unequal access to resources, population growth, poverty, military intervention, political interference and inadequate policy and incentive systems. These factors create incentives for kaingin-making, improper mining procedures, over-harvesting of forest products, forest/grassland fires and illegal logging, which are the identified major causes of destruction of natural forests in the country.

Population Pressure

Rapid population growth and the resulting pressure on land provide the driving forces for high rates of deforestation through the conversion of forest lands to agricultural and other uses.

Table 3 shows that higher densities of population correspond to regions with high deforestation rates (Regions V, VII and IV). Generally, this pattern holds true in the forest zone area, supporting the conclusion that the level of population in the upland and forest areas directly affects the deforestation rate.

The attraction of upland and forest areas to lowland migration is evident in Table 4. The table shows that upland areas receiving major net inflows of migrants are also the remaining areas with larger forest lands (Regions 2, 4, 10, 11 and 12). Those areas with large out-migration, on the other hand, have already lost a considerable portion of their forest cover (Regions 1, 3, 5, 6 and 7).

The threat increasing population presents to the remaining forest resource is made more alarming by the fact that upland areas generally have high dependency ratios. According to Cruz and Zosa-Feranil (1988), 43% of the upland population are in the young age bracket of 0-14 while 54% are of working age (15-64). Regions with high dependency ratios and density levels, like Bicol and Western Visayas, have thin remaining forest lands. The greater the dependency burden of an area, the higher the need to exploit resources in order to provide for the consumption requirements of the population.

If the present trends in population growth (currently 2.6% in the uplands, 3.5% in logging concession areas and 2.3% national average) continue, more forest lands will be converted to agricultural lands to meet food needs. It is estimated that the rate of forest conversion from 1988 up to year 2025 will be 1.56% per year, which is significantly faster than the rate of conversion from logging operations of 1.2% per year for 1960-1980 (Hernandez 1989).

Balangue's study (1989) on alternative land-use scenario from year 1990-2015 clearly showed that population pressure would be a major factor in land use in the future. He predicted that with the current population growth trend, it would be very difficult for the country to feed its growing population starting 1995. Moreover, if the situation is left unattended, a serious land use conflict between agriculture and forestry will likely arise. This may result in the further deterioration of the people's living conditions, as well as the environment.

Cruz and Soza-Feranil (1988) also projected what would happen if the remaining forest lands in the country would be distributed to the people. At the rate of one hectare per household (current ISF Program distributes 2.5 ha/family), it was shown that only 3,080,000 ha would be the remaining forest area in the country in the year 2000. In 2025, this would further diminish to 1,700,000 ha.

The pressure on land would be alleviated if enough employment could be provided to the population. However, Philippine unemployment rate seems to worsen, over the years, implying that employment opportunities could not cope with the increase in working age population.

Impact

Forest destruction sets into motion a series of causes and impact that has resulted in soil erosion, siltation, hydrologic impairment, reduction in biological diversity, displacement of cultural communities, low income, poverty and malnutrition.

Soil Erosion

Deforestation-induced erosion was estimated by DENR to be 100,000 hectares at 1 m depth or about 1 billion cubic meters of materials every year. This problem has already caused massive and irreparable loss of fertile top soil, deterioration of productive

arable lands and low crop yield. Soil erosion has also contributed to the increased occurrence of floods, as well as siltation and sedimentation of rivers, lakes and waterways. Ultimately, these problems lead to the shortening of the lifespan of hydroelectric dams and irrigation canals, water shortage and destruction of standing crops in the lowlands.

Loss of Biodiversity

The progressive decline of Philippine wildlife has been largely a result of deforestation. People's continued encroachment on the forests destroy and alter the natural habitats of wildlife population, which brings about their decline, even their extinction. Today, out of the 1,657 known wildlife species, no less than 46 species are possibly threatened based on the 1988 IUCN Red List of Threatened Animals. At present, 18 species are already in the endangered list. The list included the Philippine eagle, tamaraw, Philippine tarsier, dugong, Philippine crocodile, monitor lizard and the famous monkey-eating eagle. There are 25 others classified as threatened.

The partial list provided by Tan et al. (1987) showed five immediately endangered plant species, including the Philippine Teak (*Tectona grandis*), 28 vulnerable or potentially threatened species and 34 rare species. The molave gigantic trees (*Vitex parviflora*) used for railroad ties, and as construction material for churches, convents and large houses during the Spanish period are now practically extinct (Philippine Daily Express, 23 Jan. 1981). Other endangered plant species today are kalantas (*Toona calantas*) used for cigar boxes; gubas (*Endospermum peltatum* and *Rauwolfia amsoniaefolia*) used for matchsticks; batikuling (*Paralstonia clusiacea*), batsan (*Litsea microphylla*) and sanglo (*Pistacia chinensis*) all used for carving and other wood handicrafts (Philippine Daily Express, 23 Jan. 1981).

Hydrologic Impairment

A study done in the mid-1970s for the Agency for International Development found that deforestation had reduced rainfall in Northwestern Luzon (Hickey and Flammang, 1977). Deforestation induces drought by lowering the amount of water retained in forest soil and quickly depleting them during the dry season.

Much of Philippine floods could be traced to forest denudation. For instance, a study by a United Nations organization in 1982 traced the increased frequency of flooding from Northern Luzon to Southern Samar to watershed degradation (FAO 1982).

Displacement of Cultural Communities

Migrations into the uplands have created much pressure on tribal inhabitants because of the failure of society to recognize property rights associated with ancestral lands. Putting up of national parks, logging concessions, illegal logging, agribusiness, hydroelectric development projects are some of the documented causes of displacements.

Poverty and Malnutrition

The poor socio-economic conditions of the upland dwellers are evident in their low annual income of around ₱10,000 to ₱15,000 compared to ₱34,933 for the entire country in 1988 (at 1985 prices). This is way below the poverty line placed at ₱36,000 a year. As the Department of Agriculture reported in 1989, "Poverty is rife in the rural areas. Over 40% of rural families live on incomes below the poverty line in contrast to only 20% of urban families. What is even more disturbing is how far and fast rural incomes have fallen behind. The average rural family income is now only 40% of the urban income level, compared to 60% in 1970 and 75% in 1975. Four-fifths of the households in the bottom 30% of the income scale are in the rural areas."

Three factors explain why the income of the upland farmers is low: (a) low farm productivity and limited products gathered from the forest; (b) high cost of inputs; and (c) low prices received for their products. Because of high cost of inputs farmers seldom apply any and as a result, productivity is low. This is compounded by the farmers' inability to apply appropriate technologies in their farms. The absence of farm to market roads does not allow them to get higher prices for their produce. Prices are usually set by unscrupulous traders who pay very low prices.

High malnutrition, high rate of infant mortality due to improper health practices and lack of knowledge on proper care and lack of proper clothing are some of the dilemmas upland communities face. In Palawan uplands, the percentage of second and third degree malnutrition is from 40.7-46.9%.

RECOMMENDATIONS

From the above assessment of the status of the Philippine forest ecosystem, the following have been identified as the relevant questions for research and further study. Appropriate courses of action are also given.

A. Priority Problems

Priority should be given to the following research questions to be able to come up with a workable strategy for forest sustainable management.

1. What strategy should we adopt to protect the remaining original dipterocarp forest?
2. How do we generate basic information, which will be incorporated into the utilization of logged over areas, fast enough to safeguard this remaining forest?
3. How do we bring together successful lessons to establish community-based participatory forest development cum conservation efforts?
4. How do we begin to tie-up forest development and protection with lowland, coastal and town development integrated planning?

B. Courses of Action

1. Legislation of an Integrated Protected Areas Network and provision of the necessary institutional support system for the preservation of the remaining original dipterocarp forest
2. Integration of all lessons learned into all phases of Forestry Development Programs for running successful programs along the following areas:
   a. Integrated Social Forestry
   b. Community Forestry Program
   c. Mangrove Rehabilitation Program
   d. Reforestation Program
3. Analysis of forestry policy formulation and implementation including appropriate management units and institutional arrangements at grassroot levels

4. Provision of adequate forestry research development priority areas related to logged over forestry utilization, forest ecology and conservation
5. Formulation of regional strengthening strategies in environment and natural resources management and training for DENR and Local Government
6. Establishment of DENR, NGO and Academe Network for Forest Management and Conservation at all levels

MARGINAL LANDS

Definition of Terms

Following the definition by the Department of Environment and Natural Resources (DENR), "Marginal lands are areas once covered with tropical moist forest, converted to plantation forests, fire-climax *Imperata* grassland and reproduction brushlands." These areas have the general characteristics of low productivity, inability to support extensive lowland type agriculture and high people in-migration rate. Those falling under the category of marginal lands are open lands, grasslands, range lands, grazing lands and pasture lands (DENR 1989).

Present Situation

The main issues and concerns in the marginal lands are population pressure, environmental degradation, rapid deforestation, lack of upland tenurial security, poverty and inequitable access to resources and inadequate soil and resource management. The interrelationship among these factors is shown in Figure 4.

The marginal lands in the country are composed mostly of converted forest, grasslands, brushlands and barren areas. They comprise 41% of the total land area in the country according to the latest DENR estimate. Their distribution, based on the 1988 satellite photo, is as follows:

cultivated mixed brushlands	-	10.1143 million hectares (mha)
grasslands	-	1.8129 mha
cultivated/open areas	-	0.3040 mha
eroded areas	-	0.0007 mha
barren areas	-	0.0103 mha
Total		11.9686 mha

Marginal lands are concentrated mainly in the degraded uplands (with slopes greater than 18%). Upland areas are estimated to cover 17,600,000 ha or 59%, or nearly 2 out of every 3 ha of our total land area. These were once covered with lush tropical forest vegetation but are now subject to extensive cultivation by upland inhabitants.

Native Philippine grasslands belong to four community types: *Imperata cylindrica* (cogon); *Themeda triandria* (bogokbok); *Capillipedium parviflorum* (Misamis grass); and *Chrysopogon aciculatus* (amorseku). These have been described by Sajise (1977) as marginal in productivity, undulating in topography, prone to soil erosion and remote.

Major range areas in the country are found in the hilly mountains of Northern Luzon, particularly in Nueva Ecija, Nueva Vizcaya, Isabela, Cagayan, Ilocos Norte, Abra and Pangasinan; in Mindoro and Palawan; Masbate, Panay and Negros in the Visayas; and in Bukidnon, Zamboanga and Cotabato in Mindanao (Umali and Pameron 1977).

The population now occupying marginal lands throughout the country has been estimated at 7,500,000 in 1988, about 50% of whom are national minorities (DENR 1988).

The present environmental concerns and issues with respect to the marginal lands are as follows.

1. Soil Erosion

At present, at least 22 provinces are known to have more than half of their areas eroded (Table 5). Nearly half or 36 out of 81 provinces are considered severely eroded. About 9,000,000 ha of alienable lands in the country are eroded in varying degrees and about 1,000,000 ha of agricultural lands are located on 8-15^{o} slope, making them susceptible to severe soil erosion during

the rainy season. This means that about 75% of our total alienable and disposable lands are vulnerable to erosion.

It has been estimated that the average annual erosion rate in Philippine uplands varies from 20-40 tons per hectare (t/ha) to as much as 200 t/ha in degraded grasslands (Revilla 1985 cited in Cruz 1989). Soil erosion rate in disturbed and overgrazed open grasslands has been reported to reach as high as 300-400 t/ha per year. Damage has been placed at about ₱2 billion annually as a result of nutrient loss and economic loss due in turn to sedimentation and siltation of dams, irrigation canals and coastal fishing grounds.

2. Low Productivity

The relatively low productivity of marginal lands compared to that of arable lands is shown in the fact that rice yield in the former is only 1 t/ha (20 cavans) on the average (Sajise et al. 1990). Furthermore, the productivity of upland rice was found to decline to one-third of the original value in three years' time due to soil erosion and dominance of cogon (Sajise and Magcale 1983).

3. Acid, Saline and Other Soil Pollution Problems

Another emerging problem in marginal lands concerns the occurrence of soil acidity and salinity. Many acid soils are particularly susceptible to the dispersion of clays because of the characteristic low electrolyte levels in the soil solution. This makes them relatively vulnerable to a variety of physical changes, such as erosion, compared to similar soils with higher base status (Holzhey and Kimble 1990). In addition, agricultural productivity could be hampered by the high exchangeable aluminum content, toxic levels of iron and less amount of calcium and magnesium and most essential nutrients in the acid soils.

The National Economic and Development Authority (NEDA) in 1989 reported that acid soils having $pH \leq 5.0$ occupied about 56% of the total land area of the country (Recale et al. 1990). The hectarage ranged from about 20-50% in these provinces. However, despite their productivity, acid soils are continuously being cultivated due to the limited area available for cultivation.

Soil acidity occurs naturally through the centuries during which soil development takes place as hydrogen ions carried by downward percolating waters slowly replace calcium and magne-

sium ions. This can, however, be induced by man's activities, such as excessive use of acid-forming chemical fertilizers and mining activities. In Loo Valley at the Benguet Province, for instance, intensive vegetable production with the use of inorganic fertilizers, particularly the acid forming ones, resulted in the increase in soil acidity (Alcantara undated).

Salt-affected soils, on the other hand, occur extensively in natural condition. This problem can, however, be induced by improper irrigation. Moreover, in the presence of a high groundwater table, restricted drainage could contribute to the salinization of soils (Bower and Milton 1957).

The severe intrusion of salt water in deep wells in the Central and Coastal areas of Bulacan had resulted in economic losses placed at ₱25,600,000 in only three years' time (Manila Chronicle, 28 Sept. 1988).

An adverse effect of intensified use of inorganic fertilizer in farming is the deterioration of soil structure, which makes it prone to erosion. The pesticides, herbicides and similar chemicals, on the other hand, pose serious ecological problems because of their high degree of chemical stability and persistence of their residues in the soil.

In 1987, some 11,514 mt of pesticides were imported by the country. Pesticide poisoning incidents are, however, increasing. From 1980-1988, an average of 503 cases were reported, about 15% of whom die every year.

The rate of deposition of mine tailings through the Agno River in Central Luzon (occurring since 1960) was 1 cm per day (PCARRD 1980). The problem has reduced the areas of irrigated farms, clogged irrigation canals and lowered crop yield by 50% in heavily silted areas and 20% on the average.

4. Loss of Biodiversity

Aside from deforestation, the simplification of biological communities results from extensive use of artificially created varieties, monocultures and uniform strains. While the objectives of these activities are indeed, noble, i.e., to be able to produce enough food for the growing population, there are expected negative repucussions.

In agriculture, these create greater probabilities of pest and disease outbreaks. More alarmingly, the extinction of many of the

wild strains, from which crops are developed, diminishes the potential to improve disease and pest resistance of the improved varieties. In forestry, the increasing emphasis being given on tree plantation types of activities, usually involving few, exotic, fast-growing species, increases the probability of failure of reforestation and tree-farm-based development for the same reasons. An example is the psyllid infestation on *Leucaena* spp..

5. Uncontrolled Fires

Fire is a regular occurrence in grass-covered areas in the uplands that prevents the establishment of reforestation species. This usually occurs during the dry season when water is almost lacking (Sajise 1983). Fires go on uncontrolled at times for weeks especially during this season. For instance, an uncontrolled fire in 1988 destroyed a vast area from the Dalton Pass in Nueva Viscaya and throughout the circuitous mountain highway to Santiago, Isabela - a distance of more than 100 km (Philippine Daily Inquirer, 8 April 1988).

In 1978, there were 21,094.32 ha of forest plantations razed by 1,225 fires (NEPC 1980). During this year, 2,148,377 saplings valued at P6,700,000 were destroyed. Similarly, records show that in 1981 and 1982, forest fires were responsible for an average of 49.6% of the total forest destroyed during these years. Shifting cultivation and logging accounted for only 21.7% and 27.28%, respectively (NEPC 1983).

Repeated burnings scorch and kill tree seedlings and abort the regrowth of trees. Furthermore, the exhaustion of tree seed supplies in the soil, coupled with the decline in soil fertility as a result of exposure, ultimately lead to the establishment of grass-dominated vegetation with which the reforestation species could hardly compete.

However, the DENR lacks manpower and proper equipment to cope with the problem. As the Philippine Daily Inquirer, 8 April 1988, reports: "The standard equipment of an FMB forest proteciton officer consists of an antiquated ordinary water sprayer, fire rake and fire swatter from tree branches. And because of lack of transportation utility, they have to hike at times for several days to reach a fire area. Their common defense is to establish a fireline or isolate undamaged area."

Worst, we have at present insufficient knowledge on the natural system's capabilities and limitations with respect to fire (DENR 1988).

6. Poverty and Low Access to Production Resources

The lamentably low socio-economic condition of the people living in the uplands and other marginal areas is evident in the fact that majority of them realize only an annual income of between ₱800 and ₱3000 (Philippine Panorama, 1 Jan. 1990). These people, situated in remote places, are total strangers to such basic services as health, education and livelihood. Consequently, they appear to be the least educated, lowest paid, least healthy and most neglected in agricultural development (Alcantara 1985). David (1987) furthermore observes that with very poor harvest owing to the marginal nature of the land, the nutritional status of upland farmers is worse than that of their lowland counterparts.

The marginal farmers' very minimal access, or lack of it, to production inputs has been well documented. In a study of marginal farmers in Southern Leyte and Eastern Samar, it was found that more than half of the respondents (63% and 94%, respectively) did not apply fertilizers and pesticides (65% and 92%, respectively), citing as primary reason the lack of money to buy these inputs (Tabada and Dabuet 1985). Those who applied the inputs used them in their lowland rice fields. It was also revealed that the predominat source of labor was the family. Most respondents (45% in Southern Leyte and 52% in Eastern Samar) did not use hired labor due to lack of money.

The lack of infrastructural and institutional support mechanisms in the remote marginal areas hinders access to social services, financial and marketing facilities and various technical assistance programs by both the government and NGO groups. These inhibit the opportunity of bringing in sustainable development to these areas.

In addition to these, the worsening peace and order situation in the uplands serves as a deterrent to productivity. The upland farmers are often caught in the middle of the war between the government forces and the insurgents. Moreover, with their poor socio-economic condition, they are vulnerable to the recruitment by the insurgents fomenting socio-political unrest in the countryside.

Factors Affecting Degradation

There are certain conditions in our country that tend to reinforce and perpetuate the issues and problems obtaining in the marginal lands.

1. Rapid Population Increase

According to the 1990 preliminary population count of the National Statistics Office, the country has a population of 60,500,000 as of May 1990 and is growing at 2.3% per year. The population is projected to grow to 71,000,000 by year 2000 and 97,000,000 by year 2030 (Herrin 1988). Before the country attains zero population growth in the year 2075 the number of Filipinos would have reached the 125 million mark.

Although the population growth rate is now perhaps in the "transition stage towards slow growth levels", the deceleration is observed to proceed at a rate too slow for economic growth to support (Philippine Daily Inquirer, 21 Aug. 1990). The population, furthermore, is already large that yearly increments to the total, even on a declining rate, would still be considerable. In the light of this trend, it is expected that greater population-driven pressure on land will result in the conversion of more forests to agriculture and other uses.

The greatly emerging pressure on land and resources is best illustrated in the fact that about one-third (17,800,000) of the total Philippine population lives in the uplands. This segment of the country's total population grows at a very fast rate of 2.54%, which is greater than the national average. By the year 2000, the upland population is projected to increase to 24,000,000-26,000,000 (Cruz 1989). This means that an additional 5,000,000-6,000,000 people in hilly areas will be opening up more lands for cultivation. Cruz (1989) calculated, on the assumption that about 2 ha were used for cultivation per upland family of 6, that another 8 million hectares of forest lands would be converted to agriculture to be able to feed this population. This implies the clearing of about four-fifths (4/5) of the present forest land under tree cover.

Without adequate tradition of upland farming and with little knowledge of ecological constraints in the hillslopes, the upland migrants (representing 50-60% of the 17,800,000 population) practice the only way they know of hill cultivation: shifting cultivation. Unlike the practice of traditional tribal communities, how-

ever, shifting cultivation by the migrants often leads to erosion problems and land degradation.

Erosion, in turn, leads to declining yield, abandonment of fields and compensatory conversion of new forest areas for cultivation. In this manner, the forest environment is virtually transformed into cogonal lands. Without the necessary inputs to intensify and practice sedentary farming, this practice of extensification in the fragile uplands is expected to continue unless adequate measures are put in place.

Worse, movement into the uplands continues unabated. Although the present population growth rate in the upland areas reflects a declining trend from about 3.0 percent in the 1950s and 1970s, the fall in in-migration seems to occur only in municipalities with already high densities of about 150-250 persons/km (Cruz 1989). Economic displacement (as there are not enough industries to give them employment and income opportunities), the spiraling costs of living and stiff competition for access to lowland resources, social injustice, and other factors continue to push the poor lowland population into the uplands.

At present, millions of rural inhabitants do not have the opportunity to eke out a living on a piece of land while a few rich people own vast tracts of arable often underutilized lands. In 1987, 154 concessionaires held the right to exploit more than a third of the country's prime forest resources (National Statistics Office 1989). On the other hand, the "poorest of the poor" Filipinos languish in the degraded forest areas and marginal lands.

The DENR (1988) admits that despite current efforts to more equitably allocate forest resources, the structure of forest land disposition still favors timber concessionaires. In 1988, the Timber Lease Agreements covered about 23% of total forestlands while only 3.0% were allocated to some 152,528 beneficiaries of various Integrated Social Forestry (ISF) programs. A positive development is the plan of the DENR to reduce the number of TLA holders from 65 this year to about 50 and eventually phase out the scheme with the full implementation of the Forest Land Management Agreement (FLMA).

2. Insecurity of Land Tenure

The Department of Agrarian Reform (DAR) reported in 1987 that of the country's total population of 56,000,000, only 1,500,000 Filipino farmers owned the land they tilled. About

2,000,000 are share and leasehold tenants. Some 1,500,000 farmers in the public lands do not have land titles.

The insecurity in land tenure status of upland farmers is influenced by the prevailing land use policy, particularly the 18% slope cut-off set for public lands. Based on this, 14,108,000 ha or 47% of the country's total land area officially belong to the alienable and disposable (A & D) category while the rest (53%) are forest lands (Philippine Statistical Yearbook 1987). However, this does not take into consideration the fact that land forms in the country are mostly hilly and mountainous with about 59% of the total having $\leq 18\%$ slopes. The inappropriateness of the policy to the natural topography of the country is reflected in part by the fact that of the areas considered as uplands, about 5,600,000 ha or 39.7% constitute A & D lands. Confusions therefore arise in the classification of lands into A & D and forest lands. This is because basing only from slope consideration, some areas already declared as A & D do not qualify into the category while some forest lands have been classified as A & D. Moreover, factors such as land quality, climatic conditions, market accessibility and criticality of watershed are ignored in the prevailing land use classification based on slope criterion.

Land rights conflicts in the country also arise because of the current government policy of vesting private rights based on the Regalian doctrine. This doctrine espouses the principle that all natural wealth (except for A & D lands) belongs to the State. The doctrine has been the cornerstone of Philippine land use laws since the Spanish times. There are two natural consequences of this adherence to the doctrine.

1. **Native titles and customary laws are not acceptable or recognized by the state.**

2. **Displacement of both cultural minorities and long-settled upland migrants occur very frequently due to land grabbing and encroachment by various development and industrial projects.**

Other examples of laws, which resulted in the exploitation of tribal Filipinos, are Presidential Decree PD 410 (also known as Ancestral Land Decree) and PD 705 (also known as the Revised Forestry Code). PD 410 requires that tribal lands be registered within a specific period of time, after which all unregistered lands

will be declared public domain. PD 705 prohibits undertaking kaingin, upland farming or any other activity without government license on ancestral lands without title. These laws are entirely alien to the tribal Filipinos whose systems of ownership are rooted in some customary beliefs on property rights.

The evolution of social forestry concepts has resulted in the recognition of upland farmers' tenurial security over some period of time on designated portions of land. However, this is still short of their goal of owning and thereby securing their family's perpetual rights over the land. Moreover, the lands available under the ISF program are usually marginally productive lands not covered by various concessions, permits and leases and hence could not sufficiently support an acceptable level of development for the beneficiaries. The various land stewardship contracts, such as the Communal Stewardship Contract (CSC) afforded to cultural communities likewise show the government's apparent lack of definite stand in officially recognizing vested rights on ancestral lands. This kind of stand is rooted in Article XII section 5 in the 1987 Constitution, which provides that:

... The state subject to the provisions of this Constitution and national development policies and programs shall protect the rights of indigenous and rural communities to their ancestral lands...

This action of the government results in the undermining of the efforts of these groups to exclude migrants from their occupied territory. In many cases this has led to their displacement in the pretext of serving national interest.

Therefore, those who benefit from these laws are the foreign transnational corporations and other local agri-businesses supported by infrastructures, such as dams and processing zones.

Another problem with land allocation is the absence of an agency solely entrusted with land classification. This is because agencies other than the Land Classification and Regulatory Board can place certain portions of land under their jurisdiction and management through presidential or legislative processes. The slow process of classification of public lands, which results in the pre-emption of lands into non-forestry use, is another problem. Those lands are finally classified as forest lands. The process of classifying lands tends to be a very slow process as this involves not only physical but also ecological, economic, socio-cultural and

legal considerations. Another factor which hampers efforts is the big gap in the length of time between the time survey results come out and the proposed A & D lands are finally certified for release.

Lastly, while the government is now in the process of evolving various tenurial forms for the uplands, we do not have the sufficient time and research data to support and identify the appropriate tenurial forms and rehabilitation strategy (Sajise and Ganapin 1990).

3. Slow Rate of Reforestation

Reforestation efforts in the country had taken great strides since the launching of the Program on Forest Ecosystem Management (PROFEM) in 1976. From this year up to 1988, the area reforested averaged 54,553 ha per year compared to the 20,000-hectare average in the pre-PROFEM period. However, this figure, as stated earlier, still leaves a very big gap (about 135,000 ha annually) between deforestation and reforestation based on official DENR figures. The wide discrepancy between the size of forest utilization (by logging) and plantation establishment area from 1976-1988 is shown in Table 6.

Although considerable improvements have been made under the current DENR procedures, the reforestation effort is still being constrained by several problems. Foremost is the high state of denudation in many parts of the country vis a vis the high cost of reforestation. The FMB targets an annual reforestation rate of 100,000 ha in the critically denuded watersheds, 50,000 of which are supposed to be financed by private entities. The budgetary allocation, however, is only for 20,000 ha - - a rate well below the levels in the late 1970s. At the present reforestation cost of about ₱21,500/ha, the 100,000-hectare target, if done solely by the government, would cost ₱2,150,000,000 per year or about 0.9% of the 1990 government expenditures.

While it is true that reforestation is critical to the management and rehabilitation of the denuded marginal lands, a key factor for the success of these endeavors and the conservation of the remaining resources is protection, particularly from fire and illegal

settling in steep slopes. However, lack of manpower hampers protection and law enforcement efforts on the part of the DENR.

RECOMMENDATIONS

1. Preserve and protect the remaining primary forest, especially in critical areas like national parks, protection forest and watershed. A total log ban in selected old growth dipterocarp forests and their proclamation as preserved/protected areas should be promulgated by the legislative branch.

2. Reexamine policies regarding tenurial status of upland communities and provide mechanisms, which will improve their participation in land and resouce use planning and in the allocation of benefits.

3. Provide strong support for research on the ecological processes and regeneration of marginal areas.

4. Expand the agrarian reform program and provide mechanisms, such as more employment opportunities, which will reduce the incentive to migrate to marginal lands.

5. Review criteria on selecting sites for social forestry to include consideration of both land capability and the needs of the local communities. Evaluate carefully the performance of current permit holders to ensure success of the program.

6. Develop a rational land use plan and management, which provides the variety of habitats necessary for the maintenance of species diversity.

7. Devise a well-supported program to promote proper management and even reclamation of "problem soils." There is a great need to conduct research on appropriate management schemes and agrotechnology for these types of soil.

8. Convert reclaimable open grasslands into productive agroforestry farms. Good candidate sites for reclamation are those within the 18-30% slope condition. Those falling within the 30-50% slope should be left under forest or converted into agroforestry while those with higher slopes should be fully protected.

CROPLAND'S ECOSYSTEM

The country's croplands are now under environmental stress. Prime or productive agricultural lowlands and alluvial plains are rapidly shrinking from about 8,000,000 ha to less than 5,000,000 ha. Soil erosion is severe in 36 of the 81 provinces. Urban encroachment into prime and productive agricultural lands has reduced the country's agricultural production capacity and the decreasing man-land ratio has led the landless to occupy and cultivate ecologically unstable croplands.

Under NEDA's Medium-Term Development Plan (1987-1992), the government is adopting the following strategies for cropland conservation: (1) promotion of the efficient use of agricultural lands in order to meet the food requirements of the growing population and to provide sufficient, low-cost and nutrient-rich agricultural crops; and (2) intensification of land classification to promote optimal use of land and to prevent and/or discourage conversion of agricultural lands to non-agricultural uses.

The Department of Agriculture will be pursuing some policy reforms as expressed in its Medium-Term Development Plan (1990- 1995). These include the formulation of a land use policy, which will preserve the best agricultural lands for agricultural use, ensure the sustainability of the agricultural resource base and adhere to the spirit of the Comprehensive Agrarian Reform Law.

This segment of the paper analyzes the state of the cropland environment in relation to other ecosystems. Policy recommendations are also given to maintain the integrity of the croplands.

Extent

Of the 30-million-hectare total land area of the Philippines, about 9,900,000 ha (33%) are classified as croplands. The

classes of lands under this category based on SPOT (1987) in million hectares (Mha) are the following:

coconut plantations	-	1.1326 mha.
other plantations	-	0.0908
arable land, cereals, sugar	-	4.3923
crops mixed with coconuts	-	3.7478
crops mixed with other plantations	-	0.3652
fishponds from mangroves	-	0.1952
other fishponds	-	0.0101
TOTAL	-	9.9340 mha.

By region, the largest percentage of farm areas can be found in Regions IV (22.98%), XI (11.67%), V (10.72%) and X (9.9%) (NSCB 1989). Temporary crops occupy a large percentage of arable lands in Regions VI, XII, III, II and IV. Permanent crops on the other hand are found mostly in Regions X, XI, IX and XII.

Based on the cropping utilization data in 1983, corn (3.16 mha) and rice (3.12 mha) occupied the largest percentage of Philippine croplands planted to annual crops (Fenandez and Badayos 1986). High yielding varieties of rice occupied about 82% of total rice cropped area and 21% of total croplands. Coconut, on the other hand, was the most dominant perennial crop occupying 26% of the total cropland area. Pasture areas occupied 5.78%.

Issues

While one-third of the country's total land area is actually farmed, only 58% or 5,800,000 ha of agricultural lands are said to be suitable for crop production (Porter with Ganapin 1988). Furthermore, only 2,500,000 ha are considered to have the potential to respond to intensive agriculture or can be cropped more than once a year (NEPC 1985).

The current demand for higher production to sustain the food needs of the growing population aggravates this situation. In addition, the sustainability of the croplands is being threatened by the combined effects of degradation of soil quality due to

excessive use of agricultural chemical inputs, land pollution, unsustainable farming in the fragile uplands and climatic changes. The loss of genetic diversity due to the excessive use of artificially created varieties is also an emerging factor in sustainability.

Of great concern to small farmers, on the other hand, is the issue of land ownership and inequitable access to land resources. Another problematic area is the present land use system, which fails to control the growth and encroachment of urban, commercial and industrial land uses into prime agricultural lands.

1. Soil Erosion

The Bureau of Soils, in its 1984 surveys, has identified 8,250,000 ha (or 75%) of the country's alienable and disposable (A & D) lands as severely eroded. Of the 5,780,000 ha suited for crop production, 45% belonged to Erosion Class A (0-5% slope); 31% Class B (6-10% slope); 1% to Class C (11-15% slope); and 23% to Class D (16-22% slope). This means that all of these land types are susceptible to erosion in varying degrees.

In addition the Magat, Bicol, Agno and Pampanga River Systems, which are important to lowland irrigation and hydroelectric power generation, are in danger of drying up due to soil erosion. The irrigation system, which taps the Agno, owes 65% of the total sediment load to erosion during the rainiy season (NEPC 1977).

2. Land Pollution from Agricultural Activities

Following the introduction of HYVs of rice in the late 1960s, fertilizer consumption in the country increased from 200,000 mt in 1960 to 878,000 mt annually in 1983. Higher consumption of chemical fertilizers and pesticides was encouraged by the government by subsidizing their purchase through the Masagana 99 Program, which started in 1975.

Fertilizers are important to enhance productivity. Rola (1988), for instance, estimated that 25% of total production gains in the country have been due to fertilizers. Herdt and Capule's (1983) study has shown that use of fertilizers increased yields of modern rice varieties by about 31%. The major increase in fertilizer and chemical use and the increase in irrigation during the Masagana year's Phases V and VI finally made the Philippines self-sufficient

in rice for the first time in 1976. By 1977, the Philippines started exporting rice (Bernardo 1985).

On the other hand, it has been commented that "the apparent stability of rice yields through the 1950s and 1960s masked the fact that yields in areas not benefiting from irrigation and fertilizer were declining and that newly opened rice lands were consistently less than those in established rice growing areas. Fertilizer consumption quadrupled and irrigated land tripled between 1950 and 1969, while average rice yield stagnated." This observation is supported in Table 7 and Figure 5, which reveal that the increase in fertilizer consumption since 1986 was accompanied by a sharp decline in efficiency of producing palay and corn. In contrast, the period from 1983-1985 when fertilizer consumption declined was the period of highest production efficiency in palay and corn.

There are also ecological problems concomitant with chemical fertilizer use. Excessive use of this input is known to cause acidification of soil. Acid soils are vulnerable to erosion because of the characteristic low electrolyte levels in the soil solution. Acidity also depletes soil fertility through the development of toxic levels of iron and by lowering the amount of most essential nutrients in the soil. In addition, soil microbiota, which are partly responsible for nutrient release, are adversely affected (Medina 1990).

The use of chemical fertilizers also poses some health problems. Contamination of drinking water with nitrate concentration greater than 45 parts per million (ppm) can cause metheglobinemia disease, which affects both livestock and human infants (Rola 1990). Another health hazard results from the carcinogenic nitrosoamines formed when nitrates in the food or in the digestive system combine with protein.

Nitrogen and phosphorus nutrients from fertilizers are washed down by run-off water into freshwater bodies creating eutrophication problems. One glaring example is the much eutrophied Laguna Lake. Of the 3,600 mt of nitrogen, the major determinant of the recurrent algal blooms that enter the lake, 77.2% is agricultural in origin (LLDA 1978).

The emergence of the pesticide industry in the country in the late 1940s coincided with the introduction of DDT; 2, 4-D;Endrin and Malathion (Elazequi 1989). With the launching of the Green

Revolution in 1965, the government embarked on a crop protection program based on chemicals. The Bureau of Plant Industry, in pursuance of this objective, treated half of the total irrigated rice lands with pesticides free of charge. Various government programs in crop production, like the Masagana 99, Masaganang Maisan and Gulayan sa Kalusugan gave rise to the massive use of pesticides and the expansion of plantation areas in the country.

The use of pesticides is claimed to be responsible for increasing marketable surplus by minimizing crop losses due to pests. It has been measured, for instance, that yield losses range from 0.5 to 1.7 t/ha if no pesticide is used in agricultural production (Herdt et al. 1984).

However, the use of pesticides as crop protection agents has many negative effects. One cause of alarm concerns the health hazards they pose to farmer-users. Statistics from rural health clinics have shown an increasing trend of pesticide poisoning cases, especially on the working group population (Rola 1989). Loevinsohn's study in 1987 showed that widespread use of pesticides by farmers in Central Luzon was followed by 27% increase in deaths among them from causes other than physical injury. Human milk in some towns of Laguna was found to contain DDT (Barril et al. 1974).

On the other hand, Dr. Teodoro Mendoza of the UPLB Department of Agronomy pointed out that the combined effect of rice ideotypes, namely, high photosynthetic productivities and photosysthetic partitioning in favor of grain yield, tied the farmers to greater needs for off-farm inputs (PESAM Bulletin 1985). To increase crop yield requires four bags of fertilizer per hectare. To double the yield therefore means a tenfold increase in fertilizer input.

3. Tenurial Issues

The problem of land ownership and distribution is being addressed by the government through the implementation of the Comprehensive Agrarian Reform Program (CARP). Under this program, the Department of Agrarian Reform (DAR) officials have claimed the distribution of 430,000 ha of land all over the Philippines from July 1987 to May 1990 (Philippine Daily Inquirer, 8 Oct. 1990).

Critics, however, said that only 1.2%of CARP's 10-year hectarage target has been achieved so far. Moreover, it is said that

79% (341,149 ha) of those distributed so far were distributed under Presidential Decree (PD) 27 of the late President Marcos.

Budgetary constraints cripple CARP implementation. Of the $10- billion budgetary requirement to carry out the program, 50% will be raised from foreign donors in grants and loans. However, due to DAR's frequent leadership turnover, the coordinating council of the Philippine Assistance Program (PAP) recommended the deferment of CARP's presentation to PAP foreign donors last July 1990 (Philippine Daily Inquirer, 8 Oct. 1990).

4. The Land Constraint

The emerging pressure on the fixed land resource becomes more alarming in the light of several claims by several studies that as early as the beginning of the 1970s, the country has already reached its land frontiers in terms of land utilization for agriculture purposes (Encarnacion et al. 1976; ILO 1974 cited in Agenda for Action for the Philippine Rural Sector 1986).

URBAN AND INDUSTRIAL ENCROACHMENT

As rural lands evolve into urban lands, the need for prime agricultural land competes with the need for urban growth. This was most obvious in Metro Manila where a 35.29%- decrease in ricelands occurred from 1972-1976 as a result of the combined growth of residential, commercial and industrial areas (Pascual and Silonga 1978).

Diminution of agricultural lands also resulted from infrastructure development projects. For example, hydroelectric dams require flooding of thousands of acres in impounded areas, which silt up in time and become less useful (Stein 1973). Controversial examples are the Chico and Magat Dams in Luzon.

RAPID POPULATION GROWTH: IMPLICATIONS IN AGRICULTURE AND FORESTRY

Porter and Ganapin (1988) observed that: "Until 1960, the high population growth rates did not reduce the total ratio of cultivated land to population because new land was being brought into cultivation even faster than population was growing. From the late 1950s, while population was growing at an average annual rate of 3.1%, cultivated land was increasing at an average

annual rate of 3.8%. During the 1960s, however, the average annual increase in cultivated land of only 1.4% fell far behind annual rates of population increases, which continued to average over 3%.

Fernandez and Badayos (1986) analyzed the trend in areas planted to various crops in the country from 1979-1983. The trend analysis indicated a downward trend of 9,525,000 ha per year (Table 8). With 1979 as the base year, the percent decrease in total area for all crops in 1983 amounted to approximately 4.0% equivalent to some 500,000 ha. The same study also revealed that from 1979-1983, increase in area among the major crops in the country occurred only in irrigated areas planted to HYV rice, coconut, fruits and nuts, coffee and rubber.

On the other hand, the expansion of the land area devoted to crop production between 1971 and 1980 was made possible through a decrease in the hectarage utilized for other purposes (Agenda for Action for the Philippine Rural Sector 1986). Table 9 reveals that the area of productive forest and open lands declined as a result of the areal expansion of plantation and croplands and urban areas.

Because non-agricultural occupation could absorb only so many workers, population increase necessitates the further expansion of agricultural areas. However, the then Ministry of Human Settlements estimated that as much as 1,000,000 ha of high quality land would be overtaken for non-agricultural purposes by the year 2000 (The World Bank 1980).

5. Low Income and Poverty

Data from the agricultural sector from 1980-1983 revealed that around 5,300,000 families representing 56% of the total number of families in the country depended on agriculture (Philippine Agenda for Rural Development 1986) and majority of them were at the bottom 30% of the income distribution brackets.

6. Climatic Factors

Adverse climatic conditions have been estimated to cause loss of 85% total annual production of rice and corn in the country.

For instance, as a result of the prolonged drought, crop production declined by 4.77% in 1990 according to the National

Statistics Coordination Board (NSCB) (cited in Philippine Daily Inquirer, 28 October 1990).

Other calamities, which have had profound effects on the productivity of our croplands, are floods and typhoons. A total of 54 incidences of flooding was counted from 1973-1989 while a total of 127 typhoons occurred from 1983-1989 (Office of Civil Defense Operations Center).

RECOMMENDATIONS

The policy and program gaps noted, as well as the measures recommended for the croplands ecosystem, are as follows:

1. **The absence of a national agricultural land use policy, which will resolve the conflict between agriculture and industry in terms of positioning for space.** At present, the NEDA Land Use Committee is confronted with the problem of conflicting land use between the agriculture sector and the industry, housing and commercial sectors. Resolving this conflict becomes difficult for the committee due to the absence of an agricultural land use policy, which sets the directions for the proper disposition of prime agricultural lands. By the year 2020, the demand for land for non-agricultural uses will be roughly about 6,000,000 ha which is almost equal to the size of prime agricultural lowlands left. Therefore, this demand for urban land use poses a tremendous threat to the remaining agricultural lands, especially the productive lowland areas. It is therefore urgent for the government to formulate a land use policy at this stage to be able to have enough lead time to plan and implement a sound land use development program.

2. **The lack of appropriate measures to protect prime agricultural lands from being converted to other agricultural uses.** The continuous encroachment of urban uses into prime agricultural lands has not been satisfactorily abated due to the absence of a national agricultural land use policy. Moreover, the protection and preservation of prime agricultural lands has not been properly implemented by the DA not only because of the absence of such policy

but also due to its failure to clearly define the characteristic properties of prime lands and to provide information on their geographic locations or spatial distribution. The NEDA Land Use Committee has recently come up with a definition of prime agricultural lands. If this definition would nevertheless be officially accepted, the next most important matter is to assess the status of prime agricultural lands through mapping and delineation and to formulate measures to protect and preserve these lands.

3. **The need for a well-coordinated and well funded national soil conservation program covering both lowlands and uplands.** Efforts to conserve soils and minimize erosions are undertaken by the Department of Agriculture - Bureau of Soil and Water Management (BSWM) in agricultural lands under 18% slope, and by the DENR in forestlands (i.e. above 18% slope). The BSWM soil conservation program has its limited focus on Soil Conservation Guided Farms project and Small Water Impounding project. Considering, however, the extent and magnitude of the soil erosion problem in the country, these efforts of the BSWM still seem limited. While the DA-BSWM undertake soil conservation in agricultural lands, the DENR is doing a similar job in the uplands/forestlands by virtue of its mandate. The forestation and ISF efforts of DENR, aside from their still limited scale, do not fully address the soil erosion problem in cultivated forestlands due to the lack of technology, knowledge and experience of DENR extension workers on upland soil conservation farming systems. The DA extension workers, on the other hand, also lack the knowledge and experience in agro-forestry. A national soil conservation program, which integrates and closely coordinates the efforts of these two agencies in developing soil conservation farming system technologies and undertaking soil conservation extension and technology transfer, is therefore necessary to fill the institutional gaps which constrain and limit their efforts in achieving an effective soil conservation program nationwide. Furthermore an intensification of efforts in the conservation of critical upland areas requires the collabo-

ration of the government, the NGOs and the local communities.

4. **The need for agricultural planners of the DA to acquire the necessary skills in agroenvironmental planning/program implementation and the need for extension workers of the DA and DENR to acquire the knowledge and technology in upland soil conservation.**

5. **The need for the DA, the DENR and the DAR to collaboratively establish a coherent program for sustainable agroenvironment development.** The Philippine Strategy for Sustainable Development (PSSD), formulated by the DENR, is somewhat biased toward the natural resources sector. It has overlooked some important aspects of the agroenvironmental sector, such as the protection and conservation of prime agricultural lands, the development and transfer of agrotechnologies for hilly and highland agroecosystems, the promotion of highly efficient agricultural land uses and the reclamation of problem soils. Likewise, the PSSD still has to be translated by the DA and the DENR into a viable operational plan. In particular, the existing programs and projects of the DA and the DENR should be organized coherently under the PSSD operational plan in order to avoid overlaps and redundancies or even conflicting programs and projects. An Integrated Sustainable Environment Development Program would serve as a means of complementing and reinforcing the efforts of the DA and the DENR. This kind of program should be pursued through the creation of a special inter-agency technical working committee.

6. **The need for the DAR and the DENR to provide security of tenure to the uplanders or those occupying areas classified as forestlands.** A potential solution to this problem is to reclassify lands suitable to agriculture within the 18-30% slope into A & D lands and issue titles to deserving beneficiaries. This may, however, pose some legal questions and create jurisdictional debate between DA and DENR. Worst, this will serve to attract more upland migration. Perhaps a better solution would be to revise some of the conditions governing the CSC, such as making it transferable to other upland occupants,

automatically renewable. And for those holders who satisfactorily achieve the objectives of the ISF, a longer renewable period of 50 years would be awarded.

7. **The lack of a program to monitor and control pesticide and fertilizer pollution.** Pesticide and fertilizer residues are not properly monitored by concerned agencies, such as the Fertilizer and Pesticide Authority and the Environmental Management Bureau, due to the lack of necessary laboratory facilities and equipment, as well as manpower and technical capability. Another factor that contributes to the problem of pesticide misuse, in addition to the lack of users' access to technical assistance, is the lack of information provided by the sellers and distributors on the hazards of pesticide use. Labels on pesticides are sometimes not fully understood by users. With the government's target to meet 100 percent self-sufficiency in rice, it must correspondingly increase its extension efforts to promote the proper use and management of fertilizers and pesticides, and to develop and expand its program in organic fertilization and biological pest control.

8. **The need to pursue vigorously an integrated population and environmental resources development program.** The DENR is at present unaware of the maximum sustainable population that could be supported by available upland resources. Likewise, there is no clear-cut land use plan for the upland areas in general and the settled areas in particular. The populations and ecological upland carrying capacities should be assessed by the DENR toward the formulation of a sound land use plan and the promotion of a population program in the uplands. The DENR and the DA should actively participate in this population control campaign by integrating population programs in their development and extension works and by collaborating closely with concerned agencies of the government adiministering population programs. One promising approach is to integrate population concerns with development programs, such as actively involving women in upland development; providing better education, nutrition, health services and more diversified livelihood opportunities in the uplands; and improving security of tenure. Meantime, the government should fully develop lowland

agriculture and support agro- industrialization as a prelude to full-scale industrialization. This strategy is consistent with the agrarian reform vision of transforming tenants into productive tillers of their acquired lands, and former landowners into agro-industrial entrepreneurs. This development strategy worked for Taiwan in absorbing surplus labor and accumulating capital resources, which provided the take-off point for industrialization.

CONCLUSIONS

As a conclusion, the state of the terrestrial environment has caused:

- Low income and poverty;
- Displacement of cultural communities;
- Social conflicts and instability;
- Declining productivity of natural resource base;
- Poor health and nutrition; and
- Hydrologic instability and siltation.

Environmental problems during the next two decades will be exacerbated by:

- High foreigh debt burden;
- Poverty;
- Rapid population growth;
- Inequity;
- Weak institutional capacity;
- Non-participation of local communities; and
- Possibly a stagnant economy and non-responsive political set-up.

There are successful examples of locally-evolved and implemented natural resource management projects that we can draw lessons from, i.e. Bontoc system, Ifugao, Mangyan, Kalahan, SALT, Jose Panganiban, RRDP and many more.

This is the background upon which an environmental agenda for the next decades must be based.

THE ENVIRONMENTAL AGENDA

* Developing a rational land use plan adopted at all levels, (national, regional, provincial, local) to ensure sustainable natural resources use and avoid conflicting use of resources

* Protecting the remaining primary forest areas, especially in critical areas (national parks, protection forest, nature reserves, watersheds) and endangered flora and fauna for maintaining biodiversity and ecological stability

* Providing strong support for environment cum population related programs

* Promoting and enhancing implementation of community-based, participatory and integrated approaches to natural resource management, taking off from successful examples

* Providing adequate research and development support for long term and priority concerns related to agricultural sustainability/ management of logged over areas; upland, coastal and urban rehabilitation and management

* Promoting environmental education and extension programs for all sectors of society

* Establishing line agency (DENR, DA, DAR) - NGO - academe network for natural resource management at the national, regional, provincial and grassroots levels

* Evolving a comprehensive national policy of environmental ethics and management, which would encompass promotion of environmental awareness, formulation and enforcement of legislation to protect and enhance the environment and giving support to NGOs to encourage their participation

* Recognizing the scale and nature of current urbanization and assessing the extent of deterioration so that the resource base can be rehabilitated and preserved

* Adopting low-waste and environmentally sound technologies utilizing recycling, rehabilitation and renewability

* Adopting agriculturally sustainable technology and practices, such as organic agriculture, integrated farming and integrated pest management

* Promoting economic incentive schemes for regulating use of natural resources and pollution control measures

* Developing and adopting an environmental master plan for major urban centers and the 179 Community Environment and Natural Resources Offices throughout the country reflective of the Philippine Strategy for Sustainable Development (PSSD)

* Developing strong and effective preventive and anticipatory environmental management mechanisms, such as an Environmental Impact System (EIS), at all levels (national, regional and local) on hazardous biological and chemical handling and regulation, etc.

These agenda must be pursued vigorously through policy reforms, legislation and programs if we hope to pursue a direction of sustainable development instead of short term growth and even probably national suicide by installment.

Table 1. Land use/land cover classification of the Philippines

Land Use/Land Cover		Area (000 ha)	% of Total
A.	Forest	7,226	23.92
	Pine	81	0.27
	Mossy	246	0.81
	Dipterocarp	6,629	21.94
	Closed	2,435	8.06
	Open	4,194	13.88
	Mangrove	149	0.49
	Other	-	-
B.	Extensive Cultivation	11,958	39.58
	Open in forest	30	0.09
	Grassland	1,813	6.00
	Mixed[a]	10,114	33.49
C.	Intensive Cultivation	9,729	32.20
	Plantation	5,336	17.67
	Coconut	1,133	3.75
	Other Crops	91	0.30
	Coconut and Cropland	3,748	12.40
	Other Crops and Cropland	365	1.20
	Cropland	4,392	14.54
D.	Fishponds	205	0.67
	Fishpond from Mangrove	195	0.64
	Other Fishponds	10	0.03
E.	Non-vegetable Areas	101.4	0.34
	Eroded areas	0.7	0.002
	Quarries	8	0.02
	Riverbeds	81	0.27
	Other Barren Land	10	0.03
F.	Others	439	1.45
	Built-up Areas	131	0.43
	Marshy Areas	103	0.34
	Lakes	205	0.68
G.	Unclassified	546	1.80
	TOTAL	30,205	99.96

[a] Mixed grass, brush, plantation and other crops

Source of Data: Swedish Space Corporation, Mapping of the Natural Conditions of the Philippines, Solna, Sweden, (1988).

Table 2. Forest cover by Philippine Regions

Region	Land Area (ha)	Forest Cover (%)	Total Reproductive** Dipterocarp forest (ha)
1	2,156,845	14	337,300
2	3,640,300	42	862,400
3	1,823,082	16	178,600
4	4,756,016	19 *	675,200
Palawan		54	
5	1,763,249	7	58,500
6	2,022,311	7	56,400
7	1,495,142	3	12,100
8	2,143,169	26	351,000
9	1,868,514	20	187,800
10	2,832,774	37	703,400
11	3,169,275	31	720,000
12	2,329,323	34	257,200
Philippines	30,000,000	100	4,399,900

* data exclude Palawan

Source of Data: Swedish Space Corporation, Mapping of the Natural Conditions of the Philippines, Solna, Sweden (1988)

** Consolidated SPOT (1988) and RP-German Forest Inventory (1988)

Table 3. Deforestation rate, forest and cultivated land by Region in the Philippines, 1988

Region	Deforestation rate (%)	Total Land ('000 ha)	Total Forest ('000 ha)		Total Cultivated ('000 ha)	
1	0.8	2157	289	(13.4%)	810	(37.55%)
2	0.6	3640	1470	(40.38%)	928	(25.49%)
3	2.3	1823	294	(16.13%)	815	(44.70%)
4	2.3	4840	1334	(27.56%)	1692	(34.96%)
5	5.2	1763	118	(6.7%)	1000	(56.72%)
6	3.9	2022	140	(7.0%)	974	(48.17%)
7	4.6	1495	45	(3.0%)	685	(45.82%)
8	3.4	2142	559	(26.097%)	782	(36.508%)
9	4.2	1868	318	(17.02%)	729	(39.02%)
10		2833	1040	(36.71%)	862	(30.427%)
11		3169	975	(30.76%)	1108	(34.96%)
12	3.2	2329	523	(22.45%)	932	(40.017%)

Source: Swedish Space Corporation (1988)

Table 4. Upland Migration Data

	Intra-regional	Interregional		
Region	Migrants to upland areas from other provinces of the same region	In-migrants to upland areas from other regions	Total out-migrants lost to upland areas in other regions	Regional upland net migration
1	11,657	17,279	18,017	-738
2	8,680	17,670	8,912	8,758
3	5,855	17,792	15,775	2,017
4	11,361	40,216	12,101	18,115
5	5,684	11,094	13,487	-2,393
6	6,644	9,951	23,934	-13,983
7	4,959	20,332	39,950	-19,618
8	2,860	10,056	18,985	-8,929
9	2,881	8,354	14,668	-6,314
10	21,781	48,228	23,088	25,140
11	23,653	47,120	21,863	25,257
12	5,247	26,195	16,147	10,048

Source: Ma. Concepcion Cruz and I. Zosa-Feranil, "Policy Implications of Population Pressure in the Philippine Uplands," (unpublished 1988)

Table 5. Provinces with more than half their areas eroded

Provinces	Eroded area (% of total area of province)
Ilocos Sur	60 - 70
La Union	60 - 70
Batangas	80 - 85
Marinduque	75 - 80
Capiz	50 - 60
Aklan	50
Antique	50
Iloilo	50
Cebu	80 - 85
Negros Occidental	50
Negros Oriental	50
Misamis Oriental	50
Bukidnon	50
Davao del Norte	50
Davao del Sur	50
Davao Oriental	50
Zamboanga del Norte	50
Zamboanga del Sur	50
Lanao del Norte	50
Lanao del Sur	50
North Cotabato	50
South Cotabato	50

Table 6. **Forest utilization and forest plantation areas(1976-1988) (1988 Philippine Forestry Statistics)**

Year	ITP/TF/AFF	TLA (ha)
1976		800,500
1977	2,000	827,900
1978	11,000	706,000
1979	39,000	677,600
1980	98,000	650,000
1981	140,000	653,900
1982	232,000	670,900
1983	305,000	539,200
1984	386,000	587,800
1985	407,000	609,300
1986	432,000	567,500
1987	428,000	434,000
1988	464,000	365,000

Note:
ITP - Industrial Tree Plantation
TF - Tree Farm
AFF - Agro-Forestry Farm
TLA - Timber License Agreement

Table 7. Efficiency of production

(1) Year	(2) Area of Palay and Corn ('000 ha)	(3) Ave. Mean Yield of Palay and Corn (mt/ha)	(4) Fertilizer Consumption ('000 mt)	(5) (2)/(4) (ha/mt)	(6) (5)x(3)
1976	6,836.3	1.29	890.7	7.68	9.90
1977	6,868.1	1.34	946.2	7.26	9.73
1978	6,731.0	1.43	1,103.4	6.10	8.72
1979	6,795.8	1.51	1,191.0	5.71	8.62
1980	6,670.0	1.60	1,152.4	5.79	9.26
1981	6,714.0	1.67	1,107.0	6.07	10.13
1982	6,734.0	1.74	1,192.3	5.65	9.83
1983	6,186.0	1.68	1,241.7	4.98	8.37
1984	6,389.0	1.73	982.0	6.51	11.25
1985	6,817.0	1.86	993.4	6.86	12.76
1986	7,059.0	1.89	1,316.2	5.36	10.14
1987	6,938.0	1.85	1,667.7	4.16	7.70
1988	7,138.0	1.88	1,745.2	4.09	7.69

(5) - area planted to corn and rice per metric ton of fertilizer used

(6) - tons of produce (palay & corn) per ton of fertilizer

- Trend: decreasing - which means that a ton of fertilizer then produced more than what a ton could produce today

- Implication: To produce the same yield, more fertilizer should be used.

Table 8. Cropped area, Philippines 1979-1983

Year	Area ('000 ha)	% change
1979	12,041.0	100.00
1980	12,133.4	100.77
1981	11,961.8	99.34
1982	12,182.3	101.17
1983	11,540.3	95.84
Trendline slope - 95.25 (ha/yr)		

Source: Fernandez and Badayos (1986)

Table 9. Status of Land in the Philippines, 1970-1984 (in million ha)

	1970		1977		1980		1982		1984	
	Hectares	% to total	Hectares	% to total	Hectares	% to total	Hectares	% to total	Hectares	% to total
Total Philippines	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00	30.00	100.00
1. Forest	15.90	53.00	13.10	43.67	12.50	41.67	12.00	40.00	11.50	38.33
Productive	14.10	47.00	11.30	37.67	10.70	35.67	9.70	32.33	9.30	31.00
Unproductive	1.80	6.00	1.80	6.00	1.80	6.00	2.20	7.33	2.30	7.67
2. Non-Forest	14.10	47.00	16.90	56.33	17.50	58.33	18.00	60.00	18.40	61.33
Open Land	2.60	8.67	1.00	3.33	0.80	2.67	1.00	3.33	1.80	2.67
Managed pastures	0.80	2.67	1.00	3.33	1.00	3.33	0.60	2.00	0.70	2.33
Wash and small water	0.20	0.67	0.10	0.33	0.10	0.33	0.10	0.33	0.10	0.33
Plantation			6.80	22.67	7.20	24.00	247.30	24.33	7.50	25.00
Cultivated cropland	9.80*	32.67*	7.20	24.00	7.60	25.33	8.00	26.67	8.20	27.33
Urban & other	0.60	2.00	0.80	2.67	0.80	2.67	1.10	3.67	1.10	3.67

* The figure combines plantation and cultivated croplands.

Source: Bureau of Forestry Development

CONCEPT OF SUSTAINABLE DEVELOPMENT (WCED, 1987)

Sustainable development is a dynamic process
in which the development and utilization of resources,
orientation of technological development, institutional change
and direction of investments are all in harmony and enhance both
current and future potentials to meet human needs and aspirations.

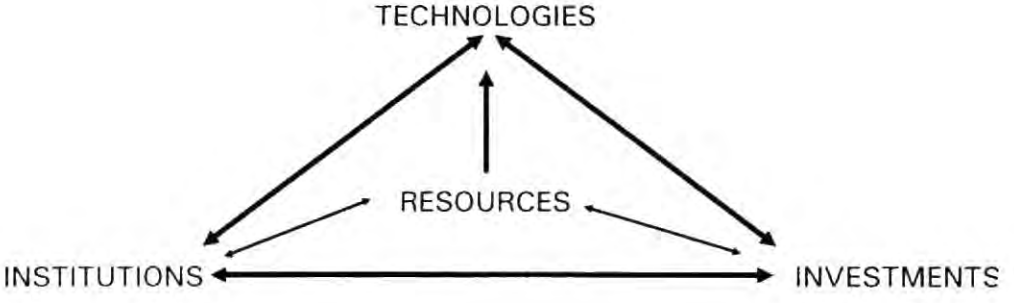


Figure 1. Conceptual framework for sustainable development

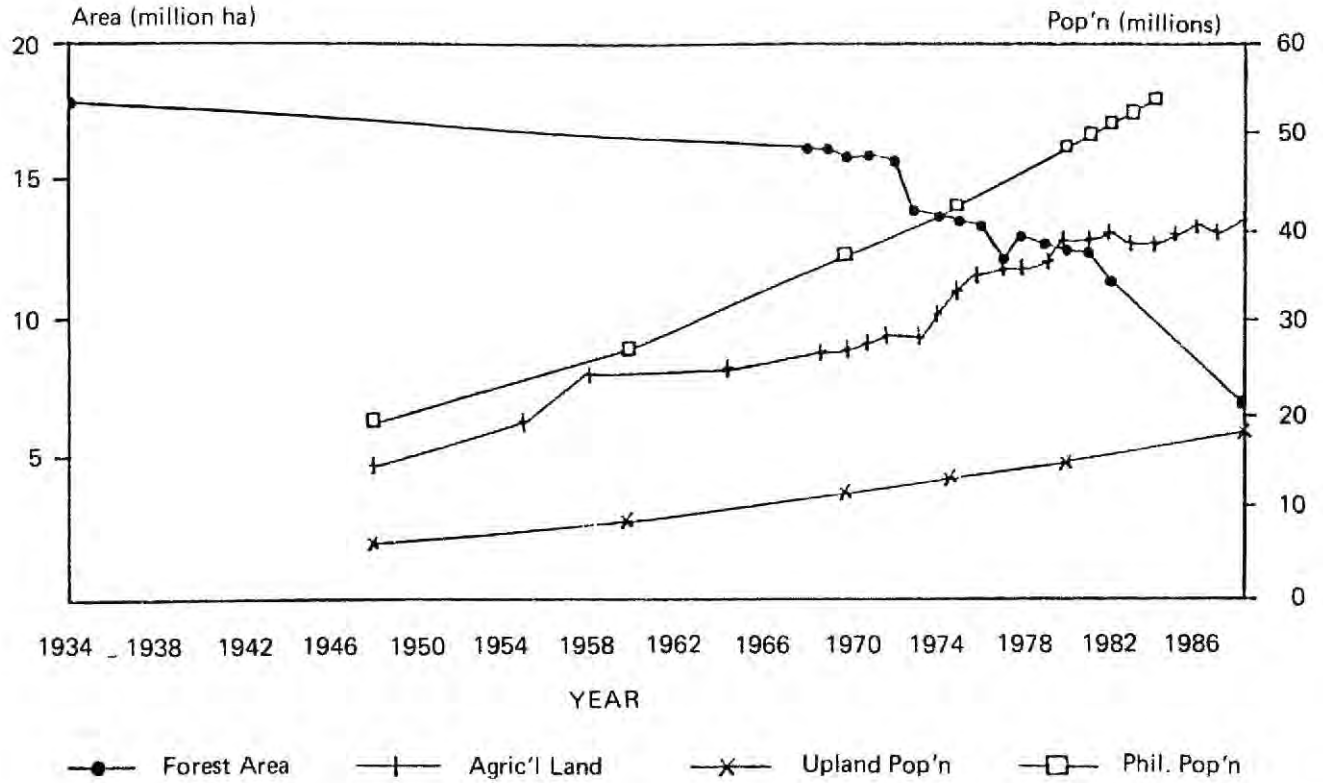


Figure 2. Forest and Agricultural Land Use vs Population growth

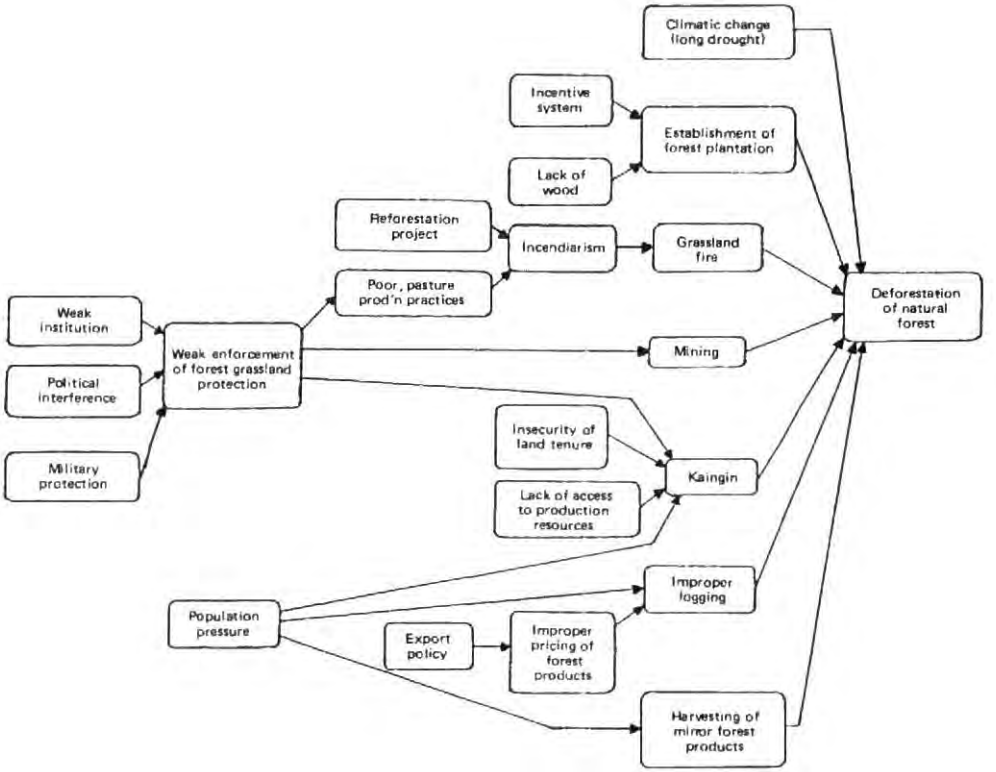


Figure 3. Problem Analysis: Causes and Impacts in Forestry

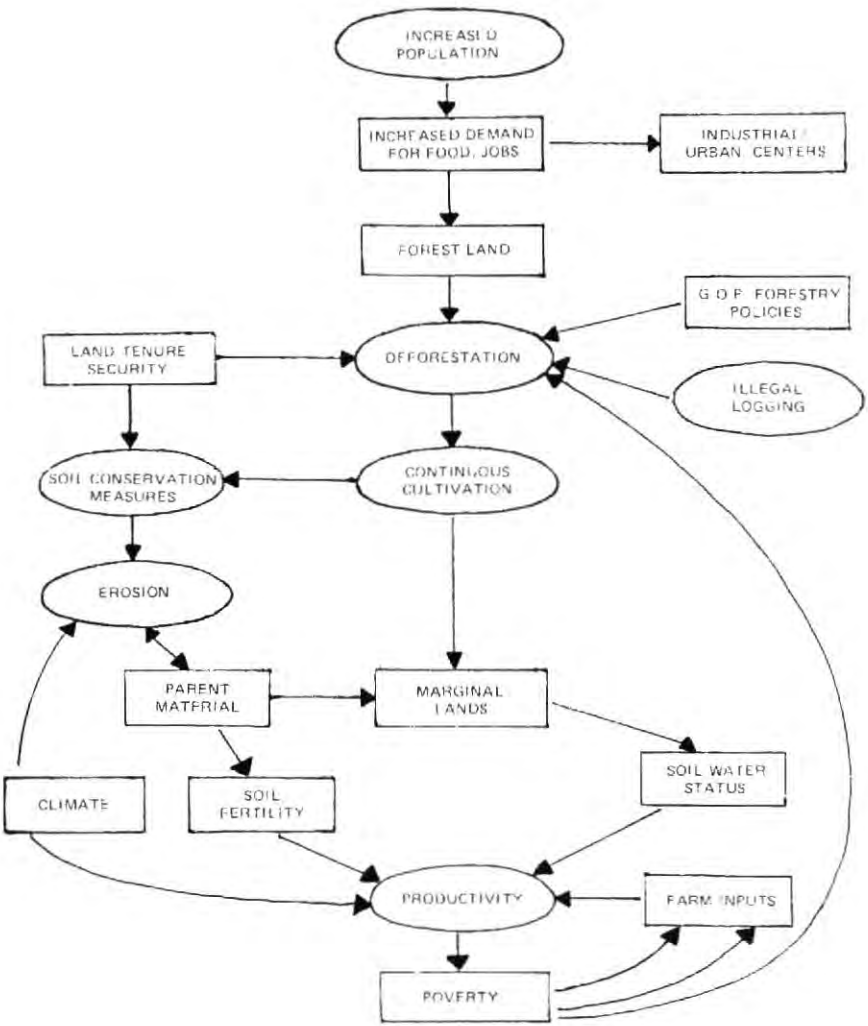
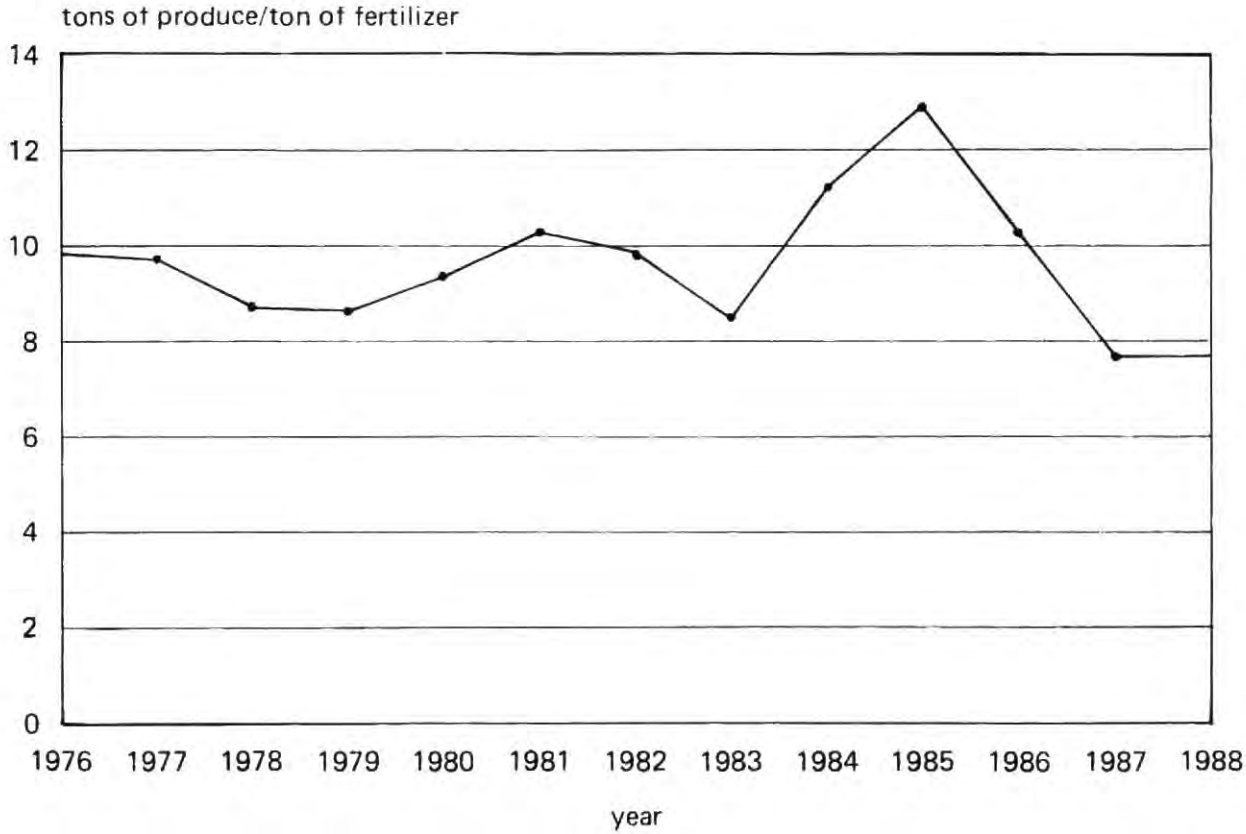


Figure 4. Analytical Framework for Marginal Lands



Note: Palay and corn data were averaged.

Figure 5. Efficiency of Production (Palay and Corn)

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Some Social Insights on Philippine Programs in the Forestry Sector

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First, I would like to congratulate Dr. Percy E. Sajise and Dr. Enrique P. Pacardo for presenting a very comprehensive status of the Philippine Terrestrial Environment. The data presented are indeed very revealing and it is very apparent that in the next few decades, the Filipino environmentalist will have a lot of items in his environmental agenda. I honestly believe at this stage that there is no turning back... as delayed reactions could be very detrimental to all of us.

As a social forester, let me just concentrate on the items that have greater relevance to the social aspects of forestry and the areas we popularly call the uplands. Honestly, these are the points that I feel more comfortable to discuss.

The historical trend of the Philippine upland forest ecosystem, as presented in the paper, means only one thing: we no longer have sufficient forest cover to meet the demands of the continuously growing population in terms of productivity and ecological stability. The fact is that our forests are continuously decreasing while our population is continuously increasing (Fig. 1). We have reached that point in time where the critical impacts of forest denudation, such as accelerated soil erosion, loss of biodiversity, impairment of hydrologic cycle, displacement of cultural communities and poverty and malnutrition have brought about severe damages to lives and properties of our people.

Two of the more popular reasons cited for the depletion of the forest resources and the consequent degradation of the environment are commercial logging operations and shifting

cultivation (kaingin). While we cannot separately delianeate the extent of destruction caused by each, history will tell us that, to a great extent, commercial logging immediately followed by shifting cultivation aggravated the problem of forest renewal.

As early as the 1970s, the government and the private sector had already realized that the problems in Philippine forestry were not purely technical but were also social and economic in nature. With more and more people engaged in the conversion of the forestlands into other land uses like agriculture, we have recognized that the punitive means of addressing the problem is not the solution. We have tried it for quite sometime but it did not work.

A GLIMPSE OF THE INTEGRATED SOCIAL FORESTRY PROGRAM (ISFP)

In the late '70s, the government, through the then Ministry of Natural Resources, initiated a number of "socially-oriented" forestry programs in its attempt to improve people's participation in government efforts on forest rehabilitation. Such programs included the Communal Tree Farming (CTR), the Family Approach to Reforestation (FAR) and the Forest Occupancy Management (FOM). Through Letter of Instruction (LOI) 1260 and Ministry Administrative Order (MAO) No. 48, these programs were lumped into one - the Integrated Social Forestry Program (ISFP) which was institutionalized in 1982.

Based on the Philippine Forestry Statistics of the DENR Forest Management Bureau (FMB), we have a total of 2,715 ISF projects all over the country as of 1989, covering an area of 525,619 hectares and involving 204,999 family beneficiaries. Of these, 128,772 have been issued their Certificate of Stewardship Contracts (CSCs; Table 1). It has been claimed that around 176,244 hectares were already developed. Fourteen Community Forest Stewardship Agreements (CFSAs) have also been awarded to cultural communities. These CFSAs cover a total of 37,672 hectares and 8,256 beneficiaries as of 1989 (Table 2).

The basic issue of whether or not the ISFP has really been a factor in the rehabilitation, development and management of the Philippine terrestrial ecosystem always lingers. It was DENR Assistant Secretary Bernardo C. Agaloos himself who admitted that the social and economic impacts of the program are still

insignificant. Most program beneficiaries have not attained self-reliance as they have remained dependent on and have anticipated government assistance to improve their way of living. Strong, active and community organized associations that are expected to manage their own concerns are still very few.

Some participants even claim that they are becoming poorer and poorer despite the program. The lack of capital, credit and other support services has contributed most to this very slow socio- economic development of the upland population.

Similarly, the ecological impact of the ISFP is still a question. While Assistant Secretary Agaloos claimed that the physical structures of soil and water conservation measures have improved the degraded hillsides of the ISFP sites, there are actually very few of them because of the physical, financial and time constraints of contructing them on the hillside farms by the farmers themselves.

An off-hand assessment of the ISFP reveals that after more than eight years of existence, it has not significantly produced concrete outputs that would have improved the conditions of the forest ecosystem and the socio-economic being of the so-called "poorest of the poor" sector of the society. To an extent, it has created an extensive awareness of the social problem in Philippine forestry, but its contribution to forest rehabilitation efforts is still close to nil. In fact, some sectors believe that the Program somehow aggravates the upland occupancy problem. This may be true as more and more lowlanders are attracted to migrate to the uplands in the hope of getting a share of the ISF Program benefits. Due to lack of a reliable basis for actual forest occupancy and an effective forest occupancy protection activity, we have continuously accommodated everybody who claims to have occupied a piece of the public forestland sometime ago. The will to prevent future occupancy does not seem to be in our social forestry agenda.

In terms of the over-all program implementation strategy, we seem to have forgotten one of the major goals of the program: to lessen the destructive pressure on the critical uplands and the forest ecosystem. The ISFP should not be treated as an end, but as a means to address the social problems of forestry in the country. The Program should emphasize the development of economic alternatives **outside** the forestland domain without sacrificing the people's access to the resource. As I have always thought in the past, a major bulk of the social problems of forestry

in the Philippines could be solved in the lowlands, not necessarily in the uplands.

Finally, by this time, we should have already integrated and internalized the lessons that we have learned from the numerous "socially-oriented" forestry programs of the government and the private sector. After more than eight years, we can no longer make the excuse that we still have very little understanding of the origin of social forestry and the context in which it was developed. I believe that we now have more than enough lessons to work with considering the big number of social forestry projects and efforts so far done by the government as well as the private sector.

EQUITY AND TENURE IN THE CONTRACT REFORESTATION PROGRAM

So much has been said about the critical condition of the forests in the Philippines. Since the last decade, the government has undoubtedly taken serious steps to rehabilitate our degraded forestlands through various strategies of reforestation.

In 1986, the DENR launched the centerpiece of an all-out multi- sectoral effort to avert an environmental holocaust resulting from forest destruction and forest resource depletion. This is called the National Forestation Program (NFP), which puts the private sector in a frontline position as the vanguard of the country's reforestation efforts. The program is boosted by two soft loans negotiated by the DENR with the Asian Development Bank (ADB) and the Overseas Economic Cooperation Fund (OECF) in 1988.

Part of the NFP is the Contract Reforestation scheme wherein an individual or an entity agrees to implement a series of activities required to reforest denuded areas, with the DENR paying for duly accomplished acitivities. This scheme provides incentives to the private sector, NGOs and local government units to become DENR's partners in forest resources conservation, development, management and protection.

Under the DENR Memorandum Circular No. 11, there are three major typologies of the Contract Reforestation sheme: the family contract, the community contract and the corporate

contract. The family contract is awarded to the bonafide local community household heads within the immediate vicinity of the identified reforestation area. The community contract is a negotiated contract entered into by the DENR and duly recognized local entities such as associations, cooperatives, NGOs, foundations and civic and religious groups **in behalf** of the community located in or adjacent to the reforestation site. This typology includes the local government unit (LGU) contract where LGUs having jurisdiction over the area could likewise enter into agreement with DENR. The corporate contract involves private corporations entering into agreement with the DENR to conduct reforestation through competitive bidding.

As of 1990, a total of 5,907 contracts covering an aggregate area of 72,251 hectares have been awarded to the three types of contractors by the DENR. It is significant to note that the family and the community contracts involve more local community people as contractors based on records.

A macro-level study conducted this year (1991) by the Asian NGO Coalition (ANGOC) showed that maximum participation by the local community people was achieved under the family approach. The same study, however, revealed that most community contractors are not really community organizations, but private individuals disguised as representatives of the people in the locality. Their "entrepreneurial" set-up reflects a tremendous inequity in terms of the distribution of financial benefits derived from the contract. Most of the financial benefits accrue to a limited group of individuals (usually the head of the organization and a few other officers) while the local community people are merely hired as daily reforestation laborers or are not involved at all. In fact, many of those interviewed feel that they are being short-changed by their "entrepreneur employer-contractors" in terms of daily payments for their reforestation efforts. To some of them, the contract reforestation program is just an ordinary government program and not truly designed for maximum local community participation as the government claims.

As this suspicion continues, the credibility of the government continuously suffers and the poor local communities are continuously forced to engage in forest destructive forms of economic activities because they have no other choice. Forest development has not provided enough opportunities and the benefits of reforestation have been inequitably shared. As Kummer (undated)

had put it: "Upland degradation is actually a reflection of uneven Philippine development in general. For those in poverty, environmental preservation is a luxury; as such, the poor have very little choice but to accept the continuing destruction. And those who benefit from the destruction will certainly allow it to continue."

In the first two years of the contract reforestation program, the issue of tenure came out with a big bang. There are actually two tenure-related issues involved in the activity: (a) private claims on public forestlands identified as reforestation areas; and (b) the access rights to the trees (and possibly the land) after the actual contract reforestation has been completed.

The identification of reforestation areas, which are subjects of private claims, has given rise to problems with serious repercussions on the sustainability of the program. This is particularly true in places where most of the public forests are already claimed. In Cebu for example, there were instances when privately claimed lands in the public forest were identified as contract reforestation areas by the DENR and subsequently contracted out to NGOs without the knowledge of occupants. As such, the implementation of the contract reforestation project was adversely affected.

A concomitant tenure-related problem concerns the issue of protection and maintenance of the reforested areas. The Forest Land Management Agreement (FLMA) came out in response to this. Here, the DENR enters into an agreement with the communities, NGOs and other qualified entities who will protect, maintain and benefit from the trees covered under the reforestation project. Priority is given to the individual or group that contracted the reforestation activity with the DENR.

The FLMA is actually an approach to guarantee the sustainability of the renewed forest resource. It is premised on two valid grounds: (a) that the community has to participate in forest protection; and (b) that to ensure sustained community participation, tenurial security coupled with economic incentives have to be provided for. Yet empirical reality may dissipate the good and valid intentions of the FLMA. Since a large number of reforestation projects are contracted out to secondary groups like private entrepreneur NGOs, this means that there will be a larger number of secondary groups than base/community groups that will directly benefit from FLMA. In such cases, the community will only benefit indirectly through employment as they did in the contract reforestation project.

A possible serious complication concerns the reforestation areas that are occupied and contracted out to an NGO group instead of the community. The grant of the FLMA to the NGO contractor will provide a legitimate basis for the displacement of occupants whose status has not been legally recognized.

The FLMA, as a response to the tenurial issues posed against contract reforestation, has another weakness. Prospective FLMA grantees contend that the economic incentives attached to the agreement are long-term and not attractive enough to induce those groups to enter into it. It is further claimed that their financial motivations are primarily short-term, hence they would rather concentrate on garnering more short-term contracts such as the three-year contract reforestation project.

In summary, the family approach of the contract reforestation program exemplifies direct participation of the community people in all phases of the activity. The community approach, on the other hand, which is supposed to be pursued in behalf of the community does not necessarily enhance genuine community participation. With its loose definition and operationalization, the gains could be dissipated. Generally, community contracts to real and organized groups composed of community residents will have a more direct impetus than those granted to secondary groups.

SOME REMINDERS ON THE COMMUNITY FORESTRY PROGRAM (CFP)

In another attempt of the government to include the local communities in forest development and management, the Community Forestry Program (CFP) will soon be launched on a pilot basis. In general, the Program aims to transfer the major responsibility of forest resource management and utilization from the commercial timber licensees to the local community within or adjacent to the area.

The Program's operational framework reflects that its success rests primarily on the shoulders of a capable NGO which will be entrusted to develop the local community into an eventual forest resource manager. The effort, as expected, will be complemented by a strong local community organization which will actually take charge of the development, protection, management

and utilization of the forest resource equally among its constituents.

The over-all goal of providing equity concerning access to the forest resource is the salient feature of the Program. Caution must, however, be observed in its implementation because at present, both the NGOs and the local communities still lack the experience and the preparation to undertake such a gigantic task. While community experiences on reforestation and forest protection may be claimed as sufficient, forest harvesting (or logging) and forest product utilization may not be as easy to handle. These are specialized economic ventures requiring a different set of experience and expertise for the implementors. For the community to acquire these, it may require a long process of creating awareness and instilling the value of forest conservation in the mind of every community member before actual operation. For how long? -- It is not easy to tell.

Table 1. Integrated social forestry projects, individual CSCs, 1989

REGION	PROJECT (No.)	TOTAL AREA (ha)	BENEFICIARY (No. of Families)	CSCs Issued
CAR	183	20,740	18,243	7,901
1	193	26,248	13,319	8,313
2	182	34,175	15,569	15,569
3	147	25,021	11,324	9,611
4	293	83,619	34,621	15,347
5	116	32,699	8,000	5,691
6	299	54,953	18,170	15,179
7	226	22,664	14,466	10,386
8	111	22,516	7,824	4,133
9	103	44,486	12,643	data not available
10	257	34,307	12,563	12,127
11	501	87,271	28,550	18,702
12	104	36,920	9,707	5,813
TOTAL	2,715	525,619	204,999	128,772

Table 2. Community forest stewardship agreements, 1989

Region	Name of Group	Location (Province)	Area (ha)	Beneficiaries (No.)
2	Kalahan Educational Foundation	Nueva Vlzcaya	14,672	3,000
	Bayagong Asso. for Community Dev't. Inc.	Nueva Vizcaya	1,213	250
3	Siglakas ng mga Negrito sa Canawan	Bataan	165	103
4	Pundasyon ng Bagong Buhay ng Gubatnon	Mindoro Occ.	1,340	400
	Pundasyon Hanunuo Mangyan, Inc.	Mindoro Or.	3,980	781
	Samahan ng Nagkaka-isang Mangyan, Inc.	Mindoro Or.	2,065	415
	Pinagsurutan Foundation, Inc.	Palawan	1,335	746
	Domadoway Foundation	Palawan	2,531	604
	Tagbanua Foundation of Coron	Palawan	7,748	994
	Tagbanua Educational Foundation of Lamani, Inc.	Palawan	1,425	174
	Kapisanan ng Huyon-uyon Mabuhay Asso. Inc.	Quezon	512	130
6	Malay Highlanders, Inc.	Aklan	79	528
10	Temple of Eternal God, Inc.	Agusan del Norte	49	31
12	Bage-bage Muslim Returnees, Inc.	Cotabato	500	100
TOTAL			37,672	8,256

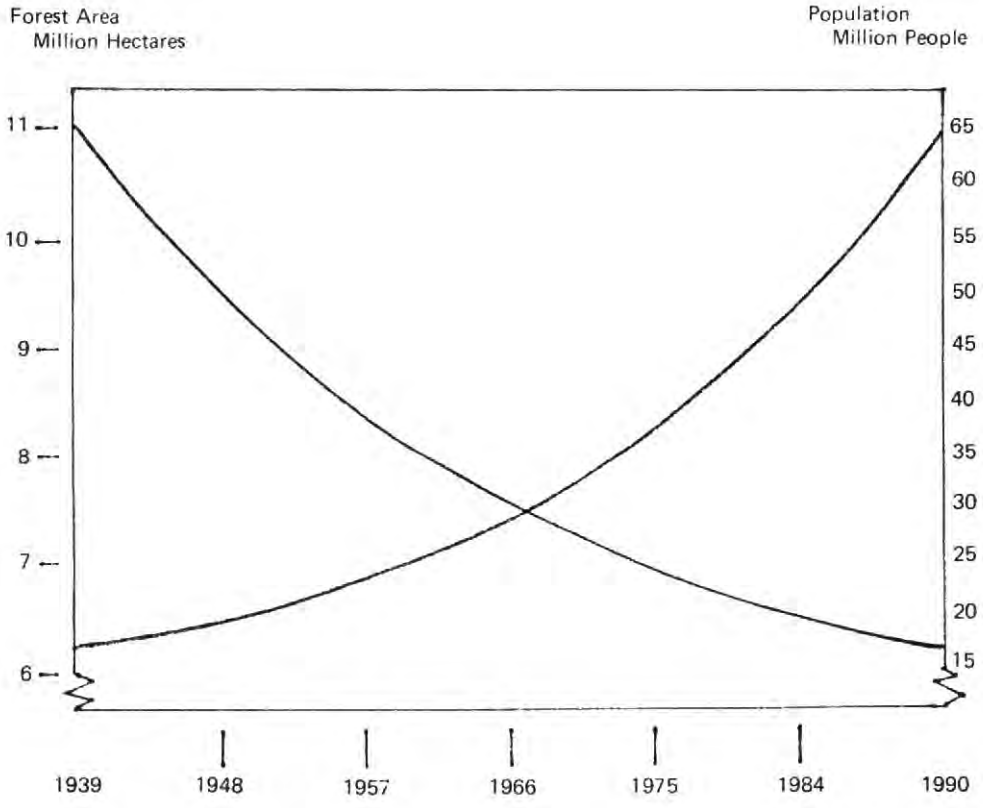

Figure 1. Decrease in forest area compared to population increase, 1939-1990

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PLENARY IV

Managing Our Urban Ecosystems for Survival

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ABSTRACT

An urban ecosystem is man-made. It is not natural in the way that mountains, forests and oceans are. Nonetheless, its four elements -- land, air, waters and people -- maintain a dynamic balance the poor management of which results in pollution, environmental deterioration and social problems.

Metro Manila is confronted with the environmental and social problems that accompany rapid industrialization and increase in population density. A 1990 study commissioned by the Asian Development Bank identified eight leading environmental problems. These are:

1. *solid waste;*
2. *slum areas;*
3. *flooding;*
4. *water pollution;*
5. *air pollution;*
6. *hazardous/toxic waste;*
7. *destruction of natural resources; and*
8. *noise pollution.*

These environmental problems occur for a variety of reasons; but oftentimes, these reasons include underlying social problems that extend beyond the city. For example, the increase in the number of squatters and slum areas is not only associated with inadequate housing facilities or the high cost of urban real estate but it is also a consequence of rural poverty, rural unemployment and the migration of the rural poor into the cities to seek their livelihood. Once established, slum areas in turn spin an ever-evolving web of social consequences.

To solve the environmental problems of Metro Manila, we must address both the physical and social determinants which give rise to these problems. Resources need to be found to finance the infrastructure improvements for solid waste disposal, sewage, transport, social services and many others. Without doubt, long- term solutions must take into account the larger issues of poverty, social equity and good government.

INTRODUCTION

In 1962, Loren Eisely wrote in **The Epic of Man** (Time-Life International 1962):

> *"Man is paradoxically the supreme generalized animal by reason of a supremely specialized brain. It is his cities that are now his true specializations, his cities that lie vulnerable to extinction under the silent winging of the satellites."*

In the years after Eisely's words were published, man successfully built his spaceships, landed them on the moon and sent them circling around the earth. It is intriguing to think that beneath their quiet orbits, another of man's creations -- the city -- was tracing its own life cycle of growth, development and decay.

This article takes an ecological view of the city. It surveys its problems and suggests certain approaches to solve them. It focuses on Metro Manila, the premier city of the Philippines, whose growth and development may provide lessons for other growing cities within this country.[1]

Speaking figuratively, the ecological view is like a satellite view of things below. It involves the "study of living things, their interrelationships and their relationships with the environment" (Basic Facts: Geography 1983). The environment refers to man's physical surroundings: the soil, vegetation, wildlife and atmosphere. Man's impact on it is of growing concern worldwide: soil erosion, global warming, pollution, the extinction of species, the spread of urban areas (Sadik, Brown and Jacobson 1987). But in

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[1] Metro Manila, also called the National Capital Region, is composed of the cities of Manila, Quezon City, Pasay and Caloocan, and the 13 municipalities of Las Piñas, Makati, Malabon, Mandaluyong, Marikina, Muntinlupa, Navotas, Parañaque, Pasig, Pateros, San Juan, Taguig and Valenzuela.

a broader sense, the environment can include the social surroundings: culture, traditions, language, the economic and political life. Physical and social events influence each other. The world is witnessto situations where social turmoil has spawned environmental disaster: in the recent Mideast war, Iraqi soldiers discharged more than 6 million barrels of crude oil into the sea (Newsweek Feb. 4, 1991) and set fire to hundreds of oil wells in Kuwait (Horgan 1991).[2] But the reverse is also true. Here in Luzon last year we saw how an earthquake destroyed the lives and livelihood of hundreds of our countrymen (The Manila Chronicle July 22, 1990).

To look at the city ecologically then, is to look at it as an ecosystem, to examine its parts and how they interrelate, to study the balance within the whole.

URBAN ECOSYSTEM -- A DEFINITION

An ecosystem is defined as a natural system composed of living organisms and their environment. All elements of the ecosystem are intricately linked by flows of energy and nutrients. A change in one element has repercussions throughout the system (Collins 1983).

For the urban ecosystem to be healthy, its four elements -- land, water, air, people -- must enhance one another; the products and byproducts of one become nutrients or inputs for the others. For example, the atmosphere produces rain; rain replenishes groundwater and surface waters; it revitalizes land and stimulates plant growth; through evaporation water returns to the atmosphere. Here is another example. Man produces excrement which is collected in the sewage systems of the city. Sewage can be treated and piped out to sea where it becomes nutrient for marine life. Part of it can be used as fertilizer for land. Man benefits when land and sea provide him with food for his nourishment.

There are many such cycles in nature and it should be obvious that disruption of any of these causes ecological imbalance. Byproducts which cannot be recycled accumulate and pollute the

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[2] John Horgan writes: " . . . Six million barrels of oil weighing a million tons are going up in smoke daily. If the figure is accurate, 50,000 tons of sulfur oxide – the chief constituent of acid rain – and 100,000 tons of carbon as soot and more than 800,000 tons of carbon in the form of carbon dioxide go into the atmosphere everyday." (Scientific American May 1991)

environment. Such is the case with plastics, styrofoam and toxic chemicals commonly used by urban man (Hayes 1979). Plastics pile up in garbage dumps or clog sewers impairing the drainage of city streets and causing floods. Pesticides are frankly hazardous to people. Conversely substances necessary for the maintenance of a cycle might be excessively depleted. Water shortages in the city are common. Long rainless summers reduce the city's water supply and dry out the wells leaving city people thirsty, hot and miserable (Manila Bulletin, March 21, 1991).

The city, strictly speaking, is a man-made system. In this respect, it is not "natural" as mountains, forests, seas and rivers are. However, as a city draws from the terrestrial and aquatic ecosystems within and around it, and incorporates these unto itself, it will have an impact on these natural ecosystems. Similarly, the city will affect people's lifestyle and health.

The city has its own dynamism: population growth and movements within and across its boundaries, the rise of factories and workplaces, the trading of real estate, the insatiable demand for energy, food and water, the inexhaustible capacity to produce waste. These dynamics often determine whether or not people have decent homes, whether or not there are jobs for jobseekers, whether or not there is enough water in the tap, whether or not commuting on city streets is pleasant.

SOME BASIC QUESTIONS

Man has lived in cities since the earliest of times. The first cities arose from the agricultural villages and towns of Mesopotamia around 3500 B.C. Later they flourished in the fertile valleys of Egypt and India. By 1500 B.C., the Yellow River nourished a thriving urban civilization in China. These early cities had centralized governments controlled by powerful elites. They had systems of law, commerce and taxation. They were fed by large cultivated areas. They maintained armies for protection and conquest (Stuart 1988). The Athens of Socrates in 450 B.C. had a population of 50,000, about the size of a large municipality here in the Philippines. Cities, whether ancient or modern, have had to cope with the same problems inherent in human settlements. For example, this description of the sanitary conditions in a medieval city in Europe around the 1400s, with a population of about 100,000, fits contemporary life in some of our urban districts (Bishop 1970).

"The medieval streets were unquestionably foul. Butchers slaughtered animals at their shop fronts and let the blood run into the gutters. Poulterers flung chicken heads and feathers into the streets. Dyers released noisome waters from their vats. City officials in Italy would throw the fishmongers' unsold fish into the street for the poor, to make sure it would not sicken honest purchasers.

"Sewage disposal was an impossible problem. Only the big cities had sewers, which emptied into rivers below the laundry area. At Strasbourg malefactors were ducked where the sewer joined the river. Pollution of the streams became a serious concern; everyone agreed that something should be done about it. Street cleaning and removal of wastes to rural dumps were usually left to individual householders, who were apt to toss their refuse over the city walls or abandon it just outside the

Today there are about 270 cities with over half a million people and 17 with populations over 10 million people (Ward 1979). By the turn of the century, at its present rate of growth, Metro Manila would move into the second category. According to the statistics of the United Nations Population Fund, Metro Manila ranks 23rd in the world in population size (See Table I).

We might ask ourselves two sets of questions. First, can Metro Manila sustain its present rate of growth? Is it ecologically sound for Manila to continue to expand its size, its population, its industrial and commercial activities? If so, how might we manage this growth? Second, what cost, to ourselves and to our environment, will such growth exact? Is there an alternative to the development road we are taking?

Perhaps, we can already arrive at provisional answers to these questions by surveying our current situation.

MANILA'S ENVIRONMENTAL PROBLEMS -- AN OVERVIEW

A recent report of the Asian Development Bank enumerated eight environmental problems of Metro Manila. ADB ranked them according to how severely (degree of importance) and extensively (geographical spread) they damaged socio-economic activity and physical surroundings. The higher the score the more serious the

problem. Each item could rate a maximum of 100 points (ADB 1990).

The top 8 environmental problems were identified to be:

Problem	Score
1. Solid Waste	53.4
2. Slum Areas	51.7
3. Flooding	51.7
4. Water Pollution	51.0
5. Air Pollution	39.9
6. Hazardous/Toxic Waste	36.5
7. Destruction of Natural Resources	34.4
8. Noise Pollution	28.1

There is no doubt that the above list reflects problems that Metro Manilans are familiar with. Already there are government plans to address some of them, like the opening of new landfill areas for solid waste (Manila Times February 26, 1991) and the building of a new sewage outfall in Manila Bay (ADB I-36). But these problems are only by-products of the pervasive and relentless processes of urbanization and industrialization going on around us. Let us take a closer look at them.

"METRO MANILA ON THE GO" -- A LOOK AT TRENDS

The pressures forcing Manila to go and to grow are staggering. They are driven by the imperatives to modernize, to make the Philippines an NIC (newly industrialized country) by the turn of the century.

Today, Metro Manila has an estimated eight million people. This count excludes transient workers and students. The average growth rate of Manila's population over the last five years is 2.8% per year (See Tables II and III). This means that the city grows by approximately two million people every 10 years. In 1980, there were six million people in Manila; by the year 2000, there will be 10 million. Sixty percent (60%) of the growth is attributed to natural increase and the remaining 40% to in-migration.

Metro Manila's economy dramatically expanded by 35% from 1985 to 1987 after overcoming negative growth rates in the early 1980s (See Figure 1). At the end of 1988, Metro Manila

registered a gross domestic regional output of ₱31 billion measured at 1972 prices. This was about 30% of the total gross national product. Hand in hand with the growth of the economy, the labor force of Metro Manila has steadily grown. In 1989, it was estimated at three million people (See Figure 2).

PATTERNS OF LAND USE

The surge of the economy is reflected in the way land is used and traded. Metro Manila is like an insatiable amoeba stretching its boundaries ever outwards. With the building of the north and south exspressways in the 1970s and '80s, new factories have risen along these main transportation routes. Old factories remain cramped in their original locations along the Pasig River and its tibutaries in places like Pandacan, Punta, Mandaluyong, Pasong Tamo, Pasig and Marikina and the Port Area fronting Manila Bay. It is estimated that of the 636 square kilometers which is the total land area of Metro Manila, 5% is given to industrial use (ADB C-16).

On the other hand, commercial centers have sprouted in several locations. While business has been revitalized by the Light Rail in the old business district on the north bank of the Pasig River (Escolta, Binondo, Quiapo, Sta. Cruz and Divisoria), new commercial centers have flourished elsewhere: Sta. Mesa, Cubao, Balintawak-Monumento, Makati, Greenhills, Las Piñas, Pasig and many others. Business establishments have also invaded residential areas adjacent to growing commercial districts. Along main thoroughfares of residential areas, we find offices, restaurants, dress shops, beauty parlors, video rental counters. We see this in San Lorenzo Village in Makati, Wilson Street in Greenhills, Tomas Morato Street in Quezon City, BF Homes in Parañaque and even the once genteel Malate area. Commercial establishments occupy 8% of Metro Manila's land area (ADB C-15).

The lion's share, 37% of the land in Metro Manila, is used for residential housing (ADB C-14). There are several types. One type is the low-density-low-rise, single detached dwelling units found in well-to-do neighborhoods and residential villages. Another is the high density, generally poorer housing found near the old central commercial district and poorer areas throughout the city. A third type, which is of fairly recent origin, consists of the high-density-high-rise condominiums and the high-density-low-rise town houses in Makati, Sta. Mesa, Greenhills and other

areas. On the intersection of EDSA and Ayala Avenue, for example, stand several high rise condominiums which epitomize the shape of buildings to come. They speak eloquently of how real estate values have risen in Makati as in other parts of Manila.

The trends in residential housing show rising land values and for those who can afford it, the rush towards suburban subdivisions which are a short drive from a bustling commercial center. For the poor who must seek their niches where they can easily find work or some marginal means of livelihood like selling newspapers or sampaguitas, to stay in the city means to live in slums or squat on somebody else's property. Slum and squatter colonies are increasing and most everywhere in the city -- along the reclamation area in Roxas boulevard, beside railroad tracks, under bridges and along riverbanks, on government-owned land -- they take root.

In 1987, the National Statistics Office estimated there were about 415,000 squatter families in the whole of Metro Manila living in 591 different slum and squatter settlements. Squatter population is 2.5 million or 34% of the total population of Metro Manila (See Table IV). By 1992, there will be about 3.0 million squatters in Metro Manila.

As the city grows, population pressure on the land will increase; so will competition for its different uses (ADB C-17) [3] Metro Manila with its many autonomous local governments and its Metro Manila Authority does not appear headed into the future with clear policies and plans for further urban development (ADB E- 2).[4] Without a comprehensive ecological plan for the city, industries and commercial areas will mushroom where they may and so will slums and squatter areas. Inappropriate development has occurred and will continue to occur. As has happened along

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[3] There are three other general categories of land use. One is land occupied by institutions including military installations, educational institutions, health and hospital facilities and government institutions. These constitute about 5% of the total urban area. Another is agricultural land covering roughly 10%. Finally, there is the 35% classified as open area consisting of undeveloped areas, golf courses, parks, playgrounds, and even cemeteries (ADB 1990).

[4] The Metro Manila Authority or MMA serves as the link between the local government units (LGUs) and the national agencies like the National Economic Development Authority (NEDA) and the Department of Finance. MMA's mandate is to act as the central coordinating body of development planning for Metro Manila. The MMA has the power to tax through passage of various revenue-raising ordinances. Local government units contribute 15% of their gross income as contribution to MMA but the national government accounts for 90% of MMA revenues (MMR Environmental Improvement Study Volume 1 1990).

the coastal areas or in the lowland margins of Laguna lake, industries will be set up on land inappropriate for industrial use (ADB C-16).

Metro Manila's growth is an interplay of order and disorder. We have clashing images: well planned and engineered residential subdivisions versus make-shift, congested squatter colonies; food terminals and shopping malls versus muddy, fishy street-markets (talipapa). In a situation of disordered growth, basic services to people are bound to be patchy.

WATER AND SANITATION SERVICES

Only 15% of the city is properly sewered: 12% by the MWSS (Manila Waterworks and Sewerege System) and 3% by other small independent sewerage systems (Fig. 3). This translates to about 1.2 million people served. As 1.2 million was Manila's population count in the mid-1940s, we might say that the city's sewerage infrastructure is at least 45 years behind schedule. The MWSS says that at the planned rate of completion of present projects to expand sewerage services, it will take 25 years to cover 50% of Metro Manila (ADB I-22). At present, 85% of Metro Manila residents rely on inadequately maintained septic tanks; in very poor areas, no waste disposal system exists (Environmental Management Bureau 1990).

Without a pipe sewerage system, the sewage and sullage[5] from households flow into storm drains, then into the esteros and rivers and out to Manila Bay; all watercourses and shoreline waters become polluted as a result. With a sewage outfall 5 to 6 kilometers from shore, shoreline pollution is lessened. There is at present one outfall which serves the existing sewerage system. There are plans to build another (Feachem 1983).

City water services have not been able to serve the whole of Metro Manila either. MWSS provides water to 70% of the population. The areas that do not have city water have resorted to deep wells for their water supply. This for example is the case in the south of Manila in Parañaque and Las Piñas. Experts say

[5] Sullage, also known as graywater, is domestic wastewater not containing excreta -- the water discarded from baths, sinks, basins and the like that may be expected to contain considerably fewer pathogenic microorganisms than sewage.

that the water level of the aquifer has been declining due to overextraction of ground water. This decline has actually been estimated to be about 4 to 10 meters per year in some parts of the city. Near the coastline, it was found that groundwater extraction has exceeded the rate of groundwater recharge. The consequent depletion of the aquifer has caused seepage of sea water into the aquifer. Significant increases in the salinity of wells have been detected within 1.5 km of the shoreline in most coastal areas (ADB I-12, I-13).

Improvement of the water distribution system in Metro Manila is hampered by poor revenue collection. In 1989, 55% of city water was lost in leaks and unbilled water consumption. While MWSS aims to reduce these losses to 32% by 1995, progress to remedy the situation has been slow (ADB I-40).

At present MWSS is expanding water services to unserved areas of Metro Manila. There are also plans to tap Laguna de Bay as a source of drinking water by the year 2000 (ADB J-24). Expansion of water services must be coupled with parallel growth of the sewerage system. Otherwise the amount of wastewater pollution increases. Because of the 65% increase in water supply without the concomitant increase in sewerage facilities in 1980-85, there was a 53%-net increase in domestic wastewater discharge into rivers and the coastal shorelines of Manila Bay (ADB I-24).[6]

For Laguna de Bay to be converted into a source of potable water for Parañaque, Muntinlupa and Cavite, its present water quality must be upgraded from a fishery (Class C) to a public water supply (Class AA) (See Table V) (ADB I-4). This requires the construction of sewage systems and treatment plants for both domestic and industrial wastes. This is necessary because the Laguna lake basin particularly its western portion is densely populated and highly industrialized.[7] Open fishing and fishpen

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[6] Volumes of domestic sewage depend on quantities of water used in the home. Houses connected to sewers must also be connected to water systems; they usually have comprehensive plumbing fittings. Such houses may, rarely, use as little as 30 liters per capita daily. If daily use falls below 50 liters per capita, however, the sewers lose their self-cleansing flow and become blocked. At the other extreme, households with many water-using appliances (such as washing machines) may use 300 or more liters per capita daily (Feachem 1983).

aquaculture would also be phased out. The conflict of interests makes this plan highly controversial (ADB J-16).[8]

PUBLIC TRANSPORTATION SERVICES

These last few years have seen a worsening of the public transportation situation in Metro Manila. It has been more and more difficult for a commuter to get around the city.[9] In recent years the number of public transport utilities has not kept pace with the growth in population. Government has tried to ease the transport crisis by importing new buses to travel city and provincial routes. In 1989, some 1,000 buses were procured by the Department of Transportation and Communication (DOTC). The DOTC has also activated rail transport into the city as well as ferry boats on the Pasig River. The trains service 20,000 passengers per day while the ferry boat, 5,000 (Ibon Facts and Figures, March 1990). The long queues and waiting times, especially during rush hours, depict a system under severe strain.

The number of motor vehicles in Metro Manila has more than doubled from 231,000 in 1975 to 500,000 today (Nierras 1979; EMB 1990). As in most cities, the trend has been for the urban dweller to buy a car if he can afford it. In the third quarter of 1989, for example, 39,685 new motor vehicles were registered, 87% of which were private cars (Ibon, March 1990). As early as 1974, studies showed that 41% of the estimated 1.7 million

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[7] The Laguna lake basin covers an area of 382,000 ha; the lake itself covers 90,000 ha. Eighty percent of industries are located on the western part of the lake. In the early 1980s, the Laguna lake region had a population of 6.2 million and a growth rate of 3.7%. This population would include the towns and municipalities south of Metro Manila. Only a portion of Metro Manila, mainly its southern municipalities including Taguig, Pateros, Muntinlupa, are considered part of the Laguna watershed (Environmental Management Bureau 1991.).

[8] In her book **Blanco** where she presents the painting of a family of artists from Angono, Alice G. Guillermo writes: "In years past, the early morning air resounded with the voices of fishermen hauling in their nets and vendors haggling for the piles of leaping silvery freshwater fish, the *kanduli* which thrives in the waters of Laguna de Bay. Of late, although the village is yet unaffected by urban taint, the fisherfolk of Angono are menaced by the pollution of the waters from the new factories. The other more immediate threat to the livelihood is the powerful fishermen who have come to exploit the lake with launches and larger nets and who have set up rows of fishpens which effectively crowd out the small fishermen from their traditional ground." (Guillermo 1987).

9 Travel times by public transport have been one of the slowest in the world. In 1975, it took an hour to travel 10-20 kilometers within Metro Manila in a jeepney or a bus (Nierras 1979).

vehicle- trips per day were made by private vehicles (Nierras 1979). With the increasing number of private cars on the road, this percentage is now probably larger. This increase translates into more traffic on the road, actual longer travel times on the average, more gasoline consumption and more motorcar exhaust pollution.

Of the urban transport problem, an expert wrote in 1979:

> *Supplying mobility to Metro Manila's - 7 million residents has become a dilemma. On one hand, the combined capacity of all public vehicles -- about 431,000 seats – is not enough to meet the 8.5 million person-trips per day, especially during the rush hours. On the other hand, there are not enough vehicles to meet the travel demand, the roads are too few and too narrow to accommodate the increasing number of vehicles. Solutions lie somewhere between providing more roads and transport infrastructure, providing more and better public vehicles, and reducing the population and travel demand (Nierras 1979).*

ENERGY

There were frequent power shortages in Metro Manila during summertime over the last couple of years. Ironically, the shortfall of energy has slowed down economic growth. A 1989 survey of 60 firms by a metropolitan daily showed production losses amounting to ₱27 billion (Ibon, March 1990). The shortage of power has been attributed to a number of factors. One is the decreased capability to generate hydroelectric power during summer when the water levels in the dams fall. Another is the increased demand for electric power as a result of expanded economic activity.

The country is dependent mainly on imported petroleum for energy. In 1975, the energy use patterns of the various sectors were: transportation, 35.8%; industry, 34.2%; residential commercial, 6.6% and others 23.4% (See Fig. 4). The high consumption of energy in the transportation sector has much to do with use of motor vehicles in Metro Manila where half of all motor vehicles in the country are found. The increasing urban sprawl and increased travel times as a result of longer distances to and from work, the increasing number of private cars which consume more

energy per capita compared to buses and other forms of mass transport, the worsening traffic situation and the poor maintenance of vehicles all contribute to a higher energy bill.

With increasing industrialization and commercialization, construction of more residential subdivisions, more demand for transport services, the energy requirements of Metro Manila will continue to rise.

HEALTH SERVICES

Services for personal health care are widely available in the city for those who can pay. For those who cannot, there are the government hospitals and clinics.[10]

Government provides the bulk of public health services, which include programs and health interventions with broad community focus. Some examples of these are: Dengue fever control by fogging against the mosquito vector; regulation of sanitary practices in restaurants; prevention and control of infectious disease epidemics like typhoid and cholera. Some public health services, like immunization and tuberculosis treatment, seek community impact (eg. prevention of measles, polio, or TB) by servicing as many eligible clients as possible. In so protecting large segments of the population, the spread of illness is either slowed down or stopped.

The increase in the number of slum and squatter dwellings has made it difficult to cover large portions of urban populations susceptible to disease. Measles epidemics have often erupted in urban poor areas where measles immunization coverage is low. The very recent measles outbreak in Novaliches in May 1991 where eight children died is a case in point (The Manila Bulletin, May 30, 1991). In places like these, immunization coverage of children is only 30-60% (Unpublished data of Maternal and Child Health Service, Department of Health, 1990). In Barrio Magdaragat, better known as "Smokey Mountain", which is the site of the biggest dumping area (15 ha) in Tondo, only 39% of the 216 children surveyed had measles vaccination. Furthermore, 96% of

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[10] Personal health care refers to direct medical service to an individual for a specific ailment. In the country as a whole, public hospitals, and clinics account for 26-36% of personal services provided. In Metro Manila, where private services are concentrated, this percentage is much less (Intercare: Health Care Financing in the Philippine: A Country Studio).

these children harbored intestinal parasites and remained untreated (Auer 1990).

Crowding and the lack of water and sanitation facilities are known to be associated with the spread of cholera (Velimirovic, Subramanian, Sadek 1975). Studies by the Department of Health have associated cholera with water hoses used to collect water from public faucets in urban poor areas along the coastline (Benabaye unpublished). Seepage of sewage into water lines through illegal pipe connections is common. A recent epidemic in Barangay Escopa, Quezon City illustrated this (Lopez et al. unpublished).

The above situations amply confirm what is widely known: that many urban health problems stem from the squalor of the environment compounded by the lack of basic services.

TYPES OF URBAN POLLUTION

One of the consequences of industrialization and urbanization -- particularly when rapid -- is pollution. This is common in many parts of the world. Mexico and China, two countries that have rapidly industrialized in the last 25 years, have seen their industrial centers suffer from smog, acid rain and toxic sludge (National Geographic, May 1973 and Time Magazine, April 29, 1991). A city poisons itself in many ways. Let us examine the pollution picture of Metro Manila.

POLLUTION OF THE URBAN AQUATIC ECOSYSTEM

Pasig River. Like many ancient cities whose civilizations sprung from a magnificent river like the Nile in Egypt or the Tigris-Euphrates in what is now Iraq, Manila had its beginnings at the mouth of a river: the Pasig. A Filipina writer-historian wrote: "The first street of Manila was the Pasig, if by street is meant a passage or thoroughfare. From the waters of the South China Sea up through the Pasig -- an old Malayan word pertaining to the coast or strand -- rowed the early Manilans." (Luning 1977).

The Pasig River flows through Manila from east to west for 25 km from its origins at the Laguna de Bay to its mouth at Manila Bay. It is estimated that 60% of Metro Manila comprise the watershed of the river involving some 5 million residents in the cities of Manila and Quezon City and the municipalities of San

Juan, Mandaluyong, Makati, Pasig, Taguig, Marikina, San Mateo and Montalban (ADB J-2).

The Pasig has suffered the accumulated abuses of garbage dumping, discharge of industrial effluents, mismanagement of domestic waste, siltation due to deforestation and bank erosion and proliferation of squatters along its banks (See Fig. 5). It is estimated that 60-70% of pollution of the Pasig River is due to domestic waste. The Pasig is so badly polluted that some parts of it are biologically dead. The most polluted portion is the Navotas-Malabon-Tullahan-Tenejeros tributary which is a 26-km water-course that runs from the La Mesa reservoir in Novaliches towards Manila Bay. It is polluted by over 1,000 industries and wastes from 11,000 squatter homes on its watershed (ADB I-10).

The Department of Environment and Natural Resources regularly monitors the Pasig River for certain parameters which include: color, temperature, turbidity, pH, dissolved oxygen, biochemical oxygen demand, suspended solids, total and fecal coliforms, nitrate, phosphorus, oil and grease. Dissolved oxygen levels, which give an indication of the capacity of the aquatic environment to sustain living things, have consistently been measured at less than 2 mg/l in many monitoring sites (ADB I-9) This is below the standard for surface water classified only for navigational use (Class E) (See Table V). Biochemical oxygen demand, which is a measure of the amount of organic matter in wastewater, has occasionally been recorded as high as 500 mg/l (ADB I-10); this is much like the BOD level of wastewater which contains heavy amounts of human excreta. For a river where fish and aquatic resources are propagated, the recommended level is 20 mg/l. Industrial pollution, as measured by mercury levels above acceptable limits, has also been documented.

Laguna de Bay. Laguna de Bay, a 90,000-ha freshwater lake, which contributes to the waters of the Pasig, traditionally provided the livelihood of thousands of fishermen in lakeside towns. Today, it has also become shallow from siltation as a result of deforestation of the land around it. To prevent lakeshore flooding, the Napindan hydraulic control structure permits discharge of lake water into Manila Bay via the Pasig River. The channel can be opened or closed to control the flow of water coming into or leaving the lake. Fishermen want the channel open for saline water from Manila Bay to enter the lake and provide nutrients for fish. However, when this happens, it is polluted saline water from the Pasig and Manila Bay which enters the lake. Coupled with the

industrial effluents from about 1,000 industries in its watershed, pollution now threatens the lake. Dissolved oxygen levels measured in 1986-87 showed levels ranging from 6.1 - 9.3 mg/l which is above the standard set for Class A waters (ADB I-12). This is encouraging as the lake is being considered a source of drinking water. However, quite recently, disease in fish associated with *Aeromonas hydrophila* has been attributed to pollution; these occurrences herald a worsening of the lake's degradation (Environmental Management Bureau 1990).

Manila Bay. Like Laguna de Bay and the Pasig River, Manila Bay has also become shallow and heavily polluted. Fourteen sampling stations in different parts of the bay detect signs of eutrophy which means the depletion of oxygen because of excessive algae and bacteria. Dissolved oxygen levels ranged from 3mg/l to 8.5 mg/l in various parts of the bay indicating some variation in the extent of pollution within the bay area. In the Navotas area, for example, pollution is such that the geometric mean of fecal coliforms averaged 0.6 million per 100 ml for 1982-89; this is 100 times less than the number found in raw sewage but nowhere near the acceptable level for bathing or recreation. In the Luneta area, coliforms were measured at 77,000 per 100 ml and near Corregidor Island (P. Grande sampling station), fecal coliforms had fallen to 500 per 100 ml (ADB I-17). A Hong Kong study showed that at the levels of pollution like that measured near Corregidor, swimmers were still more likely than non- swimmers to develop infections of the eyes, ears, gastrointestinal and respiratory systems (Cheung, Chang and Hung 1990).

Data collected at the sampling stations indicate that the level of pollution in Manila Bay as indicated by the numbers of coliforms has gradually worsened over the last decade. At Luneta and P. Grande near Corregidor, fecal coliform counts went up over a hundredfold from 1982 to 1989 (See Table VI). At Bacoor, where shellfish (oysters, mussels) are farmed, levels have fluctuated probably because of the proximity of the sampling point to the outlet of the Imus River.

The pollution in Manila Bay has great public health implications for the shellfish industry in Cavite as oysters and mussels are known to be associated with infections, like cholera, hepatitis A and paralytic shellfish poisoning. The eventual confidence of the public in these sources of shellfish could erode what is now a ₱100-million industry. This already happened in 1988 when the

public stopped buying mussels because of the Red Tide phenomenon.[11]

To control the spread of illnesses, like cholera and typhoid that result from sewage poisoning, sanitation -- more than any other intervention (eg. vaccines or drugs) --- is most effective (See Fig. 6).

THE GARBAGE SITUATION

The rise of the garbage pile has accompanied the growth of the city. Metro Manila is said to produce close to 4,000 tons of solid waste everyday, only an estimated 70% of which is collected and transported to dumpsites (Environmental Management Bureau 1990). The uncollected waste is scattered on city streets, thrown or washed down drainage pipes, esteros and rivers, and contribute to the flooding problem. A recent television advertisement declared, "Basurang itinapon mo, babalik rin sa iyo" while cinematically portraying the rush of flood waters and accompanying debris into the family living room.

Several reasons have been identified for the garbage problem. One is indiscriminate and unmindful disposal of refuse in streets and empty lots by people. Another is the poor system of collection. A third is the inability of garbage trucks to collect garbage in squatter and slum areas where streets are narrow and inaccessible. A fourth is the inability of city authorities to adequately plan and provide resources for the proper collection and disposal of waste (ADB F-2). Recently the mayors of Metro Manila have made garbage collection one of their main priorities but the permanent solution to the problem will require a far-ranging and comprehensive approach that goes beyond improvement of garbage collection.

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[11] In the Philippines, Red Tide is cause by a marine dinoflagellate, *Pyrodinium bahanense* which, because of unpredictable blooms, causes sea water to turn red. Filter feeders, like mussels, accumulate high concentrations of the toxin produced by the dinoflagellate. When contaminated mussels are ingested by man, an illness called paralytic shellfish poisoning results. Symptoms include numbness and respiratory paralysis. These might lead to death. Outbreaks of Red Tide occurred in various parts of the Philippines, including Manila Bay, in 1988-89 (Department of Health 1989).

The exact causes of the dinoflagellate blooms have not been fully determined. Some factors thought to contribute to their occurrence include enrichment of marine waters from land run-off, upuvelling of colder nutrient-rich oceanic water and temperature rises (Environmental Management Bureau 1990).

For example, Metro Manila's garbage is transported to open dumpsites which do not fulfill the standards for an environmentally acceptable refuse disposal system (See Fig. 7). The areas are not fenced. They breed rodents, flies and other vectors of disease. The lack of earth cover for the daily pile of refuse allows rainwater to seep through the heap causing anaerobic decomposition, gas production and bad odors. The result is a "smokey mountain": on top of it, poor people scavenge; under it, contaminants threaten to leak into nearby aquifers (ADB F-2).

A recommendation has been made for Metro Manila's dumpsites to be closed and for two new landfill areas to be opened, one to serve the north, and the other the southern part of the city. On Feb. 26, 1991, The Manila Times carried a photo of the San Mateo landfill starting its operation.

AIR POLLUTION

There are two major sources of air pollution in the city. Motor vehicles account mostly for carbon monoxide and nitrogen dioxide emissions; industries are the main source of sulfur dioxide. All these gases are hazardous to good health.

Although monitoring of air quality in Metro Manila has been erratic due to breakdown of instruments and changes in technique, data show that air quality in the city deteriorated in the last decade. High readings for total suspended particulates, which include dust, smoke, fumes, and metallic and mineral particles, have been found in several areas like Valenzuela, Malate, Ermita, and Pasay. Carbon monoxide levels have been rising with the increase in motor vehicles and the generally poor vehicle maintenance. Although sulfur dioxide and other heavy metal pollutants (lead, cadmium, copper, zinc) still do not show dangerously high levels, the increasing industrialization of the Metro Manila area and the inadequate compliance with and enforcement of pollution control laws do not provide optimism that air quality will improve (Environmental Management Bureau 1990).

Local air pollution will have effects on the environment beyond the boundaries of Metro Manila. Air pollutants anywhere and everywhere cumulate and lead to global warming, ozone layer depletion and acid rain.

MANAGING THE URBAN ECOSYSTEM: WHERE TO BEGIN?

As described above, the problems of the urban ecosystem are complex and intractable to quick fixes. Solutions need to be applied at individual, community and societal levels, by government and private sectors working together, with local and international support.

First, we must begin to develop, cultivate and propagate among Filipinos, an attitude of respect, maybe even reverence, for our environment.

Richard F. Townsend of the Art Institute of Chicago said: "Harnessing ecological resources and expanding the economy are primary forces in the development of societies and their increasing complexities, but parallel with that is the idea of sacred places." The ancient Aztecs understood the unity of the land and the cosmos and of the gods which protected it. Because of this, they built beautiful temples and did not pollute their surroundings. The Spaniards under Hernan Cortes, accustomed to the dirt and grime of the Old World cities, marvelled at the cleanliness of the Aztec capital of Tenochtitlan in 1519 (Stuart 1988). There is much we can learn from this ancient civilization.

Respect for the environment can be taught through innovative programs at all levels of schools and colleges. Futhermore, well- crafted communication campaigns designed for home, work and public places should set a climate so that people are encouraged to behave in non-wasteful, non-pollutive ways. Simple anti- littering and proper garbage disposal practices are of immense importance when millions of people are involved. There is no underestimating the role of each individual in contributing to the total effort of conserving or rehabilitating the physical surroundings. Educational materials are needed. A book entitled "50 simple things you can do to save the earth" published by the Earthworks Group of Berkeley, California, advises on a host of things, like pesticide use, recycling of motor oil and composting. Ideas in such a publication, if widely circulated and discussed, could change people's views and behavior for the better.

Second, as a society, we must solve the problem of rural poverty from which stems the complex web of in-migration, squatting and slum areas.

History has shown that any success in fighting rural poverty will depend on a successful land reform program. Japan and the

tiger economies of Asia put land in the hands of the tiller and supported him with cooperative structures for marketing and purchasing, extension services and sustained research and innovation As the rural population prospered, they became both a market and a source of savings for industrial investment. Japan completed its land reform program in the late 1940s and early '50s, Korea, in the 1970s (Ward 1979).

Furthermore, Sixto K. Roxas has made a case against economic policies that generate mass poverty by marginalizing people in the rural areas. Such policies include the conversion of scarce prime agricultural land into non-agricultural uses (eg. export processing zones) thereby wasting the inherent productive capacity of land as soil. Roxas believes that this approach makes no sense because it increases the amount of capital necessary to generate livelihood (Roxas 1990).

Third, we must find ways to upgrade the urban infrastructure for basic services without which city living becomes unpleasant and hazardous.

The investment requirements for this upgrading have been estimated as follows (ADB p. 7-1):

Integrated Solid Waste Management		139.0
Integrated Flood Control Program		297.0
Integrated Water Quality Management		163.0
Industrial Pollution Control Program		100.0
Environmental Management and Monitoring	US$	11.3 M
Total		710.3 M
	(P	16,640.0 M)

Where will the money come from? Environment Secretary Fulgencio Factoran, in a speech at a conference convened by the Philippine Futuristics Society on May 30, 1991, lamented the cuts in the budget of the Department of Environment and Natural Resources which will jeopardize the implementation of ongoing projects (Futuristics Society, Proceedings of the Conference, unpublished). There were no clear answers to this question but alternative modes of financing were proposed.

One, cleaning up the environment raised opportunities that businesses could invest in and profit from. For example waste recycling technologies for sewage and for solid waste are being undertaken in other countries. Two, a scheme called the debt-for-nature swap could allow a non-governmental organization to

undertake an environmental rehabilitation program using local funds generated by the exchange of a foreign debt obligation with a new obligation to undertake a conservation program. Three, taxes could be raised from polluters. An emissions tax could be charged from motor vehicles discharging unacceptable levels of carbon monoxide or carbon dioxide. Factories could also be made to pay for pollution attributable to their operations.

Fourth, institutional roles and responsibilities must be straightened out. The Metro Manila Authority, the different local government units and various national agencies need to get their act together so to speak, to effectively undertake Environmental improvement projects and enforce laws and ordinances on land use, squatting, pollution controls and many other issues.

Figure 8 (ADB 6-6a) shows us how complex the organizational set- up is. This chart includes the national agencies involved in the environmental improvement projects of Metro Manila. Local government units are not indicated.

On the matter of law enforcement, a foreign consultant observed:

> *Among the phases of urban and environmental management process, the enforcement of rules, regulations, legislations and ordinances is weakest. This is primarily due to: lack of financial support to properly implement the rules and regulations; lack of specific provisions in policies or regulations which make implementation or enforcement difficult; and lack of sincere commitment on the part of implementors and enforcers. Enforcement in Metro Manila is characterized with partialities, is seasonal and is vulnerable to graft and corruption. (ADB 1990).*

There are a multitude of weaknesses that need to be addressed here. We could start by strengthening the powers of the Metro Manila Authority and perhaps making governorship of Metro Manila an elective position so that it could carry more clout. And of course, we would need to elect better public officials.

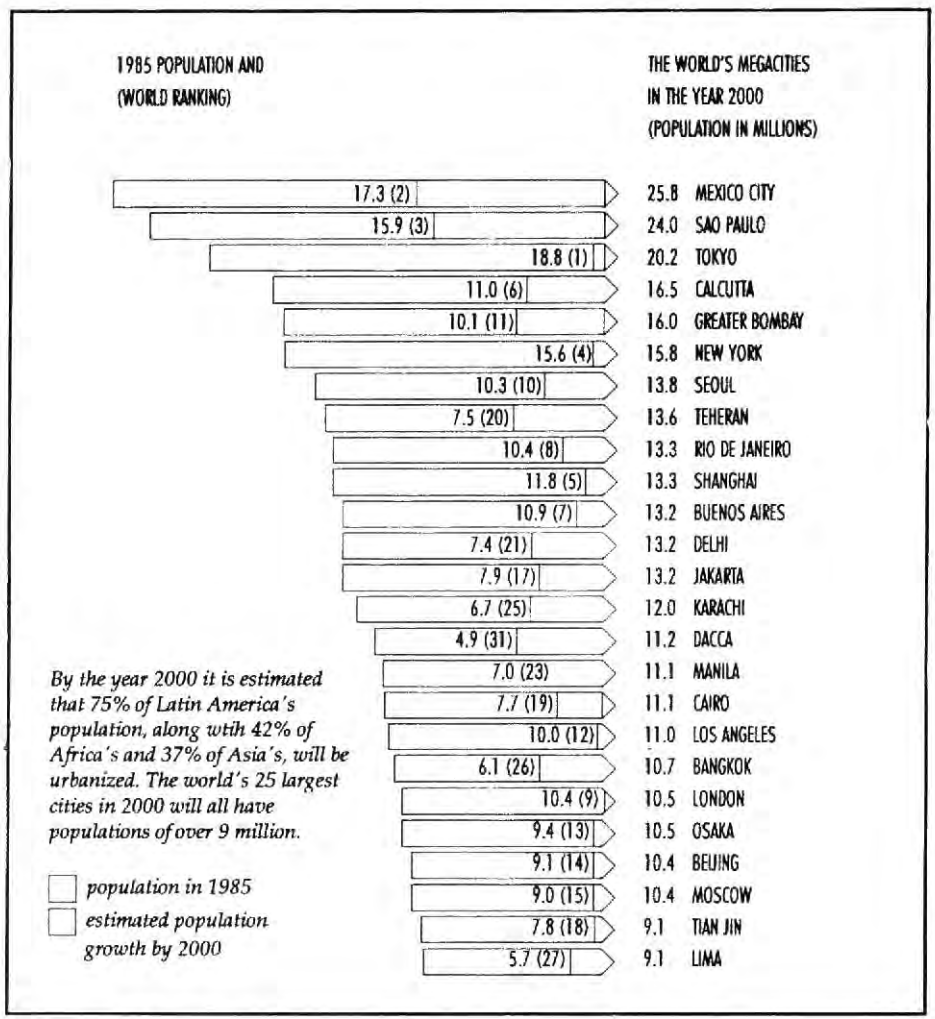
Finally, we should use the lessons of Metro Manila's development in order to plan and build better cities in other parts of the country.

The problems of Metro Manila are already happening in the cities of Cebu, Iloilo and Davao. The list is the same: poor sewage

and sanitation services, poor water supply, squatter colonies, and others.

Let me end by relating a recent conversation with the Assistant Provincial Health Officer of Guimaras Island in Iloilo. In speaking about the development of that small island, he envisioned the education of its poor people who could provide the labor for a boat building industry that might be set up there. Development, when it uplifts people, is also a vehicle for environemental conservation. It is this people-oriented development that we must continue to strive for.

Population of World's Megacities
Table I



SOURCE: UN POPULATION FUND

Table 2. Population by city and municipality (NCR, 1948-1980)

Jurisdiction	1948	1960	1970	1975	1980
NCR	1,569,128	2,462,481	3,966,695	4,970,006	5,970,310
Manila	983,906	1,138,611	1,330,788	1,479,116	1,642,708
Caloocan	58,208	145,523	274,453	397,201	471,323
Pasay City	88,728	132,673	206,283	254,999	289,927
Quezon City	107,977	397,990	754,452	956,864	1,174,605
Las Piñas	9,280	16,093	45,732	81,610	137,537
Makati	41,335	114,540	264,918	334,448	375,424
Malabon	46,455	76,438	141,514	174,878	192,432
Mandaluyong	26,309	71,619	149,407	182,267	206,905
Marikina	23,353	40,445	113,400	168,453	213,199
Muntinlupa	18,444	21,893	65,057	94,563	137,703
Navotas	28,889	49,262	83,245	97,098	127,091
Parañaque	28,884	61,898	97,214	158,974	210,115
Pateros	8,380	13,173	25,468	32,821	40,590
San Juan	31,993	56,861	104,559	122,492	131,063
Taguig	15,340	21,856	55,257	73,702	135,0142
Valenzuela	16,740	41,473	98,456	150,605	213,955
Pasig	35,407	62,130	4,156,492	209,915	270,583

Source: For 1948-1990: 1980 Census of Population by Province Municipality and Barangay, NCR, Final Report

Table 3. Population projection of cities and municipalities: .1980-2030. (Medium assumption: Moderate fertility decline and moderate mortality decline)

Province/ Municipality	1980	1985	1990	1995	2000	2005	2010	2015	2020	2025	2030
Metro Manila	5970310	6942204	7974002	8970970	9894837	10737419	11481317	12152388	12765312	13265262	13607025
Caloocan City	471323	543302	615726	680769	735657	780438	814885	842105	864376	879294	885288
Manila	1642708	1765907	1876194	1954926	2001338	2021942	2021067	2009880	1995673	1995673	1974072
Pasay City	289927	331860	373657	410663	441357	465919	484343	498582	510053	517387	519713
Quezon City	1174605	1377926	1587140	1781159	1951129	2095504	2212170	2308301	2389270	2447735	2478649
Las Piñas	137537	207770	302560	421420	562420	722333	894996	1075709	1258577	1430111	1575997
Makati	375424	421367	465896	503560	533019	554994	569895	580334	588156	591923	590775
Malabon	192432	220197	247860	272336	292621	308839	320990	330370	337921	342738	344244
Mandaluyong	206905	233843	260221	282922	301090	315031	324897	332116	337707	340819	340931
Marikina	213199	259806	309986	359340	405449	447256	483584	515347	543241	565168	579534
Muntinlupa	137703	183696	238337	298449	361343	424867	486372	545099	600230	647908	684665
Navotas	127091	147364	167919	186588	202554	215772	226127	234440	241317	246063	248219
Parañaque	210115	266740	330552	396779	462181	524743	582181	634686	682338	721779	750229
Pasig	270583	334770	405075	475673	543075	605498	660955	710318	754238	789522	813660
Pateros	40590	48346	56478	64209	71174	77262	82348	86653	90349	93131	94784
San Juan	131063	142444	152880	160783	166001	168998	170082	170160	169837	168735	166649
Taguig	135142	166308	200239	234066	266122	295595	321583	344574	363939	381182	392140
Valenzuela	213955	290551	383274	487318	598298	712421	824833	933707	1037081	1127686	1198808

Source: Philippine Population Projections, NEDA

Table 4. Metro Manila Distribution of Squatter Population, 1987

District	LGU	Total Population	Squatter Population		Squatter Families
1	Manila	1,813,064	545,496	(30.1)	90,916
2	Caloocan	572,763	223,848	(39.1)	37,308
	Malabon	231,492	73,374	(31.7)	12,229
	Navotas	155,702	102,714	(66.0)	17,119
	Valenzuela	325,958	52,682	(16.2)	8,781
3	Quezon City	1,462,327	516,000	(35.3)	86,000
	Makati,	439,747	81,612	(18.6)	13,602
	Mandaluyong	244,687	108,380	(44.3)	19,300
	Pasig	362,519	100,668	(27.8)	16,278
	Pateros	,51,605	25,530	(49.5)	4,255
	San Juan	146,856	21,972	(15.0)	3,662
	Marikina	279,729	61,692	(22.1)	10,282
4	Pasay	348,923	266,220	(76.3)	44,370
	Parañaque	291,687	76,776	(26.3)	12,796
	Las Piñas	242,716	37,578	(15.5)	6,263
	TOTAL	7,354,190	2,485,696	(33.6)	415,020

Source: NSO/LGUs

Note: Figures in parenthesis represent percentage of total population.
Figures for squatter families are based on average assumed family size of 6 persons.

Table 5. Water Usage and Classification

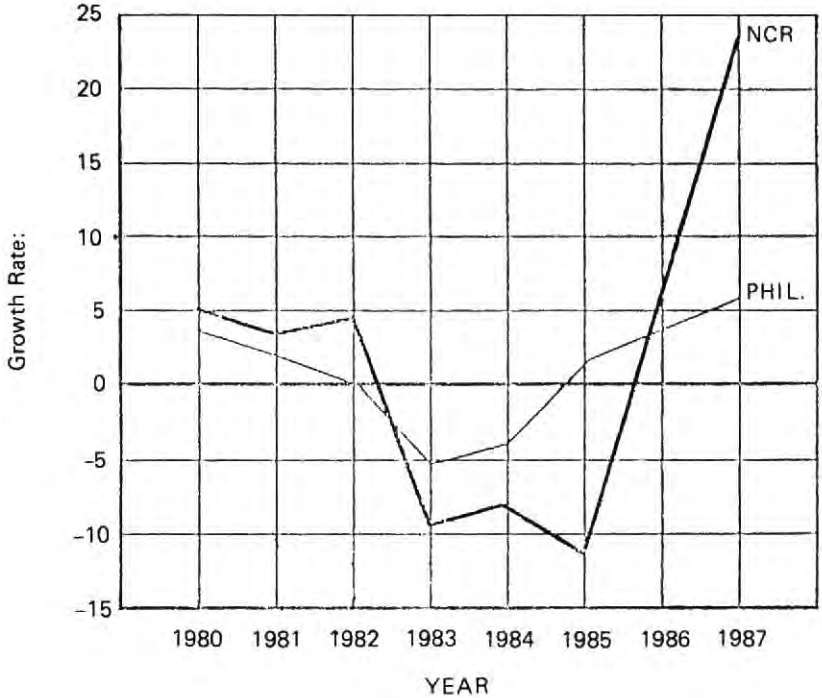
		Dissolved Oxygen (mg/l)	BOD (mg/l)
Class AA	Public water supply needing only disinfection to meet standards for drinking quality	—	—
Class A	Public water supply needing complete treatment (coagulation, sedimentation, filtration and disinfection) to meet standards for drinking quality	5	10
Class B	For primary contact recreation	5	15
Class C	For propagation and growth of fish and other aquatic resources	5	20
Class D	For agriculture, irrigation, livestock watering and industrial cooling and processing	3	–
Class E	For navigational use	2	–

Source: (ADB, I-4)

Table 6. Geometric Means for Fecal Coliforms (1982-89)

Station	1982	1983	1984	1985	1989	1982-1989
Luneta	17,833	25,246	31,007	91,435	2,223,123	611,280
Bacoor	45,605	10,987	21,982	17,086	66,169	26,240
P. Grande	137	168	384	976	16,771	670

Source: (ADB, I-17)



Source: MMC - OCP

Figure 1. Growth trends of NCR economy

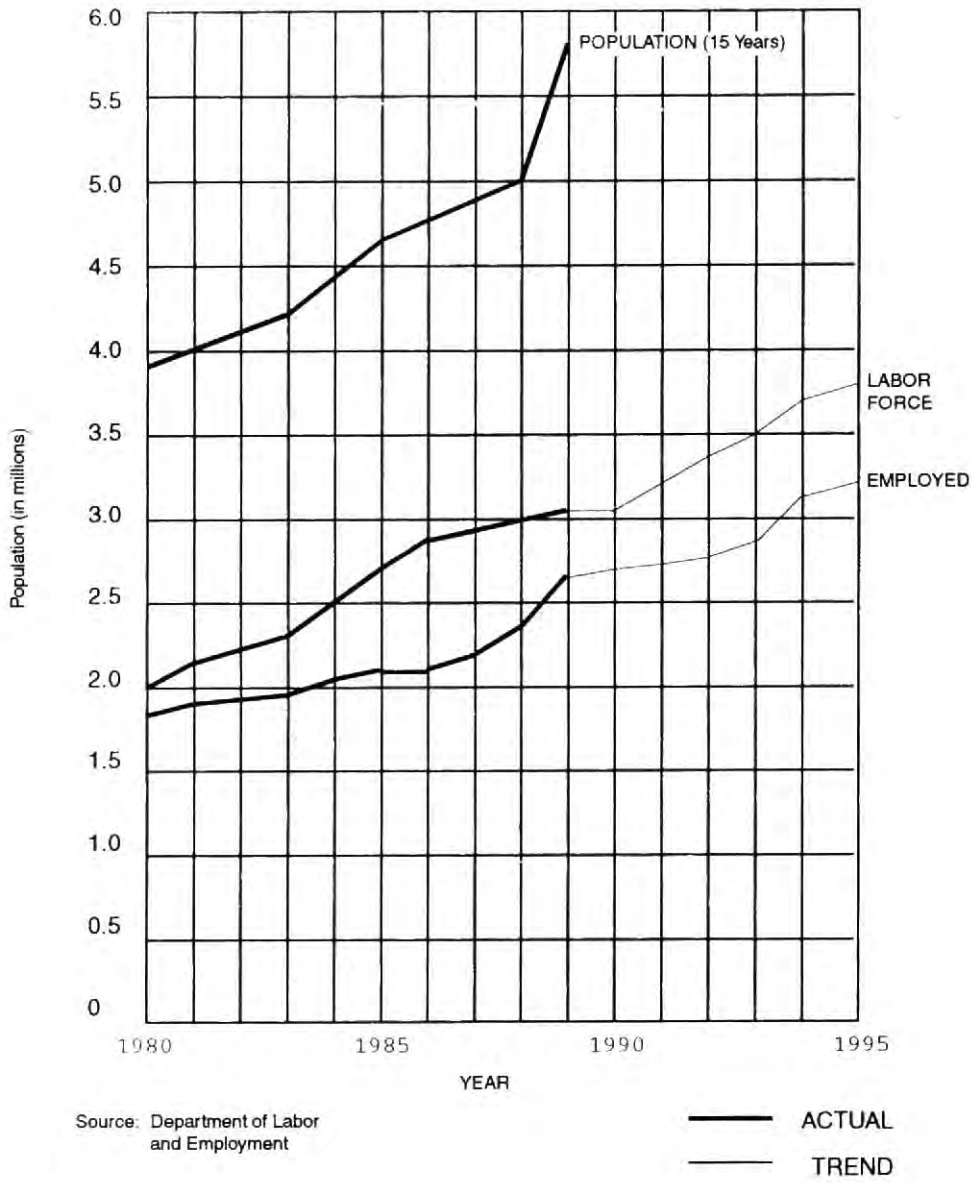


Figure 2. Employment Situation, 1980-1995

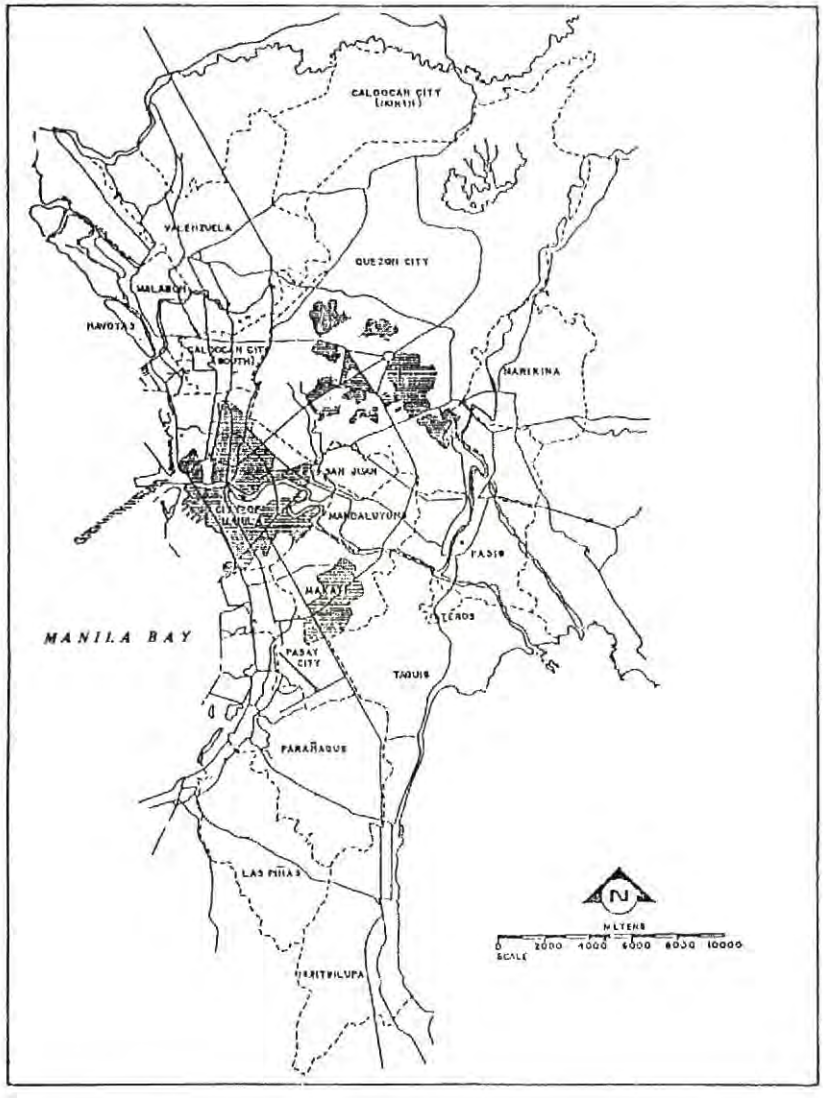


Figure 3. Areas with existing pipe sewer system

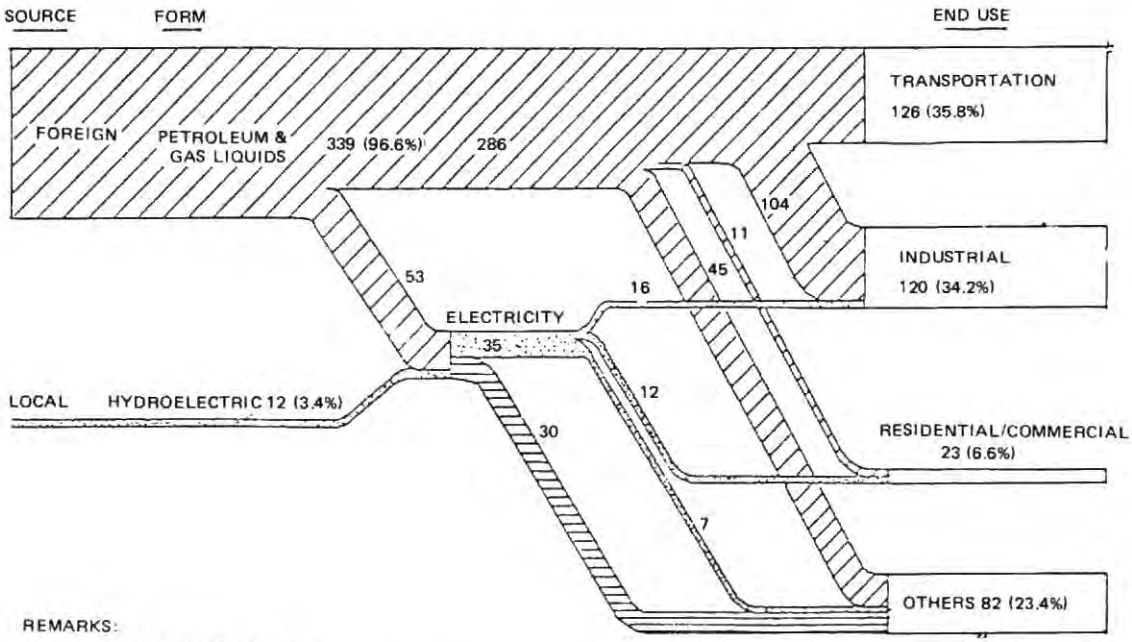


Figure 4. Energy flow patterns in the country

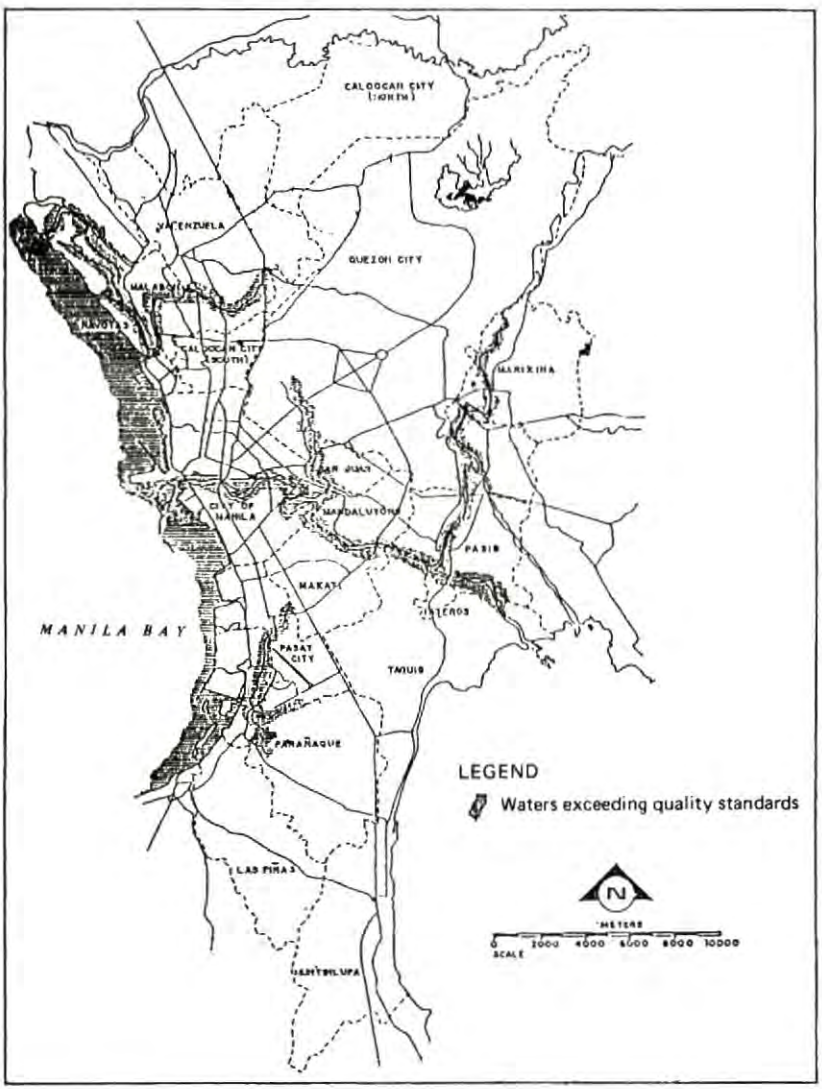
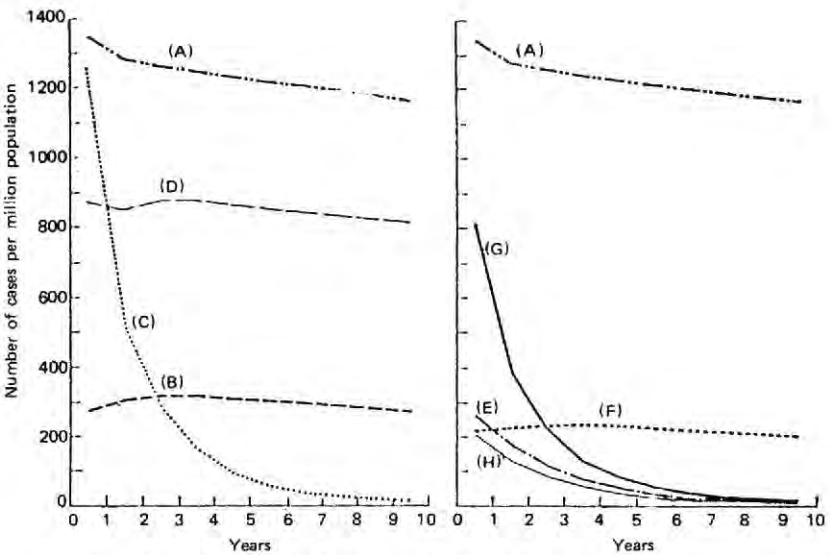


Figure 5. Surface waters exceeding quality standards



Simulated incidence of cholera during a 10-year period. (A) no control measures undertaken; (B) vaccination programme, 75% coverage; (C) sanitation programme (10 years); (D) drug prophylaxis; (E) vaccination and sanitation (B + C); (F) vaccination and drug prophylaxis (B + D); (G) sanitation and drug prophylaxis (C + D); (H) vaccination + sanitation + drug prophylaxis (B + C + D).

Figure 6. Infectious Disease Dynamics

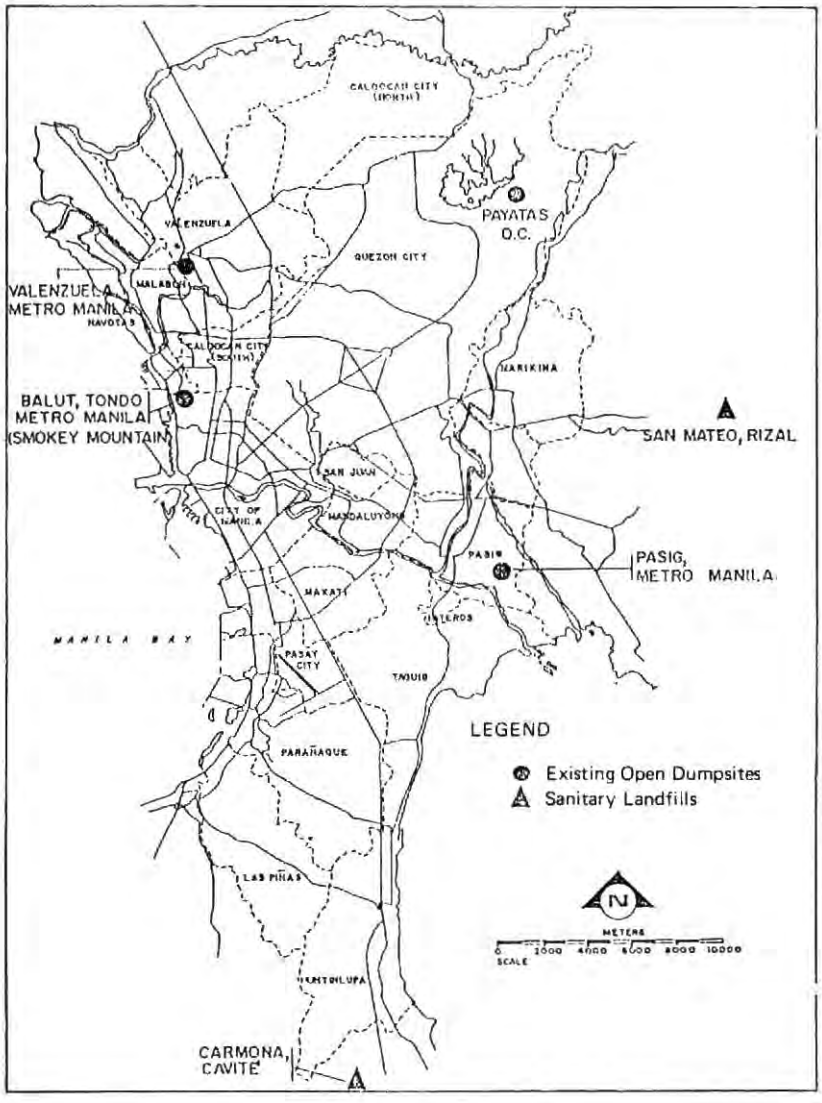


Figure 7. Solid waste disposal sites

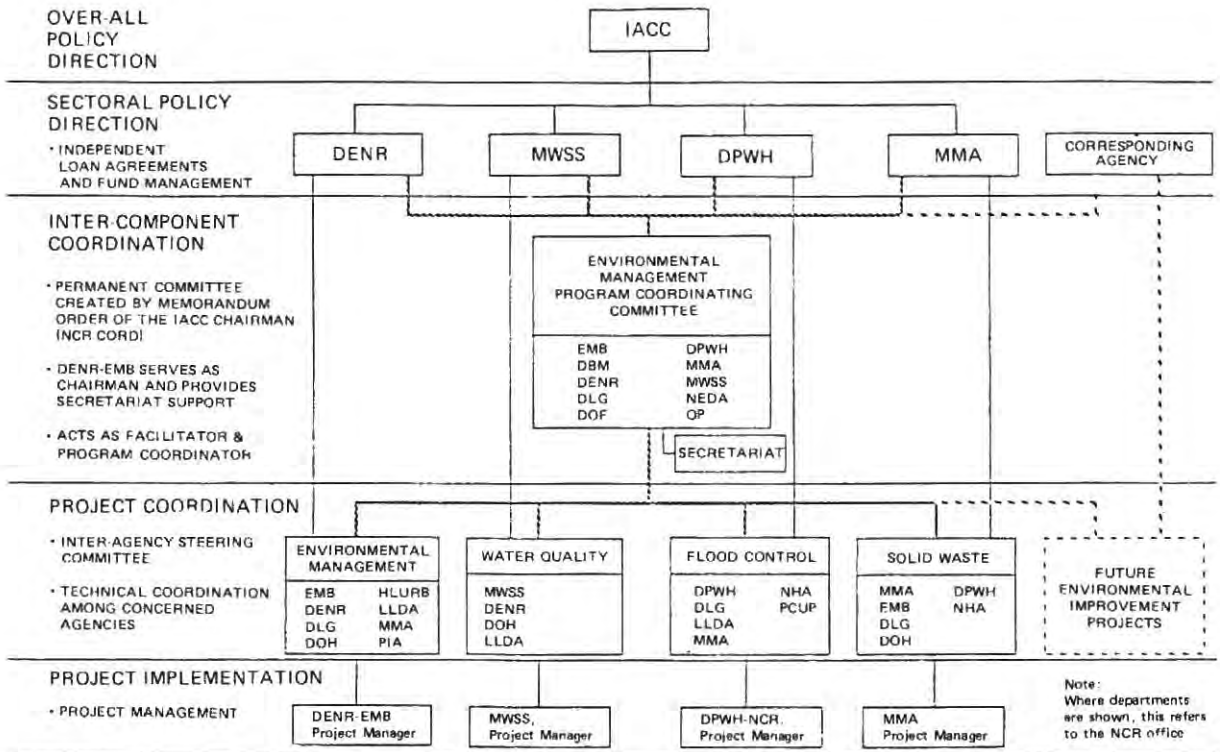


Figure 8. Institutional arrangement for separate loan packages

DISCUSSION PAPER ON: Managing Our Urban Ecosystem for Survival

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Dr. Dayrit has defined for us the problems of an urban ecosystem exemplified by Metro Manila. Needless to say the picture is a grim and depressing one. The challenge to all of us lies in the last three pages which say: "Managing the urban ecosystem: Where to begin? "There is no magic management strategy which provides us the winning formula. At best we can identify a set of solutions which have differing degrees of implementability and doability, some of which have been outlined by the author:

(1) As the saying goes: Let it begin with me; with my family; and household.

(2) Some of us still think that if squatters and slum dwellers would participate in a **Balik-Probinsiya** program, it will minimize the population pressure in Metro Manila. Studies have shown that very few of them have land to go home to. There may be land, land, everywhere but none for them to keep. A few families own them all. Residential landlessness is as much a rural as well as an urban phenomenon although not as obvious. What about rural residential land reform? They can put up their own houses somehow.

(3) If the country were to invest in the upgrading of the urban infrastructure for basic services in order to make Metro Manila pleasant for all of us, the amount involved as per ADB estimate is ₱16,640 million. The irony of this investment is that the more we make the city liveable, the more we attract rural-urban migrants and the less money we have for the rural areas. If we set our minds to it, Metro Manila can absorb most of our resources.

(4) Despite the general environmental degradation in the Metropolis, there is no equality of suffering. The rich **"air- condition"** out the garbage, the air pollution, the

heat and the poor; high-rise condos' tinted windows and draperies do the trick. One of the most interesting dilemmas in urban ecosystem management is the reality that the rich man's garbage is the poor man's livelihood. Can we apply science and technology to make this livelihood more liveable? At the moment, slums and squatters make up a third of the population in the Metropolis. Perhaps when they become 60%, change will take place because the rich will move out of the city and property values in the inner city will drop.

(5) There is one avenue for change that is going to be available pretty soon. We should use the 1992 elections to choose more accountable public officials. If we cannot even do that wisely, we deserve all the pollution we get. Incidentally, because of the systemic nature of urban problems, electing good Metro Manila officials is not enough. We need environmentally-friendly leaders throughout the country. This is one sphere of Filipino life where "recycling of garbage" is not recommended.

6) The paper mentions that 60% of the population growth in Metro Manila is attributed to natural increase and 40% to in-migration. It seems to me that we might be able to do more about the natural increase than about in-migration. With Cardinal Sin willing, why couldn't we have 3,500 natural family planning clinics all over the Metropolis? Provided we cut down ST movies that fuel the imagination, we will either succeed or have a generation of city-born rhythm babies.

(7) Finally, if we define ecology as sharing, and live our lives on that basis, Metro Manila may still be polluted but perhaps those who share will sleep better and live longer.

Our Collapsing Urban Ecosystem

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Dr. Manuel H. Dayrit has laid down an ecological view of the National Capital Region, which even if expressed in academese, is all too familiar to the harassed and hassled dweller of Metro Manila.

Said Dr. Dayrit, the ecosystem of Metro Manila is breaking down. Its four elements -- land, waters, air, people -- no longer enhance one another as they ideally should. In 1987, an estimated 2.5 million or 34% of total metro population were squatters. By 1992, that figure will soar to three million.

In our situation of disordered growth, basic services are bound to be patchy. Only 15% of the city is sewered. The metro's sewage network is outdated by at least 45 years. At the present rate of expansion, it will take 25 years for that sewage system to cover 50% of Metro Manila.

Potable water services are said to reach 70% of the population -- an optimistic estimate in my opinion. The water level of the underground aquifer has been going down by about 4-10 m a year because of overextraction of ground water, causing sea water to seep in. An estimated 55% of city water is lost through pipe leaks and unbilled water consumption. Laguna de Bay is being tapped as a source of potable water by the year 2000, but this would require treatment plants as the lake water is heavily polluted by industrial and human wastes and agricultural toxins.

Travel time by public transport in Metro Manila is one of the slowest in the world because of increasing vehicle density and inadequate road surfaces to accommodate the increase. The combined capacity of all public vehicles -- 431,000 seats -- is not enough for the 8.5 million person-trips taken by residents every working day.

Inadequate electric power translates into irrecoverable economic losses. In 1989, 60 firms in Metro Manila reported losses in production worth P27 billion due to power outages.

The spread of slum and squatter colonies is taxing the ability of the Health Department to provide adequate health services. Immunization against measles covers only 30-60% of slum children and more than 90% of these children harbor intestinal parasites and remain untreated.

The Pasig River and its tributaries are biologically dead due to industrial and human wastes. Biochemical oxygen demand has been recorded to be as high as 500 mg/l, when the safe level that would still allow aquatic life to flourish is only 20 mg/l.

Fourteen sampling stations in Manila Bay have detected signs of entrophy or oxygen depletion because of excessive algae and bacteria produced by pollution. Because of inadequate sewage in Metro Manila, waters as far as those nearing Corregidor have shown high levels of fecal coliforms that cause infections of the eyes, ears and the gastro-intestinal and respiratory systems.

Pollution in Manila Bay has affected the shellfish industry of Cavite and poses health hazards as a possible avenue for the spread of cholera, Hepatitis A and paralytic shellfish poisoning. The spread of a marine dinoflaggelate, the so-called red tide, is a direct result of water pollution.

The inability of the metro government to devise and sutain an efficient system for the collection and disposal of garbage aggravates the related problems of air and water pollution and recurrent floods in the Metro area.

As an invited reactor to the presentation of Dr. Dayrit, I am offering suggestions on what can and should be done to rescue our urban ecosystem.

1. I recommend a methodology by which a mammoth problem like our urban ecosystem can be managed and solved. Whatever the problem, it should be broken down into barangay-level components and each barangay should be graded A, B, C, D or E in relation to that problem.

The idea is to upgrade the condition of each barangay, with the active involvement of its residents, one modest grade at a time, from E to D, from D to C, from C to B or from B to A ... instead of trying to solve a problem on a mammoth metro scale, with grandiose, unrealizable objectives.

Such a methodology, aside from having modest realizable goals, lends itself to quantification and computerization on both macro and micro levels. Hence, it provides a system for effective monitoring of progress -- or lack of progress -- for the administering bodies.

2. I propose the formation of a Presidential Commission on Urban Development to strategize the management of the urban ecosystem and to set targets for local government units to accomplish within specific time frames.

3. I am in favor of a massive program to build low-cost pre-fab housing for the poorest of the poor. In my opinion, housing reform will benefit more people and is therefore more desirable than agrarian reform.

4. I propose: a) an extensive network of elevated LRTs that reaches as far as Malolos, Tanay, Los Baños and Cavite City, which will decongest the metro area by allowing low and middle income families to live far away and yet still be within one hour's commuting time to the city center via an elevated commuter rail system; and b) the conversion of existing metro thoroughfares (like EDSA, Roxas Boulevard, España, etc.) into non-stop arteries by building overpasses and interchanges at all their major intersections.

5. I propose we do away with filthy open dump sites (like Smokey Mountain) and distant landfills (like Carmona and San Mateo) for the disposal of garbage. Instead the Environmental Network Center, of which I am chairman, has proposed to the government the compacting or incineration of garbage in Tondo and the use of the compacted or incinerated garbage to reclaim land from Manila Bay.

6. Finally, I propose that the least polluted section of Laguna de Bay be identified and the appropriate are walled off from the rest of the lake. The walled water and its sources can then be protected and treated to make it potable.

MATHEMATICAL, PHYSICAL AND ENGINEERING SCIENCES

Fiber-optic Reflectometric Investigations of Acid-base Equilibria in Immobilized Indicators: Effect of the Nature of Immobilizing Agent

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ABSTRACT

The effect of the nature of the immobilizing agent on the equilibrium properties of acid-base indicators was investigated. Three sulfonphthalein dyes were immobilized on an ion-exchange resin and on a non-ionic polymer. The immobilized reagents were localized on the tip of a bifurcated optical fiber system which measured the reflectance of the reagent phase. Reflectance measurements were carried out at various pH values and were used to evaluate the pKa of the immobilized dye. The results indicate that the ionization of the indicator immbolized on a non-polar surface was less extensive than when it was immobilized on an ionic solid resin. The solvent model for adsorption on a polymer surface was used to explain the observed behavior.

INTRODUCTION

Immobilized reagents are essential components in a number of chemical sensors. Coupled with sensing elements, such as an optical fiber, a piezoelectric crystal and a membrane electrode,

these materials enable the transduction of chemical information into a suitable signal. Their interaction with the analyte results in a modulation of a property of the sensing element and provides the basis for the quantitation of the analyte.

Immobilization of reagents on a solid support could be carried out through physical and chemical means. The physical methods include entrapment in a gel matrix, adsorption on a solid surface and electrostatic attraction on ionic sites of a solid support. Chemical immobilization involves the formation of a covalent bond between the reagent molecule and functional groups on the surface of the immobilizing agent.

Physical immobilization has been observed to cause changes in some properties of the immobilized reagent. Adsoprtion on a hydrophobic polymer represses the ionization of acid-base indicators (**6**), lowers the reduction potential of oxidation- reduction indicators (**7**) and modifies the spectral and temporal characteristics of the luminescence of fluorophores (**10**). Immobilization of chelating agents on an ion-exchange resin enhances the selectivity of the reagent (**2**).

In this paper, the effect of the nature of the immobilizing agent on the modification of the equilibrium properties of acid-base indicators is examined. A reflectometric method employing optical fibers was used to characterize the equilibrium state of the immobilized reagent. The feasibility of this method has been demonstrated in previous investigations (**6, 7**).

EXPERIMENT

Instrumentation

The principle of fiber-optic reflectometry is illustrated in Figure 1. Radiation from a suitable source is focused on one end of a bifurcated optical fiber system and directed to the immobilized reagent phase at the distal end of the fiber system. The reagent phase interacts with the radiation which is then reflected back into the other arm of the bifurcated optical fiber. The reflected radiation is beamed to the photodetector system which measures the intensity of the radiation.

The instrumentation system employed in this investigation is diagrammed in Figure 2. The radiation source, monochromator and photomultiplier tube were derived from a

Beckman DU-2 single-beam spectrophotometer. A bifurcated optical fiber (Matec LG3202-048) was interposed between the monochromator and the photodetector. The detector signal was amplified using an op-amp circuit and recorded on a chart recorder (Lloyd Graphic 450).

Reagents

All reagents used were AR grade. Amberlite XAZ-2, a non-ionic styrene/divinylbenzene copolymer, and Amberlite IRA-400, an anionic-exchange resin, were chosen as the solid supports for the indicators in this study. The polymer microspheres were used without further sieving.

The indicators investigated were sulfonphthalein dyes which could be immobilized on XAD-2 and IRA-400; viz., bromothymol blue (BTB), bromocresol green (BCG) and bromocresol purple (BCP). These indicators were immobilized from ethanolic solutions (0.05- 0.10% for XAD-2 and 0.01-0.025% for IRA-400) by equilibrating about 1.0 g of the polymer with 25 ml of the solution for 24 hours. The immobilized reagent was separated by decanting off the supernatant liquid and then washed thoroughly with distilled water until the washings were colorless. After immobilization, no significant leaching of the indicator occured on both polymer supports at all pH values studied.

Buffer solutions were prepared from a solution which was 0.04M in acetic acid, 0.04M in potassium dihydrogen phosphate, 0.04M in boric acid and 0.50M in sodium chloride. The pH of the solution was adjusted to the appropriate value with 0.5M hydrochloric acid and 0.5M sodium hydroxide solution. The pH of the solutions were measured using a pH meter (Metrohm 69:1).

Measurements

The polymer microspheres containing the immobilized indicators were tightly packed on the distal end of the bifurcated optical fiber, being held in position by a nylon mesh. The resulting optical fiber probe was immersed in the buffer solutions and its reflectance spectra was measured at each pH value. The results of the measurements were expressed as relative reflectance, the reference being the XAD-2 beads without the immobilized reagent.

RESULTS AND DISCUSSION

Reflectance spectra

A distinct isosbestic point was observed in the reflectance spectra of the immobilized indicators studied (illustrated in Fig. 3 for BCG immobilized on XAD-2 and IRA-400). The occurrence of this point suggests that the equilibrium involves only two absorbing species, namely, the molecular and the ionic forms of the indicator.

The isosbestic points of the three indicator dyes immobilized on an ionic polymer occurred at a longer wavelength than that of the dye immobilized on a non-ionic solid support (Table 1). This bathochromic shift is an effect of the polar microenvironment provided by the surface of the ionic polymer and is analogous to a solvent effect on pi-pi* transitions (**4**).

The spectra of the immobilized reagents, whether held on a non- ionic or ionic polymer surface, exhibited a bathochromic and hyperchromic shift as the pH was increased. The shifting of the peaks to longer wavelength could be associated with a change in the electronic structure of the molecule upon ionization. The increase in the intensity of the peaks at higher pH values suggests the greater molar absorptivity of the ionic form of the compound to that of the undissociated reagent molecule.

The greatest divergence between the spectra at low and high pH values occurred at 580 nm for the three indicators. Reflectance readings were recorded at this wavelength to obtain a maximum sensitivity in the measurements.

Effect of pH

For immobilized reagents, the concentration of the absorbing species can be described best by the Kubelka-Munk function (**3**):

$$F(R) = (1 - R)^2 / 2R = kC \qquad (1)$$

where R is the relative reflectance and k is a constant related to molar absorptivity and scattering coefficients. The normalized Kubelka-Munk functions for the different immobilized indicators are plotted against pH in Figure 3.

The pH curves obtained were sigmoid and resembled titration curves. The variation in the Kubelka-Munk function versus pH can be correlated with the change in the relative amounts of the molecular and ionic forms of the immobilized reagents. At the lower pH values, the predominant species was the molecular form; whereas at the higher pH values, the major species was the ionized form of the indicator.

The steep portions of the curves are noted to extend through a wider range (2 to 3 pH units) compared to those observed in solutions (1 to 2 pH units). This broadening was attributed to the distribution of the dye molecules over slightly different sites on the surface of the solid support (**1**). Associated with this behavior is a widening of the linear portion of the curve and, therefore, of the dynamic range of the immobilizing reagent.

Evaluation of pKa values

A graphical method for the evaluation of pKa, analogous to that used for absorbance data, was employed. At any wavelength, the Kubelka-Munk function obtained from the reflectance data is the sum of the Kubelka-Munk functions of the two absorbing species, i.e.,

$$F(R) = k_{HIn}[HIn] + k_{In}[In^-] \quad (2)$$

where K_{HIn} and K_{In} are constants involving absorption and scattering. At all times, the total concentration, C_t, of the indicator is constant, i.e.,

$$C_t = [HIn] + [In^-] \quad (3)$$

Combining equations 2 and 3 with the definition of the indicator dissociation constant,

$$Ka = [H^+][In^-] / [HIn] \quad (4)$$

Equation 2 becomes

$$F(R) = \frac{K_{HIn} C_t [H^+]}{([H^+] + Ka)} + \frac{K_{In} C_t Ka}{([H^+] + Ka)} \quad (5)$$

Equation 5 can be shown to be consistent with the behavior of F(R), as depicted in Figure 4. Under highly acidic conditions, $[H^+] >> Ka$ then Equation 5 simplifies to

$$F(R)_{acid} = k_{HIn}\, C_t \qquad (6)$$

which predicts a constant value of F(R). Under strongly alkaline conditions, $[H^+] << Ka$, and Equation 5 becomes

$$F(R)_{alk} = k_{HIn}\, C_t \qquad (7)$$

which predicts a constant value of F(R).

At the equivalence point, $[HIn] = [In^-]$ and $[H^+] = Ka$. Equation 5 then becomes,

$$F(R)_{eq} = (k_{HIn}\, C_t/2) + (k_{In}\, C_t/2) \qquad (8)$$

At the wavelength of maximum reflectance of the acidic forms (580 nm), this expression can be rewritten as

$$F(R)_{eq} = 1/2\, [F(R)_{acid} + F(R)_{alk}] \qquad (9)$$

This equation resembles that obtained by Patrick and Svehla (**8**) for the evaluation of pK values of indicators from absorbance/pH data. The pK value corresponds to the pH at the inflection point of the sigmoidal pH curve.

Comparison of pK values

Table 2 shows the pK values of the immobilized indicators in the two polymeric supports studied. The value obtained for BTB immobilized on XAD-2 is of the same order as previously reported (**6**).

A comparison of the values reveals that the reagents immobilized on a non-ionic polymer have a higher pK value than those supported on an ionic surface. The higher pK value indicates a lower degree of dissociation on the surface of the non-ionic polymer. The neutral form is more stabilized than the charged ionic form in a nonpolar environment and is therefore the dominant species. This behavior can be interpreted as a medium effect, since the physical immobilization of reagents on a polymeric surface can be thought to take place through a "solvent action" of the polymeric matrix (**5**).

CONCLUSION

The results show that the nature of the immobilizing agent affects the equilibrium properties, as well as the dynamic range of an analytical reagent, such as an acid-base indicator. Therefore, the proper choice of immobilizing agent is a factor which should be considered in the optimization of the working range of sensing systems based on immobilized reagents.

Table 1. Isosbestic points of the immobilized indicators

INDICATOR	AMBERLITE XAD-2	AMBERLITE IRA-400
BCG	490 nm	510 nm
BCP	482 nm	494 nm
BTB	480 nm	504 nm

Table 2. The pKa values of the sulfonphthalein indicators

INDICATOR	AMBERLITE iRA-400	AQUEOUS SOLN.[9]	AMEBERLITE XAD-2
BCG	3.69	4.70	5.63
BCP	4.24	6.30	10.28
BTB	5.86	7.00	9.67

ACKNOWLEDGMENT

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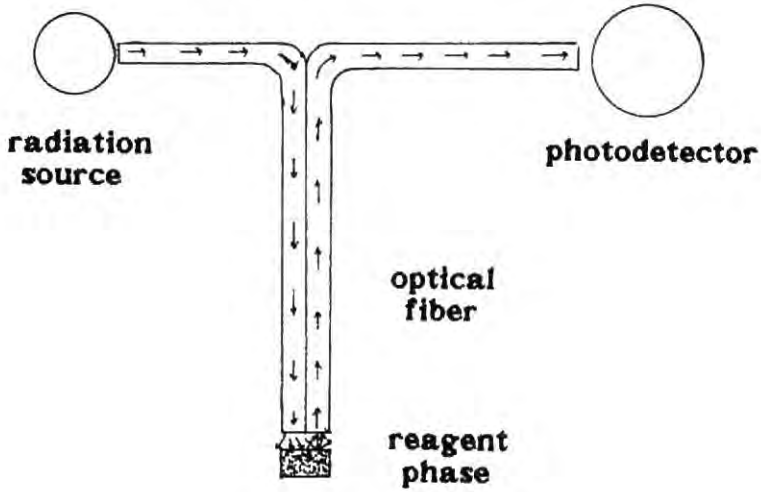

Figure 1. Principle of fiber-optic reflectometry

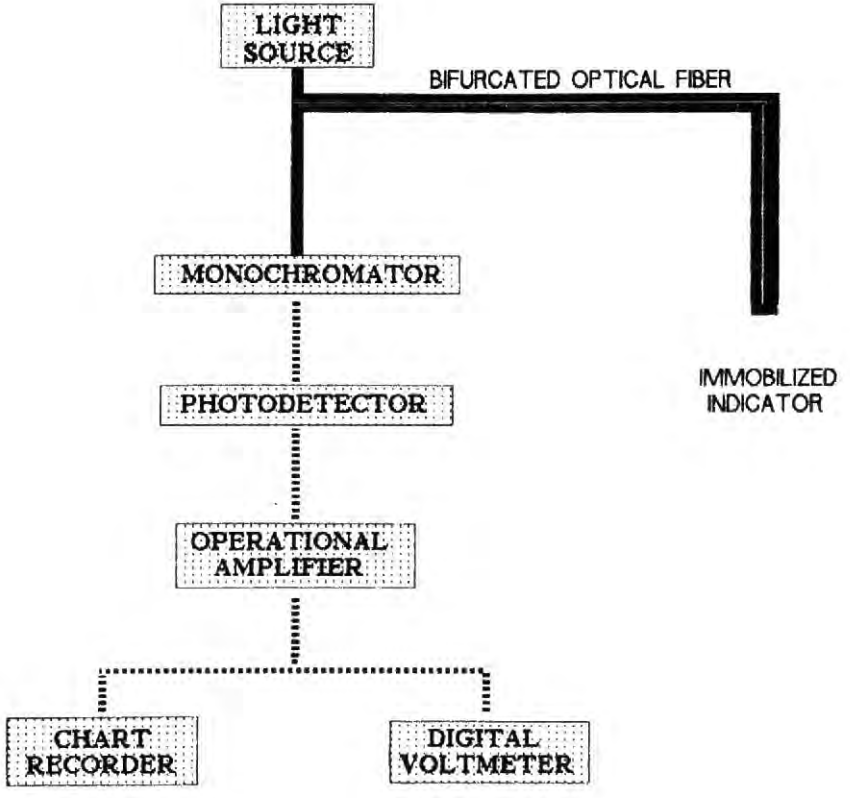

Figure 2. Schematic Diagram of Fiber-optic Instrumentation System

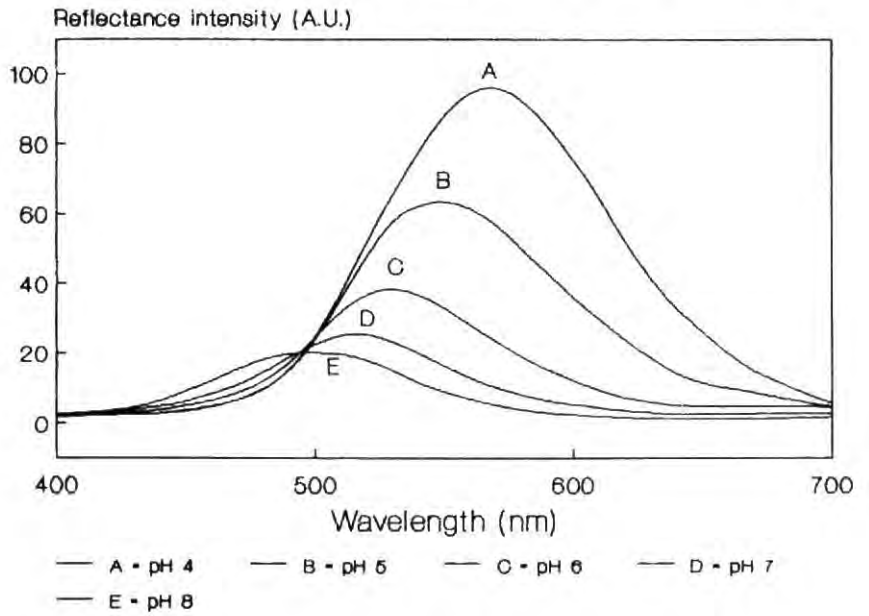

Figure 3A. Reflectance spectra of bromocresol green on Amberlite XAD-2

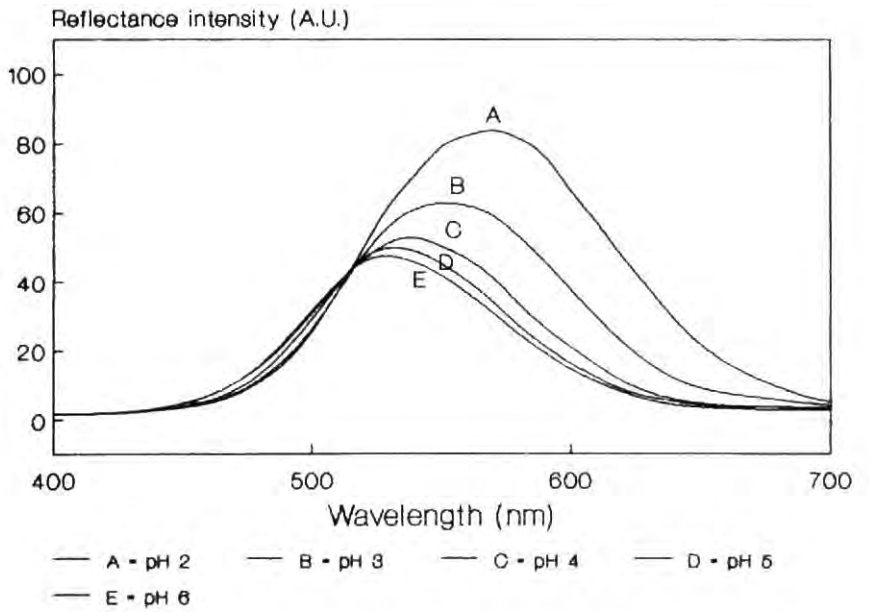

Figure 3B. Reflectance spectra of bromocresol green on Amberlite IRA-400

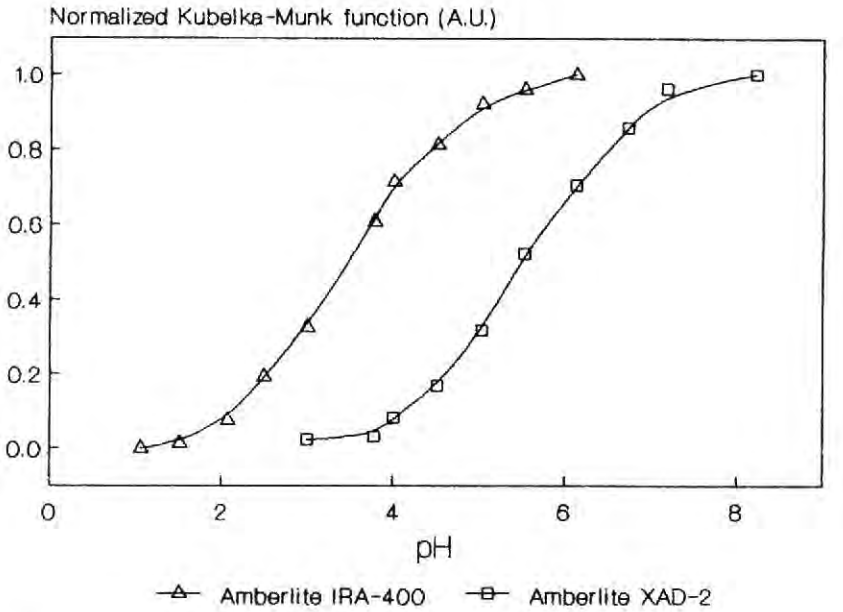

Figure 4A. Kubelka-Munk plots of immobilized bromocresol green

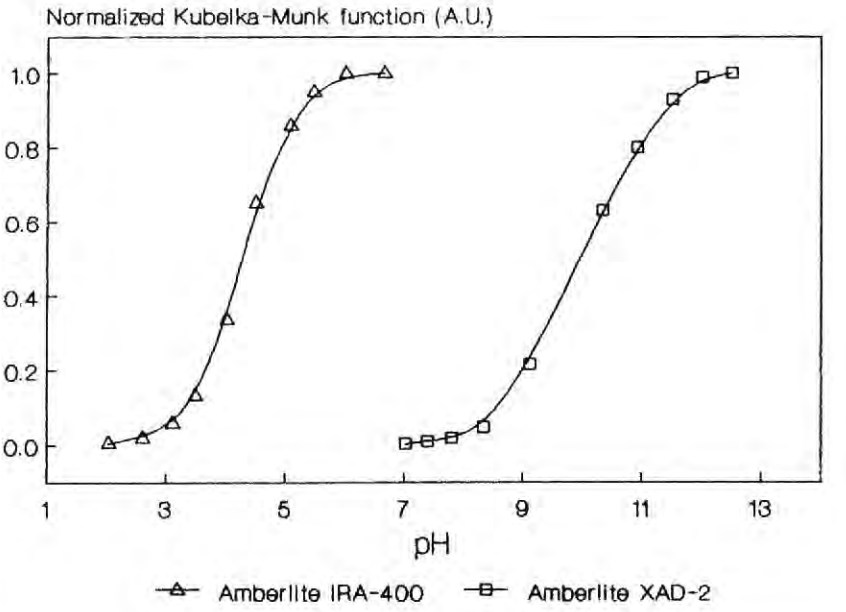

Figure 4B. Kubelka-Munk plots of immobilized bromocresol purple

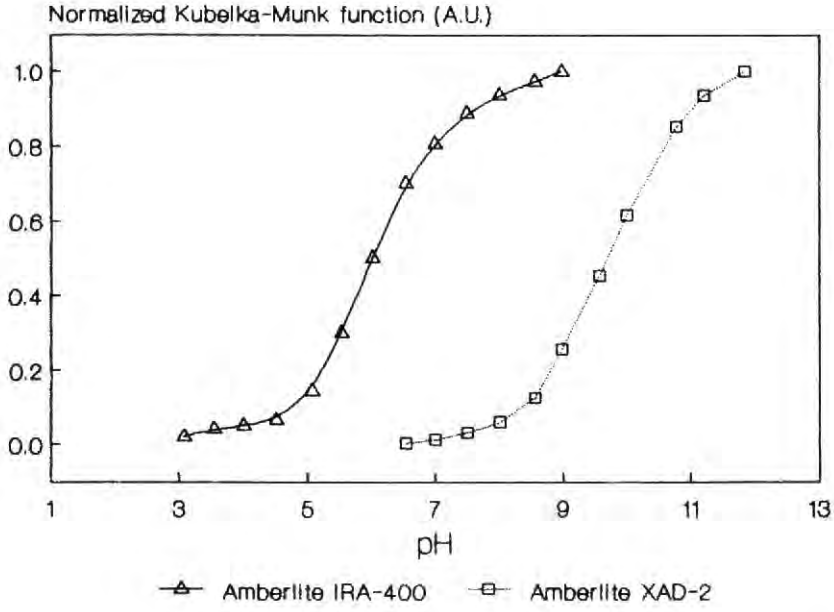

Figure 4C. Kubelka-Munk plots of immobilized bromothymol blue

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Studies on the Gasification of Biomass Fuels With Oxygen-enriched Air

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ABSTRACT

A mathematical model developed for the calculation of gas compostion and other performance parameters in the gasification of biomass fuels with oxygen or oxygen-enriched air presented results which are substantially consistent with experimental results. Good comparison of calculated gas compositions with Orsat analyses of gas samples from experimental runs on the gasification of coke, coconut shell and ipil-ipil wood validate the model.

The experiments provided evidence that biomass gasification at atmospheric pressure with pure oxygen is possible without encountering excessively high temperatures and, consequently, alleviating the problem of ash clinkering, if the gas producer is operating in the downdraft mode. High gas calorific values and high rates of gasification were observed to be marked advantages of oxygen-enrichment of the gasifying medium over ordinary air. A trend for cold gas thermal efficiency of the gas producer to improve with increasing oxygen content of the gasification medium was also observed.

INTRODUCTION

An alternative automotive fuel in the form of methanol can be produced from biomass resources. Biomass gasification is the first step in biomethanol production. Biomass is gasified in an oxygen-blown downdraft gasifier into synthetic gas consisting primarily of hydrogen and carbon oxides, and eventually is catalytically reacted to form methanol. Methanol is a good substitute fuel for gasoline engines, either straight or blended with gasoline. It could also be reacted with coconut oil to produce methyl esters which is prospectively a good fuel for diesel engines.

Design of gas producers and optimization of their operation are among recent studies undertaken in the U.P. College of Engineering (**5**). These were largely empirical processes based on experience and past experimental data. The present study focuses on theoretical considerations using basic principles of thermodynamics and chemical equilibrium (**4**). One of the problems in conducting experiments in biomass gasification, for instance, is the accurate measurement of the composition of the end products of gasification (CO, H_2, CH_4, CO_2). The common method of doing this is by gas chromatography or by absorption of the component gases by various chemicals, as in the Orsat Analyzer. However, high cost of chemicals (some of which have to be obtained overseas) or the unavailability of test equipment does not always allow complete gas analyses to be made in some laboratories. For example, in the present study, measurements of H_2 and Ch_4 components were not possible and CO analysis by the Orsat apparatus was difficult, tending to be inaccurate because of stale chemicals. It is desirable, therefore, to be able to predict a complete gas composition when gas analysis is limited to only one or two gas component measurements (like CO_2 and CO). A mathematical model simulating gas producer performance can be developed to accomplish this objective.

The objectives of this study are as follows:

1. To develop a mathematical model based on the principles of thermodynamics and chemical equilibrium for the calculation of gas composition and other performance parameters in gasification of biomass fuels with oxygen or oxygen-enriched air;

2. To study improvements in the gasification of biomass fuels with oxygen or oxygen-enriched air with the aid of the mathematical model; and
3. To validate results of the model with experimental data.

THEORETICAL

The mathematical model assumed a certain percentage approach to chemical equilibrium of the reactions taking place in the gasifier fuel bed at an equivalent reaction temperature obtained through a heat balance of the system. This reaction temperature would depend upon a number of factors, namely: the heating value of the solid fuel, its ultimate analysis, its moisture content, the mode of gasifier operation (updraft or downdraft), the temperature and composition of the gasification medium (percent O_2, N_2 and steam), the heat losses due to radiation and unburnt carbon in the ash residue.

The degree to which chemical equilibrium was attained by each of the pertinent gasification reactions, for instance, the heterogenous water-gas rection $C + H_2O = CO + H_2$ is defined by certain parameters like $\underline{y}$ in the relation:

$$\underline{y} + K_{pw} = \frac{[CO] * [H_2]}{[H_2O]}$$

where K_{pw} is the equilibrium constant for the heterogenous water- gas reaction at the calculated reaction temperature and the gas components in brackets are the experimental wet gas compositions in percent. Since actual H_2O in the gas could not be experimentally measured, it was estimated to be the same as the calculated equilibrium H_2O. In the calculation of the equilibrium, gas composition takes into account the degree of approach to equilibrium (in percent) defined as $\underline{y}$ in the above equation. The other reactions ($C + CO_2 = 2\ CO$, and $C + 2\ H_2 = CH_4$) may be assumed to have reached 100% chemical equilibrium if complete gas analyses of the producer gas cannot be measured experimentally.

If complete gas analyses data are available, then the degree of approach to equilibrium of the other two reactions above can be estimated from the following relations:

$$\underline{z} * K_{pb} = \frac{[CO]^2}{[CO_2]}$$

$$\underline{x} * K_{pm} = \frac{[CH_4]}{[H_2]^2}$$

where z is the percent approach to equilibrium of the Boudouard reaction ($C + CO_2 = 2\ CO$), and x that of the methanation reaction ($C + 2\ H_2 = CH_4$).

The mathematical model was validated by comparing its results with past experimental data. For instance, Table 1 shows the results of gasification of coke with oxygen and steam in an updraft reactor at the University of New South Wales (**2**). Tables 2 and 3 summarize experimental and calculated performance of coconut shell gasification with air in updraft and downdraft modes, respectively, at the University of the Philippines (**3**). These results show that it is possible to estimate the parameters $\underline{x}$, $\underline{y}$ and $\underline{z}$ (percent approach to equilibrium of the methanation reaction, the heterogenous water-gas reaction and the Boudouard reaction, respectively) so that the calculated gas composition is practically identical to the experimentally-measured gas composition.

A complete discussion of the mathematical model is found in the Appendix.

EXPERIMENTAL

An experimental gasifier used in previous experiments (Figure 1) was modified to allow precise measurements of oxygen and air flows supplied to the reactors during experimental runs. In this small gas producer, the small air inlets (tuveres) were plugged and replaced with a larger (1.5-inch diameter) single air inlet pipe located at the throat of the reactor. Air and oxygen flows were measured by separate rotameters before mixing and being introduced to the gasifier. During an experimental run, the gasifier was mounted on a platform balance so that continuous weight loss of fuel could be monitored during the gasification process. Various biomass fuels were gasified, such as coconut shell, corn cobs, rice husk, coconut husk and ipil-ipil wood. Orsat

analyses of four to five gas samples were made for every run and the averages of these were reported for a given run. Table 4 presents some typical results in the gasification of ipil wood which was the fuel used more extensively in the experiments. Figures 3 to 5 depict graphically the performance of the gasifier at various oxygen enrichment levels of air and total flow of the gasification medium.

DISCUSSION

Table 1 shows the performance of oxygen-steam, pulsed-blast gasification of coke as compared to that when the blast was steady (unpulsed). In the pulsed-blast mode, steam was fed in a steady stream through the reactor grate, and oxygen was introduced in intermittent blasts or pulses. In the unpulsed mode, both steam and oxygen were introduced as a mixture in a steady stream. In the unpulsed mode, therefore, even if H_2 is formed by the reaction $C + H_2O = H_2 + CO$ in the fuel bed near the grate, the presence of O_2 with the product gases will oxidize H_2 to steam again. Therefore, the degree to which the reaction could approach equilibrium will be less compared to gasification in the pulsed- blast mode when O_2 is not present with the product gases between pulses of oxygen. This is verified experimentally by the results shown in Table 1 where the values for $\underline{y}$ and $\underline{z}$ (calculated by means of the mathematical model) are higher for pulsed gasification (e.g. $\underline{y} = 28\%$ and $\underline{z} = 93\%$) than for the unpulsed mode ($\underline{y} = 2\%$ and $\underline{z} = 5\%$). Since no experimental measurement for CH_4 was made, theoretical CH_4 values were calculated by assuming a value for the parameter x of 24% obtained from literature (1).

In Table 2, complete gas analyses, including that of CH4, were made for the updraft gasification of coconut shell with air. Thus, estimates for the parameters $\underline{x}$, $\underline{y}$ and $\underline{z}$ can be calculated from the mathematical model. Approach to equilibrium for the methanation reaction was estimated at $\underline{x} = 100\%$. This was a rough estimate because in Run 1 LUD (Large Up-Draft), for example, the experimental measurement of 0.9% for CH_4 (which might appear inconsistent with the calculated value of CH_4 = 0.2%) was due to the gas sampling and gas composition

measurement techniques which could detect volumetric percentage figures to only within 0.5 percentage point accuracy.

Table 4 shows the typical results of the performance of downdraft gasification of ipil wood with oxygen-enriched air, the percent carbon in the refuse, %c and the losses due to heat radiation and convection in the gasifier. Percent losses are assumed at varying values until the experimental $\%CO_2$ and ratio F/M (ratio of lb dry fuel gasified per mol of gasification medium) equal the calculated $\%CO_2$ and ratio F/M, respectively. (See the Appendix for an example calculation.) It was noted that when the chemicals used in the Orsat Analyzer were fresh, the experimentally-measured CO values were substantially equal to the calculated values. Since there was no way of experimentally measuring the H_2 and CH_4 content of the gases at the time of the experiments, the calculated values provided good estimates of these measurements.

The performance parameters, namely, Higher Heating Value (HHV) in Btu/cubic foot of producer gas, Gasification Rate in Kg/hour and Cold Gas Thermal Efficiency (%), are correlated as functions of Gasification Medium Flow in cubic feet/hour and percent oxygen in the gasification medium. The results are presented as graphs in Figures 2 to 4.

Figure 2 shows a good correlation of HHV against $\%O_2$ in the medium, indicating a significant increase in gas calorific value as the oxygen enrichment of the air gasifying medium is increased.

Figure 3 shows that gasification rate increased markedly both with an increase in the flow rate of the gasifying medium and increase in the oxygen enrichment of the air. This is consistent with considerations of material balance and stoichiometry of reaction involved in the process of gasification.

Figure 4 shows a trend for the cold gas thermal efficiency to increase with oxygen-enrichment. The experimental points, however, are too scattered to indicate any trend as to the effect of flow rate of gasifying medium. The fact that the gasifier was operated in short batch runs, lasting from 1- 3 hours, resulted in significant ungasified carbon in the ash rejects (%c) at varying values (from 88%-97%), which accounts for the scattering of efficiency values, from a low of 26% to a high of 68%. In other words, the gasifier produced a by-product, particularly in the downdraft operation, and this was charged as a loss in the

computation for efficiency. Gasifiers continuous operation, for say at least 10 hours, would result in less unburnt carbon in the ash residue.

One of the concerns when using oxygen or oxygen-enriched air for gasification is the possibility of ash clinkering because of high temperatures reached in the combustion zone. No such problem was encountered when the gasifier was operated in the downdraft mode even when the gasifying medium was 100% oxygen. However, in a single run in the updraft mode, melting and fusion of ash were observed after 2 hours of operation at 30% oxygen content in the gasifying medium. The clinker did not form maybe because the combined water content of wood was considerable (V_{H2O} = 0.64 or 64% mol/mol gasifying medium) and most of this passed through the combustion zone in downdraft mode, cooling the combustion zone in the process. This was not the case in updraft operation.

CONCLUSIONS

The mathematical model developed for the calculation of gas compositon and other performance parameters in the gasification of biomass fuels with oxygen or oxygen-enriched air presented results which are substantially consistent with experimental results. More confidence in the validity of the model could be established, however, if more data from longer experimental runs using other biomass fuels are made. A good comparison of calculated gas composition with the complete analysis of gas samples from these experimental runs could further validate the model. Such additional data are recommended to be gathered in further experiments.

Biomass gasification at atmospheric pressure with pure oxygen is possible without encountering the problem of ash clinkering if the gas producer is operating in the downdraft mode. High gas calorific values and high rates of gasification are marked advantages of oxygen-enrichment of the gasifying medium over ordinary air. There is also a trend for cold gas thermal efficiency to improve with increasing oxygen content of the gasification medium.

Table 1. Comparison Between Pulsed and Unpulsed Blast Updraft Gasification of Coke with Oxygen and Steam

Run	33 Pulsed		27 Unpulsed	
Air Rate, cu ft/h	140		150	
% O2, Air-O2 Mix	100		100	
% Steam	75		76	
Reaction Temp., deg. C		748		84.7
% Gas	Expt'l	Calcul'd	Expt'l	Calcul'd
% CO2	8.5	8.5	19.0	18.8
% CO	55.2	54.2	42.5	41.9
% H2	36.3	36.8	38.5	39.0
% CH4	0.0	0.2	0.0	0 1
% N2	0.0	0.3	0.0	0.3
Higher Heating Value				
Btu/cu ft	313	313	277	277
Cold Gas				
Efficiency, %	90.8	90.9	81.3	81.3
Approach to Equilibrium				
x, %		24		24
y, %		24		3
z, %		100		4
Gasification Rate				
Kg/h	7.93		6.99	

Run	28 Pulsed		29 Unpulsed	
Air Rate, cu ft/h	150		140	
% O2, Air-O2 Mix	100		100	
% Steam	66		80	
Reaction Temp., deg. C		770		755
% Gas	Expt'l	Calcul'd	Expt't	Calcul'd
% CO2	7.7	7.7	32.4	32.5
% CO	60.3	59.4	31.7	31.3
% H2	32.0	32.5	35.6	35.8
% CH4	0.0	0.1	0.0	0 1
% N2	0.0	0.3	0.3	0.3
Higher Heating Value				
Btu/cu ft	315	315	230	231
Cold Gas				
Efficiency, %	87.7	87.7	59.2	59.3
Approach to Equilibrium				
x, %		24		24
y, %		28		2
z, %		93		5
Gasification Rate				
Kg/h	7.42		4.64	

Table 2. Updraft Gasification of Coconut Shell with Air (Gas Producer Grate Area = 0.9 Square Meter)

Run	1 LUD		2 LUD	
Air Rate, cu ft/h	13915		26377	
% 02, Air - 02 Mix	21		21	
% Steam	0		0	
Reaction Temp., deg. C		703		703
% Gas	Expt'l	Calcul'd	Expt'l	Calcul'd
% CO2	8.9	8.1	7.1	6.9
% CO	25.7	22.8	25.6	24.5
%H2	11.6	11.5	11.3	11.4
%CH4	0.9	0.2	0.5	0.1
%N2	52.9	57.5	55.4	57.1
Higher Heating Value				
Btu/cu ft	137	119	131	123
Cold Gas				
Efficiency, %	83.1	72.0	78.3	73.6
Approach to Equilibrium				
x, %		100		100
y, %		62		18
z, %		55		18
Gasification Rate				
kg/h	175.50		342.00	

Table 3. Downdraft Gasification of Coconut Shell with Air (Gas Producer Grate Area = 0.9 Square Meter)

Run	4 LDD		5 LDD	
Air Rate, cu ft/h	19280		21350	
% O2 Air-O2 Mix	21		21	
% Steam	0		0	
Reaction Temp., deg. C		645		673
% Gas	Expt'l	Calcul'd	Expt'l	Calcul'd
% CO2	12.0	12.7	13.3	14.1
% CO	16.0	16.9	12.7	13.6
% H2	15.7	15.5	12.9	12.9
% CH4	0.0	0.6	0.0	0.3
% N2	56.3	54.4	61.1	59.1
Higher Heating Value				
Btu/cu ft	108	117	88	94
Cold Gas				
Efficiency, %	67.7	72.8	58.8	62.8
Approach to Equilibrium				
x, %		100		100
y, %		40		14
z, %		68		20
Gasification Rate				
kg/h	250.20		233.10	

Table 4. Downdraft Gasification of Ipil Wood with Air, Oxygen-Enriched Air and Pure Oxygen

Run	1	2	3	4	5
Air Rate, cu ft/h	150	250	200	400	400
% O2, Air-O2 Mix	40	40	21	21	21
% Steam	0	0	0	0	0
Reaction Temp., deg. C	681	667	595	671	640
% Gas					
% CO2	12.6	15.3	14.8	8.4	11.1
% CO	34.3	30.7	13.5	23.7	19.5
% H2	23.4	24.6	18.0	13.3	15.1
% CH4	0.7	1.0	1.1	0.3	0.5
% N2	29.1	28.4	52.5	54.3	53.8
Higher Heating Value					
Btu/cu ft	204	200	120	129	123
Cold Gas Efficiency, %	54	67	26	63	43
Carbon in Refuse (c) %	94.5	90.0	97.0	90.0	95.5
Losses (Radiation, etc.), %	3	5	2.5	5	4
Approach to Equilibrium					
x, %	100	100	100	100	100
y, %	15	35	15	15	15
z, %	100	100	100	100	100
Gasification Rate					
kg/h	5.40	7.80	4.05	6.00	7.26

Run	11	12	13	14	15
Air Rate, cu ft/h	300	200	400	150	300
% O2, Air-O2 Mix	40	100	40	100	40
% Steam	0	0	0	0	0
Reaction Temp., deg. C	648	701	672	624	671
% Gas					
% CO2	17.1	16.3	12.9	28.8	15.5
% CO	27.4	48.4	33.4	27.4	31.4
% H2	26.3	34.2	22.8	40.1	19.7
% CH4	1.3	1.2	0.6	3.7	0.6
% N2	28.0	0.0	30.3	0.0	32.8
Higher Heating Value					
Btu/cu ft	197	295	199	270	181
Cold Gas Efficiency, %	47	66	41	44	55
Carbon in Refuse (c), %	95.5	92.0	96.0	96.0	91.0
Losses (Radiation, etc.), %	3	3	3	2.5	7
Approach to Equilibrium					
x, %	100	100	100	100	100
y, %	23	23	10	40	40
z, %	100	100	100	100	100
Gasification Rate					
Kg/h	12.00	17.20	16.00	16.40	7.00

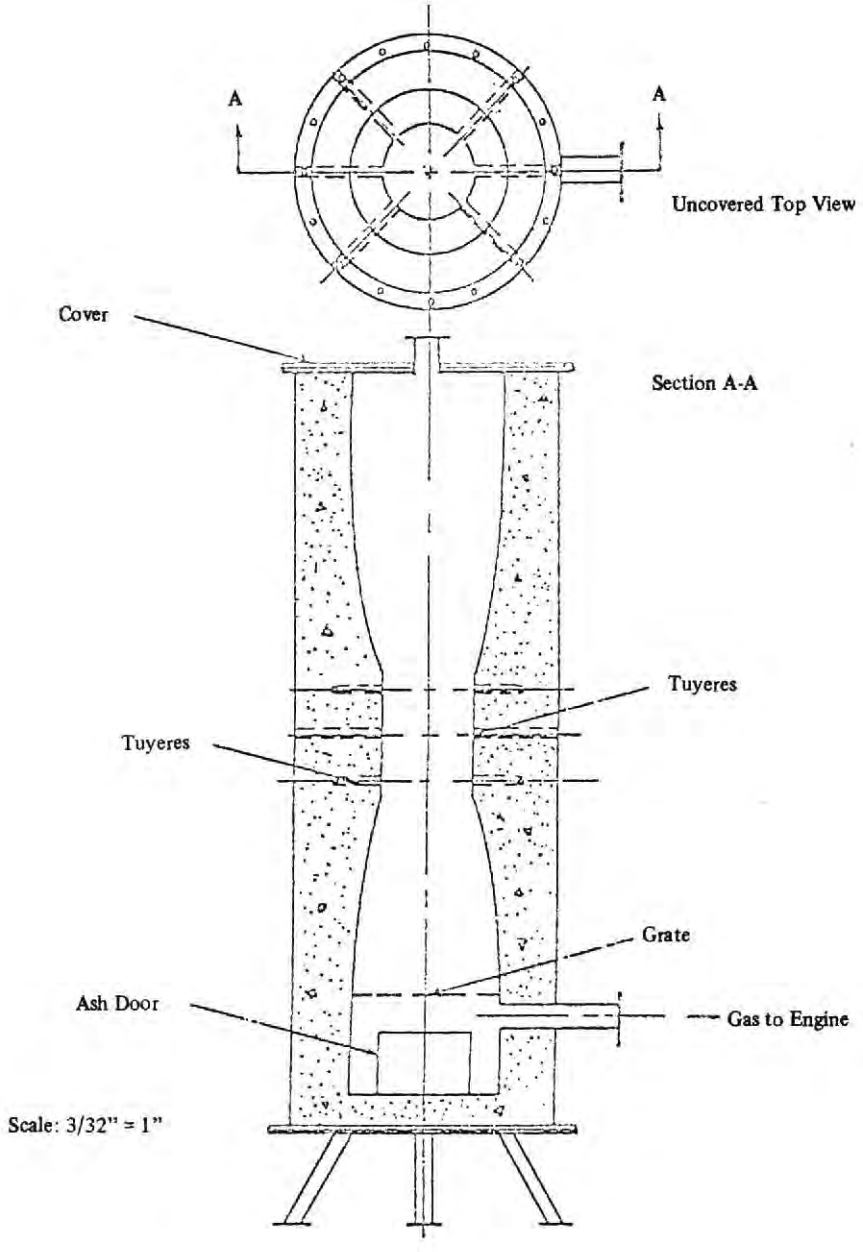

Figure 1. Suction Downdraft Gas Producer

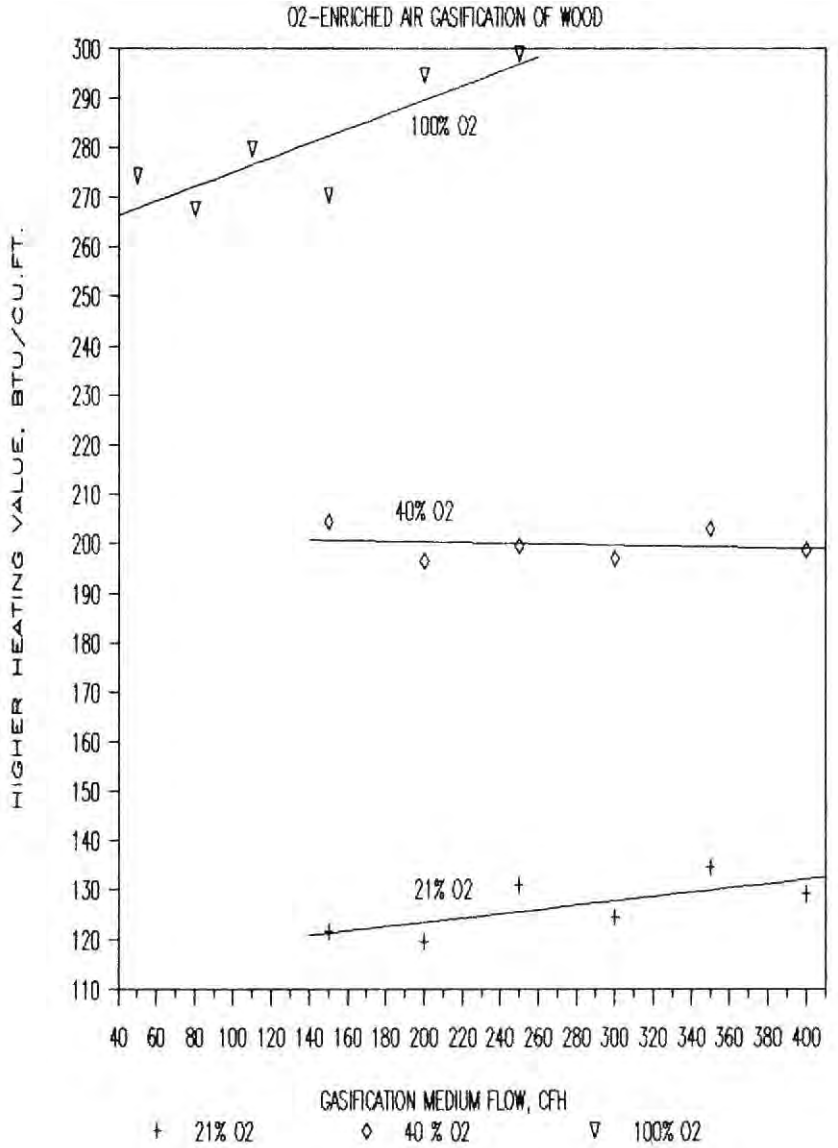

Figure 2. Higher Heating Value

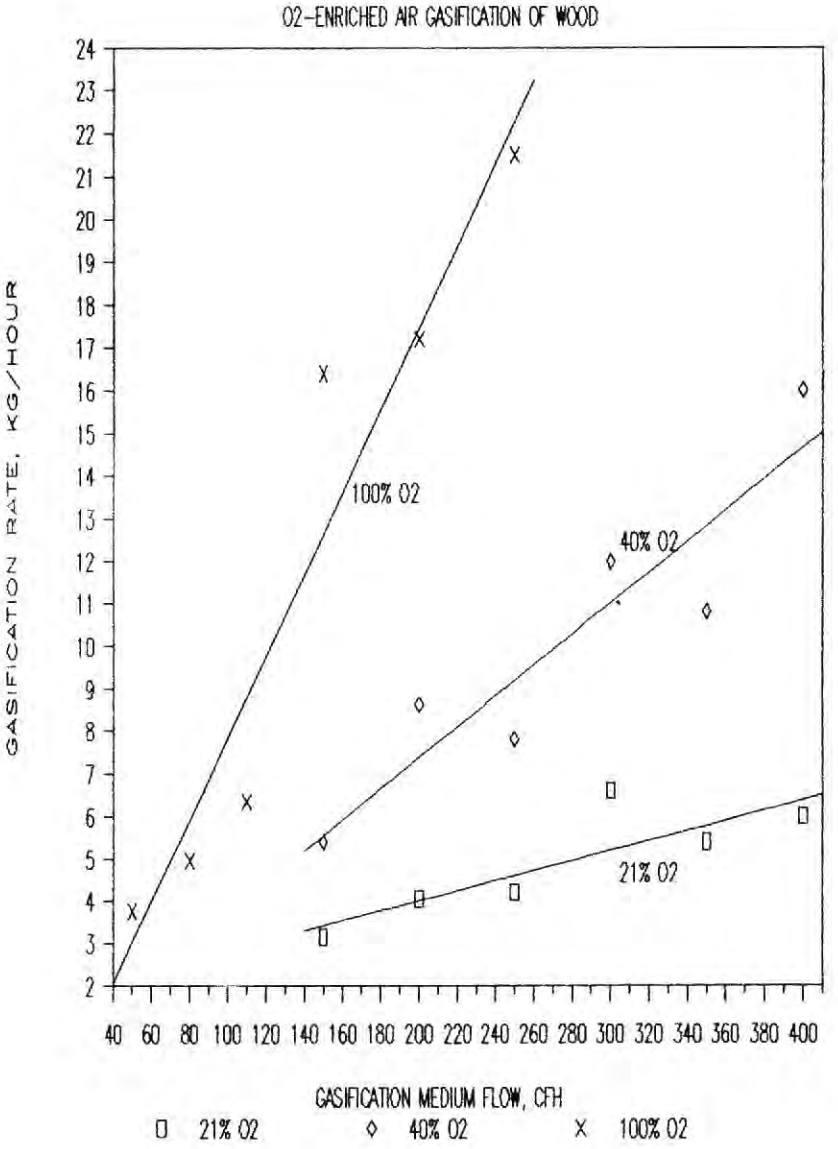

Figure 3. Gasification Rate, kg/hour

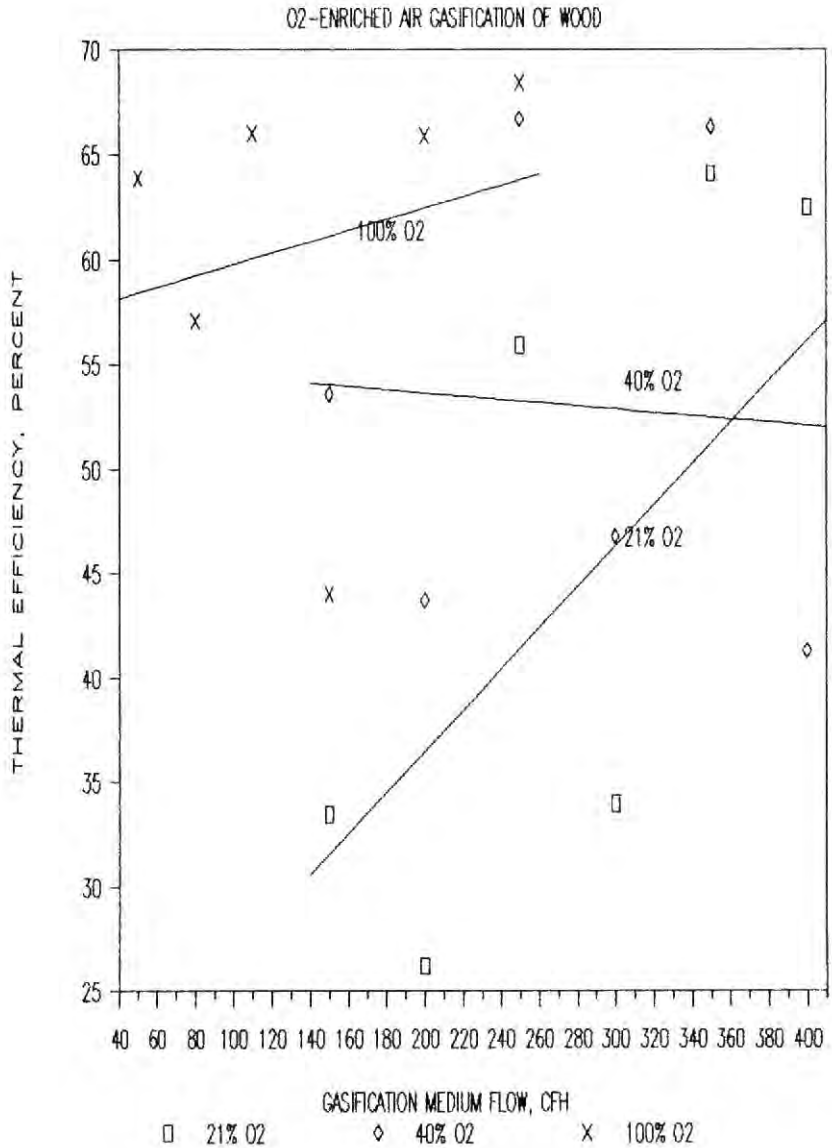

Figure 4. Cold Gas Thermal Efficiency

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APPENDIX
DEVELOPMENT OF MATHEMATICAL MODEL FOR THE CALCULATION OF GAS COMPOSITION AND OTHER PERFORMANCE PARAMETERS IN THE GASIFICATION OF BIOMASS FUELS WITH OXYGEN AND STEAM

Consider first the gasification of carbon in a deep fixed bed, where the following reactions are believed to be occurring:

(1) $C + O_2 = CO_2$

(2) $C + CO_2 = 2\,CO$

(3) $C + H_2O = CO + H_2$

(4) $C + 2\,H_2 = CH_4$

Equation (1) occurs in the so-called oxidation zone while equations (2) to (4) occur in what is termed the reductio zone of the fuel bed. Another reaction, what is known as the homogenous water-gas shift reaction (3-a0 can be obtained from (2) and (3):

(3-a) $CO_2 + H_2 = CO + H_2O$

Let

$V''H_2O, V''O_2, V''N_2$ = Volumetric composition of gasification medium entering the oxidation zone

$V'H_2O, V'O_2, V'N_2$ = Volumetric composition of gases entering the reduction zone

$VH_2O, VO_2, VN_2, VCO, VH_2, VCH_4$ = Volumetric composition of producer gas

Therefore

(5) $V'H_2O + V'O_2 + V''N_2 = 1$

Assuming that in the oxidation zone, the primary reaction is the oxidation of C to CO_2, then

(6) $V'H_2O + V'CO_2 + V'N_2 =$ or > 1
equal to 1 for carbon gasification or greater than 1 in the case of gasification of biomass fuel which contains combined water.

(7) $VH_2O + VCO_2 + VN_2 + VCO + VH_2 + VCH_4 = 1$

Consider reaction (2), (also known as the Boudouard reaction) and let

Ab = degree of conversion of CO_2 into CO, with a value varying anywhere between 0 and 1 (negative value indicates reaction in the backward direction).

Therefore

(8) $C + CO_2 = 2 * A_b\ CO + (1-A_b)\ CO_2 + (1-A_b)\ C$

If $V'CO_2$ (in mols) is the amount of CO_2 entering the reduction zone, then

$2\ A_b\ V'CO_2$ = Amount of CO formed

$(1-A_b) * V'CO_2$ = Amount of CO_2 remaining

Consider reaction (3), also known as the heterogenous water-gas reaction) and let

A_w = degree of conversion of steam to CO and H_2

Then,

(9) $C + H_2O = A_w\ CO + A_wH_2 + (1-A_w)\ H_2O + (1-A_w)\ C$

If, V'H2O (in mols) is the amount of steam entering the reduction zone, then

$A_wV'H_2O$ = amount of H_2 formed
amount of CO formed

$(1-A_w)\ V'H_2O$ = amount of steam undecomposed

Consider reaction (4), the methanation reaction, and let:

A_m = degree of conversion of H_2 to CH_4

Then

(10) $C + 2H_2 = A_mCH_4 + 2\ (1-A_m)\ H_2 + (1-A_m)\ C$

The amount of H_2 going into the above reaction comes from reaction (9). This amount is equal to $A_w V''H_2O$ mols. Therefore,

$0.5\ A_m V_w V'H_2O$ = amount of CH_4 formed

$(1-A_m)\ A_w V'H_2O$ = amount of H_2 remaining

The amount of gases in the products of gasification are tabulated as follows:

TABLE 1

GAS	AMOUNT, MOLS
CO_2	$(1-A_b)\ V'CO_2$
H_2O	$(1-A_w)\ V''H_2O$
CO	$2A_b\ V'H_2O + A_w V'H_2O$
H_2	$(1-A_m)\ A_w\ V'H_2O$
CH_4	$0.5\ A_m A_w\ V'H_2O$
N_2	$V'N_2$

Table 1 contains the amounts of gases in terms of the input gas medium ($V'CO_2$, $V'H_2O$ $V'N_2$) which are known and the three parameters A_b, A_w and A_m which are unknown. Three equations are needed to solve for these three unknowns. These are provided by the three equations corresponding to the equilibrium constants, respectively, of the homogenous water gas shift reaction (K_w), the methanation reaction (K_m) and the heterogenous water-gas reaction (K_{pw}).

Total mols of products is equal to the sum of the mols of gases in Table 1, or after simplifying:

(11) SUM $V'H_2O + V'CO_2 + V'N_a + A_b * V'CO_2$
$+ (1-0.5 * A_m)\ A_w * V'H_2O$ mols/mol blast

An expression for A_b can be obtained from the equilibrium constant equation for the homogenous water-gas reaction:

(12) $K_w = VCO * VH_2O / VCO_2 / VH_2$

Substituting the corresponding molal gas concentrations as given in Table 1 and simplifying results in:

$$(13) \quad A_b = \frac{K_w - V'H_2O/V'CO_2 * (1-A_w) / (1-A_m)}{K_w + 2/A_w * (1 - A_w)/(1 - A_m)}$$

Calculation is started by assuming values for Aw and Am and solving equation (13) for A_b. These trial values can be used to solve for gas composition as follows:

(14)

$$VCO_2 = (1\text{-}A_b) * V'CO_2 / SUM$$

$$VH_2O = (1\text{-}A_w) * V'H_2O / SUM$$

$$VCO = (2A_b V'CO_2 + A_w V'H_2O / SUM$$

$$VH_2 = (1\text{-}A_m) * A_w V'H_2O / SUM$$

$$VCH_4 = 0.5 * A_m * A_w * V'H_2O / SUM$$

$$VN_2 = V'N_2 / SUM$$

After the volumetric fractions of the gas constituents are computed using equation (14), the equilibrium constants for the heterogenous water-gas reaction are calculated as follows:

$$(15) \quad K_{pw} = PT * VCO * VH_2 / H_2O$$

$$(16) \quad K_m = VCH_4 / VH_2^2 / P_T$$

where P_T is the total pressure of the product gases, in atmospheres.

If the computed values of K_{pw} and K_m do not equal the correct values of these constants at the given temperature (obtained from data tables or from equations of equilibrium constants expressed in terms of temperature: see Table 2), the whole procedure is repeated with corrected values of A_w and A_m until a solution is obtained.

Gasification with oxygen of biomass fuel is equivalent to gasification of the carbon content of the fuel with accompanying steam equal to the combined water in the biomass. The following sample calculation will illustrate this better.

Sample Calculation

Ipil wood was gasified in a down-draft gasifier with oxygen-enriched air (40% O2) at a flow rate of 300 cubic feet per hour at 30°C. An initial charge of 27.3 Kg of ipil wood was gasified to heat up and stabilize the gasifier for 125 minutes leaving 4.9 kg of char in the reactor at the end of this time. To start an experimental run, 17.4 Kg of fresh ipil wood was added to the 4.9 Kg of char. The char's ultimate analysis (dry basis) was approximately 96% C and 4% ash; that of ipil wood: 48.6% C, 6.0% H, 44.4% O and 1% ash; the higher heating values (HHV) were 12,971 and 8144 Btu/lb.. The gasifier was mounted on a platform balance and fuel weight loss measurements during gasification were made at 5-minute intervals. The total weight loss during a 150-minute run was 17.5 Kg. Thus, the apparent gasification rate was 17.5/150*60 = 7.0 Kg/h. The other operating parameters for the 150-minute run were as follows.

1. Equivalent ultimate analysis of mixture of ipil wood and char is weighted average of 17.4 Kg (78%) ipil wood and 4.9 Kg (22%) char, or 59.02% C, 4.68% H, 34.64% O and 1.66% Ash. The weighted average of HHV is 9205 Btu/lb (dry basis). The moisture content was 13.5% for ipil wood and 10.3% for the char.

2. The total weight of ipil wood gasified was 27.3 + 17.4 = 44.7 Kg. The weight of ash accumulated in the reactor would be (0.01) * (44.7) * (1-0.135) = 0.387 Kg. The dry ash and char accumulated in the reactor at the end of the run was 3.5 * (1-0.103) = 3.14 Kg. Therefore, the percentage carbon (%c) in the dry refuse (ash + char) was 100 * (3.14-0.387)/3.14 = 88%. Carbon in the ash (p), in terms of Kg/Kg (or lb/lb) dry fuel would be: p = % ash * %c/(100-%c)/100 = 0.12173 or 12.173 lb C/100 lb dry fuel.

3. Since 12.173 lb C/100 lb dry fuel remained with the ash, the net C in the fuel that was gasified was 59.02 - 12.173 = 46.847 lb/100 lb dry fuel and the amount of refuse or rejects (ash + C) was 1.66 + 12.173 = 13.833 lb/100 lb fuel. The effective ultimate analysis of the fuel gasified, therefore, was as follows: 46.85% C, 13.83% Refuse (ash + C), 4.68% H and 34.64% O.

4. The effective gasification rate, in pounds dry fuel per hour - (apparent gasification rate) * (1-%M)/(1-%Refuse/100) or 7.0 * (1-0.135)/(1-0.13833) = 7.03 Kg/h. The gasification medium flow in mols/h was 300 ft^3/h/359 * (273/30) = 0.7529 mol/h. However, since the average Orsat analysis of 8 gas samples showed 0.66% O, (which indicated that not all the oxygen was reacted), a correction factor (less than 1

must be used to reduce the medium flow to its effective rate). If an approximate N_2 content of the producer gas is estimated as 34.3% (see item 8 below), then the correction factor is equal to (34.4 - 0.66 * 60/40)/34.3 =0.971. The effective rate of medium flow would therefore be 0.971 * 0.7529 = 0.7311 mol/h.

5. The amount of combined water in the dry fuel, in mols/lb was: 0/16/100 = 34/16/100 = 0.02165. These combined water plus the physical moisture (%M) of the fuel would constitute the effective moisture content in the gasification medium, $V'H_20$, in mol/mol of gasification medium. Thus,

(17) V'H2O = V'H2O/100 + {0/16/100 + %M/(100 - %M)/18) * F/M

where F/M is the pounds of fuel gasified per mol of gasification medium: From item 4 above, F/M = 7.03 * 2.2/0.7311 = 21.15 lb dry fuel/mol medium. No steam was added to the gasification medium ($V"H_2 = 0$) thus the steam entering the reduction zone, from the above equation would be:

$V'H_2O$ = O + (0.02165 - 13.5/100 - 13.5)/18 * 21/15
= 0.641 mol/mol gasification medium

In a downdraft reactor, the full amount of combined water and moisture in the fuel is assumed to pass through the reduction zone. In an updraft reactor, only a fraction would pass through the reduction zone because some of the moisture would be distilled off the top of the fuel bed. This fraction is estimated to be a function of the moisture content, %M, of the fuel (equal to 0.5 - 0.006 * %M).

6. The gasification medium entering the oxidation zone was 40% O_2, 60% N_2 and no steam. Thus $V"O_2 = 0.40$, $V'N_2 = 0.60$ and $V"H_20 = 0$ satisfies equation (5):

$$V"H_2O + V"O_2 + V"N_2 = 1$$

7. The gases entering the reduction zone were $V'CO_2 = 0.40$, $V"N_2 = 0.60$ and $V'H_2O = 0.641$. From equation (6):

$$V"H_2O + V'CO_2 + V'N_2 = 0.40 + 0.60 + 0.641 = 1.641$$

8. At an assumed reaction temperature of 667°C, the producer gas composition was calculated with the following results (see calculations in item 11 below).

	% Dry Basis	% Wet Basis
VH_2O	0.0	16.5
VCO_2	15.9	13.3
VCO	31.1	26.0
VH_2	18.2	15.2
VCH_4	0.5	0.4
VN_2	34.3	28.6
TOTAL	100.0	100.0

9. Orsat analyses of 8 gas samples yielded the following results:

	% Dry Basis	%Wet Basis
VO_2	0.66	0.0
VCO_2	15.5	15.9
VCO	26.1	26.7

It is noted that the experimental VCO_2 (air-free basis) is equal to the calculated or theoretical value in Item 8 above, but the experimental VCO value, 26.7%, is somewhat less than the theoretical value shown in Item 8 (31.1%). Measurement of CO by the Orsat Apparatus was, however, tedious and difficult, and it was possible that not all the CO were absorbed by the chemicals particularly when the absorbent became stale after some use.

10. The amount of fuel gasified, F, and the amount of gasification medium used, M, may be computed from the gas analysis and ultimate analysis of the fuel:

(18) $F = 12 + (VCO + VCO_2 + VCH_4)/(\%C)$ lb dry fuel/mol gas

(19) $M + (VN_2 - N_2$ from fuel$)/(V"N_2)$ mol/mol gas

Thus,
F = 12 * (26.0 + 13.3 +0.4)/46.85 + 10.16 lb dry fuel/mol wet gas M = (28.6 - 0)/60 = 0.4767 mol/mol wet gas and F/M = 10.16/0.477 = 21.29 lb dry fuel/mol gasification medium. (Compare this with the experimental value of F/M = 21.15 lb dry fuel/mol gasification medium obtained in Item 5 above.)

11. Calculations for the gas composition

Composition of gasification medium:
Entering oxidation zone:

$V''H_2O = O_1$ $V''O_2 = 0.40$ $V'N_2 = 0.60$

Entering reduction zone:
$V'CO_2 = 0.40$ $V'N_2 = 0.60$

Substituting F/M = 21.29 in equation (17):

$$V'H_2O = 0 + \{0.02165 + 13.5/(100 - 13.5)/18\} * 21.29$$
$$= 0.646 \text{ mol/mol gasification medium.}$$

Use final trial value for reaction temperature, T_r = 667°C (see calculations in Item 12 below). From appropriate tables or equations for equilibrium constants (see Table 2), obtain the constants for the water-gas reactions (3) and (3-a) and the methanation reaction (4):

$$K_{pw} = 0.9191 \qquad K_w = 0.5545 \qquad K_m = 0.1948$$

In biomass gasification, after the hydrogen content of the fuel combines with the oxygen to form the combined water, there is usually a net hydrogen left which amounts to:

(19) $H_{2not} = (H/2 - 0/16)/100 * F/M$ mol/mol gasification medium

Therefore, adding H_{net} to the corresponding equations involving hydrogen formation or depletion, the set of equations (14) becomes:

(14-a)

$$VCO_2 = (1\text{-}A_b) * V'CO_2/SUM$$
$$VH_2O = (1\text{=}A_w) * VH_2O/SUM$$
$$VCO = (2\ A_b\ V'CO_2 + A_w\ VH_2O)/SUM$$
$$VH_2 = (1\text{-}A_m) * (A_w * V'H_2O + H_{2not})/SUM$$
$$VCH_4 = 0.5 * A_m\ (A_w * V'H_2O + H_{2not})/SUM$$
$$VN_2 = V'N_2/SUM$$

Also equations (11) and (13) become:

(11-a)

$$SUM = V'H_2O + V'CO_2 + V'N_2 + A_b * V'CO_2 + (1 - 0.5 * A_m) * (A_w * V'H_2O + H_{2not})$$

mol/mol gasification medium

(13-a)

$$A_b = \frac{K''_w * C_{Hn} - V'H_2O/V'CO_2 * (1 - A_w/(1 - A_m)}{K'_w * C_{Hn} + 2/A_w * (1 - A_w)/(1 - A_m)}$$

where C_{Hn} is a correction factor which can be derived to give:

$$(20) \qquad C_{Hn} = (1 + H_{2not}/A_w/V'H_2O)$$

In biomass gasification, a factor, y, may be used to designate approach to equilibrium of the heterogenous water-gas reaction (3). A value of y less than 1 denotes less than 100% approach to equilibrium. Thus, equation (15) becomes:

$$(15 - a) \quad K'_{pw} = y * K_{pw} = P_t * VCO * VH_2/VH_2O$$

Consequently, the homogenous water-gas shift reaction equilibrium constant becomes:

$$(12 - a) \quad K'_w = K_w/y = VCO * VH_2O/VCO_2/VH_2$$

A factor x to denote approach to equilibrium of the methanation reaction (4) may also be used. Thus equation (16) becomes:

$$(16 - a) \quad K'_m = x * K_m = P_T * VCH_4/VH_3^2$$

Assuming a value for y = 0.26 (x is usually 1.00 for biomass gasification), the equilibrium constants used were:

$K'_{pw} = 0.9191 * 0.26 = 0.2391$, $K'_w = 0.5545/0.26 = 2.1325$

$K'_m = 0.1948 * 1.00 = 0.1948$

Using trial values of $A_w = 0.4640$ and $A_m = 0.0558$ and substituting into equations (19), (20) and (13 - a):

$$H_{2net} = (H/2 - 0/16)/100 * F/M$$
$$= (4.68/2 - 34.64/16)/100 * 21.29$$
$$= 0.03726 \text{ mol/mol gasification medium}$$

$$C_{Hn} = (1 + H_{2net}/A_w/V'H_2O) - (1 + 0.03726/0.464/0.646) = 1.1244$$

$$A_b = \frac{2.1325 * 1.1244 - 0.646/0.40 * (1 - 464)/(1.0558)}{2.1325 * 1.1244 + 2/0.464 * (1 - 0.464)/(1 - 0.0588)}$$

$$= 0.3058$$

From equation (11 - a)

$$SUM = 1.646 + 0.308 * 0.4 + (1 - 0.5 * 0.0558) * (0.464 * 0.646 + 0.03726) = 2.0954 \text{ mols/mol gasification medium}$$

From equation (14 - a)

$$VCO_2 = (1-0.3058)*0.4/2.0954 = 0.1325$$

$$VH_2O = (1-0.464)*0.646/2.0954 = 0.1652$$

$$VCO = (2*0.3058*0.4+0.464*0.646)/2.0954 = 0.2597$$

$$VH_2 = (1-0.0558)*(0.464*0.646+0.03726) = 0.0045$$

$$VCH_4 = 0.5*0.0558*(0.464*0.646+0.03726)/2.0954 = 0.0045$$

$$VN_2 = 0.6/2.0954 = 0.2863$$

To check:

(7) $VH_2O + VCO_2 + VN_2 + VCO + VH_2 + VCH_4 = 1$

$0.1652 + 0.1325 + 0.2863 + 0.2597 + 0.1518 + 0.0045 = 1$

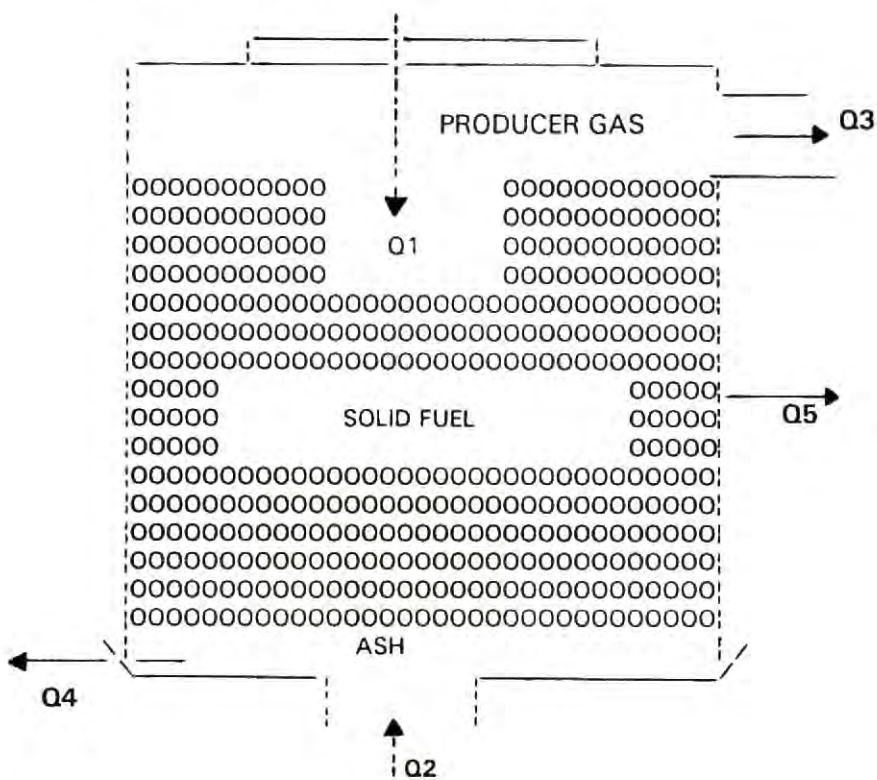
(15 - a) $K'_{pw} = P_T * VCO * VH_2/VH_2O = 0.2391$
$1 * 0.2597 * 0.1518/0.1652 = 0.2387$ (Check)

(12 - a) $K'_w = VCO * VH_2O/VCO_2/VH_2 = 2.1325$
$0.2597 * 0.1625/0.1325/0.1518 = 0.2387$ (Check)

(16 - a) $K'_m = P_T * VCH_4/VH_2^2 = 0.1948$
$1 * 0.0045/0.158^2 = 0.1945$ (Check)

12. Calculation for the reaction temperature and cold gas efficiency.

T_r = reaction temperature = 667°C (trial value)
HHV = higher heating value of dry ipil wood = 9204.6 Btu/lb
NHV_{dry} = net calorific value of dry ipil wood
= HHV-%M/(100-%M)*1040-%H*9*1040/100
= 9204.6-13.5/(100-13.5)*1040-4.68*9*1040/100
= 8604 Btu/lb or 8604/1.8 = 4780 Kcal/Kg
%M = %moisture content of fuel (wet basis) = 13.5%
NHV^{wet} = net calorific value of wet ipil wood
= NHV_{dry}*(1-%M/100) = 4780*(1-.135) = 4235 Kcal/Kg
H_f = enthalpy of fuel preheated to T_r (for updraft operation only) in Kcal/Kg
H_m = enthalpy of gasifying medium
H_{1*w} = heating value of product gas
H_w = enthalpy of product gas
%L = heat loss in % of NHV_{dry} of gasified fuel = 8.5%
F = amount of gasified fuel in lb/lb-mol or g/g-mol
Q_{exl} = external heat losses by radiation and convection
M = amount of gasifying medium in mols/mol of product gas
H_{ac} = enthalpy of as including unburnt carbon



The heat balance equation is made with the help of the preceding diagram where:

Q1 = heat input from the solid fuel
Q2 = heat input from the gasification medium
Q3 = heat content of the product gases
Q4 = heat loss in the ash including unburnt carbon
Q5 = external heat losses due to radiation and convection

$$Q1 + Q2 = Q3 + Q4 + Q5$$

(21) $F^* (H_r + NHV_{dry}) + M * H_m = H_g + H_{1,g} + F * H_{ac} + Q_{ext}$

(22) $H_r = 0.209333 * (T_r - T_o) + 0.2024 * 10^{-3} (T_r^2 - T_o^2) - 50.9333 * 10^{-9} (T_r^3 - T_o^3)$ cal/g fuel (assumed the same for all solid fuels), where T_r and T_o are fuel and ambient temperatures in °C respectively. (Reference 1).

(23) $H_m = V"H_2O * LH_{H2O} + V"H_2O * H_{h20} + (1 - V"H_20) * H_{N2\text{-}O2\ mix}$

(24) $H_{N2\text{-}O2\ mix} = H_{N2} * \{V"N_2/(V"N_2 + V"o_2)\} + H_{02} * (V"O_2/(V"N_2 + V"O_2))$

(25) $H_{1w} = VH_2 * HHV_{H2} + VCO * HHV_{CO} + VCH_4 * HHV_{CH4}$

(26) $H_g = VH_2 * HH_2 + VN_2 * H_{N2} + VH_2O * H_{H2O} + VCO_2 * H_{CO2} + VCH_4 * H_{CH4}$ cal/g.mol

(27) $Q_{ext} = F * NHV_{dry} * (\%L/100)/(\text{sqrt}(P_r)$ cal/g.mol (Reference 2).

where

Pr = total pressure in atmospheres

H_{H2} H_{N2}, H_{OC} etc. are enthalpy of gases indicated which are obtained from data tables or calculated from appropriate equations (see Table 3). LH_{H2O} = latent heat of water vapor (from steam tables) HHV_{CO}, HHV_{H2}, HHV_{CH4} = higher heating values of indicated gases.

(28) $H_{ax} = 0.209333 * (T_r - T_o) + 0.2024 * 10^{-3} * (T_r^2 - T_o^2) - 50.9333 * 10^{-9} * (T_r^3 - T_o^3) + 14,500 * p/1.8$ cal/g rejects (composed of ash and unburnt carbon), where T_r and T_o are rejects and ambient temperatures in °C, respectively, and p is the gram of carbon/gram of rejects. The value of p is obtained in Item 2: p = 0.1273

Calculated before from equations (18) and (19):

F = 10.16 lb. dry fuel/lb mol product gas (or g/g mol)
M = 0.4767 mol gasification medium/mol product gas

From appropriate data tables or equations (at T_r = 667° C):

LH_{H2O}	=	11,220.0 cal/g mol steam
NHV_{dry}	=	4,780.2 cal/g dry fuel
HHV_{CO}	=	68,030.5 cal/g mol gas
HHV_{H2}	=	68,449.3 cal/g mol gas
HHV_{CH4}	=	212,847.1 cal/g mol gas
H_{CO}	=	4,917.0 cal/g mol gas
H_2-O_2 mix	=	223.3 cal/g mol gas
H_{H2O}	=	29,012.1 cal/g mol gas
H_M	=	223.3 cal/g mol gasification medium
$H_{r,g}$	=	29.012.1 cal/g mol gas products
H_{CO2}	=	7,362.4 cal/g mol gas
H_{N2}	=	4,873.8 cal/g mol gas
H_{CH4}	=	6,017.3 cal/g mol gas
H_{H2}	=	4,688.5 cal/g mol gas
H_G	=	5,356.7 cal/g mol product gas
Q_{ext}	=	4,129.0 cal/g mol product gas
H_r	=	6.5 cal/g dry fuel
H_{ac}	=	1,010.3 cal/g dry fuel

Equation (21) can be written as:

(21 - a) $H_g = F * (NHV_{dry} + H_r - H_{ac}) - Q_{ext} + M * H_M - H_{r,g}$

Substituting into equation (21 - a), an identity is obtained:

H_g = 5,340.5 cal/g mol is practically identical to 5,356.7 cal/g mol calculated from equation (26).

Therefore, trial value of $T_r = 667^\circ C$ is the correct value. The choice of approach to equilibrium of the heterogenous water-gas reaction, y = 0.26 or 26% and percentage loss to radiation and convection, %L = 8.5% resulted in the calculated theoretical VCO_2 = 15.9% being identical to the experimentally measured (by Orsat apparatus) $\%CO_2$ = 15.9%. Otherwise, other values of y, and %L (if this is not known) would have to be tried.

The Cold Gas Thermal Efficiency is defined as the ration of the heat content of the cold product gas to the heat inputs from the fuel and the gasification medium. Thus,

Output = $H_{1,g}$ = 29,012.1 cal/g mol product gas
Inputs = F * NHV_{dry} + M * H_m = 10.16 * 4780.2
0.4767 * 223.3 = 48,673.3 cal/g mol product gas

Cold Gas Thermal Efficiency = 29,012.1/48,673.3 = 0.596 or 59.6%

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Table 2

EQUATIONS FOR EQUILIBRIUM CONSTANTS AT TEMPERATURE T
LOG K = A_0 + A_1/T + A_2 * T + A_3 * T^2 + A_4 * LOG T (T IN °K)

Constants

K	A_0	A_1	A_2	A_3	A_4
K_{PS}	3.26730	-8820.690	-1.208714 E-3	1.53734 E-7	2.295483
K_{PM}	-33.45778	-4825.986	-5.671122 E-3	8.255484 E-7	14.515760
K_w	36.72508	-3994.704	4.462408 E-3	-6.71814 E-7	-12.220277
K_{pm}	-13.06361	-4682.80	-2.09594 E-3	3.8620 E-7	3.034338

The Geometry of Uncontrolled Probabilistic Motion

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ABSTRACT

This paper upgrades classical integration to deal with set-valued functions and probabilistic motion. The notions ***generalized integral, derivative*** *and* ***pulsation*** *are introduced and applied to differential equations with plane oscillations.*

A formulation in Rn is developed and extended to Hilbert space and the Gaussian distribution is used to describe the structure of a wave packet as generalized pulsation with amplitude. It leads to a sharper form of the Heisenberg's uncertainty principle as conjugacy relationship between probability density and diffusion.

Path integration is likewise upgraded.

1. Limits of Classical Integration

Classical integration reached its apex with the introduction of the Lebesgue integral. All other integrals thereafter -- Henstock, Denjoy, Stieltjes, etc. -- were minor improvements because they differed from the Lebesgue integral, at least in their known applications, only in a set of measure zero. None of them can solve the differential equations:

(1) (a) $\dot{y} = \sin^n 1/x$ and (b) $\dot{y} = \cos^m 1/x \sin^n 1/x$,

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where m, n are positive integers, because the functions on the right side of those equations are not measurable in the neighborhood of zero; in fact, they are set-valued there. We define their set-values by:

$$(2)\quad (a)\quad \{f(x)\} = \lim_{x \to 0^+} \sin^n 1/x \text{ and}$$

$$(b)\quad \{g(x)\} = \lim_{x \to 0^+} \cos^m 1/x \sin^n 1/x$$

and denote them by

$$(3)\quad (a)\ \sin^n 1/0 \text{ and } (b)\ \cos^m 1/0 \sin^n 1/0,$$

respectively. In both cases, the limit sets are point-wise limits of sequences of arcs of the appropriate trigonometric curves. In the case of (3) (a) the graph is either of the segments [-1,1] and [0,1], depending on whether n is odd or even (See Figure 1 when n is odd).

But why are these integrals unable to solve (1)?

In its most abstract form a classical integral is a function on the space of quadruples (B,X, f(x), m) where B is a measurable subset of X, f is a function on X, and m is a measure on the measurable subsets of X. The measure is a function on sets and its composite with the integral is also a function on sets. (There is no loss of generality in taking f real-valued since if it is vector-valued we do integration on its components). This leaves f in the quadruple as the only function in the ordinary sense, i.e., single-valued. This is the fundamental limitation of the classical integral. That is why it cannot solve (1) because both functions on the right side are set-valued.

2. The Generalized Integral, Derivative and Pulsation

Given this fundamental weakness of the classical integral we now upgrade it by taking a leap to complete what was started by Lebesgue, namely, the extension of the integral to abstract spaces and its partial upgrading into a function on sets. This means replacing the single-valued function f(x) by a set-valued function {f(x)} . Since in mathematics a set is uninteresting and its elements, inessential, apart from their structures, we take one more step: introduce some structure in the form of a

function on sets, namely, a probability distribution function p_x (.) on {f(x)} such that at each x, $\dot{p}_x$ (.) is a probability distribution on {f(x)} . [The subscript x in $\dot{p}_x$ (.) means that $\dot{p}_x$ (.) is the probability distribution of the set-value {f(x)} at x in the mapping $x \rightarrow \{f(x)\}$ defining this function and the dot in (.) is reserved for the dummy variable along {f(x)} for integrating this function.] Then we define a **generalized integral** as a function on the space of quintuples (m, B, X, {f(x)} , p_x (.)) where B is a measurable subset of X, {f(x)} is a set-valued function on X and p_x (.) is a probability function on f(x) . We require X to be Hansdorff to admit probability measure concentrated at a point.

We do the formulation here for R^n and extend it to Hilbert space in Section 5.

Let Y be a compact subset of R^n with the standard subspace topology and its Borel sets. Let I be an open bounded n-cube with finite measure p(I), and containing Y, whose edges are parallel to corresponding coordinate axes. We partition I by a finite number of linear subspaces each of which is orthogonal to a coordinate axis so that, in effect, I is partitioned into n- dimensional rectangular blocks which we shall simple call blocks. Since we will be dealing here with probability measures including measure concentrated at a single point, we do not want the blocks to overlap. And so we admit only partitions such that the boundary on the face of a block belongs to one and only one block.

Suppose a block containing a point of Y has its boundary on two subspaces orthogonal to the Yi - axis, i = 1, 2,..., n, and passing through the points $(0,...,Y_{i,k},...,0)$ and $(0,...Y_{i,k+1},...,0)$, where $Y_{i,k} < Y_{i,k+1}$. We include in that block its boundary contained in the first subspace and exclude from it its boundary contained in the second subspace. The exception in each row of finite sequences of blocks induced by this ordered partitioning is the last one on the right whose boundary contained in the last bounding subspace we include in that block. Note that for any such partition the system of blocks covers Y and these blocks are non-overlapping.

Let P be a partition of I. We define the norm |P| of P as the diameter of the largest block in the partition. If P_1 is another partition of I we define the product PP_1 as the partition of I by both P and P_1. We are interested in a sequence P^i of finer partitions constructed in this manner such that $|P^i| \rightarrow 0$ as $i \rightarrow \alpha$. Sup-

pose we take a block Δi_1 containing $y \in Y$ at a particular value of i and suppose for each j, $j > i$ we take a block Δj_1, $\Delta j_1 \subseteq \Delta i_1$. Suppose further that for each Δi_1 we take a maximal Borel subset Δi_y of Y contained in Δi_1 (and, naturally, containing y because of maximality). Then we have a nested sequence of blocks Δi_1 and a sequence of maximal Borel sets Δi_y contained in Δi_1 for each i. Both sequences shrink to the point y as $|P^1| \to 0$. Since $\Delta i_y \subseteq \Delta i_1$ and $p(I) < \alpha$ then the quotient $P(\Delta i_y)/P(\Delta i_1)$ is uniformly bounded by 0 and 1 and hence has a limit point. Since both I and Y are measurable, that limit is unique and independent of the choice of partitions. We denote that limit by $\dot{p}(y)$ and call it a density or measure distribution of Y at y. In quantum mechanics this is called the probability density at that point 9. For small ΔI, $\dot{p}(y)\ P(\Delta I)$ is an approximation to $p(\Delta y)$. We denote that number by dp(y). We will normalize $\dot{p}(y)$ later and call it a probability distribution.

In the case where the measure α of a block ΔI is concentrated at the point y, i.e., $p(\{y\}) = \alpha$ and the measure of the complement of {y} in ΔI is 0, then $\dot{p}(y) = 1$ and $dp(y) = \alpha$.

Conversely, suppose we have a measure distribution on Y and let P be a partition of I. We form the sum of the measures of the blocks each of which contains an element of Y and call it an upper sum S which is bounded by the measure of I. We also take the sum of the measures of the maximal Borel sets in the blocks of the partition and call it a lower sum s. It is clear that for any partition P we have the inequality $o \leq s \leq S$. Again as $|P| \to 0$, s and S tend to some number k and, also, because of the measurability of both I and Y, that limit is unique. We call that limit the measure of Y which we denote by p(Y). We express it as an integral:

$$(3) \qquad p(Y) = \int_Y 1.dp(.) = k.$$

We normalize the measure distribution p(y) by dividing it by k and call it a probability or unit measure distribution on Y. In this case p is called a probability or unit measure of Y.

Suppose we take a point $y \in Y$ in each block ΔI of a partition and we attach to y the measure $p(\Delta y)$, i.e., we multiply

each component of y by $p(\Delta y)$ where p is a unit measure on Y. We take the sum s among all blocks in the partition each of which contains a point of Y. This sum is uniformly bounded since Y is bounded and I has finite measure. This is a rough average of the elements of Y. We let the norm of the partition approach zero and since that sum is uniformly bounded it approaches a limit which, again, is independent of the partition. We call that limit the expectation point of Y and we denote it by:

$$(4) \qquad E(Y) = \int_Y (.)dp(.).$$

We introduce an example of a **generalized integral** over an interval [a,b] in R -- a double integral defined by:

$$(5) \qquad Q_{ab}(\{f(x)\}) = \int_a^b ((.)dp_x(.))dx.$$

The inner integral on the right side of (5) maps $\{f(x)\}$ into the well-defined measurable expectation function E(x) on [a,b] so that the outer integral becomes an ordinary Lebesque integral.

(There is no loss of generality in taking $\{f(x)\}$ a plane set and E(x) real-valued since if E(x) is vector-valued we can do integration on the components.) Thus in the xy-plane the integral Q represents the area under the curve $y = E(x)$ from $x = a$ to $x = b$ (See Figure 2). If $\{f(x)\}$ is single-valued and measurable then $p_x(.)$ is concentrated at the single point f(x) at each x. In this case $E(x) = f(x)$ and the areas under the curves $y = E(x)$, $y = f(x)$ from $x = a$ to $x = b$ coincide. Then the generalized integral reduces tc a Lebesgue integral. Conversely, any Lebesgue integral can be expressed as a generalized integral. We state this as a theorem.

Theorem. The Lebesgue integral is a generalized integral with suitable integrand and probability function.

Proof. Let $\int f(x)dx$ be any Lebesgue integral, i.e., f(x) is Lebesgue integrable, and let g(x) be any bounded measurable function on [a,b], where $g(x) \geq 0$. Denote by A_x the cross section of the area under the curve $y = y(x)$, i.e., the compact vertical segment joining (x,a) and (x,g(x)). Then the function $\{g(x)\}: x \to A_x$, $x \in [a,b]$ is set-valued. Consider the set-valued function $\{h(x)\}$ defined by:

$$\{h(x)\} = f(x)A_x : = \{f(x)\ \mu/\mu \in A_x\},\quad x \in [a,b],$$

and let p_x be a probability function on $\{A_x\}$ such that at each x, p_x is concentrated at the point (x,g(x)). Then:

$$(6)\qquad Q_{ab}(\{h(x)\}) = \int_a^b \left(\int_{A_x} f(x)\ (.)dpx(.)\right)dx = \int_a^b f(x)dx.\#$$

We can also set up a generalized indefinite integral by replacing the upper limit b by x in (6) and using a dummy variable s. We can use it to solve a linear first order set-valued differential equation,

$$(7)\qquad \{\dot{y}\} = \{f(x)\}$$

where we assume the Borel sets on each $\{f(x)\}$as well as a measurable probability function $p_x(.)$ on the function $\{f(x)\}$. If the initial condition is given by $y(x_o) = y_o$ then the solution is given by

$$(8)\qquad y(x) = y_o + \int_{x_o}^{x} \left(\int_{\{f(x)\}} (.)dpx(.)\right)ds$$

which reduces to the Lebesgue integral

$$(9)\qquad y(x) = y_o + \int_{x_o}^{x} E(s)ds.$$

For well-defined derivative $\dot{y} = f(x)$ of an absolutely continuous function $y = F(x)$, we have the relationship

$$(10)\qquad y(x) = y_o + \int_{x_o}^{x} \dot{y}ds$$

or

$$(11)\qquad F(x) = F(x_o) + \int_{x_o}^{x} f(x)dx \text{ and } F'(x) = f(x) \text{ a.e.}$$

Note that the derivative of an ordinary indefinite integral is its integrand. We extend this idea to set-valued functions by defining a **generalized derivative** as the integrand of a generalized indefinite integral which is the expectation function. But, since that is the weighted average at each point x of the set-value of the integrand with respect to a probability distribution, differentiation reconstructs a probability distribution even if it is not unique. We, therefore, consider two probability distributions that yield the same expectation point E(x) equivalent and the equivalence class determined by E(x) we define as the **generalized derivative** $\dot{p}_X(.)$ of the set- valued function $\{f(x)\}$. Thus we are justified, on two counts, in the use of the dot notation $\dot{p}_X(.)$ for the probability distribution: by looking at $\dot{p}_X(.)$ as a generalization of the ordinary derivative and as the limit of the ratio of the measures of measurable subsets embedded in the nested sequence of blocks which is analogous to an ordinary derivative. And, of course, for single-valued function, the generalized derivative reduces to an ordinary derivative.

Finally, in this section, we define an n-dimensional generalized pulsation as the limit of pulsation in the ordinary sense (rapid expansion and contraction), as the latter becomes infinitely rapid. In the plane, it is an oscillation such as $\sin^n 1/x$. In the $(n+1)$ - space $R^n \times R$ a generalized pulsation is a compact subset of the subspace R^n orthogonal to R. We now drop the qualifier **generalized** for pulsation. Pulsation will refer both to the pulsation-valued function $\{f(x)\}$ and its set-value at x. We describe the structure of a pulsation by a probability distribution. If $\{f(x)\}$ is a pulsation we introduce a probability distribution function $\dot{p}_x$ such that at each x, $\dot{p}_x$ is a probability distribution on the set $\{f(x)\}$. If $\{f(x)\}$ is a singleton at x we call x an ordinary point; otherwise we call it a pulsation point for $\{f(x)\}$. At an ordinary point x the probability function $\dot{p}_x$ is concentrated at the point f(x). If there is some interval [a,b] every point of which is a pulsation point of $\{f(x)\}$ we call the latter a wild pulsation.

3. Uncontrolled Probabilistic Motion

The motion of a particle at great speed is, by definition, probabilistic since it is difficult to pinpoint its position. This is roughly expressed by the Heisenberg's uncertainty principle. For certain kinds of motion such as pulsation the calculation of

the probability distribution can be done by assuming what we shall call a pulsation probability principle which results in a sharper version of the Heisenberg's uncertainty principle.

We will apply our formulation to uncontrolled probabilistic motion, i.e., differential equation of motion with no control parameters. This means that the geometry of motion is determined by the nature of motion of that object, free from external intervention beyond the underlying electromagnetic field.

The case of differential equation with control parameters has been formulated and solved by a number of mathematicians foremost among whom was L.C. Young (**11**). Young's approach utilizes unit measure distribution on a compact control set as probability weights for evaluating the expectation function of the set-valued differential equation of motion. Thus a measurable probability measure-valued control function, called chattering control, corresponding to our probability distribution function here, generates the solution of the differential equation which Young calls relaxed trajectory.

An uncontrolled trajectory, according to the Filippov Lemna (**11**) is a controlled trajectory. Conceivably, we can reconstruct a control set and a probability distribution on it that can serve as probability weights for finding the expectation point E(x) of the set-valued differential equation of motion. This is not very promising, however. Thus we attach instead a probability distribution function that acts on the set-values of the right side of the differential equation of motion, the set-values acting as counterparts to the control set, to find E(x). That is the rationale for the formulation in Section 2.

And since uncontrolled trajectories are also controlled trajectories (**11**), all theorems about relaxed trajectories are valid for trajectories of uncontrolled probabilistic motion.

4. Application to Simple Oscillation

We take on the more challenging case of 1(a): $n > 2$ and n is even. The other cases are worked out in **(2)**. The idea is to use a wild oscillation to approximate (1) (a). We use the differential equation with wild oscillation:

$$\{\dot{y}\} = \sin^n 1/0_x, \tag{12}$$

$$\text{where } \sin^n 1/0_x = \lim^n 1/s \to x^+ s\text{-}x\,, -x \in [-\varepsilon', \varepsilon'],$$

for some $\varepsilon' > 0$, to approximate (1) (a) near $x = 0$. We will later shrink ε' to an approximate value. If $\dot{p}_0(.)$ is the probability distribution on the set $\sin^n 1/0$ then we set up the approximating differential equation to (1) (a) and its corresponding probability distribution function as follows:

$$(13) \quad \{\dot{y}\} = \begin{cases} \sin^n 1/0_x , & x \in [-\varepsilon', \varepsilon'], \\ \sin^n 1/x , & x \notin [-\varepsilon', \varepsilon'] \end{cases}$$

and the probability distribution is defined by the probability function:

$$(14) \quad p_x(.) \begin{cases} p_0(.) , & x \in [-\varepsilon', \varepsilon'], \\ \sin^n 1/x & x \notin [-\varepsilon', \varepsilon'], \end{cases}$$

which is clearly measurable. The second line of (14) means that the probability measure at each $x \notin [-\varepsilon', \varepsilon']$ is concentrated at the point $\sin^n 1/x$ and the probability distribution on the interval $[-\varepsilon', \varepsilon']$ is constant, i.e., at each $x \in [-\varepsilon', \varepsilon']$ the probability distribution on the oscillation there is equal to $\dot{p}_0(.)$.

Now we calculate the probability distribution $\dot{p}_0(.)$ using the geometry of the topologist's sine curve $y = \sin^n 1/x$. We effect, in accordance with our general formulation above, a partition by closed-open non-overlapping intervals (the blocks reduce to intervals on the segment) of the form $[s, s + dy]$, the exception being the topmost segment of any partition which will be closed with the adjunction of the upper end-point of the oscillation at $x = 0$. We effect a change of variable $w = 1/x$. This does not reproduce the entire topologist's sine curve since w is not defined at $x = 0$. But we shall use the resulting function:

$$(15) \quad y = \sin^n w$$

to approximate certain features of $\sin^n 1/0$ by a suitable subarc of (15) in some ε'- neighborhood of $x = 0$.

Consider the geometry of (14) as shown in Figure 3. At any point $W \in [0, \pi]$ whose subtending arc on the unit circle has end point P', there is some point P whose ordinate is $\sin^n w$. If $w \in [\pi, 2\pi]$, its radius vector at Q' will have a reflection P' on the upper semicircle and since n is even, $\sin^n w$ will be the ordinate of some point P on the upper semicircle. As w increases uniformly, i.e., dw/dt = constant, P oscillates back and forth from R to T along the upper semicircle and its projection y does so also along the vertical segment [0,1] at x = 0. At the same time, the point $(w, \sin^n w)$ traces an arc of the curve $y = \sin^n w$ with uniform frequency. Increase dw/dt until a half-arc corresponding to half a period of length $\pi/2$ lies within an ε'-neighborhood of x = 0. Keep dw/dt at that rate but calibrate w so that the half arc would correspond to the half period $[0, \pi/2]$ and a full sweep of y along the oscillation AB (Figure 3). The point P has a projection y on both the approximating arc and the oscillation. Consider one sweep of y along the approximating arc. We ask: given a small segment [s, s + dy) (or [s, s + dy]) in some partition, what is the probability of finding y in there? If y stops momentarily in that segment, i.e., dy/dt = 0, we want that probability to be 1. If y does not stop there and its average velocity is large then that probability must be small. Thus there is some conjugacy relationship between that probability and the speed of the oscillating point. We, therefore, assume, as an axiom, what we call the **oscillation probability principle:** the speed dy/dt represents the probability that the oscillating point y is not in the segment [s, s + dy). If we denote that number by $\dot{q}$, since it is a derivative and the probability that the oscillating point lies in [s, s + dy] by $\dot{p}$, then, after normalizing $\dot{q}$ suitably so that its values lie between 0 and 1, we must have:

$$(16) \qquad \dot{p} + \dot{q} = 1 \text{ or } \dot{p} = 1 - \dot{q}$$

We can look at $\dot{p}$ as the probability distribution along [0,1] and q as the speed distribution. They are both relative values or distribution of the value 1. Thus, division of $\dot{p}$ or $\dot{q}$ by a constant does not alter that distribution.

We have,

$$(17) \qquad dy/dt = dy/dw \cdot dw/dt.$$

Since dw/dt is constant, dy/dt is proportional to dy/dw. And we can replace dy/dt by dy/dw as the conjugate of $\dot{p}$ which we shall normalize.

From (17) we have:

$$dy/dw = n\sin^{n-1} w\cos w.$$

We normalize dy/dw by dividing it by its maximum a_n in the interval $[0, \pi/2]$. (A trivial calculation by differentiation yields $a_n = \sqrt{n}(n-1/n)^{n-1/2}$).

Set

$$(18) \qquad \dot{q} = 1 - n/a_n \sin^{n-1} w\cos w$$

and normalize $\dot{p}$ by dividing it by:

$$(19) \qquad \int_0^{\pi/2} (1 - n/a_n \sin^{n-1} w\cos w)dw = a_n \pi - 2/2a_n ,$$

using the fact that the probability that the oscillating point y is in the segment [0,1] is 1. Then we obtain the normalized probability distribution:

$$(20) \qquad \dot{p} = 2a_n/a_n \pi - 2\,(1 - n/an \sin^{n-1} w\cos w).$$

We now write the approximating differential equation and the corresponding probability distribution function:

$$(21) \qquad \{\dot{y}\} = \begin{cases} \sin^n 1/0_x , & x \in [-\varepsilon', \varepsilon'], \\ \sin^n 1/x , & x \notin [-\varepsilon', \varepsilon], \end{cases}$$

$$(22) \qquad \dot{p}_x(.) = \begin{cases} 2a_n/a_n \pi - 2\,(1 - n/a_n \sin^{n-1} w_x \cos w_x), & x \in [-\varepsilon', \varepsilon'], \\ \sin^n 1/x , & x \notin [-\varepsilon', \varepsilon']. \end{cases}$$

Since

$$dp_x(w_x) = 2a_n/a_n\pi - 2(1- n/a_n \sin^{n-1} w_x \cos w_x)dw_x = \dot{p}_x\ (w_x)dw_x,$$

we can apply the generalized integral Q on $\sin^n 1/0_x$ subject to some initial condition $y(x_0) = y_0$:

$$(23)\ \int_{x_o}^{x} 2a_n/(a_n\pi - 2)\int_{o}^{\pi/2} \sin^n w_s\ (1-n/a_n \sin^{n-1} w_s \cos w_s)\ (dw_s)\ ds.$$

This integral can be computed by a trivial algorithm. It can be shown **(2)**, **(5)** that, as expected, the expectation function of the set-valued function $\sin^n 1/0_x$, $x \in [-\varepsilon', \varepsilon']$ is some number β which lies between 0 and 1. With the transformation effected by (23), the approximate differential equation becomes an ordinary differential equation:

$$\dot{y} = \begin{cases} \beta, & x \in [-\varepsilon', \varepsilon'], \\ \sin^n 1/x, & x \notin [-\varepsilon', \varepsilon']. \end{cases}$$

We find, one at a time, the solutions in the intervals $[0, \varepsilon']$ $[-\varepsilon', 0]$, $x \geq \varepsilon'$, $x \leq -\varepsilon'$ and join together an absolutely continuous solution. For the first interval we take $y(0) = 0$ as the initial condition to obtain:

$$y(x) = \int_o^x \beta\, x ds = \beta x, \qquad x \in [0, \varepsilon'].$$

With the same initial condition and using the symmetry of $\sin^n 1/x$ with respect to the y-axis we obtain another piece of the solution:

$$y(x) = -\beta x, \qquad x \in [-\varepsilon', 0].$$

For the other parts of the solution, we note that $\int_{\varepsilon'}^{x} \sin^n 1/s\ ds$ has

a least upper bound, say α, in $x \geq \varepsilon'$. We choose, as initial condition, $y(\varepsilon) = \beta\varepsilon < \alpha$ for some small ε, $0 < \varepsilon < \varepsilon'$. Hence, the solution for $x \geq \varepsilon$ is:

$$y(x) = \beta\varepsilon + \int_{\varepsilon}^{x} \sin^n 1/s \, ds.$$

Again, using symmetry with respect to the y-axis, we obtain the full approximate solution to the differential equation: (1),

$$(24)\quad y(x) = \begin{cases} \beta x, & x \in [0, \varepsilon], \\ \beta\varepsilon + \int_{\varepsilon}^{x} \sin^n 1/s \, ds, & x \geq \varepsilon, \\ \beta\varepsilon + \int_{x}^{-\varepsilon} \sin^n 1 \quad ds, & x \leq -\varepsilon, \\ -\beta x, & x \in [-\varepsilon, 0]. \end{cases}$$

Its graph is shown in Figure 4.

5. Application to Compound Oscillation

We next apply our method to the differential equation 1(b). As before, we effect a change of variable $w = 1/x$ to obtain the approximating arcs of the functions

$$(25)\qquad y = \sin^n w \text{ and } y = \cos^m w.$$

The values of m and n affect the cycle of the compound oscillation as well as the shape of the graph of each of the factors of 1(b). To illustrate our method we take the case m, n both even. (The other cases are taken up in (**3**). The relevant graphs are shown in Figure 4, where the flatness of the graphs at their maximum points and their steepness away from those points depend on the magnitudes of m and n. It can be shown that the function $y = \cos^m w \sin^n w$ has a unique maximum at $w = \tan^{-1}\sqrt{n/m}$, has minimum at $w = 0, \pi/2$, and is strictly increasing and decreasing in the intervals $[0, \tan^{-1}\sqrt{n/m}]$ and $[\tan^{-1}\sqrt{n/m}, \pi/2]$, respectively. Thus a suboscillation which would have required a different analysis is ruled out.

Unless m = n, the function $y = \cos^m w \sin^n w$ has no symmetry with respect to the vertical line $w = \tan^{-1}\sqrt{n/m}$. The functions $\sin^n w$ and $\cos^m w$ have the same periodicity and, hence, a full cycle of their product is repeated at every interval of length $\pi/2$ corresponding to a full sweep of the oscillating point y. (We assume the non-trivial case $m \neq n$).

We now calculate the probability distribution function for the right side of 1(b) at x = 0. Note that the oscillating point y is the projection of a point t in arc C_1 and a point r in arc C_2 of the graph of $y = \cos^m w \sin^n w$. Therefore, the probability that y is in some small interval [s, s + dy) (or[s, s + dy]) is the sum of the probabilities that t and r are in the corresponding vertical intervals dy at the arcs C_1 and C_2, respectively, of the graphs of the product function.

The calculation of these probability distributions is similar to that for the simple oscillation above. The reader is referred to [3] for detailed calculation and the solution of this problem for the other values of m and n.

By differentiation we obtain the velocity functions for $y = \cos^m w \sin^n w$ in the intervals $[0, \tan^{-1}\sqrt{n/m}]$ and $[\tan^{-1}\sqrt{n/m}, \pi/2]$ which are given by:

(26)

$$\dot{q}_1(w) = n\cos^{m+1} w \sin^{n-1} w - m\cos^{m-1} w \sin^{n+1} w,\ w \in [0, \tan^{-1}\sqrt{n/m}],$$

$$\dot{q}_2(w) = m\cos^{m-1} w \sin^{n+1} w - n\cos^{m+1} w \sin^{n-1} w,\ w \in [\tan^{-1}\sqrt{n/m}],$$

respectively. Let their maxima be a_{mn} and b_{mn}, respectively. Then the normalized velocity functions are:

(27)

$$_1(w) = 1/a_{mn}\,(n\cos^{m+1} w \sin^{n-1} w - m\cos^{m-1} w \sin^{n+1} w),\ w \in [0, \tan^{-1}\sqrt{n/m}],$$

$$_2(w) = 1/b_{mn}\,(m\cos^{m-1} w \sin^{n+1} w - n\cos^{m+1} w \sin^{n-1} w),\ w \in [\tan^{-1}\sqrt{n/m}, \pi/2]$$

To simplify our notation, we write

$$(28)\qquad ./q(w) = \begin{cases} \dot{q}_1(w), & w \in [0, \tan^{-1}\sqrt{n/m}], \\ 0, & w \in [\tan^{-1}\sqrt{n/m}, \pi/2], \end{cases}$$

$$./q_2(w) = \begin{cases} \dot{q}_2(w), & w \in [\tan^{-1}\sqrt{n/m}, \pi/2], \\ 0, & w \in [0, \tan^{-1}\sqrt{n/m}]. \end{cases}$$

The probability distribution for the compound oscillation AB is given by:

$$(29)\qquad \dot{p}_o(w) = (1-./q_1(w) + (1-./q_2(w)) = 2-./q_1(w) - ./q_2(w)$$

which we normalize by dividing by the constant:

$$(30)\qquad a = \int_0^{\tan^{-1}\sqrt{n/m}} (1- ./q_1(w))dw \quad + \quad \int_{\tan^{-1}\sqrt{n/m}}^{\pi/2} (1- ./q_2(w))dw.$$

Thus, the normalized probability distribution is given by:

$$(31)\qquad \dot{p}(w) = 1/a\ (2- ./q_1(w) - ./q_2(w)).$$

For our approximate differential equation we take:

$$(32)\qquad \{\dot{y}\} = \begin{cases} \cos^m 1/0_x \sin^n 1/0_x, & x \in [-\varepsilon', \varepsilon'], \\ \cos^m 1/x \sin^n 1/x, & x \notin [-\varepsilon', \varepsilon'], \end{cases}$$

with probability distribution

$$(33)\qquad \dot{p}(w_x) = \begin{cases} \dot{p}(w), & x \in [-\varepsilon', \varepsilon'], \\ \cos^m 1/x \sin^n 1/x, & x \notin [-\varepsilon', \varepsilon']. \end{cases}$$

Applying the generalized integral Q with initial condition $y(0) = 0$ we obtain the solution in $[0,\varepsilon']$:

$$(34) \qquad y(x) = \int_0^x \left(\int_{\Gamma_s} (.)\, p\,(w_s)\, dw_s \right) ds,$$

where $\Gamma x = \cos^m 1/0_x \sin^n 1/0_x$. From the symmetry of the wild oscillation (32) with respect to the y-axis and taking also the initial condition $y(0) = 0$ we obtain the solution of (32) in $[-\varepsilon', 0]$:

$$(35) \qquad y(x) = \int_x^0 \left(\int_{\Gamma_s} (.)\, \dot{p}\,(w_s)\, dw_s \right) ds.$$

Outside these intervals the solutions are ordinary Lebesgue integrals subject to the initial conditions:

$$(36) \qquad y(\varepsilon) = \beta\varepsilon \text{ and } y(-\varepsilon) = \beta\varepsilon,$$

Where $y = \beta$ is the constant expectation function of the wild oscillation

$$\{\dot{y}\} = \cos^m 1/0_x \sin^n 1/0_x$$

and ε satisfies $0 < \varepsilon < \varepsilon'$ so chosen to obtain an absolutely continuous global solution of (32) as we did with our first application above.

The full solution of (32), which is our approximate solution to 1(b) for m, n both even, is given by:

$$(37) \qquad y(x) = \begin{cases} \beta\varepsilon + \int_\varepsilon^x \cos^m 1/s \sin^n 1/s\, ds, & x \in [\varepsilon, \infty] \\ \beta x, & x \in [0, \varepsilon] \\ -\beta x, & x \in [-\varepsilon, 0], \\ \beta\varepsilon + \int_x^{-\varepsilon} \cos^m 1/s \sin^n 1/s\, ds & x \in [-\infty, -\varepsilon]. \end{cases}$$

Its graph is shown in Figure 5.

6. Application to Quantum Mechanics

There is consensus among physicists that the motion of subatomic or elementary particles is probabilistic. The evi-

dences are their great velocities, wave characteristics and some indeterminacy of certain measurements such as position and momentun as expressed by the Heisenberg's uncertainty principle. Therefore, there is validity in assuming that such motion is probabilistic and in using ordinary pulsation in Hilbert space, the setting for quantum mechanics today, to describe such motion. Pulsation at great rapidity propagates waves in all directions including the direction of motion and is probabilistic at the same time. The frequency can be determined from the quantized nature of energy state which we will go into later.

We do some calculations on the generalized pulsation towards which the ordinary pulsation approaches. Physicists introduced the notion of probability density, i.e., the limit of the probability of finding the particle in motion in an element of volume in H. We take the limit as that element shrinks to a point x (t) $\in$ H. We will call that limit the probability distribution at that point and denote it by $\dot{G}(x(t))$. We introduce the notion of diffusion as well, i.e. the rate of flow of the points in that element of volume. We shrink that volume to a point so that the rate of flow approaches instantaneous velocity. We call it velocity distribution. In an analogous manner (to oscillation), we assume that that is the limit of the probability that the particle in motion is not in that element of volume as that volume shrinks to a point. We call it the **pulsation probability principle**. Let us denote that limit by $\dot{D}(x(t))$ and assume that it has been suitably normalized so that its value lies between 0 and 1. (This is possible since velocity is bounded, according to Relativity.) Then we have the conjugacy relationship

$$\dot{G}(x(t)) + \dot{D}(x(t)) = 1$$

which, along the direction of motion, is a sharper form of the Heisenberg's uncertainty principle for pulsation since velocity is simply momentum divided by mass. (The Heisenberg's uncertainty principle says that if Δx is the uncertainty in pinpointing the position of the particle and Δp the uncertainty in measuring momentum then $\Delta x . \Delta p \geq h/2\pi$, where h is the Planck's constant).

The probability distribution $\dot{G}$ describes the structure of the pulsation which we take to be a wave packet with a probability

distribution[1] obtained by modifying the Gaussian distribution given by:

$$\dot{G}(x(t)) = \alpha(t) e^{-\beta(t)|x(t)-\gamma(t)|^2} \tag{38}$$

where x(t) is a variable point in the subspace H of HxR orthogonal to R, $\alpha(t)$ and $\beta(t)$ are positive real numbers, $\gamma(t)$ is the point at which $\dot{G}(x(t))$ is maximum at time t and $|\ |$ is a norm in H. There is no loss of generality in assuming H to be the space l_2 of square summable sequences since this space is isomorphic to any Hilbert space (**6**). (The choice of the appropriate norm in H is left for the physicist to decide to match empirical data; one possibility is the metric $d(x,y) = \{\sum_{i=1}^{\infty}(x_i - y_i)^2\}^{1/2}$. The function $\gamma(t)$ is determined by the underlying electromagnetic field.)

We modify (38) to describe a wave packet, by a sinusoidal amplitude to obtain the modified Gaussian distribution:

$$\dot{G}(x(t)) = \alpha(t)\sin^n\sigma(t)\, e^{-\beta(t)|x(t)-\gamma(t)|^2} \tag{39}$$

which describes the structure of a wave packet consisting of a wild pulsation, with an amplitude $\sin^n \sigma(t)$ where $\sigma > 0$ determines the extent of a wave packet along the direction of motion and n is a positive integer. (The terms α, σ, β and η are introduced to obtain the desired properties of the wave packet, including extent and shape, to match empirical data.)

The wave packet is the support for $\dot{G}$ which we assume to be bounded, invoking Relativity and compact by taking its closure.

An ordinary path does not make sense here and in place of it we take the trace of the expectation point of the wave packet in motion. The quantized nature of energy state requires that this path be discrete, each point of which corresponds to an in-

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[1] This idea of Gaussian distribution I owe to the timely suggestion by Professor Dick Van Dulst of the University of Amsterdam.

tegral multiple of $\pi/2$. These points are the allowable positions of the particle in accordance with the quantized energy state requirement. Between two consecutive observable points we interpret the probability measure, i.e., the integral of $\dot{G}$, as the transition probability or the probability that the particle will jump from the first to the second point.

Finally, we indicate how path integration can be carried out in this setting.

At the subatomic level of matter and at great velocity we lose the amenities of smoothness and determinacy. Position and derivative tend to be set-valued. Even time becomes elusive.

Therefore we assume that the derivative x is some set-valued function $g(t,\Omega_t, \dot{G}_t)$ where Ω_t is the cross-section of the wave packet and $\dot{G}_t$ is the Gaussian distribution on Ω_t at time t. We find the expectation point E(t,x) of $g(t,\Omega_t, G_t)$,

where

$$(40) \quad g(t,\Omega_t, G_t) = \{ g(t,w) \mid w \in \Omega_t \}$$

and

$$(41) \quad E(t,x) = \int_{\Omega t} g(t, (.)) \, d\dot{G}_t \, (.).$$

The differential equation of motion becomes:

$$(42) \quad \dot{x} = E(t,x) \quad \text{a.e.}$$

$\dot{G}(t)$ is included in the argument of (40) only as a matter of notation to indicate that $\dot{G}$ is the probability distribution on Ω_t.

We shall not go into the requirement on g to insure existence of solution subject to some initial condition; we simply assume that E is Lipschitzian in x to insure uniqueness. Let $L(t,\Omega_t, \dot{G}_t)$ be the set-valued Lagrangian subject to the same conditions as g and let its expectation point at time t be denoted by L(t, x). Since the dimensionality of the problem is at our disposal, we can adjoin in (42) the differential equation:

(43) $\dot{x}_o = L(t,x)$

to obtain the differential equation:

(44) $\dot{z} = (\dot{x}_o, \dot{x}) = (L(t, x), E(t, x))$ a.e.

Subject to some initial condition $z(t_o) = z_o$ and suitable conditions on g(2), (48) has a unique solution which is called a generalized curve. The path integral from t_1 to t_2 reduces to the difference $x_o(t_2) - x_o(t_1)$, where $x_o(t)$ is the first component of the solution of (44).

If the path integral is a minimization problem (an optimization problem can be reduced to a minimization problem), then it reduces to the minimum of $x_o(t_2) - x_o(t_1)$, which, by a simple translation of the origin of the coordinate system to $x_o(t_1)$, further reduces to the minimum of $x_o(t_2)$.

Note that in this formulation, $\dot{G}_t$ is the generalized derivative of Ω_t and Ω_t is the compact support of $\dot{G}_t$ which coincides with the wave packet. The path integral is taken along the expectation curve z(t) whose derivative is a generalized derivative given by $\dot{G}(z(t))$.

There are many interesting properties of the solution z(t) of (44) one of which being that it can be approximated by an ordinary simplicial path (11).

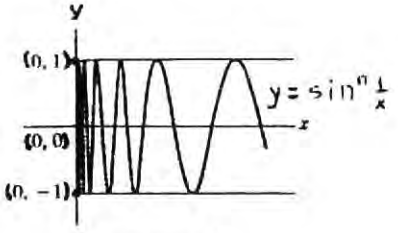

Figure 1.

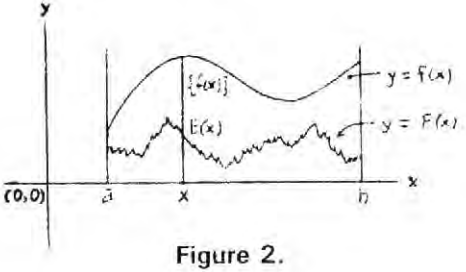

Figure 2.

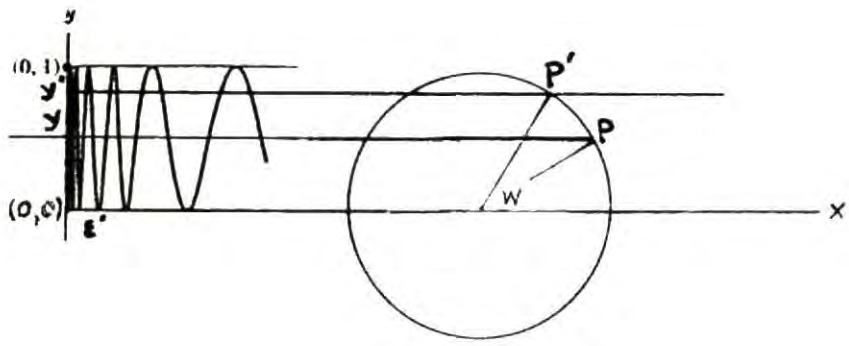

Figure 3.

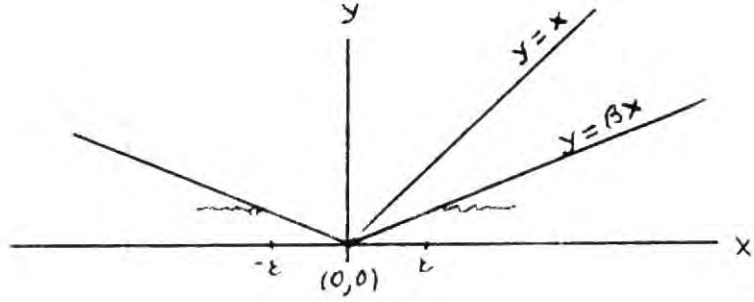

Figure 4.

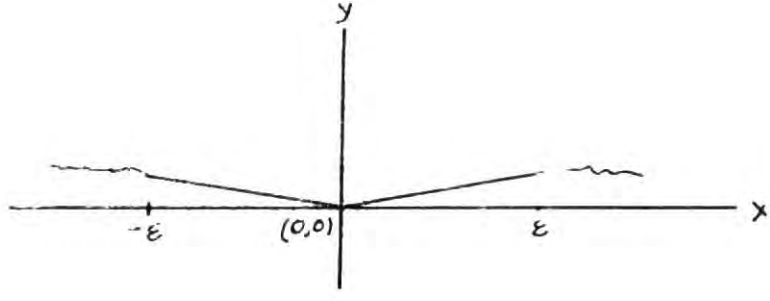

Figure 5.

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Singularity of Graphs in Some Special Classes[1]

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ABSTRACT

*A **graph** is a pair $G = <V(G), E(G)>$, where **V(G)** is a nonempty finite set of elements called **vertices** and **E(G)** is a set of unordered pairs of distinct vertices called **edges**. If $v_1, v_2, \ldots, v_n$ are the vertices of **G**, we define the **adjacency matrix** of **G**, denoted by **A(G)**, to be the nxn (0, 1) - matrix (a_{ij}), where $a_{ij} = 1$ if and only if $[v_i, v_j] \in E(G)$. The graph G is said to be **singular** if its adjacency matrix is singular, i.e., det $A(G) = 0$.*

*Singular graphs have not yet been characterized and the identification of all singular graphs seems to be a difficult problem. However, characterization of singular graphs in some special classes is possible. Here we shall completely characterize the singular graphs among the **planar grids $P_m \times P_n$**, the **prisms $C_m \times P_n$** and the **toroidal grids $C_m \times C_n$**.*

Introduction

The *path of order n*, denoted by P_n, is the graph with n vertices 1, 2, . . . , n and whose edges are [i, i + 1], i = 1, 2, 3,..., n - 1. The *cycle of order n*, denoted by C_n, is the graph obtained from P_n by adding the edge [1, n]. Figure 1 shows the path P_6 and the cycle C_6.

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[1] The results contained in this paper are taken from the NRCP-funded research project entitled "A Study of Singular Bipartite Graphs."

If $G = <V(G), E(G)>$ and $H = <V(H), E(H)>$ are two graphs, the *cartesian product* $G \times H$ is the graph with vertex-set $V(G) \times V(H)$, and two vertices (a, b) and (c, d) in $G \times H$ are adjacent if and only if either (i) [a, b] ε $E(G)$ or (ii) a = c and [b, d] ε $E(H)$. Figures 2, 3 and 4 show the planar grid $P_5 \times P_8$, the prism $C_6 \times P_4$ and the toroidal grid $C_4 \times C_6$, respectively.

In this paper, we shall determine which planar grids, prisms and toroidal grids are singular. Some reduction formulas [1] are available to handle the planar grids. However, we shall use a uniform procedure in handling all the three classes. We shall first establish one Lemma which will help us do this. The following notations are used in the statement and proof of the Lemma:

P(a, b)	denotes the point P in the plane with coordinates (a, b).
PQ	is the line segment with endpoints P and Q.
| PQ|	is the length of the line segment PQ.
gcd (a, b)	is the greatest common divisor of a and b.

PRELIMINARY RESULT

Lemma 1. Let P(a, b) and Q(c, d) be any two distinct points in the plane with integer coordinates. Then the number of points in PQ with integer coordinates (including P and Q) is equal to 1 + gcd(c-a, d-b). Furthermore, these points are evenly distributed over the line segment PQ, i.e., the distance between any two such neighboring points is | PQ | /gcd(c-a, d-b).

Proof: If the line segment PQ is horizontal or vertical, the Lemma clearly holds. We, therefore, assume that PQ is neither horizontal nor vertical. Without loss of generality, assume that $c > a$ and $d > b$ and let $g = gcd(c-a, d-b)$. Let $0 \leq k \leq g$ and $x = a + k(c-a)/g$, $y = b + k(d-b)/g$. It is easy to check that R(x, y) is a point in PQ with integer coordinates and that the distance between two such neighboring points is | PQ| /g. Since these points are g + 1 in number, it remains for us to show that there are no other points in PQ with integer coordinates. To prove this, let S(u, v) be any point in PQ with integer coordinates. Please refer to Figure 5.

Without loss of generality, assume that S is not the point P. Since $g = \gcd(c-a, d-b)$, then $\gcd(m, n) = 1$, where $m = (c-a)/g$ and $m = (d-b)/g$. By similar triangles, we have $(v-b)/(u-a) = (d-b)/(c-a) = m/n$. It follows that $u-a = kn$ and $v-b = km$ for some $0 \leq k \leq g$. Consequently, S is one of the points R(x, y).

In addition to the above Lemma, we shall use the following results on eigenvalues which can be found in [ ]:

(a) The eigenvalues of $\mathbf{A(P_m \times P_n)}$ are

$2\cos[\pi/(m+1)]i + 2\cos[\pi/(n+1)]j \quad (1 \leq i \leq m \text{ and } 1 \leq j < n).$

(b) The eigenvalues of $\mathbf{A(C_m \times P_n)}$ are

$2\cos(2\pi/m)i + 2\cos[\pi/(n+1)]j \quad (1 \leq i \leq m \text{ and } 1 \leq j < n).$

(c) The eigenvalues of $\mathbf{A(C_m \times C_n)}$ are

$2\cos(2\pi/m)i + 2\cos(2\pi/n)j \quad (1 \leq i \leq m \text{ and } 1 \leq j < n).$

SINGULAR PLANAR GRIDS

Using (a), we see that $\mathbf{P_m \times P_n}$ is singular if and only if $2\cos[\pi/(m+1)]i + 2\cos[\pi/(n+1)]j = 0$ for some i and j satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$. Using trigonometric identity $\cos\alpha + \cos\beta = 2\cos[(\alpha+\beta)/2]\cos[(\alpha-\beta)/2]$, we see that the planar grid is singular if and only if $\cos 1/2[(\pi/m+1)i + (\pi/n+1)j] = 0$ for some i and j satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$. But $1/2[(\pi/m+1) - (\pi/n+1)j]$ lies in the interval $(-\pi/2, \pi/2)$ and cosine is never zero here. On the other hand, $1/2[(\pi/m+1)i + (\pi/n+1)j]$ is in the interval $(0, \pi)$ and cosine is zero only at the point $\pi/2$. Hence, 0 is an eigenvalue of $\mathbf{A(P_m \times P_n)}$ if and only if $1/2[(\pi/m+1)i + (\pi/n+1)j] = \pi/2$ for some i and j satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$. This necessary and sufficient condition easily reduces to the following:

$[(i/m+1) + (j/n+1)] = 1$ for some i and j satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$. Observe that the equation $[(i/m+1) + (j/n+1)] = 1$ represents a straight line in the ij-plane with i- and j- intercepts of $m+1$ and $n+1$, respectively. We see then that there exists i and j satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$ if and only if there is at least one point in the line segment joining the i- and j- intercepts with integer coordinates. By Lemma 1, there is at least one such point if and only if $\gcd(m+1, n+1) > 1$. We have thus established the following:

Theorem 1. The planar grid $\mathbf{P_m \times P_n}$ is singular if and only if $\gcd(m+1, n+1) > 1$.

SINGULAR PRISMS

Using (b) and the same trigonometric identity applied in the proof of Theorem 1, we see that $\mathbf{C_m \times P_n}$ is singular if and only if $\cos 1/2[(2\pi/m)i \pm (\pi/n+1)] = 0$ for some i and j satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$. Now, $1/2[(2\pi/m)i + (\pi/n+1)]$ is in the interval $[0, (3/2)\pi)$ while $1/2[(2\pi/m)i - (\pi/n+1)]$ is in the interval $(-\pi/2, \pi)$. In both intervals, cosine is 0 only at the point $\pi/2$. Hence, $\mathbf{C_m \times P_n}$ is singular if and only if (i) $[(2/m)i + (i/n+1)j] = 1$ or (ii) $[(2/m)i - (1/n+1)j] = 1$ for some i and j satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$. The graph of (i) in the ij-plane is a straight line passing through the points $P(0, n+1)$ and $Q(m, -(n+1))$. Since PQ cuts the i-axis at $(m/2, 0)$, it follows that (i) holds for some i and j satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$ if and only if there are at least four points in PQ with integer coordinates. By Lemma 1, this is equivalent to the condition $\gcd(m, 2n+2) > 2$. Similarly, the graph of (ii) in the ij-plane is a straight line containing the points $P(0, -(n+1))$ and $Q(m, n+1)$. PQ also cuts the i-axis at $(m/2, 0)$ and so (ii) holds for some i and j satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$ if and only if there are at least four points in PQ with integer coordinates. This condition also yields the equivalent to the condition $\gcd(m, 2n+2) > 2$. Therefore, we have established the following:

Theorem 2. The prism $\mathbf{C_m \times P_n}$ is singular if and only if $\gcd(m, 2n+2) > 2$.

Remark. Theorem 2 is equivalent to the following:

Theorem 2'. The prism $\mathbf{C_m \times P_n}$ is singular if and only if $m = 0 \pmod 4$ and n is odd.

SINGULAR TOROIDAL GRIDS

By means of (c) and the trigonometric identity used in Theorems 1 and 2, we obtain the result that $\mathbf{C_m \times C_n}$ is singular if and only if $\cos[(\pi/m)i \pm (\pi/n)j] = 0$ for some i and j

satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$. But $[(\pi/m)i + (\pi/n)j]$ is in the interval $(o, 2\pi)$ while $[(\pi/m)i - (\pi/m)j]$ is in the interval $(-\pi, \pi)$. In the first interval, cosine is 0 at $\pi/2$ and $3\pi/2$ while in the second interval, cosine is 0 at $-\pi/2$ and $\pi/2$. From these, we see that $\mathbf{C_m \times C_n}$ is singular if and only if for some i and j satisfying $1 \leq i \leq m$ and $1 \leq j \leq n$, either one of the following conditions hold:

$$\text{(i)} \quad \frac{2ni + 2mj}{mn} = 1 \text{ or } 3$$

$$\text{(ii)} \quad \frac{2ni - 2mj}{mn} = 1 \text{ or } -1.$$

The numerator of (i) is always even while its righthand side is odd. Hence, (i) has no solution if m and n are both odd. The same conclusion holds for (ii). If one of m, n is even, we may assume without loss of generality that m is even. Taking $i = m/2$ and $j = n$, we will satisfy (i). Hence, we have proved the following:

Theorem 3. The toroidal grid $\mathbf{C_m \times C_n}$ is singular if and only if m or n is even.

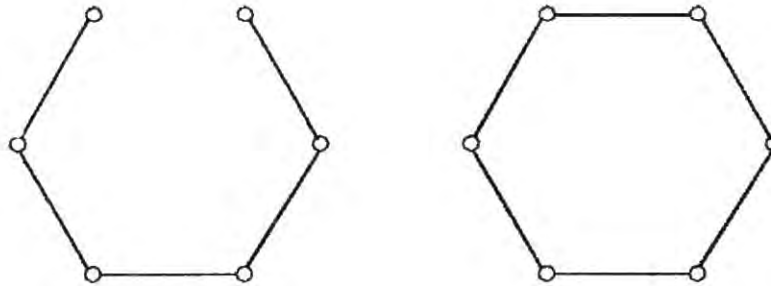

Figure 1. The path P_6 and the cycle C_6

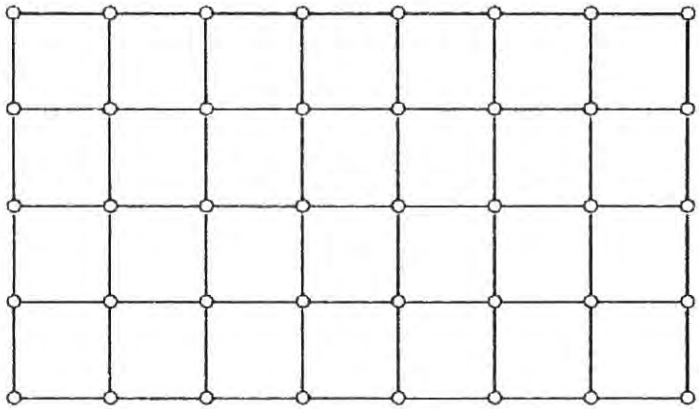

Figure 2. The planar grid $P_5 \times P_8$

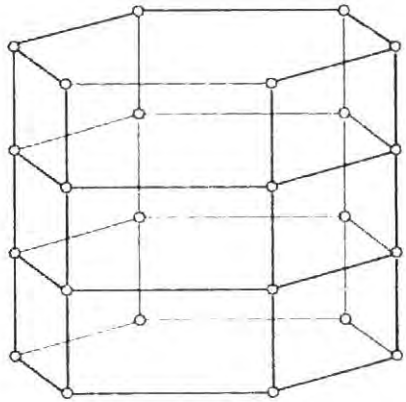

Figure 3. The prism $C_6 \times P_4$

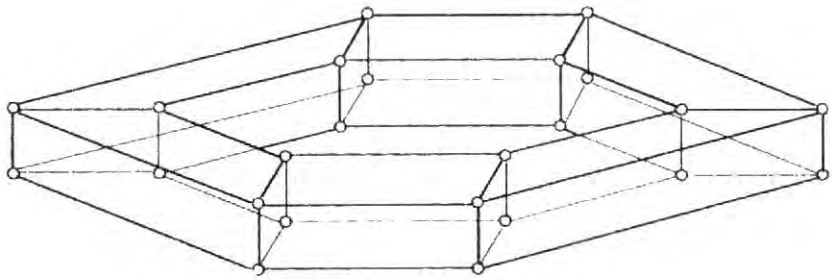

Figure 4. The toroidal grid $C_4 \times C_6$

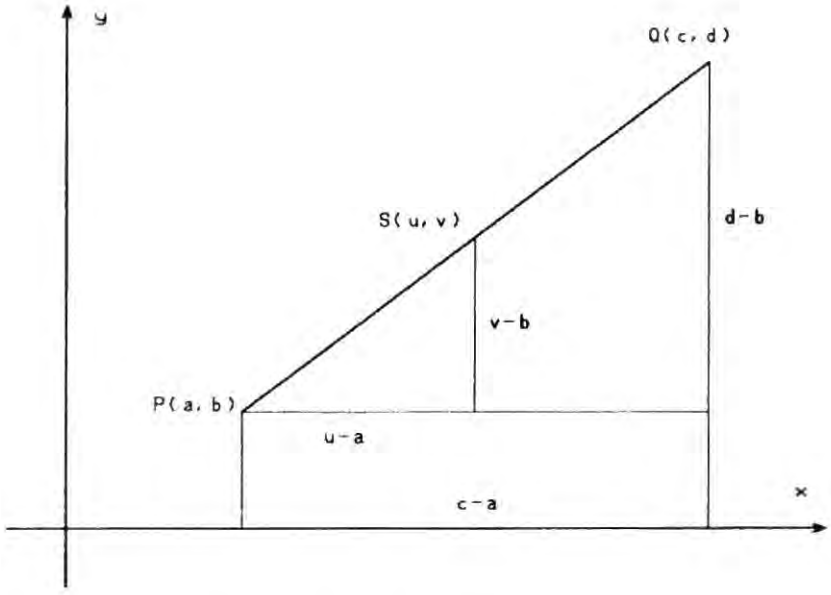

Figure 5. Three collinear points P, Q and S with integer coordinates

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Calculating Two-Dimensional Fourier Transforms I. Performance II. Normalization III. Graphics

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ABSTRACT

A scientific software for calculating the Fourier transforms of two-dimensional functions is presented in detail. It performs forward and inverse calculations, normalization and three-dimensional graphics display (modulus versus xy space). Implementation of software is achieved in a minimum environment of an IBM PC-XT microcomputer with a mathematical co-processor. Calculation of the transfer function of a grating spectrometer and high-pass image filtering are used as performance benchmarks. Considerations regarding proper normalization of result and its effective display in 3-D graphics (Floating Horizon algorithm) to minimize the number of hidden lines are also discussed.

INTRODUCTION

Analysis and processing of signals can be done both in their real (space/time) or frequency (spectrum) representation. Capability to examine the various properties in either domain is crucial in instrument design and signal/image processing. For instance,

filter design and bandwidth determination are done easily in the frequency domain of a given signal. While most signals are usually measured in the real domain, there are instances wherein their corresponding spectra are of primary interest. Such are in Fourier transform spectroscopy, image reconstruction using deconvolution techniques and data compression. The ability to transform efficiently and quickly any signal (real domain) into its equivalent spectral representation (frequency domain) and vice versa, depends on the availability of a reliable computer software that requires less complicated hardware without sacrificing speed, resolution and bandwidth.

In a linear-shift invariant (LSI) measuring apparatus, the measured signal o(**x**) results from the convolution of the original (input) signal i(**x**) with the point-spread function h(**x**), i.e. o(**x**) = i(**x**) * * h(**x**) where **x** is an N-dimensional real variable. Since h(**x**) will not have the properties of a Dirac delta function due to bandwidth limitation of real physical systems, the measured signal o(**x**) is never a one-to-one mapping from the input space to output space. Since the quantity of interest is i(x), a procedure to restore or recover it from o(**x**) must be implemented. This recovery procedure is easier done in the frequency domain because of the equivalent relation O(f) = I(**f**)H(f) where O(**f**),I(**f**) and H(**f**) are the Fourier transforms of o(**x**), i(**x**) and h(**x**), respectively and f is N-dimensional frequency variable. By calculating O(**f**)/H(**f**) and performing an inverse Fourier transform on the result, the input function i(**x**) is determined. Thus, knowledge of the transfer function H(**f**), brings about both maximum recovery of available information and complete understanding of the measuring instrument itself.

In this paper, we present a scientific software that was developed at the National Institute of Physics. It was written as a utility program in instrumentation design and requires a minimum hardware of an IBM PC-XT compatible with an 8087 math co- processor.

The paper is organized as follows: II. Fourier Transformation of Two-Dimensional Discrete Signals (Cooley-Tukey Algorithm); III. A. Implementation: Point Spread Function of a Spectrometer, B. A Scene Under High-Pass Filtering, IV. Normalization; V. Graphics Display With Minimum Hidden Lines.

FOURIER TRANSFORMATION OF TWO-DIMENSIONAL DISCRETE SIGNALS

The discrete Fourier transform in one-dimension is given by

$$X\left(\frac{m}{MD_x}\right) = A\sum_{k=0}^{M} x(kD_x)e^{-\frac{j2\pi mk}{M}} \qquad (1)$$

where A is a constant, M is the number of data points, D_x is the sampling interval and (k,m) are the indices of the discrete input data and the corresponding Fourier coefficients, respectively. For the two-dimensional function $z(kD_x,lD_y)$ the corresponding discrete Fourier transform $Z(m/MD_x,n/ND_y)$ is given by

$$Z\left(\frac{m}{MD_x},\frac{n}{ND_y}\right) = \sum_{l=0}^{M-1}\sum_{k=0}^{N-1} z(kD_x,lD_y)e^{-\frac{j2\pi mk}{N}}e^{-\frac{j2\pi ml}{M}} \qquad (2)$$

Implementation of Eqn. (2) involves the sequential calculation of one-dimensional Fourier coefficients. The number of operations required in determining the coefficients by direct calculation of Eqn. (1) obeys a square law relation with the number of data points M.

Cooley and Tukey[1] developed a more efficient (less number of operations and memory space for the same number of data points) algorithm for calculating one-dimensional Fourier coefficients for the special case when $M = 2^y$ i.e. the number of data points is expressible in powers of 2. Using the following notations:

$$X(m) = \sum_{k=0}^{M-1} x(k)W^{mk} \qquad (3)$$

where

$$W = e^{-\frac{j2\pi}{M}}$$

Equation (1) readily assumes the form of a matrix equation with the W^{mk} factorable into y separate square matrices containing only 1's, 0's, and W^{mk}'s. To carry out the factorization, a bit-

reversal of the ordering of the discrete Fourier coefficients is needed. The bit-reverse of a number is calculated by taking its binary equivalent, reversing the order of its bits and converting it back to its numeral equivalent.

Using $W^{mk} = W^P$ with $W^P = W^{P+M/2}$, then

$$x_1(k) = x_{l-1}(k) + W^P x_{l-1}\left(k + \frac{M}{2^l}\right) \quad (4)$$

and

$$x_1\left(k + \frac{M}{2^l}\right) = x_{l-1}(k) - W^P x_{l-1}\left(k + \frac{M}{2^l}\right) \quad (5)$$

Equations (4) and (5) constitute a dual-node pair and are the key to the efficiency of the FFT algorithm. Except for the sign, the values in the dual-node pair are periodic in $k + M/2^1$. Thus calculation involving dual node pairs effectively cuts the number of operations by half.

Direct calculations of the Fourier coefficients take cN^2 seconds (c is a proportionality factor) to accomplish. In the one-dimensional Cooley-Tukey algorithm, the number of necessary multiplications is reduced from N^2 to $M\gamma/2$. In two dimensions with $M = N$, the computation time for direct calculations would be proportional to $2M^3$ while the Cooley-Tukey algorithm takes only $M\gamma^2$.

We transcripted the Cooley-Tukey algorithm using Turbo-Pascal Version 5. The computer we used for calculations was a 10MHz IBM XT V6.0 equipped with an 8087 Math co-processor.

IMPLEMENTATION

Two specific cases are taken to show the performance of our software.

A. The point-spread function of a spectrometer employing phase-grating as aperture

The spectrometer functions as a coherent imaging system in which the entrance slit is imaged into the exit plane at unit magnification. The aperture of the optical system is assumed by the functional characteristics of the phase grating. Due to the finiteness of the aperture, the operational resolution (slit width,

aberrations and focal lengths are taken into consideration) of the spectrometer can not attain the resolution set by the grating relation, $\Delta\lambda = \lambda_{blaze}/pA$ where p is the diffraction order and A is the total number of equally-spaced lines in the grating.

The operational resolution is determined by calculating the point-spread function (PSF) of the spectrometer system. The PSF is calculated from the Fourier transform of the pupil function using the relation[2]; $h(x,y) = (1/f\ \lambda_{blaze})^2\ \mathbf{F}_{2D}$[grating function g(x,y)] where f is the focal length of the focusing mirror. The phase grating function (infinitely long in the x-direction) is given by[3]:

$$g(x,y) = \cos\left(\frac{\phi}{2}\right) + \frac{4i}{\pi}\sin\left(\frac{\phi}{2}\right)\sum_{q=0}^{Q}\frac{\sin(2q+1)\pi\frac{x}{a}}{2q+1} \quad (6)$$

where $\varphi = 4\pi e/\lambda_{blaze}$, e = line depth and a = width of line. Figure 5a shows the square (intesity distribution) of the Fourier transform of Eqn. (5) (line density = 1800 lines/mm or a = 0.56 μm, N = 1024, sampling distance a/4, λ_{blaze} = 550 nm, Q = 200). Since the slit function is essentially one-dimensional, the computation of the PSF simplifies into a one-dimensional exercise. Figure 5a illustrates the multiple images arising from the different diffraction orders with the zeroth-order image 60 times more intense than the first-order image. This is expected as there is no blaze angle introduced in the calculation.

Figure 5b shows a magnified view of the more useful first diffraction order. It is seen that the effective resolution of an infinitely narrow line in the entrance plane is limited due to the existence of a finite linewidth and side lobes. These are composite effects due to the finite value of Q in Eqn. (1) and the finite extent of the grating. Although gratings have been used as a dispersion optical element for years, its value as a light scatterer continues to attract researchers[4].

B. High-Pass Filtering of A Two-Dimensional Signal

High-pass filtering is a useful technique in locating the edges in the scene by suppressing irradiance of areas that are uniformly distributed [5]. Edges represent abrupt changes in the pixel-to-pixel

irradiance across the image field. Although high- emphasis filtering is also achieved through the application of a Laplacian operator on the data set (real domain), the same results can be obtained easily by deconvolution in the frequency domain.

Figure 2a shows radiance profile of the original scene (two keys, 128 x 128 pixels) taken by a CCD camera (Tektronix 1001 Video Camera, 490 x 384 pixels) and digitized using a Black/White frame grabber (Tektronix DCS01, 512 x 512 pixels). Its corresponding two-dimension spatial spectrum is presented in Figure 2b. Figure 2c shows the radiance profile of the reconstructed image done by performing an inverse Fourier transformation on the spectrum high-pass filtered with a step function ($f_{3\ dB}$ = 0.50 1/pixel size of CCD camera). Figure 2d shows a tonal profile of the filtered image.

NORMALIZATION

A waveform is normalized by assigning to its maximum amplitude the value of unity. The proportionality factor A in Eqn. (1) is introduced to make the analytic and discrete FT equivalent, i.e.

$$x(f) \cong AX\left(\frac{m}{MD_x}\right) \qquad (7)$$

Well-known references [6-8] have assigned different values for this constant: A = 1/M [*Brigham*] ; A = 1/sqrt(2p) [*Bracewell*] ; A = D_x [*W. Press et al*]. We have observed that different waveforms require different values of the normalization constant. Figure 3 illustrates the normalization constants for some waveforms (a: sinc, b: $sinc^2$ i, c: cos d: plane, and e: sombrero) commonly encountered in image/signal processing.

GRAPHICS

The ultimate goal in the graphics display of a three-dimensional field with two independent variables is always the full visualization of all its points. This is an impossible task to achieve

with the usual coordinate systems due to the depth of field requirement. The practical goal, therefore, is to find the coordinate orientation that displays the field with a sense of volume with the least number of points (or lines) hidden.

The plot of the set of one-dimensional Fourier coefficients is symmetrical with respect to M/2 with the points from M/2 to M-1 comprising the negative half of the graph.

In two dimensions, the negative and the positive half of the output are interchanged in both dimensions. Thus the output is quartered and appears at the corners. Besides bit-reversing, a procedure to interchange the quarters of the output must be included in any software implementation of the two-dimensional FFT.

The graphics output of the program is a three-dimensional surface in which the two space coordinates (x,y) are oriented, respectively, by angles α and β from the horizontal. The modulus of the Fourier coefficients constitute the vertical axis. Three-dimensional surface graphics draw the surface by planes. From the plane, which is apparently closest to the observer to the farthest plane, curves of the surface in that plane are drawn by drawing lines between sample points. To preserve the depth as well as to prevent confusion, curves hidden by other curves of closer planes must not be plotted. To accomplish this, a part of the Floating Horizon Algorithm was used[8].

This part of the floating horizon algorithm works as follows: if, at any given horizontal position, the vertical value of the curve in the current plane is larger than the maximum vertical value, or smaller than the minimum vertical value for any previous curve at that horizontal position, then the curve is visible; otherwise, it is hidden. In order to check whether a vertical value is smaller or larger than all previous values, the largest vertical value -- the upper horizon -- and the smallest vertical value -- the lower horizon -- are stored in arrays with horizontal position indices.

The graphics routine is implemented by two nested loops of two steps. The outer loop runs planes from the plane apparently closest to the observer to the farthest plane. The inner loop runs the horizontal positions from lowest to highest. Inside the inner loop are two steps: the first step computes the screen horizontal and vertical position of the current curve, from the modulus and the space coordinates via simple trigonometric conversion of the three dimensional data to the flat screen and simple screen scaling; the second step does a floating horizon test on the current screen value of the curve and plots the appropriate segments. An

unscrambling procedure first fixes the order of the bit-reversed output and then interchanges the halves in x and y.

In general, the discrete Fourier coefficient is a complex quantity. What we plot as output is the modulus of the real and imaginary FT. Figure 4a-c illustrates how the visual perception a one-dimensional $sinc^2$ function varies with the values of (α,β): A. $\alpha = 45°, \beta = 0$; B. $\alpha = 0°, \beta = 45$; C. $\alpha = 30°, \beta = 15°$; and D. $\alpha = 20°, \beta = 20°$. Note that the choice of optimal information display depends on the symmetry of the function.

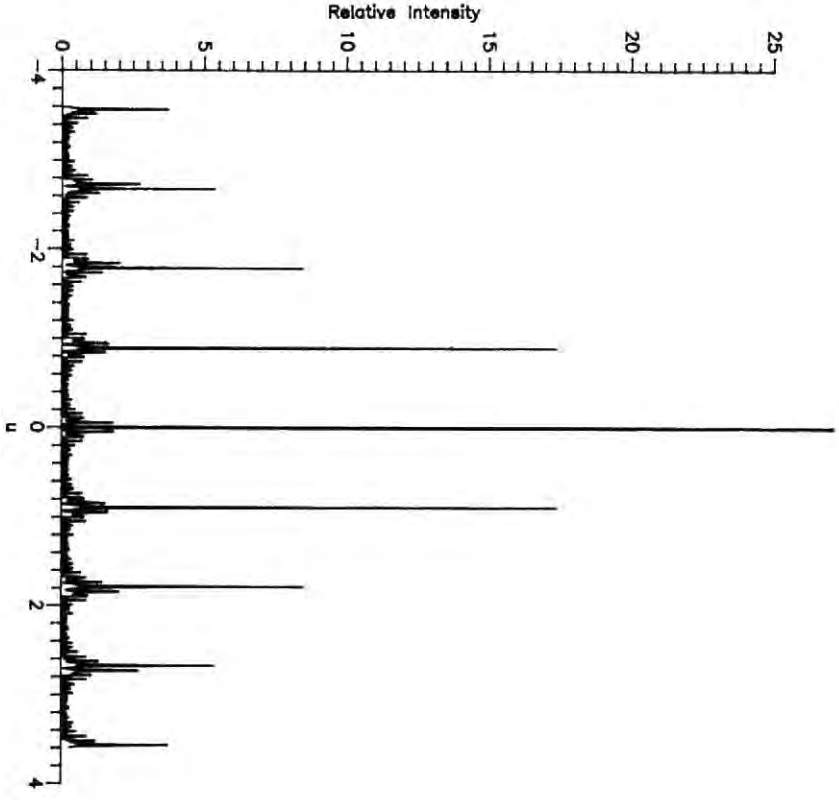

Figure 1a. Multiple images of a Dirac δ (infinitely narrow slit) when the aperture is a phase grating (line density = 1800 lines/mm, N = 1024 points, sampling distance = 0.14μm, period = 0.56 μm, λ=550nm, Q = 200)

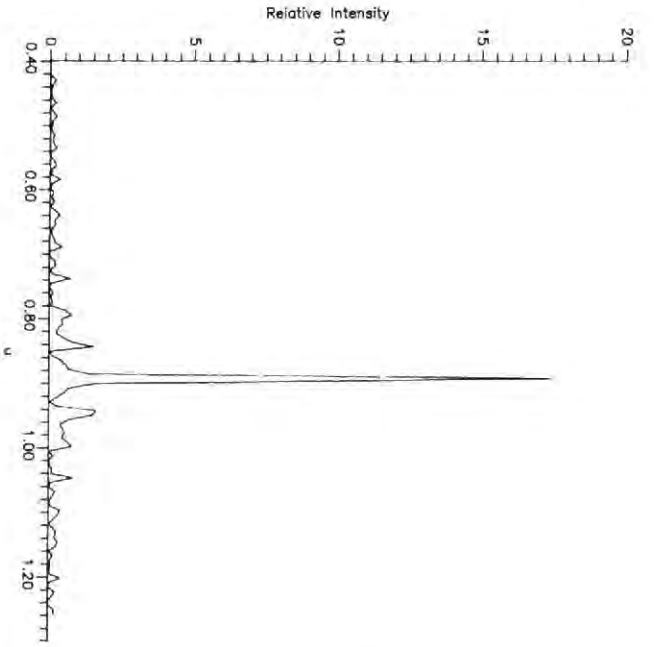

Figure 1b. Magnified version of the first order image

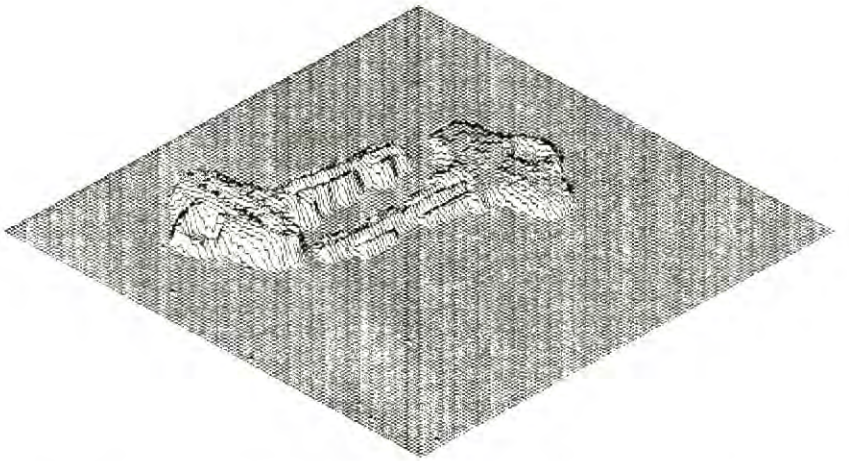

Figure 2a. Intensity profile of original image (two keys)

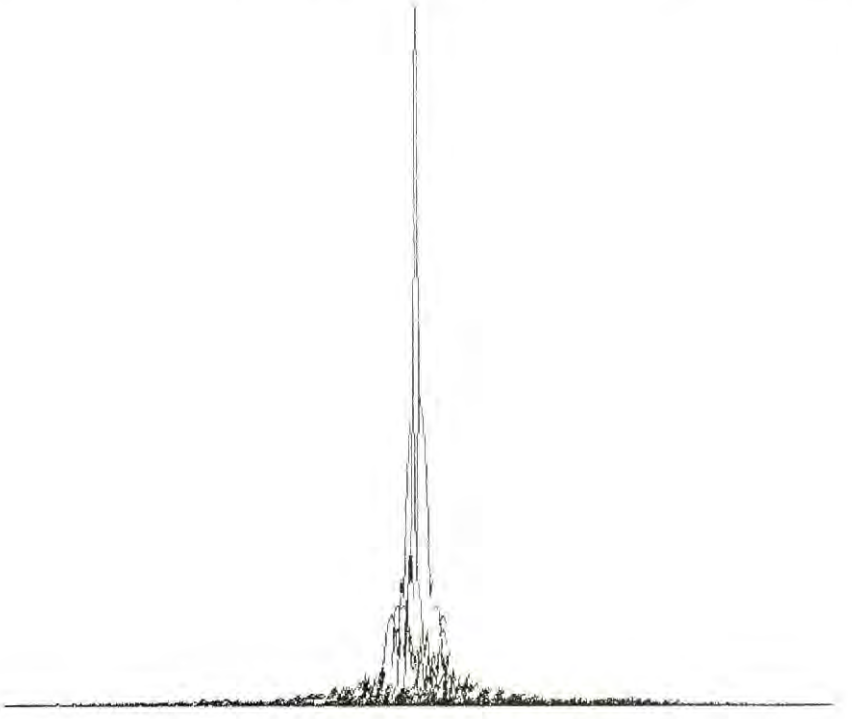

Figure 2b. Two-dimensional Fourier transform of image

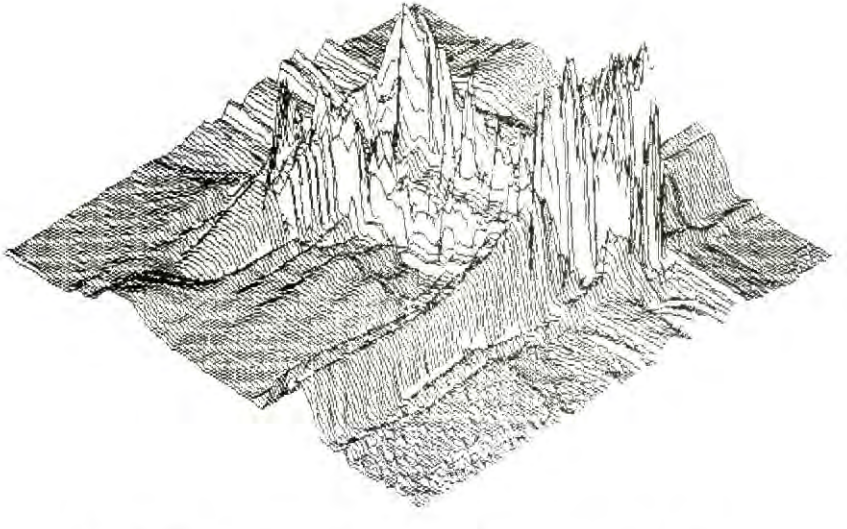

Figure 2c. Intensity profile of high-pass filtered image

ii. filtered image

i. original image

Figure 2d. Tonal version of original and filtered image

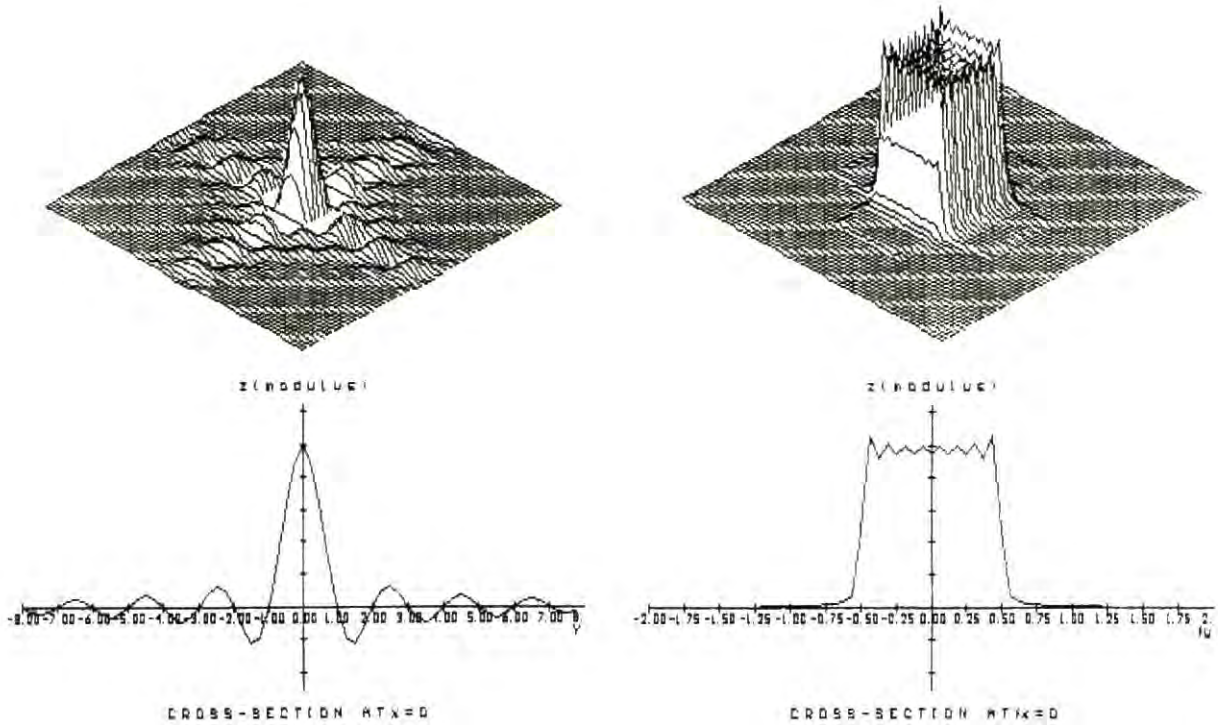

Figure 3. Normalization constants for various waveforms: a) sinc, A = DxDy. Left: Input Function, right: FT of Input Function, top: 3-d graph, bottom: 2-d view at x + 0

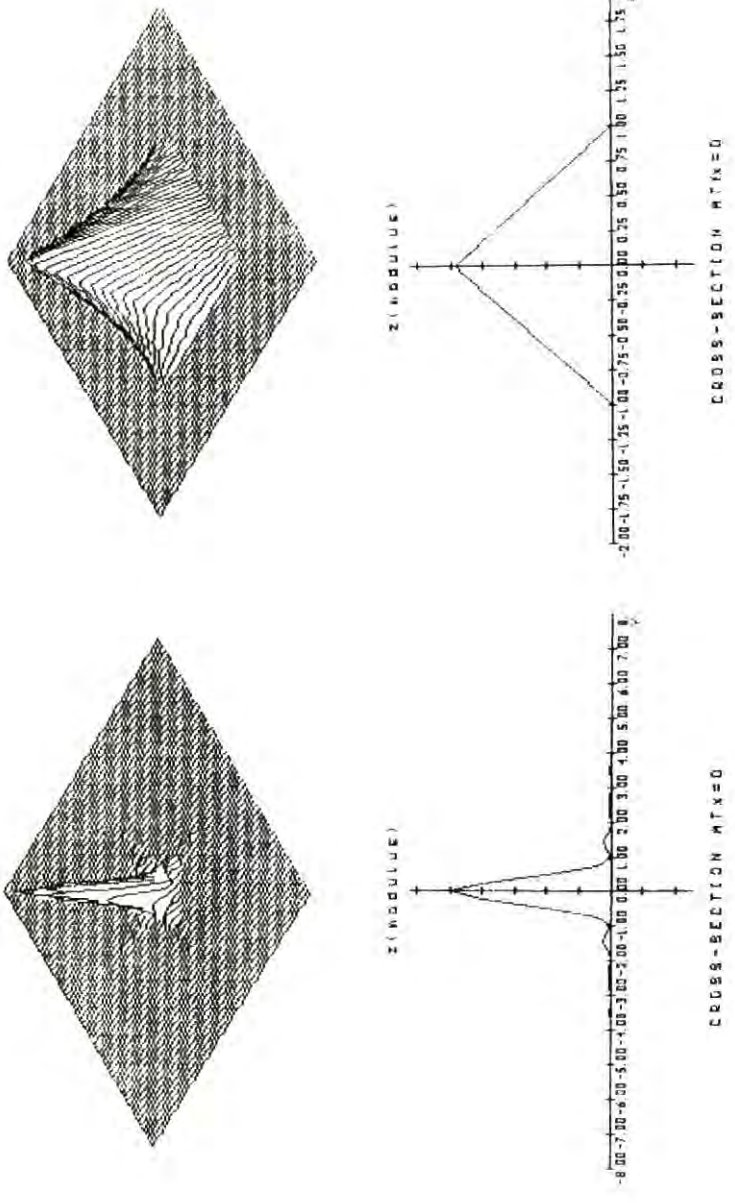

Figure 3. b) sinc^2, A = DxDy

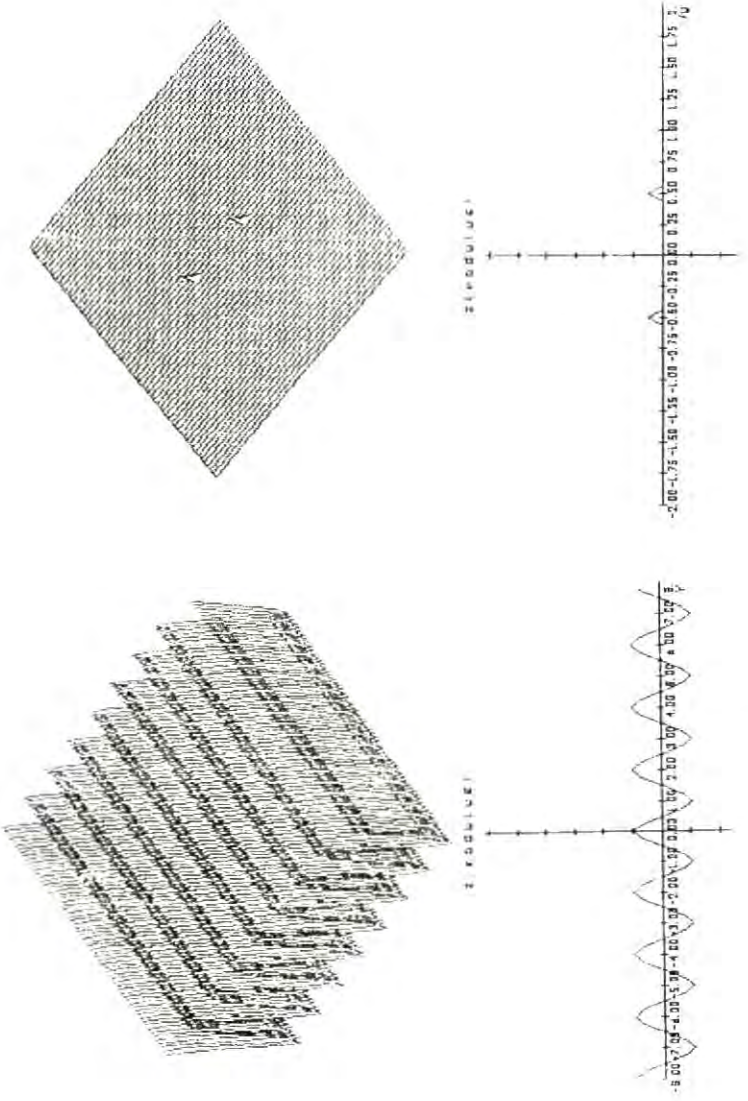

Figure 3. c) cos(y), A = 1/(NxNy)

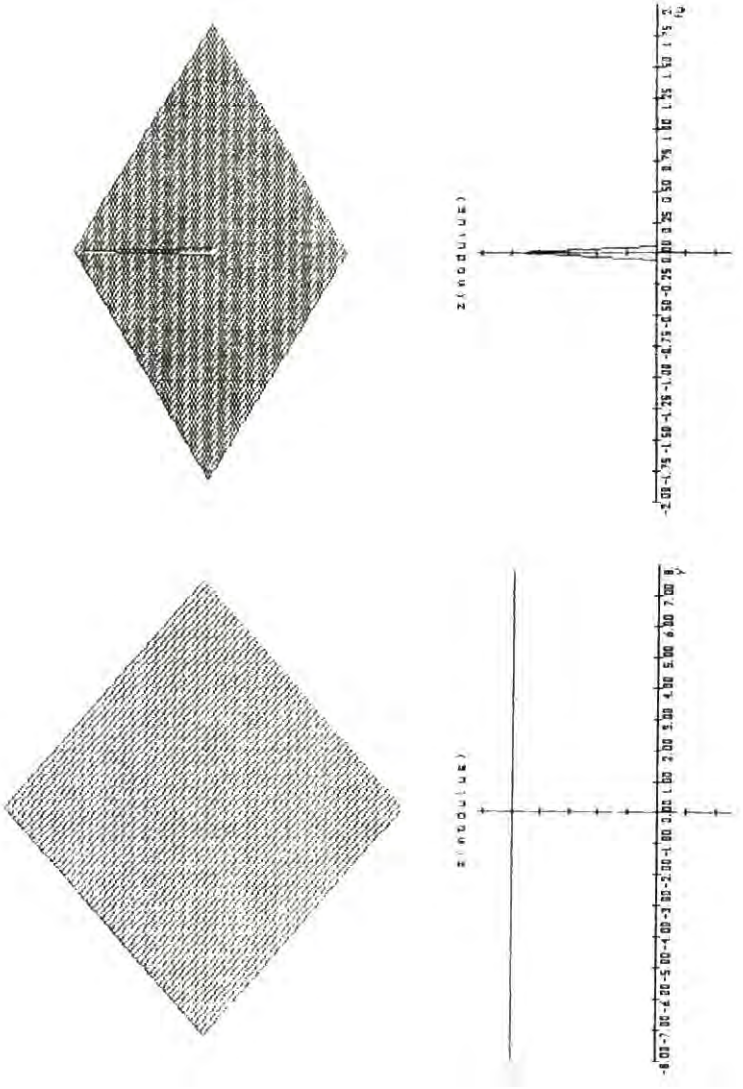

Figure 3. d) plane at z = 5, A = 1/(NxNy)

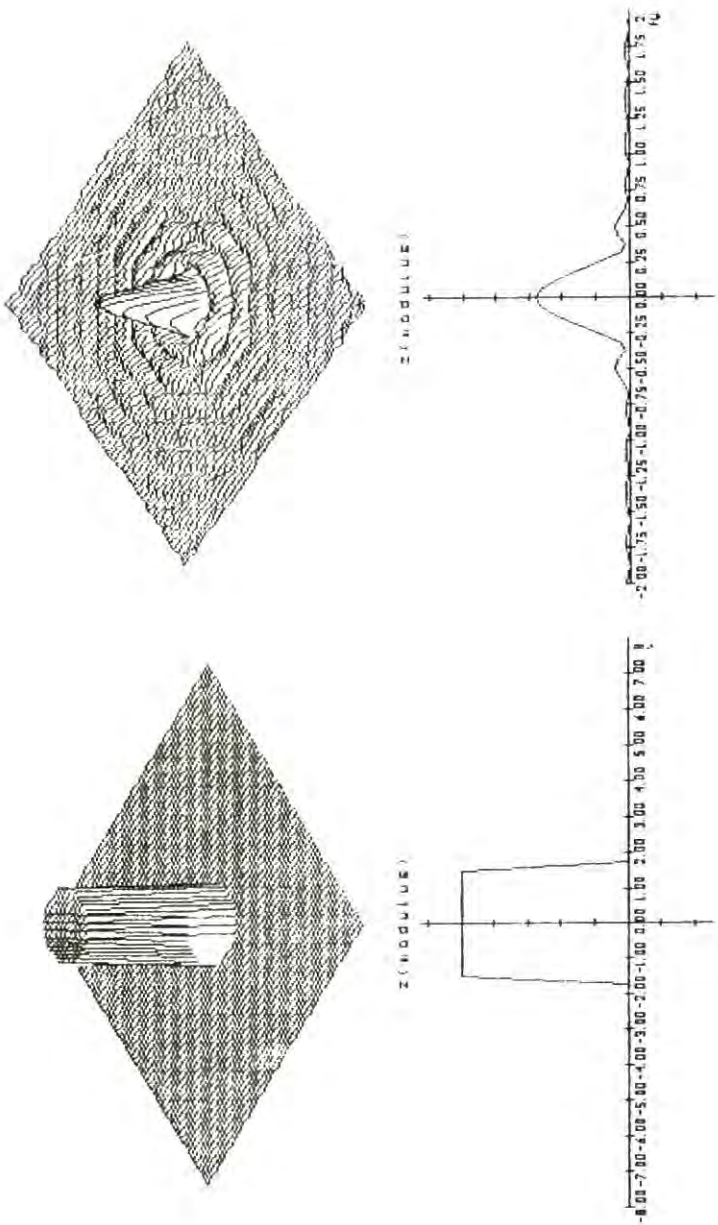

Figure 3. e) sombrero function, A = Dx/Nx

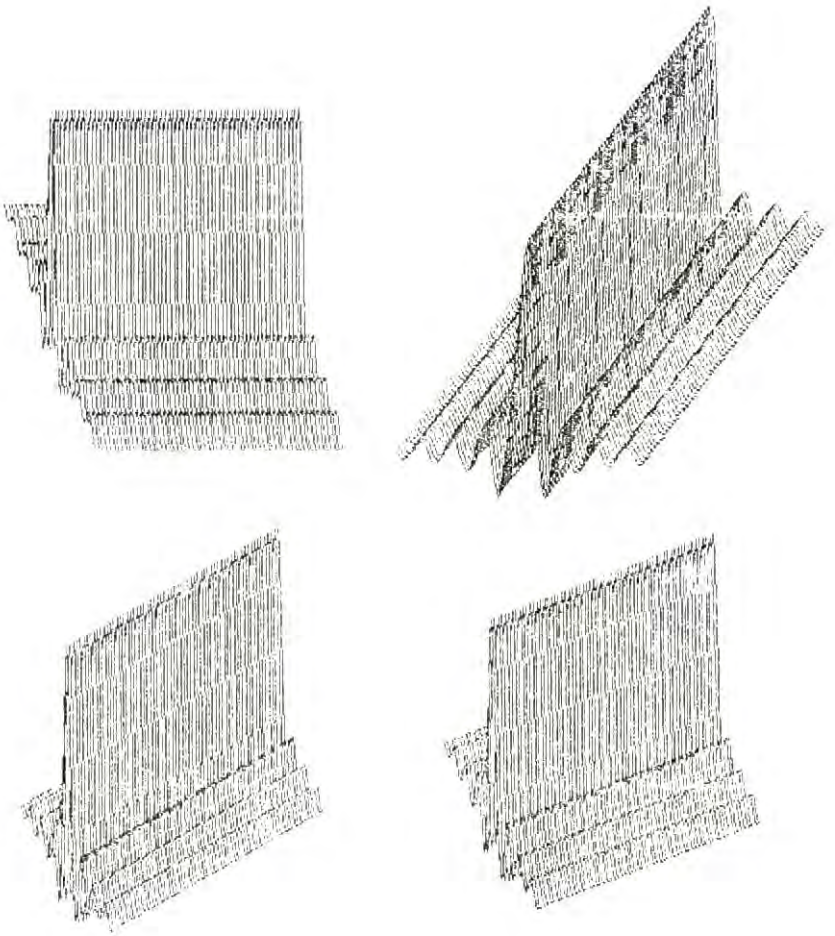

Figure 4. View of a one-dimensional sinc funtion from different perspectives: clockwise from top-left, $\alpha = 45$ deg, $\beta = 0$ deg; $\alpha = 0$ deg, $\beta = 45$ deg; $\alpha = 30$ deg, $\beta = 15$ deg; $\alpha = \beta = 20$ deg.

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Electrochemical Synthesis of Electronic and Ionic Conductive Polymer Composite Polyaniline/PEO Network

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ABSTRACT

A bilayer composite of conducting polyaniline was prepared by electrochemical polymerization of anilinium salt (dissolved in appropriate solvent like DMF, methanol or water) in poly(ethylene oxide) PEO network. When washed, dried and doped with $LiClO_4$, the bilayer showed electrochemical activity corresponding to doping and undoping reactions in the solid state. The bilayer was characterized by FT-IR (reflection), UV spectroscopy and microscopic examination while the electrochemical activity in the solid state was observed by cyclic voltammetry. The electronic conductivity was measured using a four-point probe tester while the ionic conductivity of the polymer electrolyte was measured by AC impedance. Cyclic voltammetry of the composite conductive polymer and polymer electrolyte in the solid state against Pt produced oxidation peaks at -0.26 and +0.26 V. Against Li, broad oxidation peaks appeared between 0.5 and 4.5 V; doping efficiency in the Li cell reached 95% between 2-4 V at a scan rate of 100 mv/s. The color of the conducting poly-

mer as anode changed from transparent yellow to green and then to blue during the doping process. Cole-cole plots of the composite obtained by AC impedance measurements showed an arc at high frequency region (> 2.82 MHz) due to polymer electrolyte impedance. Another arc due to impedance of the doping reaction, at lower frequency (> 20 Hz) followed as doping of the conductive polymer proceeded; the arc is then joined by a Warburg line, which characterizes diffusion controlled kinetics at the low frequency region (< 20 Hz).

INTRODUCTION

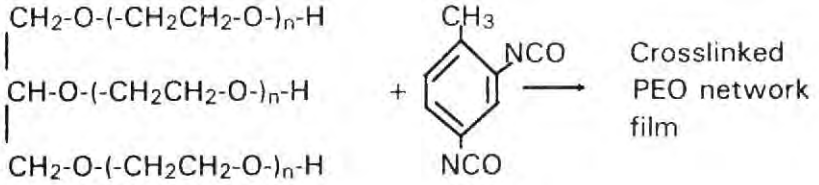
Since the discovery of the conducting properties of polyacetylene by Shirakawa (**11**), conductive polymers have caught the attention of many scientists because of their fundamental significance and their commercial application. Besides conductivity, they have several advantages over metals such as their lightness, and their interesting optical, mechanical and chemical properties. Conducting polymers like polyparaphenylene, polythiophene, polypyrrole and polyaniline have received considerable attention because of their particular combination of properties. However, a common disadvantage of these conducting polymers is their insolubility such that they can not be melted and can not be cast in molds because they are mechanically weak and brittle. Most of them slowly decompose on exposure to air or water, with the exception of polyaniline which was reported to be very stable (**3**). But like the other conductive polymers, polyaniline is brittle and insoluble in most common organic solvents due to stiffness of its backbone.

In order to improve the mechanical property of polyaniline we fabricated a composite of polyaniline and a mechanically strong poly(ethylene oxide) network polymer. This provided a lightweight, self-supporting and conducting bilayer film with a particular advantage over other composite films; the PEO network polymer can be doped with $LiClO_4$ as electrolyte and function as a solid ion-conducting polymer. The bilayer, therefore, can be used as a component in an electrochemical solid state device like a secondary battery.

Doped PEO network is an example of a polymer electrolyte which is solvent-free, ion-conducting and is capable of replacing normal electrolyte solutions. Most polymer electrolytes are solid solutions of alkali metal salts, in which polymers behave like solvents toward incorporated salts. They have a relatively high ionic conductivity of about 10^{-5}S cm^{-1} at room temperature. Unlike inorganic solid electrolytes, polymer electrolytes can absorb electroactive molecules whose redox could be investigated *in situ*, e.g. pyrrole converted to polypyrrole in PEO (**12**). In general, polymer electrolytes can be processed into thin films and have a wide potential window and good compatibility to electrodes.

EXPERIMENTAL

Synthesis of the PEO network film. The network polymers were synthesized according to the method of Watanabe et al. (**17**). Thus, the triol-type PEO (Dai-ichi Kogyo Seiyaku Co., average mol wt = 3080) was reacted in stoichiometric amounts with freshly distilled 4-methyl-1-1, 3-phenylene diisocyanate at 75°C, cast on glass, kept at the reaction temperature under dry nitrogen atmosphere for 72 hours and then *in vacuo* for 24 hours.



Preparation of crosslinked PEO network polymers

The cross-linked polymers were then washed with distilled acetone several times to remove the unreacted reagents. The resulting PEO network films with about 0.4 mm thickness were dried *in vacuo* at 80°C for 72 h. The weight swelling ration toward water at 27°C averaged about 3.0.

Preparation of network PEO-lithium salt complexes. Preweighed films (13 mm dia) were immersed in 0.1M solution of the

lithium salts in acetone for 15 minutes then dried under reduced pressure at 80°C for 72 h. The concentrations of the lithium salts in the PEO-LiX complexes were calculated as the molar ratio of LiX to the repeat unit of PEO ([LiX]/[EO unit]). The PEO-LiX complexes were kept *in vacuo* at room temperature before the experiments were done. The solid state cell of lithium/polymer electrolytes was handled in all cases under dry argon atmosphere.

Preparation of the Polyaniline/PEO network bilayer. The PEO-LiX complex films were applied with: (a) first method: distilled aniline and equilibrated in nitrogen atmosphere saturated with acetonitrile to regain their original shape, the slightly swollen films were then sandwiched between Pt as cathode and ITO as anode and polymerization allowed to proceed by galvanostatic or potentiostatic conditions; (b) second method: solution of CF_3SO_3H:aniline (1:11) and also equilibrated in acetonitrile vapor; (c) third method: solution of CF_3SO_3H:aniline or HCl:aniline (1:1) in dimethly formamide, methanol or water. Besides galvanostatic and potentiostatic preparations, the polymerization was also followed by cyclic voltammetry in a cell with platinum electrodes and Ag as quasi-reference electrodes (Fig. 1).

The elctrochemical activity was correlated with electronic conductivity of the PAN layer using a four-point probe tester (Loresta MCP-Tester). Film thickness was controlled by monitoring charges in coulombs consumed during electropolymerization.

All reagent grade salts were dried under a vacuum for 24 h, at 160°C for LiCl, LiBr, LiI (Kishida), LiSCN and NaSCN (Kanto), at 120°C for KSCN (Kanto), $LiBF_4$ (Alfa), $N(Bu)_4BF_4$ (Tokyo), LiPicrate, $LiCF_3SO_3$ (Aldrich) and at 140°C for $LiClO_4$ (Chemeleon), $N(Bu)_4ClO_4$ (Tokyo).

Cyclic voltammetry experiments were performed with a potentiostat/galvanostat HA-301 coupled with a function generator HB-104, coulomb/ampere-hour meter HF-201 and IR compensation instrument HI-203 from Hokuto Denko Ltd. Data were recorded with a Rikadenki X-Y recorder. The electrochemical cell was housed in a constant temperature oven with Elecon temperature controller from Yashida Works. Polymerization at potentiostatic conditions was done with a DC voltage standard type 2851 (Yokogawa Electric Works Ltd.) coupled with a Digital Multimeter TR 6843 (Takeda Riken). The uv-absorption spectra of the bilayer were recorded with a UV Shimadzu Graphicord UV-240. The fourier transform infrared spectra of the two sides of the bilayer were determined by reflection technique. Micro-

graphs were taken with Nikon FX- 35A on a slice of a bilayer formed at 1.3 V by passing 0.15 C/cm^2. The lithium cell (Li/$LiClO_4$ [PEO-network]/PAN) was prepared in a dry box (Eicoh Shokai Co.) under argon atmosphere. AC impedance measurements were made on the cell with a Yokogawa Hewlett-Packard 419 A impedance analyzer over a frequency range of 5 to 1.3 x 10^7 Hz at 12 mV.

RESULTS AND DISCUSSION

Solid PEO network is known to function as a polyelectrolyte only when it contains a salt that dissociates into cations and anions. The rate of polymerization of aniline, therefore, in a PEO network would greatly depend on the ionic conductivity of the polyelectrolyte. A study on the best concentration of a salt like $LiClO_4$ for high ionic conductivity polymer electrolyte suggested a concentration of around 0.02 [$LiClO_4$]/[EO unit] in PEO network (**17**). The polymerization of the monomers in the polymer electrolyte as reported in the preparation of a polypyrrole bilayer composite (**12, 18 15**) can be followed by cyclic voltammetry (**7**). However, in our experiment, after application of freshly distilled aniline on the PEO network and equilibration in acetonitrile atmosphere, the redox potentials could not be observed even after 66 cycles in the potential range -1 to +1 V (Ag as quasi reference electrode) at 30°C as shown on Fig. 2. The redox potentials only appeared at 60°C and a clearer voltammogram was observed at 70°C. This might be due to the low ionic conductivity of the polyelectrolyte at ambient temperature which consequently affected the elctrochemical activity of aniline. Even if there was some evidence of polymerization at 70°C, this method of bilayer preparation is nevertheless impractical and inefficient.

Electroactive polyaniline is generally prepared from aqueous electrolyte solution containing strong acid (**8, 4**). The absence of acidic protons in the system could be one reason for this low electrochemical activity at ambient temperatures. In fact it has been observed that PAN films deposited from non-aqueous solutions such as $LiClO_4/CH_3CN$ were electroinactive (**14, 6**), while PAN deposits in non-aqueous propylene carbonate solution with some organic acids were reported to be electroactive (**7**). The addition of a small amount of CF_3SO_3H to aniline (1:11) followed by its application on the PEO network film containing

$LiClO_4$ salt resulted in the appearance of redox potentials with large anodic and cathodic currents even at 30°C as seen in Fig. 3. The figure also shows that PAN polymerization increased proportionately upon cycling and the doping-undoping process of anions observed between -0.5 and +0.6V (Ag). The anodic peak current at about 0.1 V also increased with an increase in cycle number, which induced the growth of PAN. This result indicates that an electroactive PAN deposited from the polymer electrolyte even at ambient temperature conditions.

The ionic conductivity of polymer electrolytes, as well as the rate of PAN polymerization, is known to vary with the kind of electrolyte salt complexed. Table 1 shows a qualitative estimate of the rate of PAN polymerization along with the different salts used, as well as an indication whether or not the system is electrochemically active. Fast polymerization indicated the formation of a dense dark spot just below the surface of the Pt anode after cyclic voltammetry. With slow polymerization, only a portion directly below the Pt anode appeared dark but the dark spot did not appear dense. Very good electrochemical activity signified the appearance of redox peaks with increasing anodic current during cycling. As the table shows, fast polymerization coupled with high electrochemical activity was observed when the salts used were $LiClO_4$, $LiCF_3SO_3$, $LiBF_4$, LiPic and LiSCN; fast polymerization but low electrochemical activity were observed when the salt used was LiBr; and slow polymerization and low electrochemical activity were observed when the salts used were KSCN, NaSCN, LiCl and LiI. The difference in properties could be partially explained by the difference in ionic conductivity of the PEO network/LiX polymer electrolytes. For example, the ionic conductivity of the PEO network polymer electrolyte containing $LiClO_4$ or $LiBF_4$ is in the order of 10^{-5}S cm^{-1}; LiBr, in the order of 10^{-6}S cm and LiCl, in the order of 10^{-7}S cm^{-1}. This result also parallels that of pyrrole poly- merization in the same polymer electrolytes (**18**).

However, the resulting data on the electronic conductivity of the PAN layer were unexpected. Fast polymerization and good electrochemical activity did not mean high conductivity of the PAN layer. Surprisingly, the PAN layer with slightly high conductivity was produced when polymerization was slow and elctrochemical activity was very low. Furthermore, the slightly conductive PAN was made of a dark spot surrounded by a violet shade. The expected color of the PAN layer was dark green, the usual color of conductive PAN obtained from electrochemical prepara-

tions in acidic solutions. Non-conducting violet PAN is known to be produced in aprotic or basic solutions. So the most probable reason why no conducting PAN was produced is perhaps the basicity of the applied aniline solution where basic aniline was in excess, although CF_3SO_3H was present as a salt.

A solution to this problem is to increase the concentration of the acid. The ratio of molar concentration of the acid to aniline in a study (**7**) using aprotic solvents was 2:1. However, when this solution was applied to the PEO network, it lost its plasticity and disintegrated. This is most probably due to the hydrolysis of the amide bonds in the PEO network catalyzed by the acid. So a 1:1 molar ratio of aniline and CF_3SO_3H, which is practically a salt, was prepared and dissolved in an appropriate solvent for easy application on the PEO film. It turned out that the solvent was also important in ensuring the production of a conductive PAN layer. When acetonitrile was chosen as the solvent, no redox peaks could be seen in its cyclic voltammogram and no polymerization could be detected. This was not the case when dimethyl formamide was used. The PAN film that resulted was blue green (prepared potentiostatically at 2.0 V) and was much more conductive than the previously prepared PAN. Its electrochromic property was observed as the applied voltage was changed from -1 to + 2.5 V, where changes in colors from transparent yellow to green and then to blue took place. This result showed that what was really needed was an aniline salt dissolved in a non-basic solvent to produce a green and conductive PAN layer.

The most available aniline salt is aniline•HCl which is soluble in DMF, methanol or water. The green PAN layers made from aniline•HCl in the above solvents were similar in electrochromic property and possessed high conductivity as the PAN made from aniline CF_3SO_3H. Electronic conductivity of the PAN layer as high as 4 S cm^{-1} could be obtained from aniline•HCl by cycling from -1 to + 1 V(Ag). The cyclic voltammogram of aniline•HCl polymerization is shown on Fig. 4. When methanol was used as solvent, a high electrochemical activity was observed with aniline•HCl even in the absence of the alkaline salts.

The voltammogram showed two pairs of redox current peaks: the anodic peaks at + 0.33 V and + 0.54 V, and the cathodic peaks at 0.22 V and 0.45 V,with Ag as a quasi-reference electrode. Similar behavior of redox current peaks of polyaniline was reported in the literature where two processes were proposed

by Watanabe et al. (**16**) for the redox reactions. One process associated with the first oxidation involved the formation of a radical cation at N-position and the other associated with the second oxidation involved the formation of a diimine structure passing through an intermediate dication structure.

To prepare the bilayer composite for further study, electrochemical polymerization was carried out in galvanostatic conditions. A scheme of this preparation technique is shown in Fig. 5. The quantity of current used, measured in coulombs, was assumed to be proportional to the weight of PAN deposited. Fig. 6 shows that as the amount of PAN measured in coulombs increased, the electrical resistance of the PAN layer decreased. However, when the bilayer film was vacuum dried much of the original conductivity was lost. Even more was lost when the film was vacuum dried at an elevated temperature. This is due to the loss of the solvent which improves the electronic conductivity of the PAN layer, and the loss of protons and chloride ions from the evaporation of HCl molecules which act as dopants of PAN.

Another way by which the original conductivity of PAN was lost was by washing PAN with water in the process of removing the unreacted aniline•HCl. The original green color of PAN also changed to violet with corresponding loss in conductivity. Figure 7 shows the different uv absorptions of the green and violet bilayer films. Similar observation was reported on the behavior of PAN prepared from acidic conditions on Pt or ITO substrate (**4, 2, 8**). To return the green color and electric conductivity to the PAN layer, the bilayer was immersed in a 0.1 N HCl solution and vacuum dried at 80°C for three days.

The surface and a cross-sectional view of the bilayer film is shown on Fig. 8. The PAN layer was prepared by passing 0.15 C cm^{-2} at a constant current density of 75.3 uA cm^{-2}. From the cross-sectional view, the thickness of the green PAN layer deposited was estimated to be about 0.01mm on top of the much thicker transparent PEO network (~0.4mm). The surface view shows the PAN layer as a crumpled sheet on top of the smooth and clear PEO network film. In fact, one side could be distinguished from the other side with the naked eye by noting which side is shiny and which side is dull. This surface state of the PAN layer could be due to the shrinking of the bulk of the PEO network which formerly expanded from the presence of the solvent while PAN was deposited. While the PEO network swelled and shrank without affecting its surface features too much, the thin PAN layer

above it did not exhibit equal flexibility. One other reason perhaps for the lower conductivity of the vacuum dried film may be the increased distance that the electrons would have to travel from one electrode to the other electrode of the conductivity probe along the warped surface of PAN compared to that along the stretched PAN surface on a solvent swollen PEO network. It was also observed that the PAN layer could not be easily scraped off from the PEO network film which could mean that the PEO polymer strands might be interspersed among PAN strands on the surface. Electron microscopy of the sample would be able to clarify this structural relation between the PAN layer and the PEO network film.

More evidence of the bilayer structure of the composite was exhibited by reflection FT-IR spectra of the two sides of the film shown on Fig. 9. While the PEO network side showed principally the C-O-C absorption (1100 cm^{-1}), the PAN side showed characteristic absorptions due to PAN vibrations (1565, 1480, 1300 and 1155 cm-) similar to the FT-IR spectra during the first oxidation process (conducting form) of PAN prepared in acidic aqueous solutions (**9, 5**). The 1560, 1480 and 1300 cm^{-1} absorptions corresponded to quinoid rings of the polaronic intermediate (**9**). The FT-IR spectra of PAN doped with ClO_4- ions [after CV in the cell Li-$LiClO_4$(PEO network)/PAN] showed a more intense absorption peak at 1155 which compares well with the presence of an intense peak at the same region of the PAN prepared when $LiClO_4$ was used as electrolyte in non-aqueous solvent (**7**).

The principal application of this bilayer composite PAN/PEO network is in the fabrication of solid state electrochemical cells of which the secondary battery is the best example. Addition of a lithium salt to the PEO network of the bilayer and lithium metal as cathode would already constitute a lithium secondary battery in the solid state. Two main factors influenced the behavior of this kind of solid state cells. They were the temperature of operation and the nature of the dopant. The former was related to the fact that the conductivity of the PEO network-based polymer electrolyte was quite low at room temperature and became appreciable only at above 40°C, i.e. after transition into the amorphous phase. The ionic conductivity of the polymer electrolyte $LiClO_4$/PEO network generally increased with temperature as shown in Fig. 10. However, the ionic conductivity of the bilayer composite prepared at different current densities, was greater than the bare polymer electrolyte in the range of tempera-

tures determined. This shows that the ionic conductivity could even be improved by the presence of the PAN layer.

The second factor is related to the kinetics of the electrochemical doping process which is controlled by the diffusion of the counter ions X^- in the bulk of the polymer as the experimental results show. The cole-cole plot of the composite obtained by AC impedance measurement shows three regions (see Fig. 11). The first region is a high frequency arc (>2.82 MHz) attributed to the impedance of Li-polymer electrolyte interface. As the doping of the conducting polymer proceeded, a second arc (>20 Hz) appeared at medium frequencies, which may be attributed to the impedance of the doping reaction at the PAN- polymer electrolyte interface. The arc was then followed by the onset of a Warburg line, which characterized diffusion-controlled kinetics and indicated that at low frequencies, the kinetics of the electrochemical process were controlled by the diffusion of the counterions. The electrode process for the composite can plausibly fit a typical Randles-type equivalent circuit as shown in the figure where R_u, R_{ct}, Z_w and C_{dl} correspond to the polymer electrolyte resistance, the reaction resistance, the Warburg impedance and the double layer capacitance, respectively.

Cyclic voltammetry of this solid lithium cell prepared in a dry box under argon atmosphere is shown in Fig. 12. The appearance of redox peaks between 2-4 volts indicated the doping and undoping process of the PAN by the ClO_4- i ons and the transformation of lithium ions to metal and back to ions. From the cyclic voltammogram of the cell, the % efficiency of the doping process was determined from the ratio of the number of coulombs that passed during the anodic process over that of the number of coulombs that passed during the cathodic process. On the other hand, the % doping level of the doping process was obtained from the number of coulombs passed during the anodic process over that of the number of coulombs used during the preparation of the PAN layer. The results summarized on Table 2 were obtained from voltammetric data at 70°C and 100°C. The doping level is an important parameter in polymer elctrode batteries since it is directly related to the cell capacity which is in turn related to the cell energy density. As seen from the results on Table 2, the performance of this Li/PAN cell with PEO network/$LiClO_4$ as polymer electrolyte would be slightly better at a higher temperature because of the improvement of its doping level. This improvement of doping level could be attributed to the higher

mobility of the elctrolytes and thus, to the ionic conductivity of the PEO network at higher temperatures as previously discussed above.

The cyclic voltammogram above had broad features when compared with that made when the PAN film was thinner (0.15 C cm^{-2}) in the cell configuration Pt/$LiClO_4$ (PEO network)/PAN where both oxidation and reduction peaks were seen in Fig. 13. Thinner conducting polymer in a cell is known to elicit clearer cyclic voltammogram as thicker films are subject to uncompensated ohmic resistance **(10)**. UV absorptions of the bilayer film as the cell potential was changed from -1 to + 1 V (against Pt), corresponding to the undoped to doped state, are shown in Fig. 14. A large change in the base line of the spectrum of the undoped state was observed as the film was being doped. There was also a corresponding decrease in the intensity of absorption in the reigon from 300 nm to 500 nm as the doping of the PAN film progressed. Similar uv behavior was reported when doping of PAN was studied (**13**). The doping stages from the undoped to doped states corresponded to changes in color of the film from transparent yellow to green and finally to blue.

CONCLUSION

Finding a way of preparing a bilayer composite of conducting PAN with PEO network is not as easy as preparing the polypyrrole/PEO network film. Simple application of aniline on polymer electrolyte $LiClO_4$/PEO network followed by electro-chemical method of preparation does not produce the bilayer because of the very low electrochemical activity of aniline at ambient temperatures even in the presence of $LiClO_4$. Neither is the electrochemical activity of aniline at room temperature in the presence of a small amount of acid CF_3SO_3H a guarantee for a conductive PAN layer. Only when an aniline salt, e.g. aniline•HCl or aniline•CF_3SO_3H was dissolved in appropriate solvent prior to application was the preparation of the bilayer composite of conducting PAN/PEO network successful. The solvent was also critical , as the use of acetonitrile did not lead to polymerization as when the solvents used were DMF, methanol or water. Once the bilayer could be prepared and the conductivity of the PAN layer maintained, the study for its application in solid state

electrochemical cells could be started. Thus, the ionic conductivity of the cell with $LiClO_4$ as electrolyte at different temperatures could be determined. The kinetics of the electrochemical doping process could be assessed by ac impedance. Electrochemical data on the cell could be collected for practical evaluation and spectroscopical instruments could be used to characterize the doping processes. These preliminary data on the electrochemical activity of the bilayer composite open the way for its application to solid electrochemical devices.

ACKNOWLEDGMENT

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Table 1. Effect of electrolyte salts on polymerization, electroactivity and conductivity of polyaniline (PAN) prepared from Aniline:CF_3SO_3H (11:1)

salt	polymerization[a]	electroactivity[b]	resistance[c] ($x10^6$S cm^{-1})
$LiCF_3SO_3$	fast	very good	1.0
$LiBF_4$	fast	very good	1.2
LiPic	fast	very good	$>>10^6$
LiClO4	fast	very good	$>>10^6$
LiSCN	fast	very good	1.2
LiBr	fast	good	$>>10^6$
KSCN	slow	good	0.004
NaSCN	slow	not observed	1.2
LiCl	slow	not observed	$>>10^6$
LiI	very slow	not observed	0.59

[a] Polymerization is indicated by the presence of a dark spot just below the Pt anode; fast, dense dark spot; slow, only a portion appeared dark or the dark spot did not appear dense; very slow, dark point or points only.

[b] Electroactivity was deduced from the cyclic voltammogram; very good, large redox currents; good, small redox currents.

[c] Surface resistance was determined with a four-point probe tester.

Table 2. [a]Efficiency and doping level of the doping process of PAN in a solid state cell with $LiClO_4$/PEO network as polymer electrolyte

Temperature	Scanning Rate	Efficiency %	Doping level
70°C	30 mv/s	81	0.8
	50 mv/s	71	0.5
	100 mv/s	95	0.3
100°C	30 mv/s	84	1.1
	50 mv/s	83	0.7
	100 mv/s	88	0.4

[a] obtained from voltammetric data

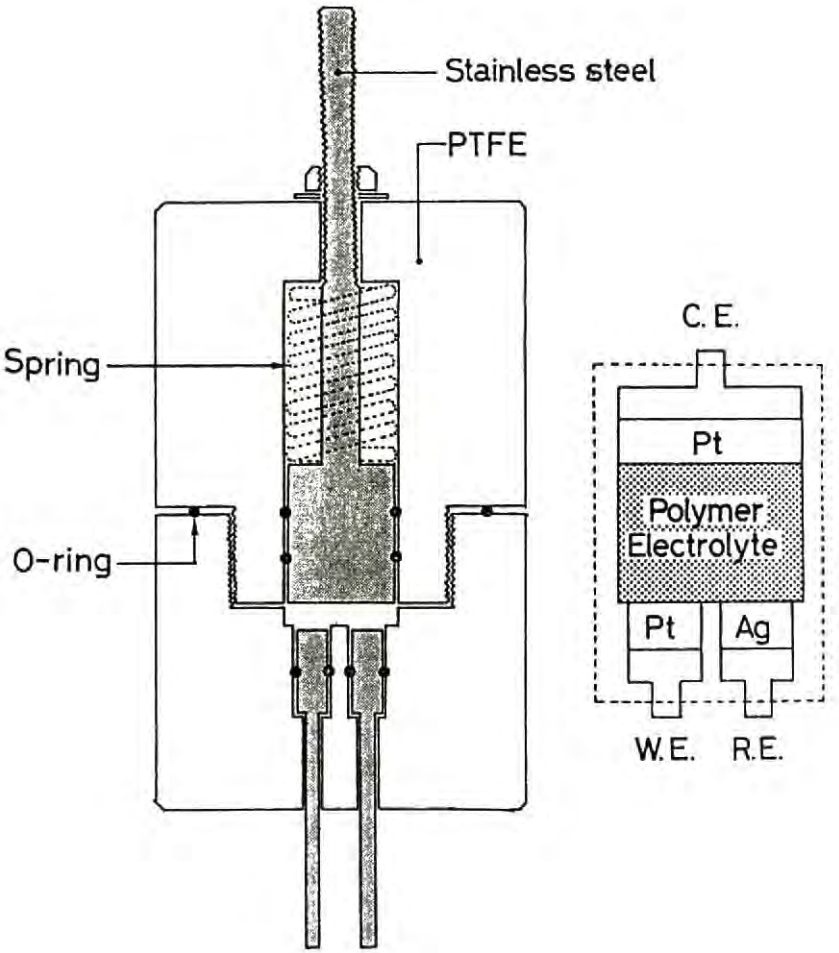


Figure 1. **Structure of homemade cell for investigating redox reactions in polymer electrolytes**

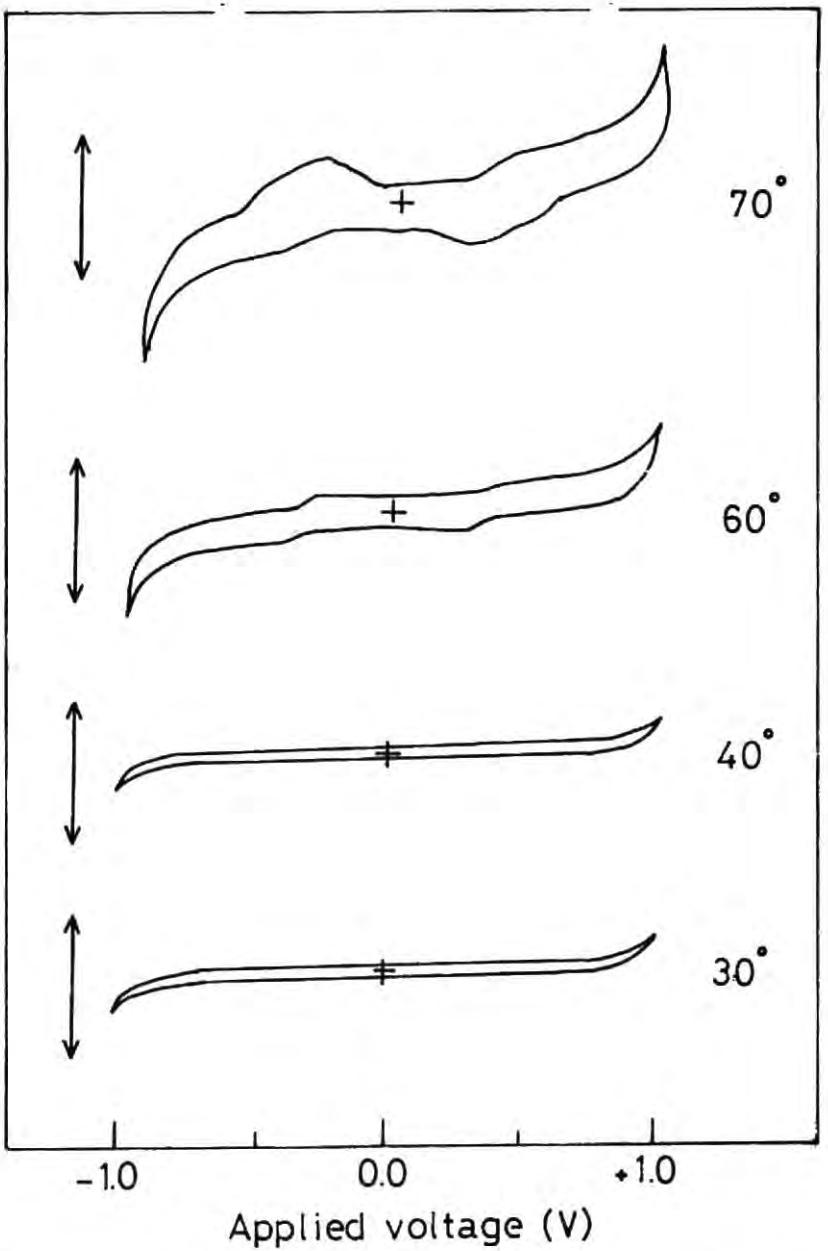


Figure 2. Cyclic voltammograms during polymerization of aniline in $LiClO_4$/PEO network polyelectrolyte at different temperatures. Electropolymerization conditions: potential range (-1 to +1 V), potential is the applied voltage between Pt electrodes without reference electrode. A cursor corresponds to 50 υA.

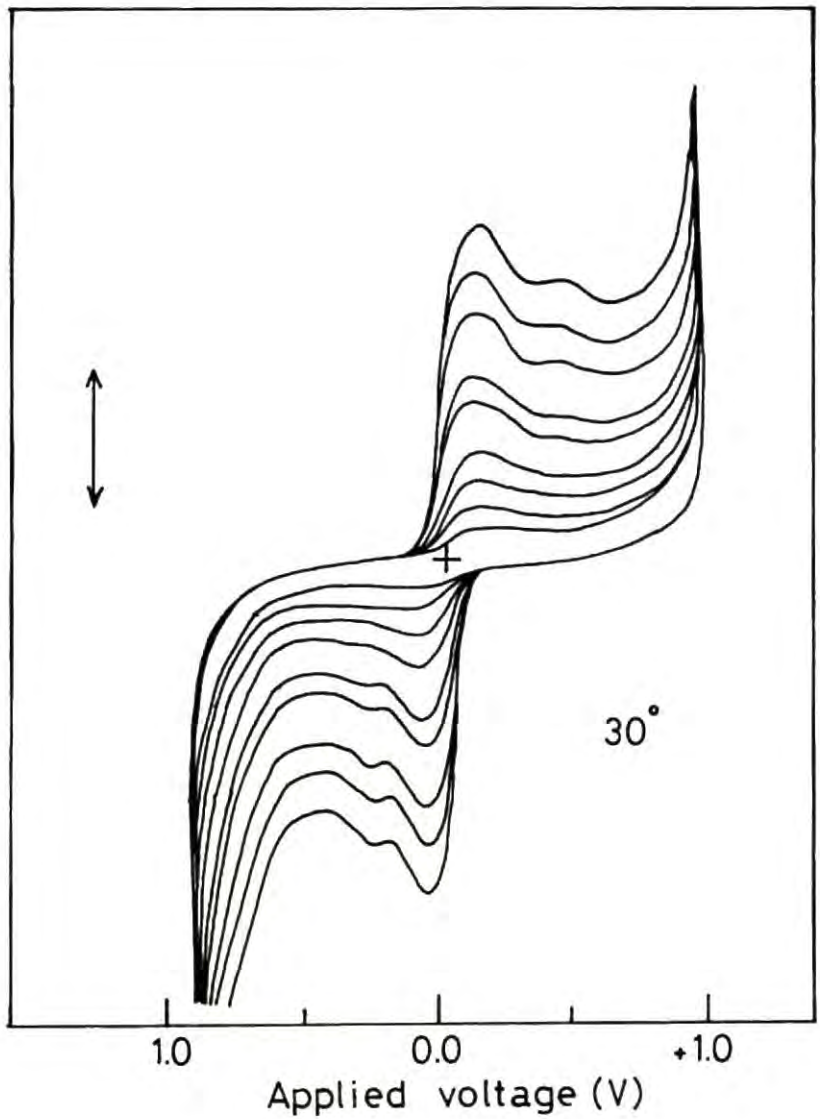


Figure 3. Cyclic voltammogram during polymerization of aniline: CF_3SO_3H (11:1) in $LiClO_4$/PEO network at 30°C. Electropolymerization conditions: potential range (-1 to +1 V), potential is the applied voltage between Pt electrodes; scanning rate (50 mv/s); the cursor corresponds to 50 υA.

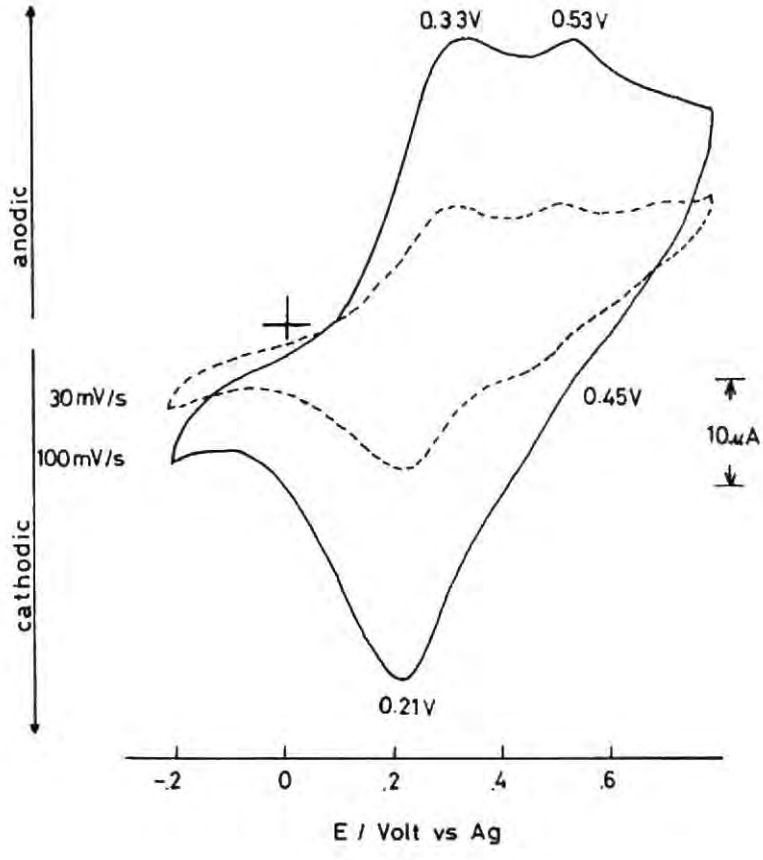


Figure 4. Cyclic voltammograms of aniline (methanolic soln. of aniline●HCl) in poly(ethylene oxide) network at 30°C

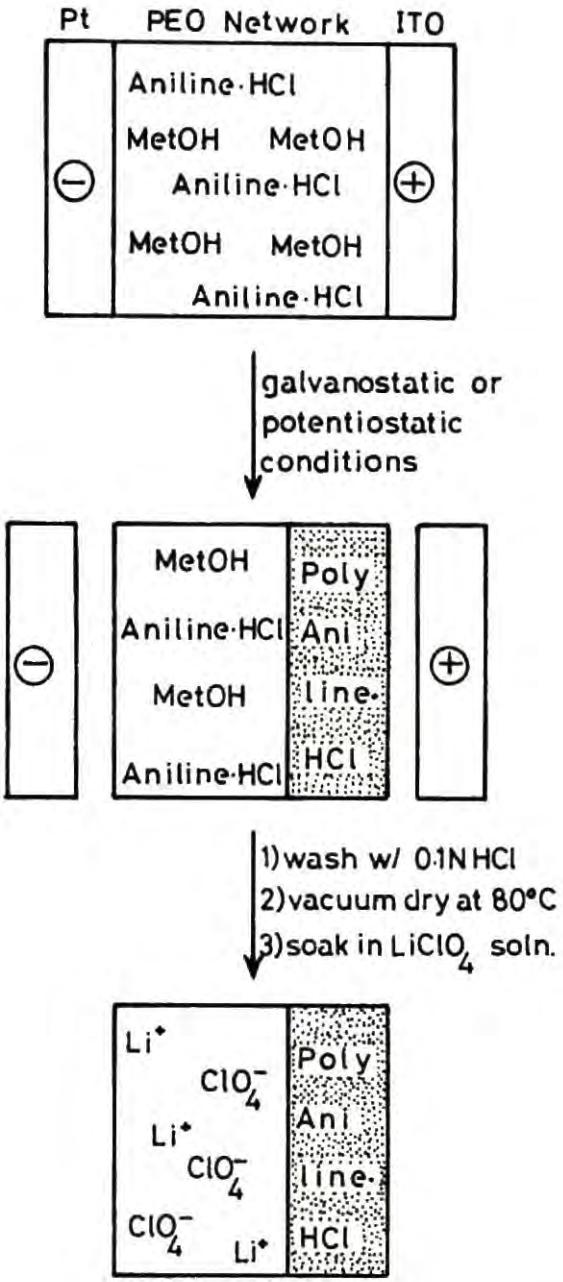


Figure 5. Fabrication scheme of the bilayer composite consisting of a conducting polymer (polyaniline) and ion conducting polymer (PEO, $LiClO_4$)

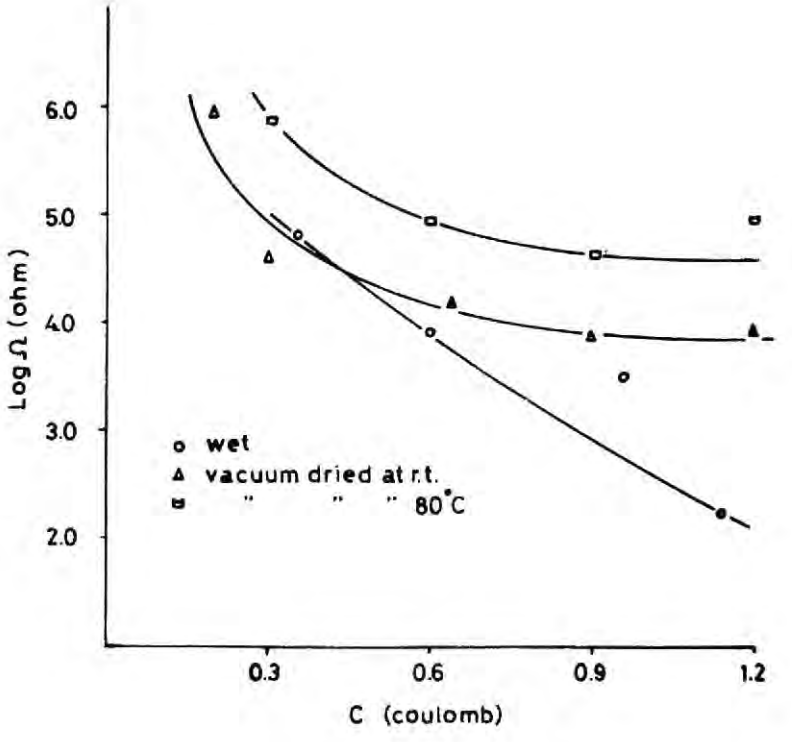


Figure 6. Resistivity of the PAN film at different stages of preparation

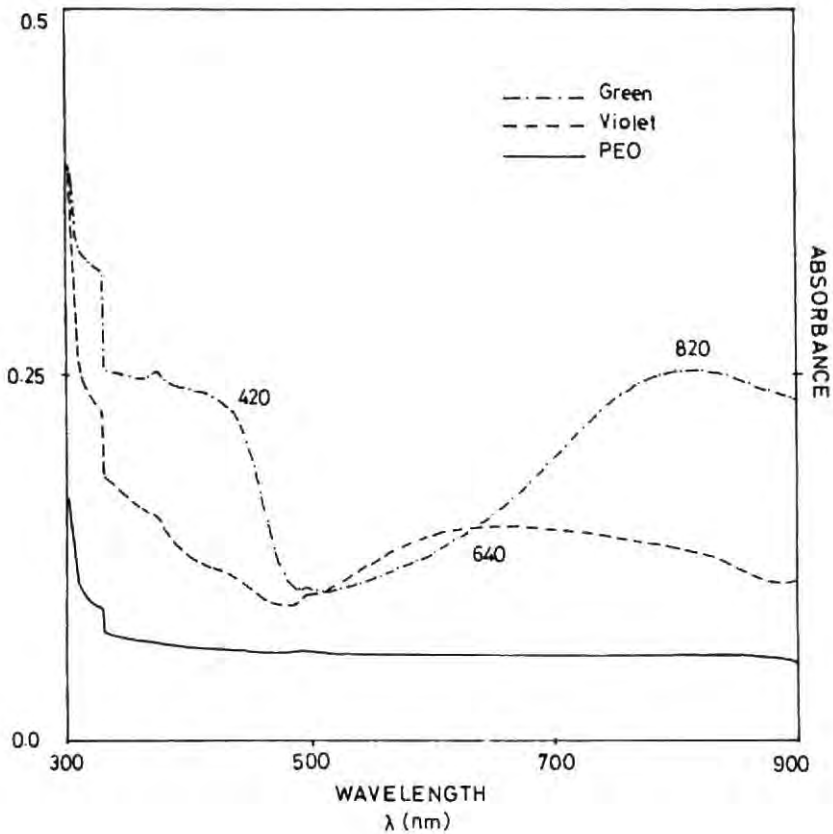
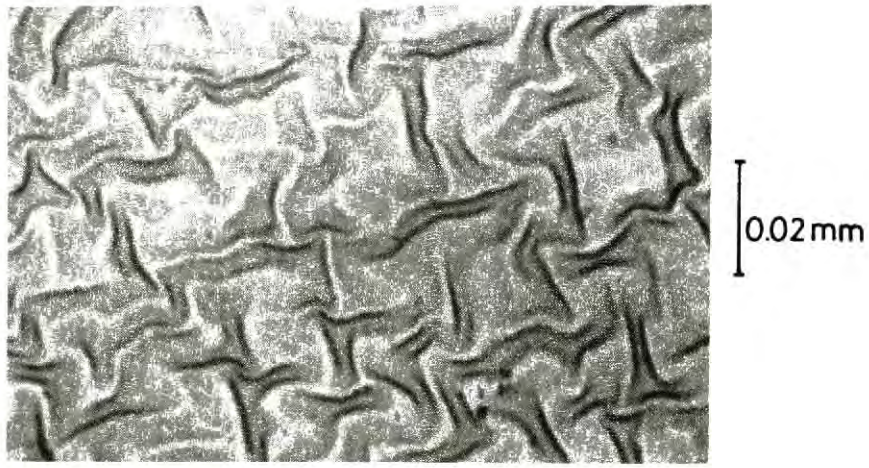


Figure 7. **UV Absorption of the 0.1N HCl (green film) and H_2O (violet film) washed bilayer PAN/PEO composite**

(a) Cross sectional view



(b) Surface view

Figure 8. Microscopic views (x 10) of polyaniline/polymer electrolyte composite

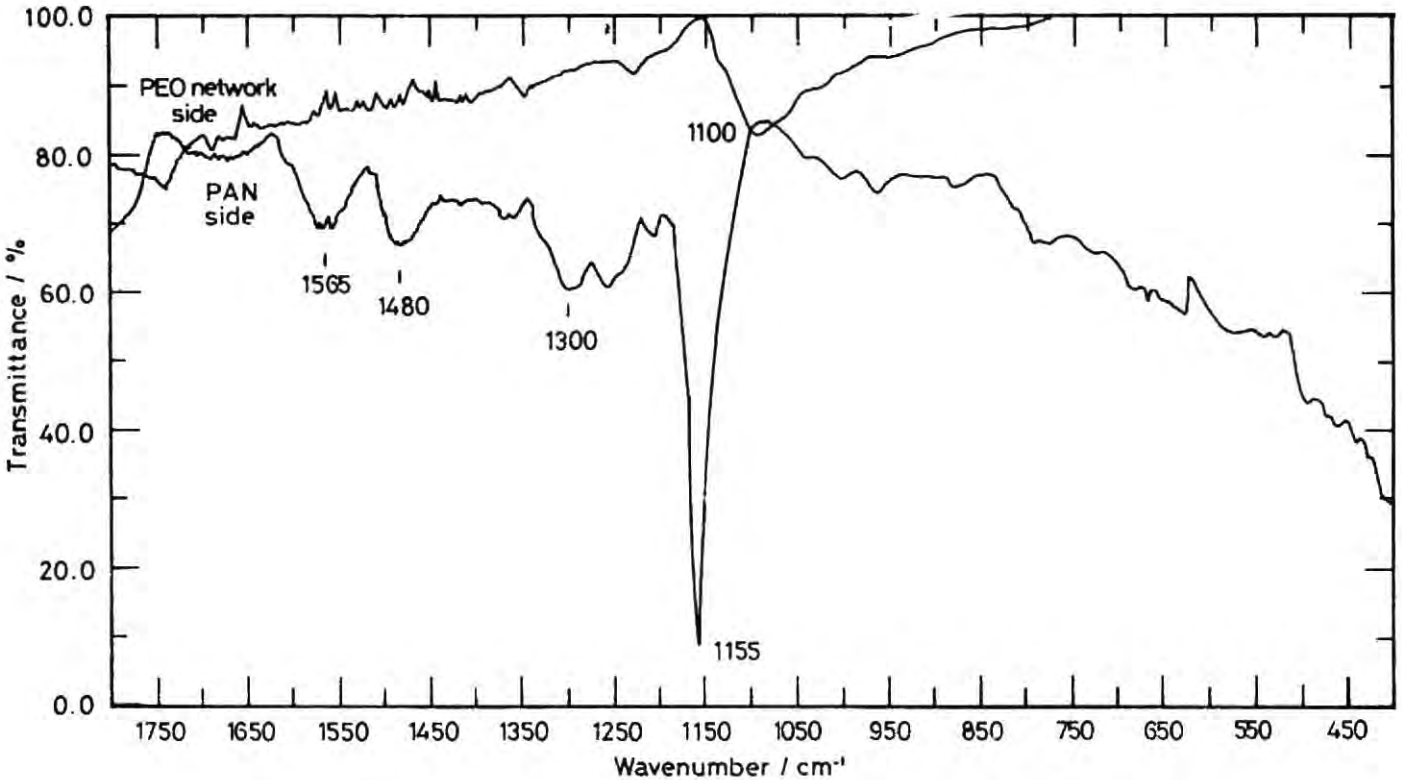


Figure 9. FT-IR reflection spectrum of PAN film deposited on PEO network

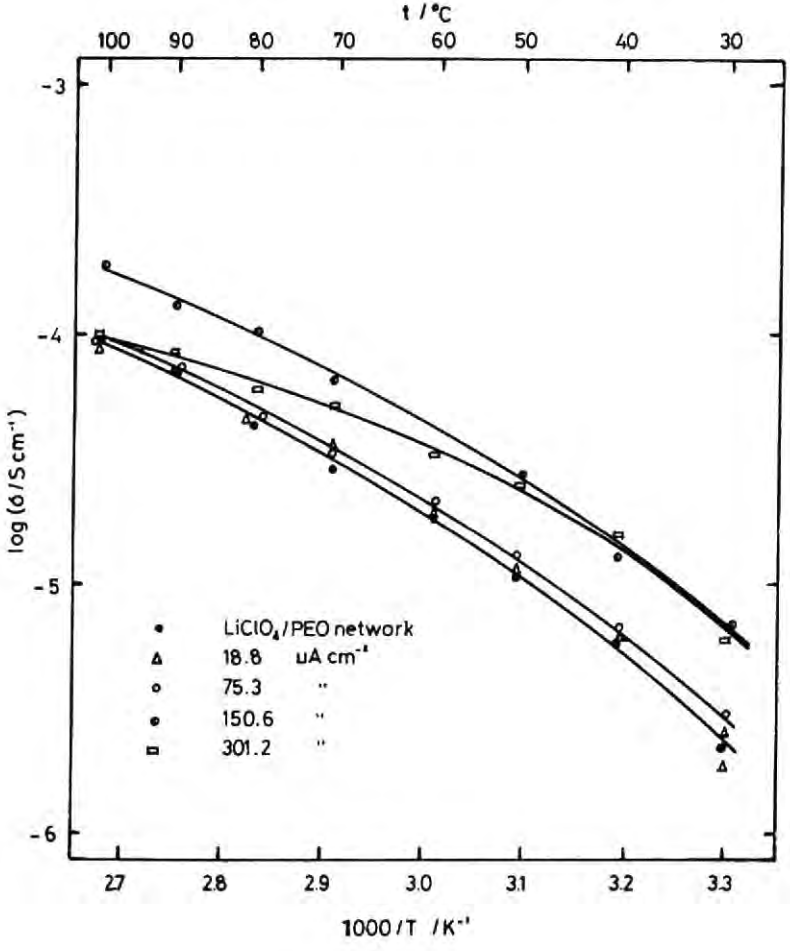


Figure 10. Temperature dependence of ionic conductivity of polymer electrolyte and bilayer composite; PAN layer prepared by passing 0.15 cm^{-2} at different current densitites; [$LiClO_4$]/[EO unit] = 0.03

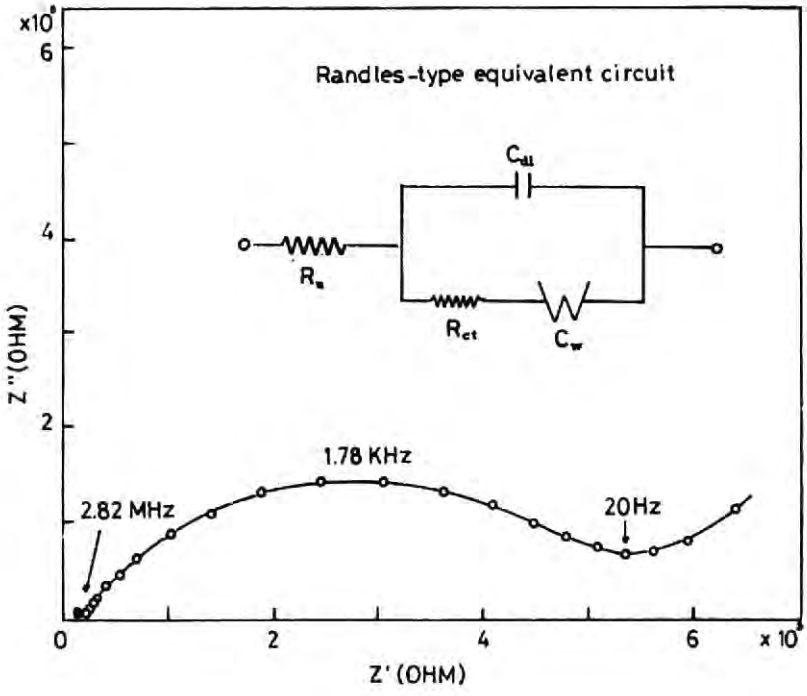


Figure 11. Complex impedance diagram of the cell Li/LiClO4 (PEO network)/PAN at 70°C and its Randles-type equivalent circuit

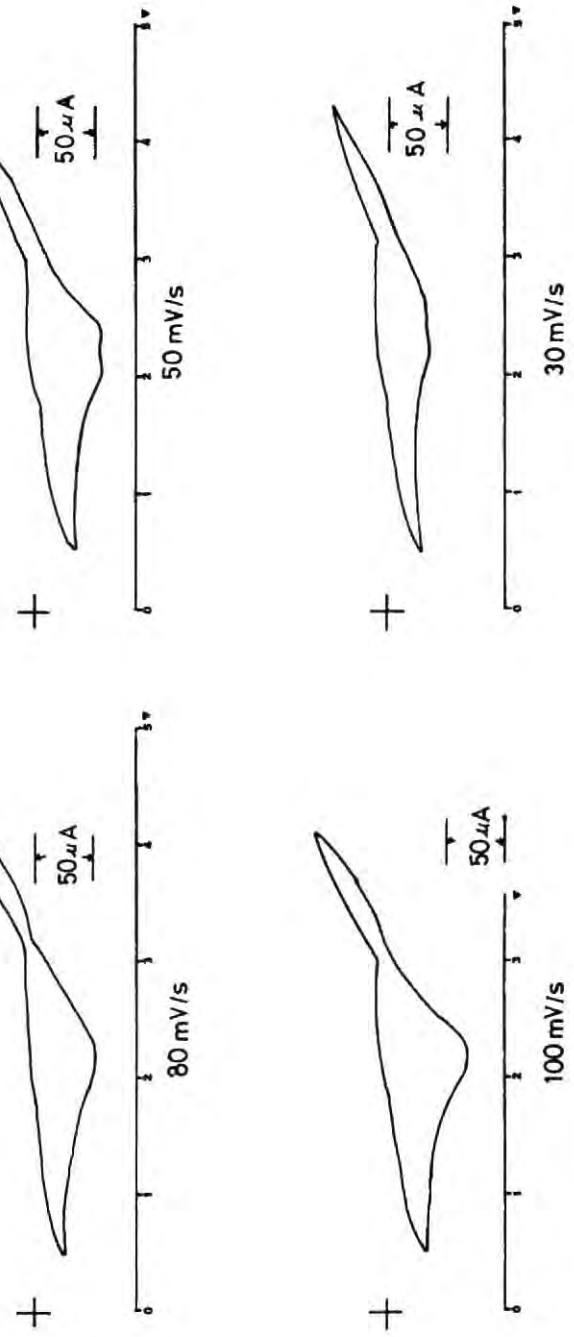


Figure 12. Cyclic voltammograms of Li/$LiClO_4$ (PEO)/PAN cell at 70°C

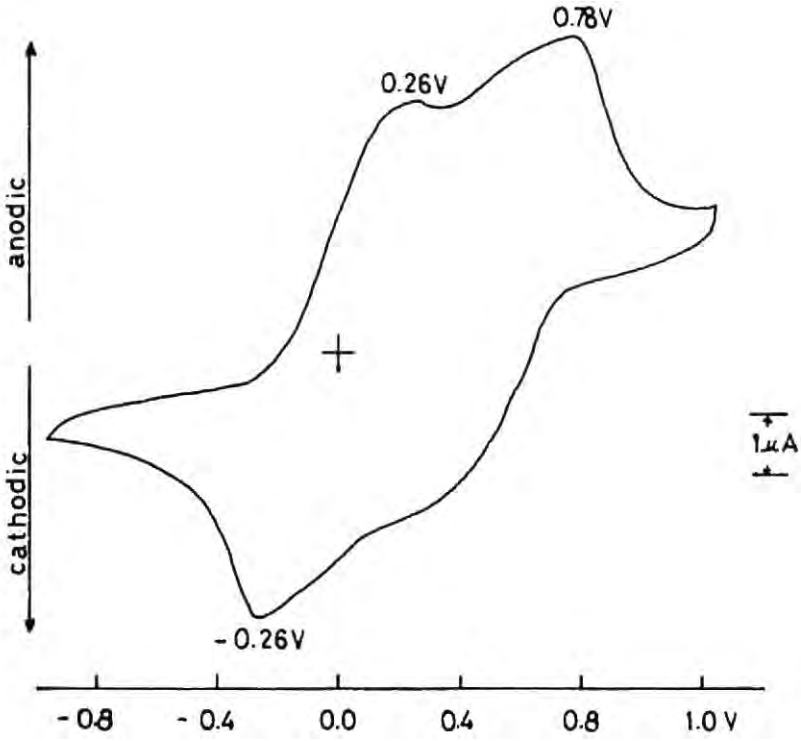


Figure 13. Cyclic voltammogram of Pt/$LiClO_4$ (PEO)/PAN cell at 70°C

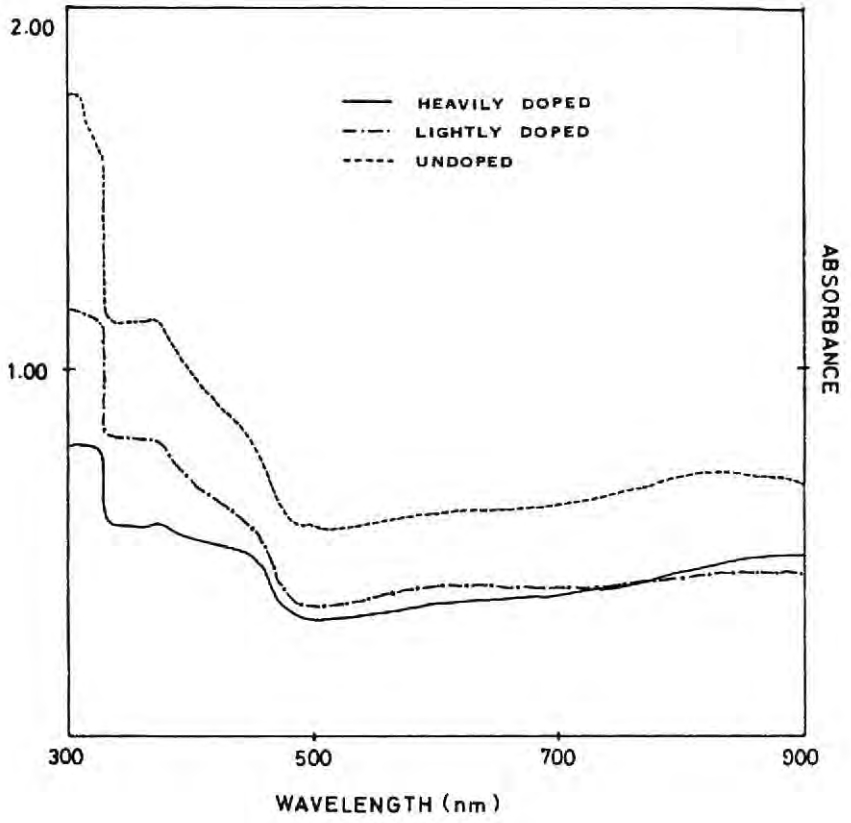


Figure 14. Change in UV absorption of the polyaniline/polymer electrolyte bilayer composite film corresponding to redox reaction of polyaniline in solid state

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A Mean Field RVB Theory For Copper Oxide-Based High T_c Superconductors in Terms of Auxiliary Bosons

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ABSTRACT

A mean-field theory for Anderson's resonating valence bond (RVB) model of high temperature superconductivity in terms of auxiliary boson fields is discussed in this paper. Contact with the conventional Bardeen-Cooper-Schrieffer (BCS) theory in the low temperature limit is achieved through an appropriate choice of order parameters. By devising an effective Hamiltonian in momentum space, holon condensation is shown to occur. This phenomenon triggers superconductivity.

PRELIMINARIES

Sometime in 1986 (1) a synthesis of a complicated ceramic compound of four elements (La-Ba-Cu-O) and the subsequent detection of the appearance of superconductivity at a temperature of 35K generated feverish interests in the scientific world. The raising of the critical temperature to around 95K by Chu and collaborators (2) by working on another ceramic compound (Y-Ba-Cu-O) intensified these interests. Hundreds of scientists all over the world are racing to reach higher critical temperatures by investigating other ceramic compounds; the highest obtained so far is 120K with thallium as the main element. But reports on other superconductors made of organic compounds seem to indicate that the critical temperature could be raised some more. There is no doubt that a new vista in the field of physics has been opened for exploration.

Superconductivity (4) at low critical temperature is well understood. The BCS theory consistently explains the properties of low T_c superconductors through the attractive electron-phonon interaction that provides the mechanism for the formation of bound cooper pairs. But it seems there is an upper bound critical temperature for the BCS theory to be workable.

There are remarkable features of high T_c superconductors that require explanations other than the conventional electron-phonon mechanism. For instance, some experiments seem to indicate that electrons are paired with an energy gap but that no isotopic effect was detected. This certainly could not be explained by the electron-phonon interaction. The mechanism involved might be due to the electron-plasmon attractive force as speculated by some investigators. In the subsequent sections we shall see some more of these as we unravel a mechanism which we believe might be a viable explanation for the behavior and characteristics of high T_c superconductors.

A direct offshoot of investigations of high T_c superconductors in the copper oxide-based ceramics is that it gives us insight into the unexpected magnetic and transport properties of pure as well as doped Mott insulators. Mott insulators, which insulate solely through the coulomb interactions, have never been quite understood for sometime. The resurgence of interest in the Mott localization for strongly correlated systems appears to confirm that this phenomenon has something to do with high T_c superconductors.

Several mechanisms have been advanced as possible explanations for the remarkable behavior of high T_c superconductors. In this paper, we shall explore the resonating valence bond (RVB) (2), (3) model in the slave boson formulation. We will subsequently treat a mean-field theory to explain some of their peculiar characteristics.

THE RESONATING VALENCE BOND (RVB) THEORY

Experimental data on insulating (undoped) La_2CuO_4 show that the Cu^{2+} is an $S = 1/2$ orbitally nondegenerate state with the Cu: $3d_{x^2-y^2}$ orbital strongly hybridizing with the oxygen O: $2p_x$, $2p_y$ orbital in the Cu-O plane. Anderson hypothesized that the insulating state of pure La_2CuO_4 is the resonating valence bond state. According to this hypothesis, there is a fermi liquid

state with lower energy than the antiferromagnetic order wherein the nearest neighbor electrons tend to form singlet pairs. These singlet pairs resonate among various singlet configurations (and thus the name resonating valence bond) (2).

The RVB state is a liquid because it has quantum transport of spin excitations. Anderson reasoned out that, although ordinarily, the ground state configuration of systems with large quantum fluctuations is antiferromagnetic, there are pre-existing spin singlet pairs in the RVB state which become charged superconducting copper pairs by strong enough doping.

When applied to superconductors, the nearly half-filled strongly correlated Hubbard model may be appropriate. In fact, the source of high Tc superconductivity may be due to the spin correlations induced by a superexchange mechanism between electrons on the nearest neighbor lattice sites.

The starting point of the RVB model is the nearly half-filled Hubbard Hamiltonian (5) (6) (7):

$$H = -t \sum_{\langle ij \rangle \sigma} (C^{+}_{ij} C_{j\sigma} + h.c.) + \mu \sum_{i} \eta_{i\alpha} \eta_{i\beta} - \mu \sum_{i\sigma} \eta_{i\sigma}, \qquad (1)$$

where $c^{+}_{i\sigma}$ ($C_{i\sigma}$) is the electron creation (annihilation) operator, t is something like a transfer integral, U is the Hubbard potential and $\eta_{i\sigma}$ is the number density operator. The chemical potential μ is introduced for the doping process. Inasmuch as the site energy is measured from the chemical potential, it is normally set equal to zero. The first term in the above Hamiltonian describes a system of free band electrons while the second term characterizes the strong onsite coulomb repulsion of two opposite spin electrons.

Generally, a many-body Hamiltonian contains a one-particle kinetic energy operator and a two-body potential energy operator. A complete set of Wannier functions could be used as a basis for second quantization. A single-band Hamiltonian that could be possibly constructed is:

$$H = \sum_{ij,\sigma} C^{+}_{i\sigma} \langle i | T | j \rangle C_{j\sigma} + \frac{1}{2} \sum_{ijR\iota,\sigma\sigma'} C^{+}_{i\sigma} C^{+}_{j\sigma'} \langle ij | V | R\iota \rangle x$$

$$x C_{\iota\sigma'} C_{R\sigma}, \qquad (2)$$

where

$$< i \mid T \mid j > \equiv \int d^3r \; w^* (\vec{r} - \vec{R}_i) \; T (\vec{r}) \; W (\vec{r} - \vec{R}_j),$$

and

$$< ij \mid V \mid R\iota > \equiv \int d^{3r} \int d^3r' \; w^*(\vec{r}-\vec{R}_i) \; w^*(\vec{r}'-\vec{R}_j) V(\vec{r},\vec{r}') w(\vec{r}'-\vec{R}_\iota) w(\vec{r}-\vec{R}_R).$$

In the above relations $W(r-R_i)$ are the single-band Wannier functions. **Hamiltonian** introduced a constant potential $U = < ii/V/ii >$ as the only non- vanishing component of the two-body potential. Furthermore, by using the kinetic energy function in the tight-binding approximation

$$< i \mid T \mid j > = \quad \epsilon \; \delta_{ij} - t \; \delta_{(ij)},$$

where ε is the site energy, $\delta_{(ij)} = 1$ for (ij) nearest neighbors bands and zero otherwise, the single-band Hubbard Hamiltonian takes the form

$$H = \epsilon \sum_{i\sigma} \eta_{i\sigma} - t \sum_{<ij>\sigma} (C^+_{i\sigma} C_{j\sigma} + C^+_{j\sigma} C_{i\sigma}) + \mu \sum_{i} \eta_{i\alpha} \eta_{i\beta}, \qquad \textbf{(3)}$$

where Σ is a sum over nearest-neighbor bands, while $\sigma = (\alpha, \beta) = (\uparrow, \downarrow)$ is a spin index. The last term tells us that the two electrons with opposite spin experience a strong repulsive force when they are in the same site. When $\varepsilon = \mu = o$, the Hamiltonian simply represents a system of free band electrons. In the event that $\mu >> t$, each electron will localize itself at each site in order to avoid the strong repulsive force. This is what happens in a Mott insulator where each site is populated by an electron of spin 1/2. Equation (1) then is obtained by setting lattice site energy to zero while introducing the chemical potential for the doping process.

After using canonical transformations, we get an effective Hamiltonian defined in the non-doubly occupied subspace

$$H = -t \sum_{\langle ij \rangle \sigma} (1 - \eta_{i,-\sigma}) C^{+}_{i\sigma} C_{j\sigma} (1 - \eta_{j-\sigma})$$

$$+ \mu \sum_{i\sigma} C^{+}_{i\sigma} C_{i\sigma} + J \sum_{\langle ij \rangle} (\vec{S}_i \cdot \vec{S}_j - \frac{1}{4} \eta_i \eta_j), \qquad (4)$$

where we introduced the antiferromagnetic spin coupling constant $J = 4t^2/U$ and the spin angular momentum operators $\vec{S}$. The above Hamiltonian is too difficult to handle. For all practical purposes, we use the hopping approximation to rewrite the effective Hamiltonian as (7)

$$H = -t\delta \sum_{\langle ij \rangle \sigma} (C^{+}_{i\sigma} C_{j\sigma} + h.c.) + \mu \sum_{i\sigma} C^{+}_{i\sigma} C_{i\sigma}$$

$$+ J \sum_{\langle ij \rangle} (\vec{S}_i \cdot \vec{S}_j - \frac{1}{4} \eta_i \eta_j), \qquad (5)$$

where δ is the hopping parameter. In the insulating phase ($\delta = \mu = 0$) and with the restriction that we confine our system to a half-filled band in the singly-occupied site subspace, we get the Heisenberg antiferromagnetic Hamiltonian,

$$H_0 = J \sum_{\langle ij \rangle} (\vec{S}_i \cdot \vec{S}_j - \frac{1}{4}). \qquad (6)$$

This describes an exactly half-filled band Mott insulator in a simple square lattice. Normally, this H_0 ground state configuration is the antiferromagnetic order. Anderson showed that by introducing the boson singlet operator,

$$b^{+}_{ij} \equiv \frac{1}{\sqrt{2}} (C^{+}_{i\sigma} C^{+}_{j\beta} - C^{+}_{i\beta} C^{+}_{j\alpha}), \qquad (7)$$

the Hamiltonian H_0 could be rewritten as

$$H_0 = - J \sum_{<ij>} b^{+}_{ij} b_{ij} . \qquad (8)$$

From this, Anderson hypothesized that there is an RVB state that is a quantum liquid state in which the spins form singlet pairs rather than the long-range antiferromagnetic order.

It was shown that by starting from (8) the development of the RVB correlations and the subsequent superconducting order in the high T_c oxide superconductors could be described by a U(1) lattice gauge theory. In fact, the insulating state ($\sigma = \mu = o$) has an almost local gauge symmetry which is spontaneously broken at low temperatures. This results in superconductivity.

With the expression of the valence bond singlet operator in (7), the effective Hamiltonian now is of the form

$$H = - t \delta \sum_{<ij>\sigma} (C^{+}_{i\sigma} C_{j\sigma} + h.c.) - J \sum_{<ij>} b^{+}_{ij} b_{ij}$$

$$+ \mu \sum_{i\sigma} C^{+}_{i\sigma} C_{i\sigma} . \qquad (9)$$

The negative sign in the second term of this Hamiltonian suggests that the singlet objects are approximate bosons which could undergo Bose condensation into zero center-of-mass momentum state. Every superconductivity practitioner knows that this state triggers superconductivity. We shall see this in detail when we discuss the RVB mean-field theory.

THE SLAVE (AUXILIARY) BOSON FORMULATION

Since the development of the resonating valence bond model of Anderson for high temperature superconductivity, several works have been made extending the said model. It was shown that in the RVB state, there exist three kinds of particle excitations: charged boson solitons which we now call holons, neutral fermion solitons which we now call spinons and true

electrons or holes. In this section we shall review (7), (9) a mathematical formalism treating these particles initially as simple mathematical objects. Later we shall render some physical explanations to show that they could be physically observable particles.

Let us consider a lattice site i in a lattice of electrons. In this site are associated four possible quantum states, namely: $|0>$, $|\alpha>$, $|\beta>$, and $|\alpha\beta>$. These states correspond with an empty site, a spin up state for an electron, a spin down state and a double occupied site (two electrons in one site) respectively. These four possible states associated with an electron lattice site form a completeness relation. Consequently, any operator associated with an electron at a particular site could be expanded as a linear combination of the above states. Thus

$$\Sigma \left| ip >< ip \right| = \left| 0 >< 0 \right| + \left| \alpha >< \alpha \right| + \left| \beta >< \beta \right| + \left| \alpha\beta >< \alpha\beta \right| = 1,\ p = (0,\alpha,\beta,\alpha\beta). \tag{10}$$

This is quite interesting in the light of the mapping

$$\left| i_p >< i_p \right| \longrightarrow \text{[fermion - boson operators or like combinations]}. \tag{11}$$

Thus associated with the possible states in an electron site are internal projection component operators in which a physical electron could be imagined to be constituted of. For example, for each particular state of $|ip><ip|$, we have such associations as

$$\left| 0 >< 0 \right| \longrightarrow e^{+}_{i} e_{i} \qquad \left| \alpha >< \alpha\beta \right| \quad S^{+}_{i\alpha} d_{i}$$

$$\left| \alpha >< \beta \right| \longrightarrow S^{+}_{i\alpha} S_{i\beta} \qquad \left| \beta >< 0 \right| \longrightarrow S^{+}_{i\beta} e_{i}, \text{ etc.} \tag{12}$$

An interesting property of the projection operators associated with the four possible states in an electron lattice site is that some follow the commutator algebra while others follow the anticommutator one. For example, projection operators associated with the states $|0>$ and $|\alpha\beta>$ follow the commutation rules while that for $|\alpha>$ and $|\beta>$, the anticommutation relations. Our mapping then convinced us that ei and di must be bosonic fields satisfying the commutation rules:

$$[e_i, e^{\dagger}_j] = \delta_{ij} \qquad [e_i, e_j] = [e^{+}_i, e^{+}_j] = 0$$
$$[d_i, d^{+}_j] = \delta_{ij} \qquad [d_i, d_j] = [d^{+}_i, d^{+}_j] = 0, \tag{13}$$

while the $S_{i\sigma}$ follows the anticommutation relations:

$$[S_{i\sigma}, S^{+}_{j\sigma'}]_{+} = \delta_{ij}\delta_{\sigma\sigma'} \qquad [S_{i\sigma}, S_{j\sigma'}]_{+} = [S^{+}_{i\sigma}, S^{+}_{j\sigma'}]_{+} = 0. \tag{14}$$

and are therefore fermions. It is obvious that different particle operators commute.

As pointed out earlier, an operator associated with the physical electron could be constructed out of a linear combination of lip><ipl. In particular, for the electron annihilation operator, we have

$$C_{i\sigma} \longrightarrow |0><0| + \sigma |-\sigma><\sigma\beta|,$$

where $\sigma = \alpha,\beta$ or **(1,-1)** Transcribed in terms of boson and fermion fields this is

$$C_{i\sigma} = e^{+}_i S_{i\sigma} + \sigma S^{+}_{i-\sigma} d_i, \tag{15}$$

and for the electron creation operator,

$$C^{+}_{i\sigma} = S^{+}_{i\sigma} e_i + \sigma d^{+}_i S_{i-\sigma}. \tag{16}$$

Based on equations (15) and (16) we can conjecture that a physical electron could be a composite object.

Certainly, the $C_{i\sigma}$'S follow the anticommutation rules:

$$[C_{i\sigma}, C^{+}_{j\sigma'}]_{+} = \delta_{ij}\delta_{\sigma\sigma'}, [C_{i\sigma}, C_{j\sigma'}]_{+} = [C^{+}_{i\sigma}, C^{+}_{j\sigma'}]_{+} = 0. \tag{17}$$

But for these to be satisfied, the following constraint must be imposed:

$$e^{+}_{i} e_{i} + d^{+}_{i} d_{i} + \sum_{\sigma} S^{+}_{i\sigma} S_{i\sigma} = 1. \qquad (18)$$

The mapping (11) shows that (18) corresponds with the completeness relation (10).

A straightforward calculation of the current densities associated with the (e_i, d_i, $S_{i\sigma}$)- fields by using the effective Hamiltonian shows that the total charge densities of the e_i and d_i fields could completely account for the charge of the physical electron. This implies that spinons ($S_{i\sigma}$)have neutral charge. The spin, on the other hand, could be assigned to the spinon so the charged e_i - fields (holons) and the d_i - fields are spinless.

We can thus associate the e^+- operator to create an empty site while d^+ creates a doubly occupied site. It is also possible to associate a fundamental charge called S-charge on top of the electric charge. Thus, e^+ carries one unit of positive S-charge while d^+ carries one unit of negative S-charge ($-e_s$) . The spinon has zero S-charge (7) (9).

The important role being played by the slave boson fields could be clarified by looking at the symmetries of the effective Hamiltonian. The original Hubbard Hamiltonian (1) is invariant under a global or phase transformation of the electron field $C_{i\sigma}$. This U(1) global symmetry corresponds with the fermion number conservation. On the other hand, this Hubbard Hamiltonian has also a global SU(2) symmetry in spin space (7).

Replacing the physical electron operator with the holon and spinon operators through the slave boson transformation, and subsequently getting rid of the doublons, we will find that this effective Hamiltonian still carries the global U(1) symmetry of the original Hubbard Hamiltonian. In fact, we can make the symmetry local (by putting in a spacetime variation in the global parameter) and still find the new effective Hamiltonian invariant. Because of this symmetry we can associate a charge conservation otherwise known as the S-charge.

The local SU(2) symmetry, however, is not carried by the original Hamiltonian as well as the new effective Hamiltonian. It is the exchange parts of both Hamiltonians, H_o (the Heisenberg antiferromagnetic Hamiltonian) that is found to be local SU(2) invariant.

A MEAN-FIELD RVB THEORY IN THE SLAVE BOSON FORMULATION

We can get an effective spinon-holon Hamiltonian from the original Hubbard Hamiltonian by making use of the slave boson transformations, (7)

$$H = -t\delta \sum_{\langle ij\rangle\sigma} (e_i e^+_j S^+_{i\sigma} S_{j\sigma} + h.c.) - \frac{1}{2} J \sum_{\langle ij\rangle} b'^+_{ij} b'_{ij} + \mu \sum_i e^+_i e_i, \quad (19)$$

The above Hamiltonian has been simplified by imposing the constraint relation (18) and simultaneously throwing the doublons. The first term is the coupling between the holon kinetic energy and the spinon kinetic energy with a coupling strength $t\delta$; it likewise represents the spinon-holon scattering term with a large matrix element for the localized spinon-holon scattering. Comparing this with the single-band Hubbard Hamiltonian might mislead us into concluding that the spinons will bose condensate because the b_{ij}'s in (19) are proportional to $S_{i\sigma}S_{j-\sigma}$. But there is something in (19) that will prevent this from occuring, that is, the coupling of the holon kinetic energy with the spinon kinetic energy.

The spinon-holon Hamiltonian (19) is a direct offshoot of the slave boson transformation. It is indeed very revealing to see if the spinon-holon scattering term could prevent the Bose condensation of spinons. A mean-field RVB theory to this effect will shed light on this interesting aspect.

A mean-field theory is basically something like a classical approximation. Operationally, this is roughly done by transforming the model Hamiltonian into momentum space and subsequently making a Hartree-Fock factorization. Tnis closely resembles the scheme of getting fermionic ensemble averages in accordance with Wick's Theorem. In our case, we simply identify the appropriate order parameters and then apply Bogoliubov transformation to diagonalize the resulting BCS-like Hamiltonian.

BZA (5) have done this for the Hubbard Hamiltonian (9). In momentum space they obtained

$$H = \sum_{\vec{R}\sigma} (\epsilon_R - \mu) C^+_{\vec{R}\sigma} C_{\vec{R}\sigma} - J \sum_{\vec{R}} (\Lambda \gamma_R C^+_{\vec{R}\sigma} C^+_{-R\downarrow} + h.c.) + N(\Delta^2 + P^2), \quad (20)$$

where the self-consistent order parameters were defined as $\Delta = \sqrt{2}\ \langle bij \rangle$ and $p = \langle c^{\dagger}_{i\sigma}\ cj\sigma \rangle$. In two dimensions the ϵ_R and γ_R were found to be related as $\gamma_R = -\ \epsilon_R\ (2t\delta + pJ)^{-1}$ with $\epsilon_R = -\ (2t\delta + pj)\ (\cos kxa + \cos kya)$. Observe that (kx, ky) are the components of the wave vector $\vec{R}$ and a is the square lattice constant. After Bogoliubov transformation, the qu. siparticle energy spectrum was obtained as well as the resulting expressions for the gap and chemical potential relations. In the insulating phase wherein $\delta=\mu=o$, and with the order parameter p being likewise set to zero, the excitation energy spectrum becomes $E_R = \Delta\ \tau x\ |\gamma_R|$. From here on, BZA obtained the RVB state originally constructed by Anderson. In addition, BZA confirmed the existence of a pseudofermi surface and at the same time obtained a linear temperature dependence of the low temperature specific heat (because of the femionic nature of the quasiparticles). In short, a satisfactory mean-field theory for the treatment of copper oxide-based superconductors was developed.

Let us do a mean-field theory, but this time in the slave boson formulation. We rewrite the electron operators in momentum space as

$$C^{+}_{R\sigma} = e_R\ S^{+}_{R\sigma} + \sigma\ S_{R-\sigma}\ d^{+}_{R}$$

$$C_{R\sigma} = S_{R\sigma}\ e^{+}_{R} + \sigma\ d_R\ S^{+}_{R-\sigma}. \qquad (21)$$

In an exactly parallel way we get rid of all terms that count in the doublon operator since we do not want the doubly occupied sites. Consequently, we have

$$\sum_{R\sigma} C^{+}_{R\sigma} C_{R\sigma} = \sum_{R} e_R e^{+}_{R} \sum_{\sigma} S^{+}_{R\sigma} S_{R\sigma}\ ;\ C^{+}_{R\alpha} C^{+}_{-R\beta} = e_R e_{-R} S^{+}_{R\alpha} S^{+}_{-R\beta}\ ,$$

On the other hand, the order parameters could be conveniently chosen as

$$\Delta_R = \langle e^{+}_{R}\ e^{+}_{R'} \sum_{\sigma} S_{R\sigma}\ S_{R'-\sigma} \rangle\ ;\ P_R = \langle e_R e^{+}_{R'}\ S^{+}_{R\sigma}\ S_{R'\sigma} \rangle\ ,$$

so that we could finally obtain the effective Hamiltonian in momentum space as

$$H = \sum_R (\epsilon'_R - \mu) e_R e^+_R - \frac{1}{2} J \sum_R \Delta_R \chi'_R e_R e_{-R} + N (\Delta^2_R + P^2_R). \quad (22)$$

In the first term of the above Hamiltonian we used the equation of constraint $\sum_\sigma S^+_{R\sigma} S_{R\sigma} = 1 - e^+_R e_R$ The expressions for the site energy ϵ'_R and χ'_R are proportional to those obtained in the conventional way. The second term clearly tells us that the holons will bose condensate into zero momentum state as they form an effective cooper pair. In effect, this holon condensation will trigger the superconductivity of the system.

We mentioned previously that the apparently deceptive form of the effective Hamiltonian (19) might yield the unexpected bose condensation of the spinons but that the scattering term would prevent this from occurring. Actually, this has been convincingly shown in a recent work by Baskaran where he formulated the problem by using functional integrals.

Our treatment is a bit different. We observed first all that the choices of our order parameters couples the holon kinetic energy and the spinon kinetic energy. This is true for both order parameters. In effect, we trivialized the so-called scattering term and, hence, ignored the dynamics involved in it. This is one reason why the Hamiltonian given by (22) is expressed entirely in terms of the holon field. The constraint equation (18) also contributed to this.

CONCLUSION

A mean-field theory for the RVB in the slave boson formulation was also tried by some authors. The theoretical framework runs like this. Starting from the original Hubbard Hamiltonian a

$$H = - t \sum_{<ij>\sigma} (C^+_{i\sigma} C_{j\sigma} + h.c.) + \mu \sum_i \eta_{i\sigma} \eta_{i\beta} - \mu \sum_{i\sigma} \eta_{i\sigma} , \quad (23)$$

slave boson formulation was used together with the constraint $\eta e + \eta d + \eta s = 1$. By eliminating the e^+, d^+, ed terms perturbatively as in the Rice transformation and, furthermore, by projecting out the pure d-terms ($\eta_d = 0$) he got the projected Hubbard model

$$H = t \sum_{<ij>\sigma} e^+_i e_j S_{j\sigma} S^+_{i\sigma} + J \sum_{<ij>} \vec{S}_i \cdot \vec{S}_j , \qquad (24)$$

where $\vec{S}_i \vec{S}_j$ are spin operators written purely in terms of the spinon operators. Attempting a mean-field theory with a mean value of $< e_i^+ e_j >$ and $< S^+_{i\sigma} S_{j\sigma} >$, he obtained an effective spinon Hamiltonian,

$$H = t \sum_{<ij>\sigma} < e^+_i e_j > S^+_{j\sigma} S_{i\sigma} + J \sum_{\sigma\sigma'} < S^+_{i\sigma} S_{j\sigma} > S^+_{i\sigma'} S_{j\sigma'} \qquad (25)$$

This gives unphysical results because it leads to spinon propagators of the order of $^1/_t$ and $^1/_J$.

Our comment runs like this: Maybe it should have been better if the original Hubbard Hamiltonian is used and not worked with the canonically transformed Hubbard Hamiltonian, and then used the slave boson formulation to obtain

$$H = -t\delta \sum_{<ij>\sigma} (e_i e^+_j S^+_{i\sigma} S_{j\sigma} + \text{h.c.}) - \frac{1}{2} J \sum_{<ij>} b^+_{ij} b_{ij} + \mu \sum e^+_i e_i ,$$

with $b_{ij} = \sum S^+_{i\sigma} S_{j-\sigma}$. An appropriate choice of the order parameters might lead to the phenomenon of holon condensation and obtain expressions for propagators that are physically acceptable.

Of course, there are also some drawbacks in our formulation. For one thing, we almost entirely ignored the dynamics of the spinon operators and instead concentrated on the holon field. Our motivation for this is understandable. We wanted an effective Hamiltonian that will describe holon condensation and this is what we exactly did.

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BIOLOGICAL SCIENCES

Cell Biology of the Philippine Amoeboflagellate, *Naegleria philippinensis*

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INTRODUCTION

Naegleria is a free-living amoeboflagellate whose trophozoite stage may differentiate reversibly into either a non-reproductive flagellate stage or a resistant cyst (Page 1967). A species of *Naegleria* known as *N. fowleri* causes fatal meningoencephalitis in humans (Julio-Martinez et al. 1983). Cell morphology, culture medium preference, temperature tolerance, lecting sensitivity, isoenzyme patterns, DNA restriction patterns, mouse pathogenicity and immunology are the criteria used to differentiate the various *Naegleria* species, as well as pathogenic from non-pathogenic species (De Jonckheere 1987; Milligan and Band 1988; Graham- Clark et al. 1989; Pernin and Cariou 1989).

Naegleria can be isolated from soil, freshwater habitats, as well as from natural or industrial thermally-polluted water systems (Marciano-Cabral 1988). An amoeboflagellate has been isolated in the Philippines by Enriquez et al. (1984). One isolate was obtained from a thermally-polluted stream, another from the cerebrospinal fluid of a young patient with encephalitis and the third from a heated swimming pool. Initial investigations place these isolates in the genus *Naegleria*. The present study further characterizes the isolates.

MATERIALS AND METHODS

Cultivation and Maintenance of Organisms. The Philippine *Naegleria* isolate was first cultured in non-nutrient agar plates lawned with *Escherichia coli.* The plates were incubated at 37°C

and microscopically examined daily for amoeba growth. Axenic cultures were prepared by transferring amoebae into Serum-Casein- Glucose-Yeast-Extract (SCGYEM) medium supplemented with 10% FBS.

Naegleria fowleri (Kul ATCC 30808), *N. lovaniensis* (Aq/8/1/45D), *N. australiensis* (NK11) and *N. gruberi* (CCAP 1518/1e) were obtained from Dr. Rolf Michel, Ernst Rodenwaldt Institute, Koblenz, Germany. Except for *N. gruberi,* all *Naegleria* strains were cultured at 37°C in SCGYEM. *N. gruberi* was maintained at 27°C in Balamuth medium.

Morphological Studies. Trophozoites and cysts, both from axenic and bacterized cultures, were examined under a phase contrast microscope. The trophozoites were further stained with trichome and haematoxylin for further studies. The cysts were stained with PATAG r for identification and classification. Trophozoites, cysts and flagellate stages were also processed for electron microscopic examination.

Lectin Sensitivity Studies. For lectin agglutination, fluorescence tests and electron microscopic visualization of receptors, 72-hour axenic cultures were harvested and washed free of the culture medium. Cells suspended in PBS were made to react with different lectins. The degree of agglutination was scored from weak (+) to strong (+ + +). To determine the specificity of the lectin reaction, a 0.2 M solution of an appropriate inhibitory sugar was added to the lectin solution one hour before incubating it with the cells. The degree of agglutination was scored as above.

Amoebae cultured on coverslips were fixed with 1% gluteraldehyde and incubated with the appropriate lectin-FITC conjugate for one hour. The unbound conjugates were washed off and the stained cells were observed under the fluorescense microscope. Gluteraldehyde- fixed cells were also incubated with lectin-gold conjugates, embedded in Lowicryl and processed for electron microscopic studies. Ultrathin sections were contrasted with uranyl acetate and lead citrate.

Naegleria philippinensis soluble and insoluble protein extracts were subjected to Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The separated protein bands were transferred to nitrocellulose membranes according to the method of Kyhse-Andersen (1988). The membranes were reacted with the appropriate lectin-peroxidase conjugate. The bound lectin was visualized using diaminobenzidine (DAB) as a substrate.

RESULTS AND DISCUSSION

Naegleria trophozoites can easily be recognized by the characteristic lobose monopseudopodium and a very prominent nucleus with a centrally located nucleolus. A section of the trophozoite under the electron microscope shows a clear separation of the pseudopodial hyaloplasm and the granular cytoplasm which in turn contains all the membrane-bound organelles such as the elongated dumb-bell shaped mitochondria, numerous vacuoles of varying sizes, rough endoplasmic reticulum, golgi and nucleus. Ribosomes may be observed free in the cytoplasm or attached to the endoplasmic reticulum. The nucleus contains a large, prominent centrally located nucleolus.

Naegleria trophozoites, when trasferred from culture dish to distilled water or saline solution, readily form pairs of flagella originating from the tip of a pear-shaped cell body. At the electron microscope level, the flagellate stage can easily be recognized when the flagellar apparatus is found in the plane of section. This consists of the flagella and their supporting structures, basal bodies and associated fibrils that run parallel to each other, as well as the rhizoplasts. Both flagella exhibit the typical $9+2$ arrangement of microtubules and are enclosed in a membrane which is continuous with the cell surface plasma membrane.

Numerous cysts can be observed in agar plates that have been kept for more than three days. Phase contrast microscopic studies show ovoid to oblong cysts with varying numbers of pores. A new cyst within an old cyst can occasionally be observed. The *Naegleria* cyst wall is made up of two layers: a fragile loosely-associated ectocyst and a very thick endocyst. The cytoplasm inside the cyst contains several mitochondria and membranous vesicles. As in the trophozoite, the nucleus of the cyst contains a prominent nucleolus and condensed chromatin material.

The reactions of the different *Naegleria* species to lectins with various sugar specificities were studied. *N. philippinensis,* as well as *N. lovaniensis,* were readily agglutinated by the lectins used, whereas *N. fowleri* did not react with any of the lectins. With the exception of *N. fowleri,* all species were agglutinated by Con A and *Triticum vulgare* lectin. *N. philippinensis, N. lovaniensis* and *N. australiensis* were strongly agglutinated by *Helix pomatia* but weakly agglutinated by *Lathyrus odoratus* lectin. *N.*

philippinensis showed weak agglutination with *Bauhinia purpurea, Bandeirea simplicifolia* and *Sambucus nigra* lectins. Furthermore, *N. philippinensis* did not agglutinate with *Glycine max* and *Arachis hypogea* before pronase treatment, but weak agglutination was observed thereafter.

To visualize the bound lectins on the surface membrane, lectin-FITC conjugates were employed. The Con A-FITC conjugate was found to be uniformly distributed throughout the surface of the trophozoites. This was also true for *Helix pomatia, Triticum vulgare* and *Ulex europaeus I.* Patching, capping was also observed when live trophozoites were stained with *Helix pomatia*-FITC. No difference was observed with the appearance of the fluorescence patterns among the different *Naegleria* species.

The surface membrane of the trophozoites incubated with *Helix pomatia*-gold conjugate was uniformly covered with colloidal gold particles. No difference was observed in the distribution of gold particles between regions of contact and free areas or areas immediately adjacent to contact points. On the other hand, the gold conjugate was randomly distributed and discrete clusters were observed on the surface membrane of the flagellate stage. No gold conjugate was found in the cytoplasm of the trophozoites or flagellates.

Cysts stained with PATAG r revealed a carbohydrate-rich cyst wall, as well as a collar surrounding the cyst pore. Mannose-specific Con A-FITC reacted with the cyst wall and the collar surrounding the cyst pores. However, galNAc-specific *Helix pomatia*-FITC did not stain the cyst wall as intensely as Con A, though the collar surrounding the pores was clearly detected.

Soluble surface membrane lectin receptors were further characterized on the basis of their molecular weights. Con A receptors separated by SDS-PAGE were found to be within the 20-60 kd range, whereas *Helix pomatia* lectin receptors were within the 30-120 kd range. The reaction was completely inhibited both by a-methyl-mannopyranoside and N-acetyl-galactosamine, respectively, indicating specificity of binding. Insoluble Con A receptors were further detected within a similar molecular weight range as that of the soluble Con A receptor. No insoluble *Helix pomatia* lectin receptor was detected.

N. philippinensis reacted with galNAc and mannose-binding lectins suggesting that these two sugars are predominantly found to be on the surface membrane. The difference in the distribution pattern of these receptors in the cyst, trophozoite and flagellate

stages indicates that modifications in membrane structure occur during transformation. The flagellate stage is usually rigid, has a smaller surface area, does not undergo endocytosis and has a completely different mode of locomotion. On the other hand, the trophozoite has a very fluid surface membrane as evidenced by its ability to patch and cap and internalize surface structures. These carbohydrate moieties may function in relation to cell recognition or cell-substrate adhesion. Other mechanisms of cell recognition may be employed by the flagellate forms.

Table 1. Rections of the various *Naegleria* species to the different Lectins used

LECTIN	SUGAR	phil isolate	N. lovan	N. austrl.	N. gruberi	N. fowleri
Concanavalin A	α–D-methyl manno-pyranoside	++	+	++	++	0
Triticum vulgare	gluNAc	+	++	++	++	0
Helix pomatia	galNAc	+++	+++	+++	0	0
Lathyrus odoratus	α-D-Mannose	+	+	+	0	0
Ulex europaeus I		+	0	+	0	0
Limulus plyphemus	NeuNAc	0	++	+	0	0
Bauhinia purpurea	galNAc	+	++	0	0	0
Bandeira simplicifolia	galNAc	+	++	0	0	0
Sambucus nigra	gluNAc	+	++	0	0	0
Mycoplasma gallisepticum		+	+	0	0	0
Glycine max	galNAc	0	+	0	0	0
Glycine max + pronase		+	+	0	0	0
Arachis hypogea	galNAc	0	++	0	0	0
Arachis hypogea + pronase		+	0	0	0	
tragonolobus purpureus	fucose	0	+	0	0	0
Anti- A		0	+++	0	0	0

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Multiproduct Fermentation: Production of Gluconic Acid and Glucose Oxidase by *Penicillium funiculosum* Thom 4072

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INTRODUCTION

The concept of multiproduct fermentation wherein more than two commercial products are derived from the same fermentation process is an economically attractive concept. Rising energy cost and increasing value of money make the system worth investing in. The concept is similar to the beer fermentation process wherein one has a main product, beer, and a by-product, yeast cells. However, multiproduct fermentation involves the production of at least two products for which the fermentation conditions are optimized for both. This is possible with the system we are studying since the two commercial products synthesized are directly related. The first product, a product of microbial synthesis is the enzyme, glucose oxidase, which catalyzes the oxidation of B-D-glucose to D-glucose-d-lactone, which hydrolyzes rapidly to D-gluconic acid, the second commercial product.

Gluconic acid and glucose oxidase are commercially produced by *Aspergillus niger.* Gluconic acid, its salts and gluconolactone are used for various medical and industrial purposes. Calcium gluconate is used as Ca supplement in human and animal

diets. Sodium gluconate is an ingredient of alkaline bottle washing preparations for cleaning equipment to prevent scale formation, iron deposition and for alkaline derusting operations. It is also an additive in concrete manufacturing. Gluconic acid is used in dairies to prevent the deposition of milkstone and for cleaning equipment. D-gluconolactone is used in meat processing and in making soybean curd. The world annual gluconic acid production is about 45,000 MT (Bronn 1976 as cited by Rohr 1983).

Glucose oxidase, on the other hand, is a higher value product. In the food industry, it is used as a safe preservative where glucose is oxidized and in the process produces hydrogen peroxide which has an anti-bactericidal effect. Hydrogen peroxide is easily transformed into water. The addition of the enzyme is known to improve the color and flavor of foods. Glucose oxidase is a known reagent used to determine the presence of glucose (White & Secor 1957). Glucose oxidase is the active component of diagnostic kits for diabetics since the enzyme is highly specific to glucose and its oxidation of glucose is easily detected with a color reaction.

There are both economic and public health reasons for the production of gluconic acid and its salts and derivatives in this country. Hydrolysis of starches derived from cassava and other root crops can be used for their manufacture. Early results also show the use of distillery slops, an obnoxious by-product of alcohol fermentation for glucose oxidase. The market for gluconic acid and its derivatives already exists since we have a growing manufacturing industry that could use non- corrosive cleaning preparations based on sodium gluconate. More importantly, the availability of low-cost gluconolactone could provide a safer alternative to the present use of gypsum in soybean curd preparations. A more affordable diagnostic kit for diabetes could save millions of lives as diabetes is a major killer disease among Filipinos.

Rohr, Kubicek and Kominek (1983) reviewed the history and production of gluconic acid from various fungal and bacterial species. Although the commercial processes described used *Aspergillus niger*, a commercial process utilizing *Penicillium luteum* has also been patented. Other *Penicilium* species reported to produce gluconic acid were *P. chrysogenum, P. divaricatum, P. citrinum* and *P. puberulum.* Kusai et al. (1960) crystallized the

glucose oxidase of *P. amagasakiense*. No mention has been made about the use of *P. funiculosum* and the possible multiple production of gluconic acid and glucose oxidase. This could be because the commercial utilization of glucose oxidase as a diagnostic tool came at a later date, whereas most of the fermentation studies on gluconic acid were done in the '20s and '50s.

This paper reports the production of extracellular gluconic acid and glucose oxidase from a single fermentation process using *Penicillium funiculosum* Thom 4072. This was identified as a high producer using a rapid screening procedure developed in the study. Results show the possible use of industrial wastes for production.

MATERIALS AND METHODS

Development of a rapid screening procedure

Fourteen *Penicillium* strains were obtained from the NSRI Microbial Culture Collection. These were grown in a liquid glucose medium which was primarily used for producing gluconic acid from *Aspergillus niger* (May et al. 1927). The medium contained 0.5 g $MgSO_4.7H_2O$, 0.1 g KH_2PO_4, 0.1 g KCl, 1.0 g $NaNO_3$ and 100 g glucose per liter. The pH was adjusted to 5.5 - 6.0 after sterilization. The culture filtrates were assayed for glucose oxidase activity and gluconic acid production. These strains were classified into three categories and classified as high, medium and zero producers of gluconic acid. Six strains representing these three categories were selected and used to test the rapid screening procedure developed.

The rapid screening procedure was designed based on the observation that the production of gluconic acid decreases the pH of the culture medium. It was assumed that a rapid decrease in pH indicated high production of gluconic acid. The decrease in pH of the medium was monitored using the non-toxic dye, bromphenol blue which changed from blue to yellow within the pH range of 4.6-3.0, the pH range found to indicate the presence of gluconic acid. Bromphenol blue solution was prepared and filter-

sterilized before it was added to the medium at a final concentration of 120 mg per liter.

The three categories of strains were grown in agar-solidified malt yeast glucose medium for 3-5 days. Approximately, 1 cm^2 of mycelial mat was transferred to agar solidified bromphenol blue glucose medium. The change in color of the area surrounding the mycelia was observed after five hours. Yellow halo around the colony was taken to indicate a pH change to a more acidic level and the production of gluconic acid. Based on this screening method and the correlated high glucose oxidase activity and high gluconic acid produced, *Penicillium funiculosum* Thom 4072 was used in subsequent experiments with *A. niger* NRRL3 as control.

Comparative performance of *P. funiculosum* 4072 and *A. niger* ATTC 9024

P. funiculosum 4072 was compared with *A. niger* ATCC 9024-(NRRL3), a standard strain known to produce high amounts of gluconic acid (Linko 1981) from the American Type Culture Collection. The presence of extracellular proteases which could destroy extracellular glucose oxidase was tested in both strains. Their production of glucose oxidase was also compared.

The extracellular proteolytic activities of *P. funiculosum* 4072 and *A. niger* ATCC9024 were detected using sterile skim milk broth prepared with 350 grams of skim milk per liter distilled water. The isolates were incubated at room temperature and observed after seven days of growth. Proteolysis of the media was indicated by the clearing of the colloidal suspension due to precipitation of milk proteins (peptonization).

The two strains were grown at 30°C in 10% glucose liquid medium with pH adjusted to 6.0 and incubated for 36 hours. The culture filtrates were filtered in ordinary Whatman No. 1 filter paper to remove the mycelia and assayed for glucose oxidase activity.

Studies on fermentation conditions

Gastrock and Porges (1978) identified three stages of growth of *A. niger* in the fermentation of gluconic acid, maintenance stage, germination stage (inoculum) and production stage, and developed corresponding media for each stage. This same princi-

ple was used in developing fermentation conditions for *Penicillium funiculosum* 4072.

Inoculum production

The inoculum was produced by taking spores from agar slant cultures which were then grown in shake flask cultures and transferred to the final fermentation medium. Two media compositions were tested for spore production in slant cultures: Gastrock and Porges agar slant medium (1938), consisted per liter distilled water of 30g glucose, 0.10g $MgSO_4.7H_2O$, 0.12g KH_2PO_4, 0.225g NH_4NO_3, 0.25 peptone, 20g agar and 4g $CaCO_3$; and modified Czapek Dox wherein sucrose was replaced by glucose. Since spore production in the latter was better, subsequent experiments used modified Czapek Dox in producing spores.

Cultures were grown for seven days in Modified Czapek slants. Sterile 3 ml deionized water was added to the tube and the surface was gently scraped using a wire loop. From this tube, 1 ml was withdrawn and transferred to a 50-ml shake flask medium (Gastrock & Porges 1938). The medium consisted per liter distilled water of 100g glucose, 0.25g $MgSO_4.7H_2O$, 0.3g KH_2PO_4, 0.80g $(NH_4)_2HPO_4$ and 0.02 g peptone. Two 50-ml 36-48-hr old shake flask cultures were used to inoculate a one-liter fermentation medium. The fermentation medium consisted per liter distilled water of 100g glucose, 0.156g $MgSO_4.7H_2O$, 0.188g KH_2PO_4, 0.388g $(NH_4)_2HPO_4$ and 26g $CaCO_3$. A one-liter capacity Cole Palmer stirred tank fermentor was used.

The following fermentor conditions were used:

rpm	:	250
aeration	:	2.0 vvm
temperature	:	room (26°C)
duration	:	0-60 hrs
pH	:	not maintained

Sucrose and glucose were used separately as substrate to determine which would support better enzyme and gluconic acid production. The effect of adding calcium carbonate to the fermentation medium on glucose oxidase and gluconic acid production was observed. Glucose oxidase activity level and gluconic acid production at different fermentation times were

studied by obtaining aliquots every six hours from the fermentation medium.

Calcium gluconate recovery

Gluconic acid was recovered from fermentation broths as calcium gluconate. Cells and other large particles were filtered out from the fermentation broth. Calcium carbonate was added to the filtrate in treatments without calcium carbonate. Measured amounts were allowed to cool down in a refrigerator. After cooling, the precipitate was quantitatively weighed after residual sugars were washed off. The precipitate was over-dried and submitted for analysis to the Analytical Services Laboratory of the Institute of Chemistry, University of the Philippines, Diliman which used an infrared spectrophotometer and the reagent grade, calcium gluconate, as standard. The yield of calcium gluconate was calculated as follows:

$$\text{Yield} = \frac{\text{amount of calcium gluconate}}{\text{amount of substrate used}} \times 100$$

Production ot glucose oxidase using industrial waste

P. funiculosum 4072 was grown in shake flask cultures in sugarcane juice and various food processing wastes: stale mango puree diluted to 50%; sugarcane processing intermediates and by- products diluted to 20%; and affination syrup, molasses, cane syrup and distillery slops stored at 30°C for 36 hrs. The culture filtrate was obtained by filtration and the glucose oxidase activity assayed.

Glucose oxidase assay, gluconic acid determination and protein and glucose content determination

Glucose oxidase activity was assayed using a glucose oxidase kit obtained from Sigma (Sigma Chemical Co., St. Louis, Mo.). The gluconic acid contents of the filtrates were assayed using a gluconic acid kit obtained from Boehringer Mannheim (Biochemica, Mannheim, West Germany). Protein content was measured using the Bradford test (1976). Residual glucose was measured using the Somogyi-Nelson test (1944).

RESULTS AND DISCUSSION

Development of a rapid screening procedure

Table 1 shows the amount of gluconic acid produced by various *Penicillium* isolates and the final pH of their culture filtrates. Clearly, there is an inverse relationship between the final pH of the culture filtrate and the amount of gluconic acid produced. Those culture filtrates containing the higher amounts of gluconic acid, 5.23 g/l and 3.241 g/l had low pH levels of 2.75 and 3.78; whereas those filtrates that contained low and zero levels, 1.159 and 0.453 g/l had pH levels of 3.63, 4.26 and 6.19-8.55. Gluconic acid production is also dependent on the strain. Of the two *P. funiculosum* strains tested, one, Strain 4072 had a low pH culture filtrate that corresponded with high gluconic acid, whereas the other, Strain 4076 had a high pH culture filtrate. The gluconic acid yield per glucose consumed by the strain of *P. purpurogenum* used by Herrick and May (1928) ranged from 12.6-56.7%, whereas the strain used in this study, Strain 4069 produced only 13.48%. The wide range obtained for *P. purpurogenum* is due to the range in glucose concentrations used, 100-400g/l. Only 100g was used in this study.

The glucose oxidase of *P. funiculosum* 4072 is extracellular, it being detected in the culture filtrates. Kusai et al. (1960) also found the glucose oxidase of *P. amagasakiense* to be extracellular. The glucose oxidase of *A. niger* has been reported to be both extracellular (Mischak et al. 1985) and intracellular (Van Dijken & Veenhuis 1980). The high activity of glucose oxidase detected in culture filtrates of *P. funiculosum* 4072 indicates that the enzyme could be actively excreted similar to the glucose oxidase of *A. niger* (Mischak et al. 1985).

Mycelia of six strains classified according to their production of gluconic acid as high producers (*P. funiculosum* 4072, *P. cyclopium* 3885;), medium producers (*P. sp* 3251,*P. notatum* 3602) and zero producers (*P.* 3262, *P. lilacinum* 3422) were transferred to bromphenol blue glucose mediums to assess acid production and to liquid glucose mediums to measure the amount of glucose oxidase produced. Table 2 shows that of the six strains tested, only *P. funiculosum* 4072 produced a yellow halo around its mycelia 5 hours after inoculation, whereas all the rest did not. Also, the highest activity of glucose oxidase, 170×10^{-3} was observed with the *P. funiculosum* 4072 culture filtrate. *P. cyclopium* 3885, which ranked second in the first assay based

on gluconic acid produced and acidity of culture filtrate, ranked lower in this test. However *P. sp* 3262, which did not produce gluconic acid in the first set of experiments, produced glucose oxidase in this set. The results imply that among the strains tested, *P. funiculosum* 4072 consistently produced appreciable amounts of gluconic acid and glucose oxidase and was therefore used in subsequent experiments.

Comparative performance of *P. funiculosum* 4072 and *A. niger* ATTC9024

The stability and production of the extracellular glucose oxidase is affected by the presence of extracellular proteases. In the skim milk broth test, *A. niger* ATTC 9024 tested positive for extracellular proteases as indicated by the clearing and peptonization of the broth whereas *P. funiculosum* 4072 did not. However, both strains grew on skim milk broth. This may indicate that proteases may have been produced by the *P. funiculosum* but not in amounts that could rapidly degrade the milk proteins. In subsequent experiments with *P. funiculosum,* some degradation of the glucose oxidase was evident after 36 hours of fermentation.

The amount of glucose oxidase produced by both strains was comparable. In the glucose liquid medium, *P. funiculosum* 4072 produced 0.858 uU of glucose oxidase whereas *A. niger* ATTC 9024 produced 0.823 uU.

Determination of fermentor conditions

Culture conditions during fermentation

Media composition affected spore production. In the production of spores in agar slants, two media were tested, Gastrock and Porges (1938) agar slant medium containing calcium carbonate compared with the modified Czapek Dox agar. Spore production in the calcium carbonate-supplemented Gastrock and Porges agar slant medium was very poor compared with that of the modified Czapek Dox medium.

To find out the chemical identity of the white precipitate obtained in refrigerated culture filtrates supplemented with calcium carbonate, a fermentation run was made wherein calcium carbonate was added at the rate of 26g/l. After 24 hours of fermentation, a mass of white precipitate, which was most likely excess calcium carbonate, was seen in the fermentor each time the motor was turned off. After 48 h of fermentation, calcium

carbonate was eliminated by filtering off. On standing and cooling, a mass of white precipitate appeared, as shown in Figure 1. This white precipitate was analyzed using an infrared spectrophotometer together with the standard sample of calcium gluconate. The results shown in Figure 2 show that the absorption peaks at the indicated wavelengths were identical. This result means that the two samples were the same and that the precipitate formed in culture filtrates supplemented with calcium carbonate was calcium gluconate of high purity. The process of recovering the calcium gluconate from the fermentation broth is a simple physical process that requires only additional refrigeration and drying of the precipitate. Refrigeration thus allows for the simultaneous recovery of an active glucose oxidase from the fermentation broth.

Figure 3 illustrates the growth of the fungus and the production of glucose oxidase and gluconic acid (recovered as calcium gluconate) with time. The growth of the fungi was diauxic, rapid growth occurred twice during the 60-hour fermentation run. The first growth phase started about 6 h after inoculation and leveled off 18-42 h after inoculation. The second growth phase occurred after 42 h from inoculation and leveled off again about 52 h after inoculation. Glucose oxidase was detected only after the start of the first growth phase and the highest activity detected from broths was obtained during the first stationary phase. However, the amount of the enzyme appeared to have decreased during the second growth phase and started to increase again when the second stationary phase was reached. On the other hand, gluconic acid appeared to have been produced starting at the time the glucose oxidase was detected 12 h after inoculation and increased up to 36 h after inoculation. From then on, the amount remained the same until 60 h.

The second phase of growth of *P. funiculosum* 4072 during the 60- hour fermentation was associated with the decrease in the glucose oxidase activity. This might indicate that the fungus used the glucose oxidase for growth not as carbon source but as an invigorating factor similar to the action of peptone in the germination medium. Apparently, the protease that degraded the enzyme might have appeared only at the onset of the stationary phase. This would explain the ability of the cultures to accumulate the glucose oxidase before the stationary phase. The protease might have been released from the cells due to autolysis. Van Dijken and Veenhuis (1980) attributed the extracellular glucose oxidase in *A. niger* to the occurrence of autolysis.

The occurrence of the second phase of active growth might have produced inhibitors to the proteases and, thus, again allowed for the accumulation of the glucose oxidase.

The amount of gluconic acid produced was limited to a certain proportion of the glucose in the medium. This yield of about 65% was reached when the maximum amount of enzyme was present in the medium afterwhich no increase in yield with time was observed. This could be due to the decreased concentration of glucose in the medium and to the decreased amount of enzyme. Several studies with *P. luteum purpurogenum* and *A. niger* have shown that low glucose concentrations in the medium yielded lower gluconic acid amounts, while higher glucose concentrations up to 20% yielded higher amounts of gluconic acid (May et al. 1939; Herrick & May 1928; Gastrock & Porges 1938).

The 65% yield obtained for *P. funiculosum* 4072 at 10% glucose was higher than that obtained for P. luteum purpurogenum which was 40-42% (Herrick & May 1928) at 10% glucose. However, it is possible that we may obtain higher yields with increased glucose concentrations, which we intend to do in subsequent experiments.

Effect of Calcium Carbonate on Glucose Oxidase Production

Figure 4 shows the enzyme activity and pH levels during a 60-hour fermentation time with and without calcium carbonate added to the fermentation broth. The figure shows that the enzyme activity level produced when calcium carbonate was added to the fermentation broth was highest at 36 h which decreased to almost zero at 48 h, started to increase again at 54 h and continued to increase up to 60-hour fermentation time. Enzyme activity level at 36-hour fermentation time was about 51% higher than that of the standard commercial glucose oxidase containing 0.5 U. The final enzyme activity obtained after 60 h of fermentation was also higher than that of the commercial glucose oxidase solution. The high level of enzyme activity in the culture filtrates after 36 h fermentation indicates the commercial feasibility of the process.

The enzyme produced in broths without calcium carbonate was very low compared with that produced with calcium carbonate. This could be due to the buffering effect of calcium carbonate as shown in the graph; the pH level of the broths without calcium carbonate shifted continuously to very acidic levels; whereas, those broths to which calcium carbonate was added

remained at pH 6.6-6.8. In *A. niger,* the type of organic acid formed was controlled by the pH of the medum. At pH below 2, citric acid was produced, whereas at pH above 5, gluconic acid was produced. Further, Mischak et al. (1985) have shown that by increasing the pH of citric-producing cultures to 6.5, the cultures started producing glucose oxidase. The mechanism may be similar in the case of the *P. funiculosum* 4072, that is, pH 6.6-6.8 could be optimal for glucose oxidase production. As indicated in the graph for cultures without calcium carbonate, the pH decreased to 3.0 after 18 h of fermentation, which coincided with the slight decrease in glucose oxidase activity, thereafter. Apparently, no glucose oxidase was synthesized after the 18th hour.

Figure 5 shows that sucrose did not promote glucose oxidase production with or without calcium carbonate even if it promoted mycelial growth. The inducing effect of glucose on glucose oxidase production has been observed in *A. niger* (Mischak et al. 1985; Duke et al. 1969). Although one can infer that glucose was produced by the hydrolysis of sucrose in our cultures, possibly a certain cellular level has to be reached for glucose to have an inductive effect on glucose oxidase production. Probably, that intracellular level of glucose was not reached by our cultures grown on sucrose.

Glucose oxidase production in food processing wastes

Table 3 shows the glucose activity observed with culture filtrates of *P. funiculosum* grown on food processing intermediates and by-products. Apparently, glucose oxidase can be produced better in distillery slops, molasses A, mango puree and molasses B better than in affination syrup, cane juice and cane syrup.

The reason that distillery slops was a good medium for producing glucose oxidase was the presence of dead yeast cells which could have provided hydrolyzed proteins. Also, the presence of the hydrolysis products of sucrose, starches and other carbohydrates could have provided a higher amount of free glucose in the medium. Molasses were also expected to contain hydrolysis products unlike the cane juice and syrups, hence, the higher yields of glucose oxidase. Mango puree, having been heat processed, most likely contained glucose, hence it served as a better medium.

CONCLUSION

Our results show the feasilbility of producing two commercial products from the same fermentation process, glucose oxidase and gluconic acid. The conditions for fermentation are the same and the processes of product recovery are compatible. The possible use of distillery slops, a problem waste in alcohol fermentation, adds further atrraction to the process. Overall, further studies have to be made to optimize the process and assess the market for the products.

Table 1. The amount of gluconic acid produced (g/l) by various *Penicillium* strains grown in 1% glucose and the final pH of their culture filtrates

Fungal Strains		pH of Filtrate (final)	Gluconic acid (g/l)
P. funiculosum	4072	2.75	5.23
P. cyclopium	3885	3.78	3.241
Penicillium sp.	3251	3.90	2.603
Penicillium sp.	3021	3.89	1.724
P. purpurogenum	4069	4.00	1.348
P. notatum	3602	3.63	1.159
P. lilacinum	3423	4.26	0.453
P. lilacinum	3260	6.19	0.000
P. lilacinum	3422	6.21	0.000
P. funiculosum	4076	6.40	not tested
P. lanoso-coereleum	4070	6.61	not tested
P. chrysogenum	3872	7.88	not tested
Penicillium sp.	3262	8.41	0.000
P. lanoso-coereleum	3493	8.55	0.000

Table 2. The reaction to bromphenol blue and glucose oxidase production of selected fungal agar cultures grown on 10% glucose

Fungal Strains		Presence/Absence of Yellow Halo in bromphenol blue	Glucose Oxidase Activity (uU)
P. funiculosum	4072	+	170×10^{-3}
P. notatum	3602	-	16.4×10^{-3}
Penicillium sp.	3262	-	5.20×10^{-3}
P. cyclopium	3885	-	4.10×10^{-3}
Penicillium sp.	3251	-	0
P. lilacinum	3422	-	0

* Legend
(uU) = micromolar unit

Table 3. Glucose oxidase activity (uU) in culture filtrates of *P. funiculosum* 4072 grown in various sugar cane by-products as substrates

Sugar Cane By-Product	Glucose Oxidase Activity (uU)
Affination Syrup	1.444
Molasses A*	3.433
Molasses B*	2.699
Distillery Slops*	3.800
Syrup*	1.894
Cane Juice	1.764

* diluted 1:4 with distilled water

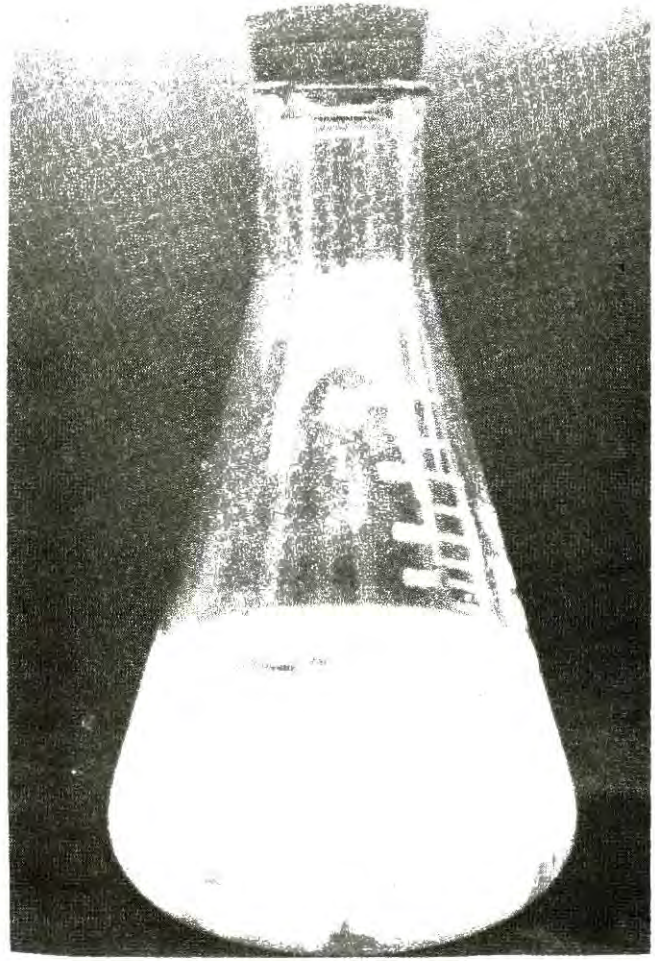
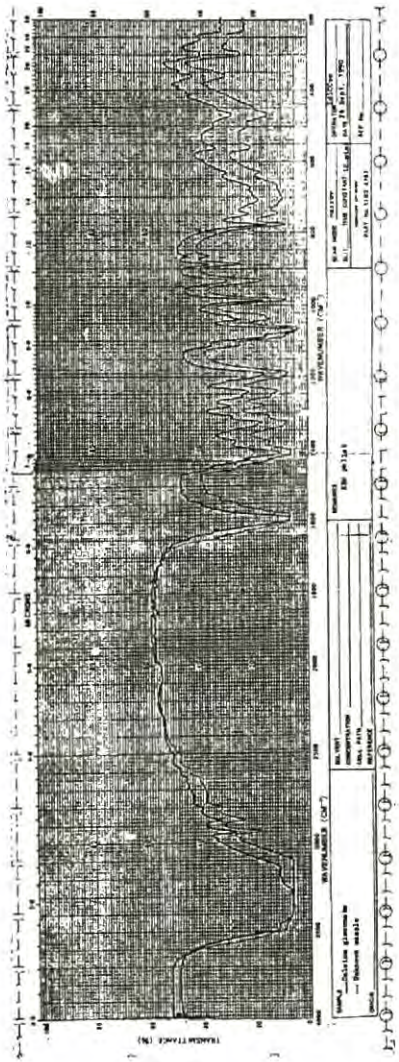


Figure 1. Fermentation filtrate upon cooling, showing Ca gluconate precipitate



Analytical Services Laboratory
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ASL (900)-98)

26 Sept. 1990

Sample: One(1) Calcium gluconate standard and one (1) unknown solid sample
Analysis Requested: Infrared Spectroscopy
Requesting Agency: NSRI c/o Mr. Benjamin C. Glorie Jr.

RESULT OF ANALYSIS

Based on the spectra obtained using the infrared spectrophotometer, the two samples run, (Calcium gluconate and the unknown sample) are identical.

Please see attached IR spectra.

(Analysis was done only on the samples submitted to this laboratory.)

Lilibeth dlC. Coo
Analyst

Noted by:

Prof. Rosendo C. Lorica
Supervisor

Figure 2. Infrared spectrophotometer analysis of product, showing identical absorption peaks with reagent grade Ca gluconate

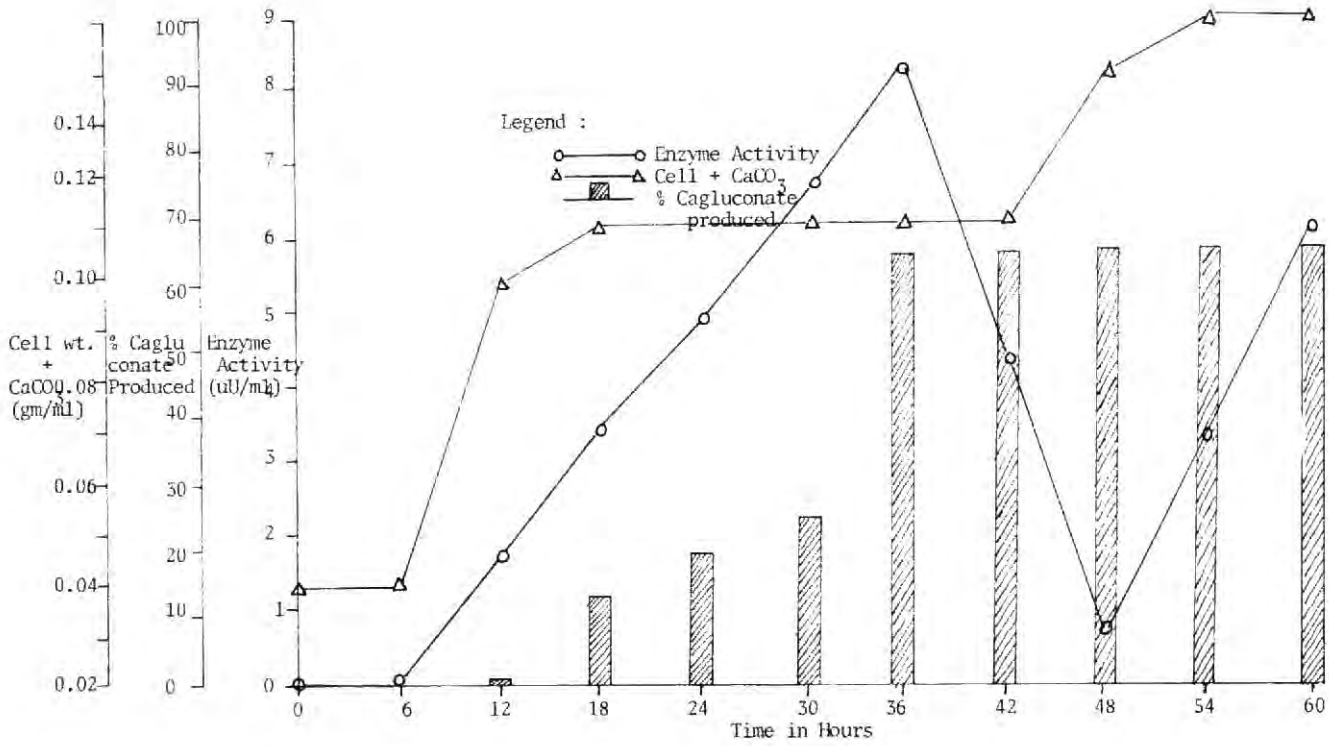

Figure 3. **Glucose oxidase activity, % Ca gluconate produced and cell + $CaCO_3$ wt., at different fermentation times, using glucose as substrate, $CaCO_3$ added to medium**

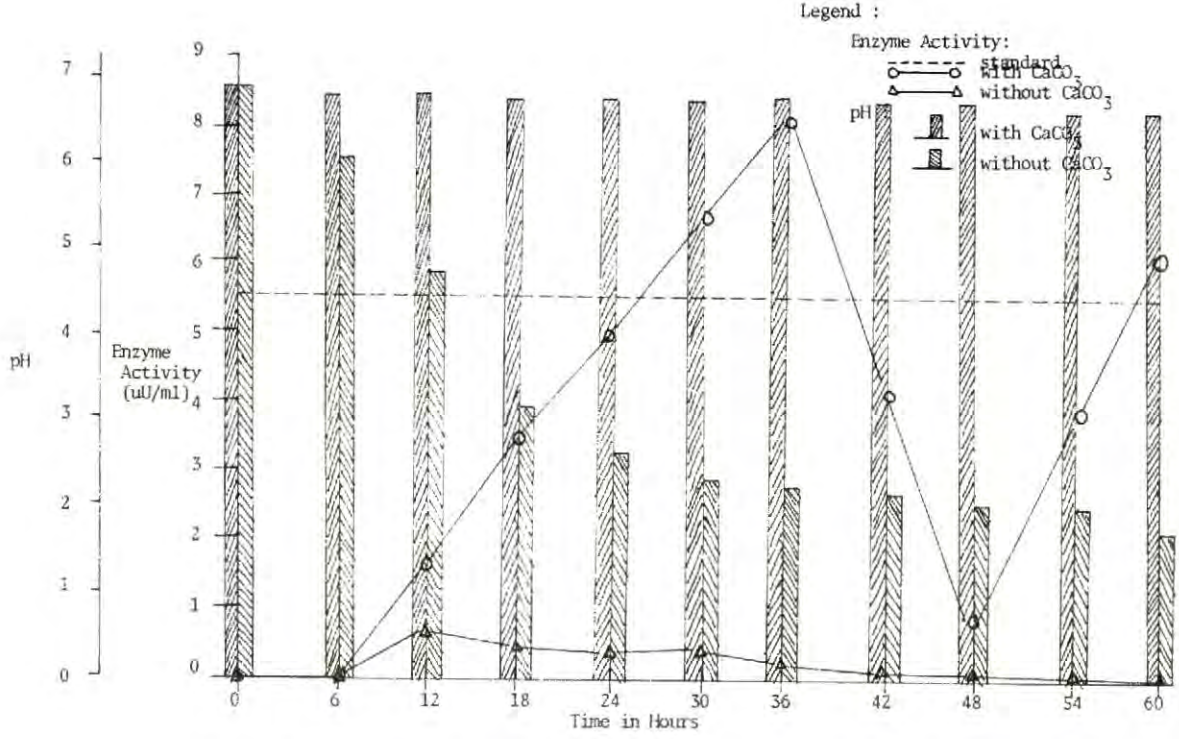

Figure 4. Effect of added $CaCO_3$ on glucose oxidase activity and pH level, at different fermentation times, using glucose as substrate

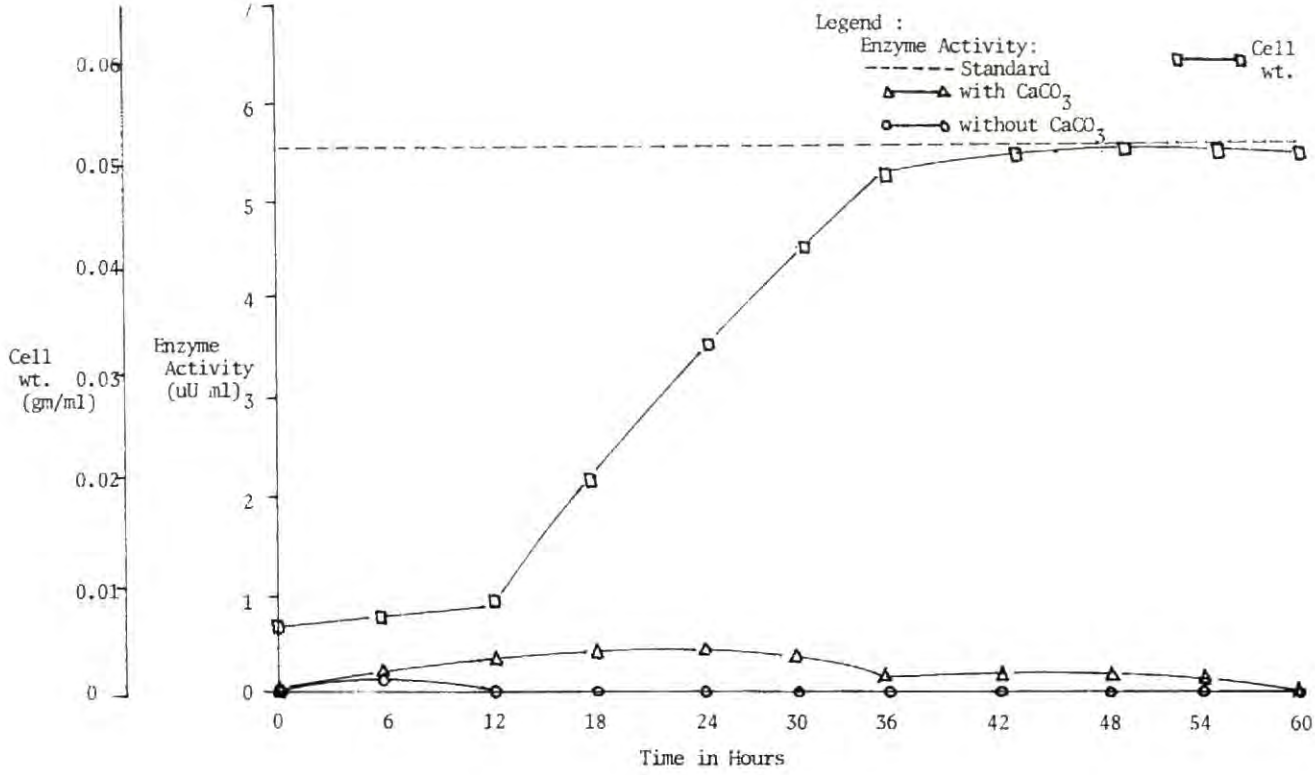

Figure 5. **Effect of $CaCO_3$ on glucose oxidase activity and cell wt., at different fermentation times, using sucrose as substrate**

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Histopathological Effects of Manganese Intoxication

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ABSTRACT

Manganese, known to be an essential and the least toxic of the trace elements, is released to the aquatic environment by the steel, paint and mining industries, among others. This study was done to investigate the effects of this metal, a potential environmental toxicant, on some organs of juvenile ***Tilapia nilotica.***

Sublethal exposure to 2000 ppm Mn for several days led to histopathological changes in the gills, liver, kidney and intestines. The extent of the damage ranged from formation of cytoplasmic vacuolations, nuclear pyknosis, fatty infiltration and epithelial hypertrophy to severe necrotic conditions.

INTRODUCTION

Manganese (Mn) is one of the metals whose use in industries has increased; the possibility of its hazardous effects has increased as well. Thus, while some metals are classified as essential nutrients, like Mn, they can also serve as environmental hazards if the mechanism which maintains them within their functional limit are unbalanced.

Manganese is among the trace elements least toxic to animals. In fact, it is even involved in many biological processes. But it was discovered that it can affect the respiratory tract (Mn pneumonitis due to acute exposure) and the central nervous system (Chronic Mn poisoning), also known as manganism (**6**).

Those who work in mines (**30**), ore crushing plants, smelters (**13**) and those engaged in occupations where exposure to the metal may be high, are the most vulnerable. Non-occupational cases of manganism have also been reported (**5**). With the introduction of unleaded gasoline, Mn is used as an anti-knock ingredient in automobile fuels (**1**). This means that, for the first time, the general population can be exposed daily to low levels of this essential but potentially toxic metal. Another source of Mn intoxication is the use of fungicide maneb (mn ethylene-bis-dithiocarbamate). Two young agricultural workers who were exposed to maneb developed the Parkinsonian syndrome which characterizes manganism (**20**).

Most studies on Mn toxicity deal with its effects on the central nervous system (CNS). Investigations have shown that the symptoms and signs of Mn encephalopathy share several features in common with Parkinson's disease and they are directly linking the metabolism of catecholamines and the concentration of Mn in the brain (**10, 31, 34, 37, 15**). They observed that it is characterized by psychiatric and neurological symptoms. Rats chronically treated with high oral load of $MnCl_2$ showed decreased concentrations of dopamine and homovanillic acid (HVA) in the brain. A return to normal values was observed after L-DOPA injections (**7**).

Neurochemical and physiological studies of Mn toxicity were mostly performed on mammals. These include lipid peroxidation inhibition in rat brain (**8, 38**); alterations of lipid synthesis (**12**); inhibition of activities of different enzymes like glutamic acid decarboxylase, choline acetyltransferase acetylcholinesterase (**25**) and monoamine oxidase (**27, 26**). It has also been reported that the metal can inhibit Na-K-ATPase and Mg-ATPase (**39, 23**). Hong and co-workers (**22**) also observed that plasma levels of some hormones and neuropeptides can be susceptible to Mn treatment.

Investigations on the toxicity of Mn on aquatic organisms have been done but they are relatively few. According to Cossarini- Dunier (**14**), the metal can be released into the water by industries like steel, mining, paint, textile dyes and fungicides from run-off water. Agrawal and Srivastava (**2**) reported a 96-hr LC_{50} value of 2850 mg/L wherein they used the freshwater fish, *Colisa fasciatus*. They observed hematological abnormalities in the fish exposed to 2500 mg/L $MnSO_4$ for 90 hrs. It was also observed that there were decreased spermatogenic activity and

also hemmorhage in the testes of *Colisa fasciatus* at the same Mn concentration (**40**). Ultrastructural changes in the gills of *Sarotherodon mossambicus* have also been observed. The fish were fed with chicken manure and significantly higher concentrations of Mn and other metals were obtained in the gills (**28**). Nath and Kumar (**32, 33**) also used *Colisa fasciatus* to investigate the impact of Mn on carbohydrate metabolism using 2584 ppm of Mn as treatment. They reported a value of 3230 mg/L as 96-hr LC_{50}. Evtushenko (**19**) studied lipid metabolism and the liver protein synthesizing function in the carp exposed to Mn.

The objective of this study, therefore, is to investigate the effects of Mn on *Tilapia nilotica,* a known hardy species of fish. Histopathological changes manifested by different tissues will be determined since there has been little information regarding this aspect of Mn toxicity. These findings are significant in understanding fully the mechanism of Mn toxicity.

MATERIALS AND METHODS

Tilapia fry from the Central Luzon State University, Muñoz, Nueva Ecija were acclimated for one week prior to $MnCl_2$ exposure. The 24-hr and 96-hr LC_{50} were determined (4000 mg/L and 3000 mg/L, respectively) after which the fry were exposed to a sublethal concentration of Mn at 2000 mg/L (30 fry/aquarium), while another group served as control. Water spiked with $MnCl_2$ was changed every other day. The fry were fed with commercial fish flakes everyday. The fry, from both the control and treated groups, were then dissected after eight days of exposure. Tissues were fixed with 2.5% glutaraldehyde, washed with 5% sucrose buffer, post-fixed with 1% osmium tetroxide, dehydrated in a series of graded acetone concentrations, infiltrated with propylene oxide and embedded in Araldit resin. Sections were then prepared for light and electron microscope observations. LM sections were stained with toluidine blue while EM sections were stained with uranyl acetate and counterstained with lead citrate. EM sections were observed using a JEOL 100 EM. Photomicrographs and electron micrographs were prepared.

Tilapia fry exposed to 4000 mg/L (24-hr) and 2000 mg/L (96-hr) Mn were processed using the paraffin method. Serial sections prepared were stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

Gills

The main target of the action of pollutants in fishes is considered to be the gills since they are the primary sites for both passive and active exchange of gases and ions (**24**). The normal morphology is shown in Fig. 1. The respiratory lamella is lined by an epithelium that is two squamous cell layers thick. Internal to the epithelium is the lamellar blood sinus, lined and spanned by pillar cells of contractile function. A marginal blood channel, lined by endothelium,occurs within the apex of the lamella. A thick stratified epithelium lines the epithelium between gill lamellae. In this interlamellar epithelium, there occurs scattered cells of two special types: choloride cells and mucous cells (**29**).

Mn-induced gill lesions investigated with light microscope include the following: hyperplasia of the lamellar epithelium which appears to begin at the bases of the lamellae (Fig. 2) and gradually fills up the lamellar troughs which lead to lamellar fusion; epithelial lifting (edematous spaces and sloughing off of branchial epithelium); necrosis and degeneration of the secondary lamellae; and the total collapse of the pillar system.

The most common gill alteration is the lifting of the epithelial cells which represents an infiltration of the epithelia with fluid. Lifting, swelling and hyperplasia of the lamellar epithelium could serve as a defense function, as these alterations increase the distance across which waterborne irritants must diffuse to reach the bloodstream. Lamellar fusion could also be protective in that it diminishes the amount of vulnerable gill surface area (**29**).

Figures 3 and 4 show normal and treated secondary lamella observed using an electron microscope. Observations revealed the sparsity or lack of microvilli, swelling or blebbing of the nuclear envelope, rough endoplasmic reticulum (ER) in the form of vesicles (Fig. 5), damaged mitochondria and widened intercellular spaces. Most of these changes indicate cell damage or death.

Liver

The hepatic parenchymal cells or hepatocytes appear as two-cell layer thick laminae, in anastomosing plates or in clumps or rosettes of several cells (Fig. 6). Each hepatocyte represents an irregular polygonal form and most contain a single spherical nucleus, with a dense nucleolus. The cytoplasm stains irregularly, alternating light and dense regions being located variably in

the cell. The nuclei are frequently masked by the dense regions. The light regions represent areas of glycogen accumulation and the dense regions represent areas of abundant rough endoplasmic reticulum (**11**).

Figure 7 shows some morphological changes in the liver in response to Mn treatment. Vacuolations and fatty infiltrations were evident. Other abnormalities included hepatocyte hypertrophy, nuclear pyknosis and extensive liver cord disarray. Necrosis and degeneration of hepatic tissues were also observed. At the ultrastructural level, mitochondrial damage was the most conspicuous. Some appeared swollen and active while others exhibited cristae which were not clearly outlined (Fig. 8). Vacuoles were present, rough endoplasmic reticulum lost its typical organization and became fragmented. Glycogen tended to accumulate in specific areas rather than disperse evenly. Manifestations of cytopathological changes in the liver clearly suggest impaired metabolic status of this vital organ (**21**). Dickinson and Hart (**16**) reported that sheep liver cytoplasmic aldehyde dehydrogenase is strongly inhibited by Mn ions. Liver glycogen was also reported to be depleted by Mn (**35**). Tolbert et al. (**42**) observed that Mn stimulated gluconeogenesis in enzymatically-isolated rat hepatic parenchymal cells.

Kidney

The normal kidney (Fig. 9) shows coiled uriniferous tubules not arranged in any specific pattern, the Malpighian or renal corpuscles and the parenchyma that form the interstitial hemopoietic tissue. The initial portion of the tubule is the neck segment where numerous cilia are present. The proximal segment that follows is characterized by columnar epithelial brush border. It leads to the distal segment having closely- packed darkly stained cells which in turn end in the collecting duct (**3**).

Renal lesions following Mn exposure included the swelling of the Bowman's capsule or space, vacuolations of the tubular epithelial cells, distended or dilated renal tubules and sometimes nuclear pyknosis (Fig. 10). Degenerative changes such as collapsed glomeruli were also observed. The renal tubules sometimes appeared to be widely separated from one another showing disorganized or hydropic condition. Endoplasmic reticulum of the tubular epithelial cells was destroyed while the mitochondria showed remnants of cristae. Electron microscope observations also revealed the presence of vacuoles and some damage to the

microvilli. Glomeruli capillaries are swollen (Fig. 11). Kramer and co-workers (**23**) determined the potential role of Mn (as trace metal) in the pathogenesis of renal diseases. They investigated the effects of various trace metals on Na-K-ATPase, the biochemical correlate of active cellular transmembrane and sodium-dependent transport. It was found that Mn can inhibit the enzyme and accumulation of this metal may present serious hazards by producing a general defect in cell membrane transport.

Intestine

The intestines show the usual four-layered structure (Fig. 12). The outermost serosa is followed by a muscular coat, consisting of an outer longitudinal and an inner circular layer. The submucosa is divisible into an outer stratum compactum, a dense connective tissue arranged in a wavy pattern and an inner stratum granulosum rich in capillary network. The latter merges with the tunica propria of the underlying mucosal coat, there being no muscular mucosa. The epithelial lining of the mucosa consists of prismatic cells with basal nuclei. The nuclei of the intestinal mucosal cells are round with 2-3 nucleoli. The intestinal mucosal cells show a serrated margin with a number of interspaced goblet cells. The mucosal layer is thrown into folds (**3**).

The intestinal mucosa showed the most severe damage manifesting degenerative changes leading to complete destruction of the cells. Most of the mucosal cells were vacuolated. Some intestinal folds were separated from the mucosal layer. Dense bodies were present in the muscularis.

In severe cases, the goblet cells were considerably enlarged (Fig. 13).

Ultrastructural studies showed the presence of numerous vacuoles and destroyed cytoplasm of mucosal cells.

Other Organs

Ovarian tissue obtained from fry treated with Mn showed decreased number of eggs as compared to the control (Figs. 14 and 15). This resulted in widened spaces between oocytes. Some oocytes even lacked lipid droplets and the granular protein inclusion which were visible in the control.

Other tissues examined did not show alterations in the treated fish.

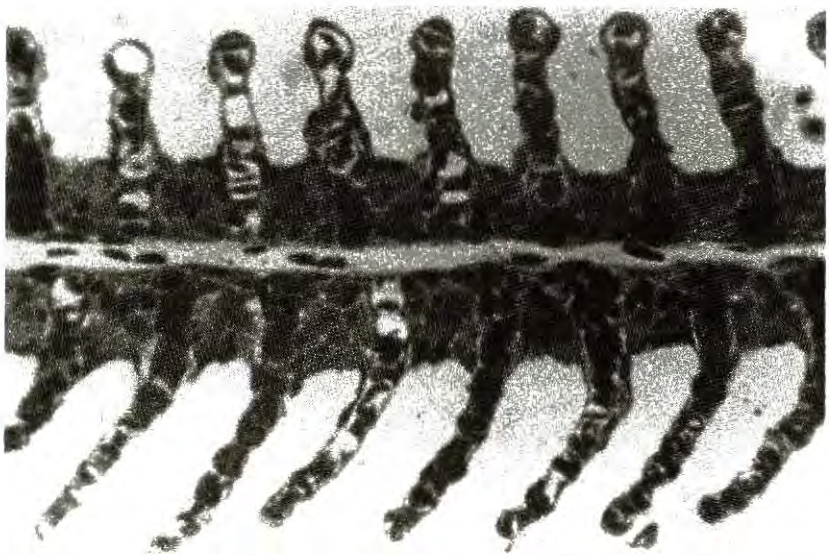

Figure 1. Respiratory lamellae of untreated fish [SG (Secondary lamella); PC (Pillar cell); MBS (Marginal blood sinus); LBS (Lamellar blood sinus); IE (Interlamellar epithelium)]

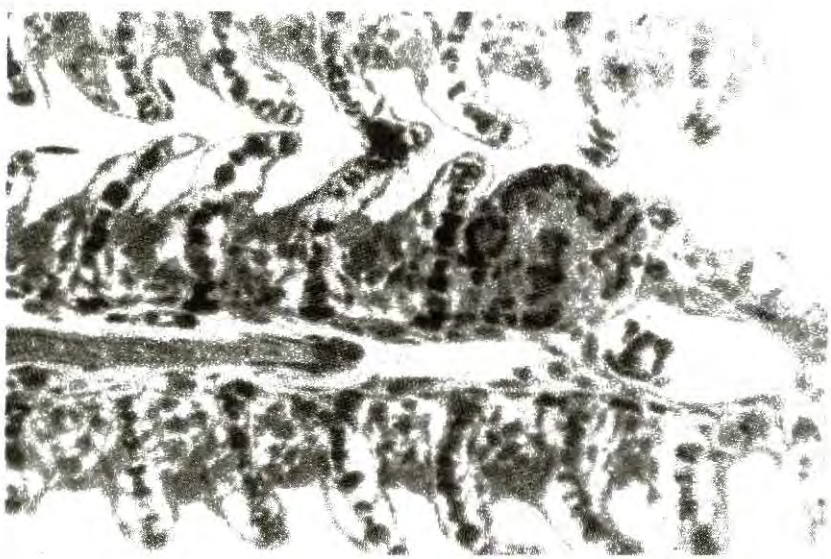

Figure 2. Respiratoy lamellae of treated fish showing hyperplastic growth of epithelial cells along the bases of the lamellae and epithelial lifting (edematous spaces)

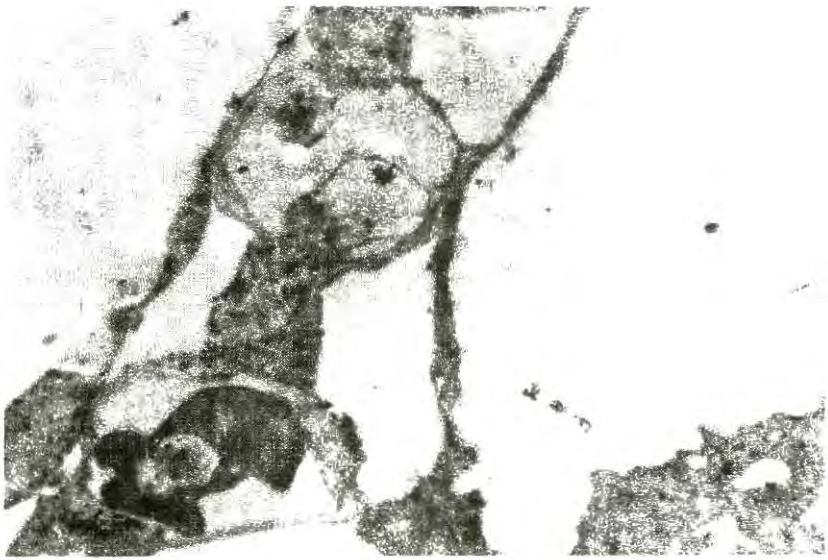

Figure 3. Electronmicrograph of secondary lamella showing intact pillar cell system [E (epithelium); PC (Pillar cell); RBC (Red blood cell); LBS (Lamellar blood sinus)]

Figure 4. Electronmicrograph of treated gill lamellae with damaged microvilli

Figure 5. Electronmicrograph of epithelial cell of treated fish with blebbing of nuclear envelope and the endoplasmic reticulum in fragments or vesicles [B (Blebs); N (Nucleus); M (Mitochondria); ER (Endoplastic reticulum)]

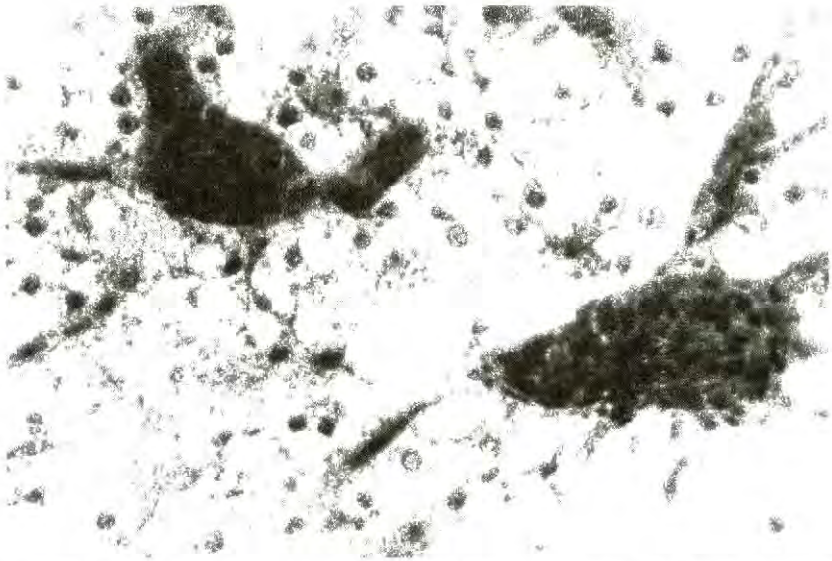

Figure 6. Liver from control fish [H (Hepatocyte); CV (Central vein); S (Sinusoids); G (Glycogen)]

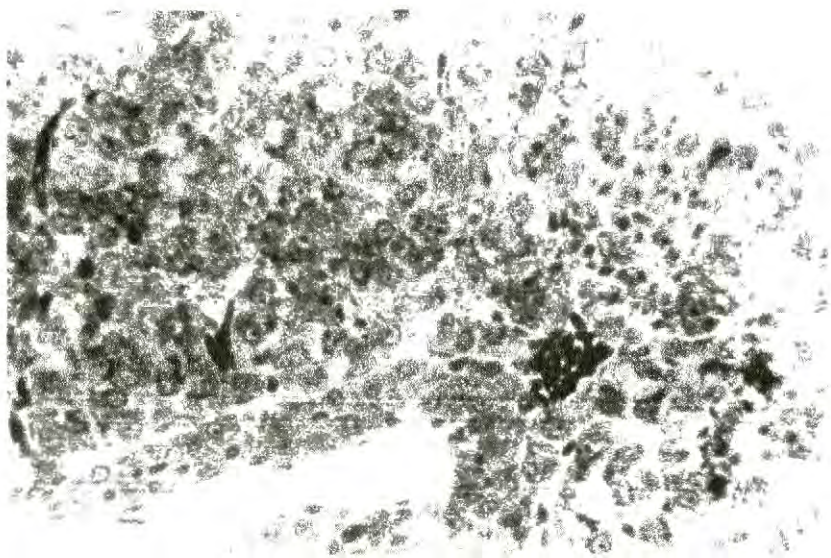

Figure 7. Liver from treated fish showing nuclear pyknosis and liver cord disarray

Figure 8. Electronmicrographs of hepatocyte with mitochondria devoid of cristae and disorganized endoplasmic reticulum

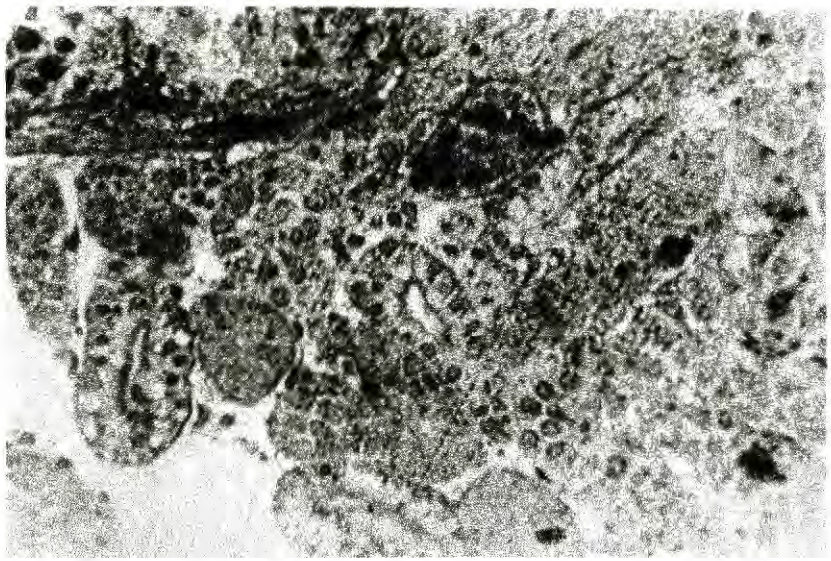

Figure 9. Renal tissue from untreated fish [G (Glomerulus); D (distal tubule); P (Proximal tubule); HT (Hemapoietic tissue)]

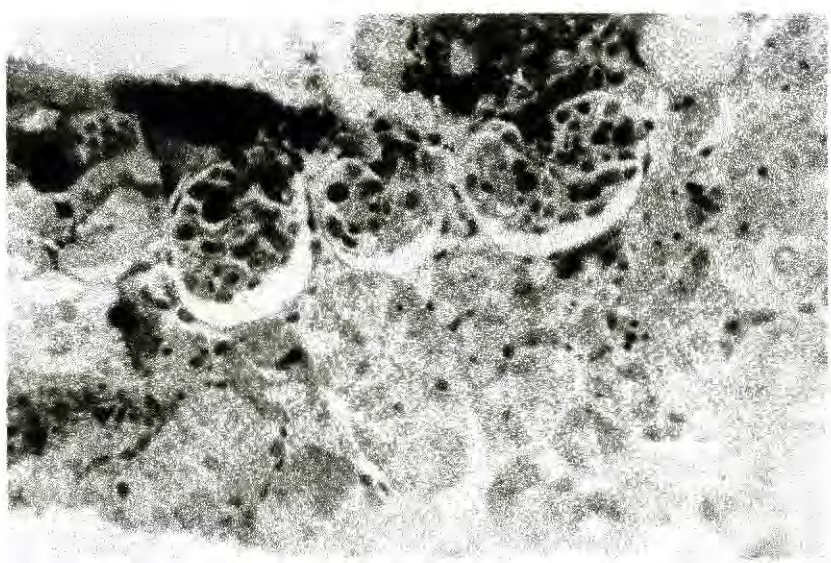

Figure 10. Treated kidney showing Bowman's capsule (BC) and hypertrophied tubular cells (HP)

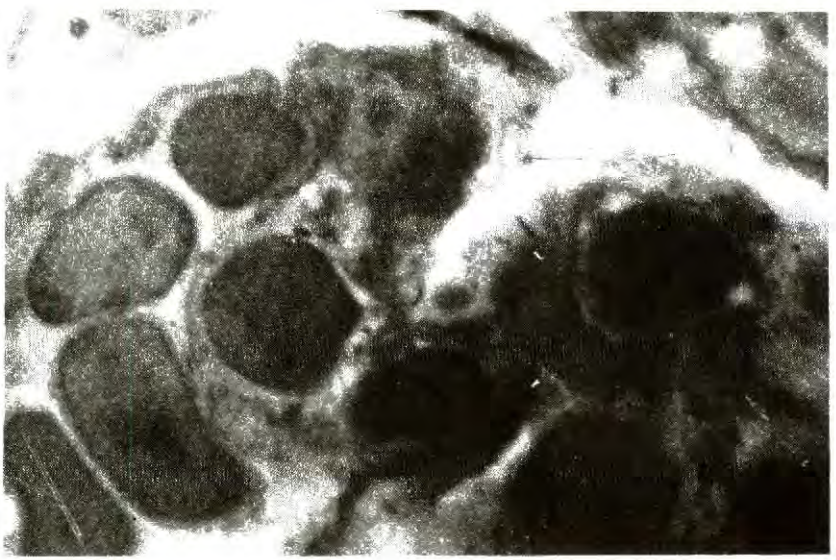

Figure 11. Electronmicrograph of glomerulus showing swollen capillaries (C); M (Mesangial cells); P (Podocyte)

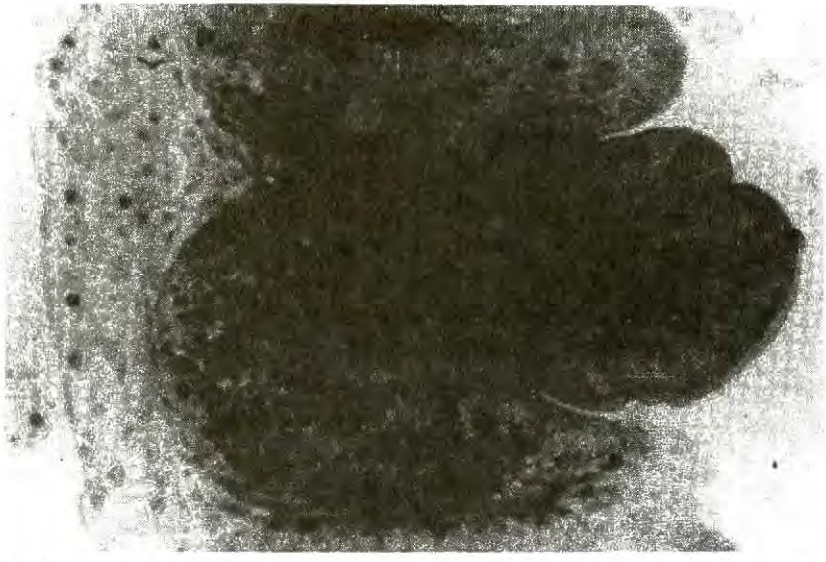

Figure 12. Normal fish intestinal structure [S (Serosa); M (Muscularis); SM (Submucosa); M (mucosa); G (Goblet cell)]

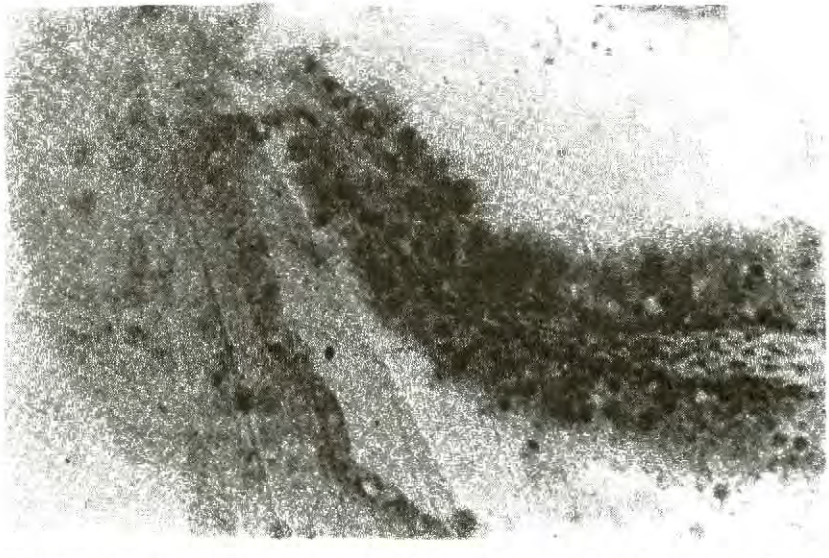

Figure 13. Intestinal mucosal folds from treated fish exhibiting degenerated epithelial lining of the mucosa (Goblet cells are enlarged.)

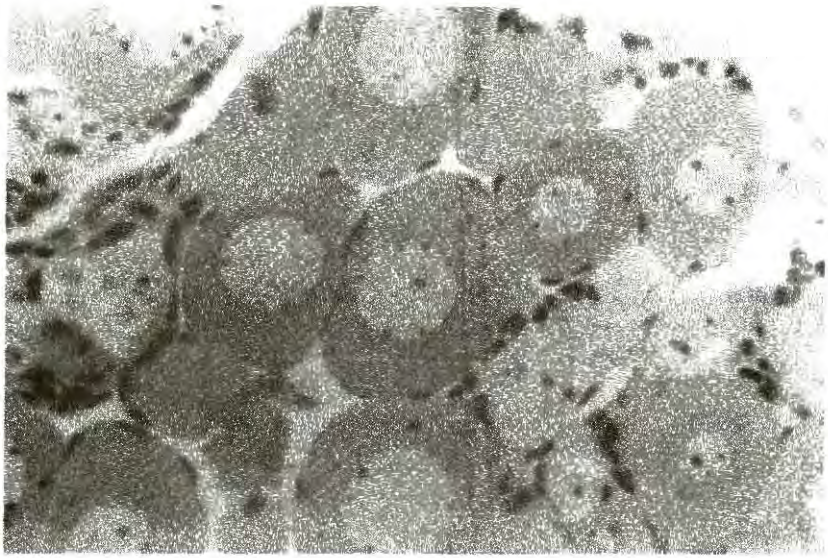

Figure 14. Oocyte from control fish (They appear closely packed.)

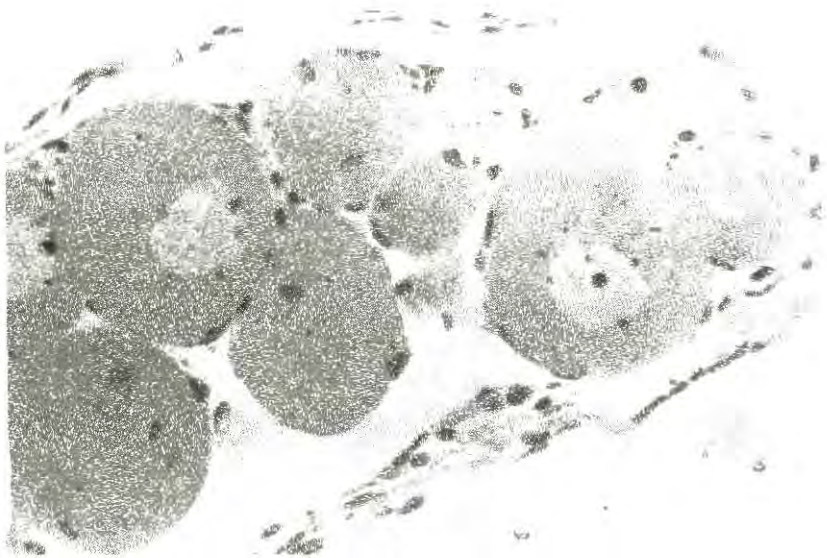

Figure 15. Ovary from a treated fish with loosely arranged oocytes and separated with thicker connective tissue

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Studies on the Effects of Gamma Radiation on *Kalanchoe Pinnata* (Pers.), Kataka-taka (Tag.), Life Plant (Eng.)

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ABSTRACT

Somatic and genetic abnormalities were induced. The percentage of regeneration of irradiated leaves was reduced markedly at a dose of 2000 r and the reduction increased linearly with increased radiation dose. Plantlet growth decreased linearly with increased dose and was evident even seven months after irradiation in mature plants. The types of chlorophyll deficiency mutant plantlets observed were dark green, yellow-green, yellow and white. The greatest frequencies of chlorophyll mutations were recorded at moderate doses of 3000 r and 4000 r. The frequency of induced morphological abnormalities, i.e., the presence of abnormalities in leaves like abnormal serrations, cordate apices, lack of serrations and presence of twin shoots, increased linearly with increasing radiation dose.

INTRODUCTION

Interest in the induction of mutation in vegetatively propagated plants began with the discovery of efficient methods of detecting mutations and the discovery of effective mutagens. Work, however, was not as extensive as that done on seed-

propagated plants. The latter has the advantage of gamethophytic screening of induced genetic and chromosome lethalities.

Experiments on the induction of mutations in vegetatively propagated plants are faced with the difficulty of coming up with uniform results. Much of the difficulty lies in the different modes of reproduction, the genetical set-up of clonar varieties, the type of organ irradiated, the mode of growth, the stage of vegetative growth and the subsequent handling of the material. On the other hand, methods of inducing mutations in vegetatively propagated plants more or less differ from those used in seed propagated plants. Thus, induction of mutations in both kinds of species has the same theoretical foundations (Gaul 1964; Nybom and KKoch 1965).

The potential of the mutagenic effects of radiation on vegetatively propagated plants has been demonstrated in many agricultural crops such as potato (Van Harten et al. 1972), grape and peach (Lapins et al. 1969) and several ornamental plants like chrysanthemum (Bowens 1965), carnation (Buiatti and Ragazzini 1964) and gladiolus (Buiatti et al. 1965). The initial experiments done on the effects of radiation on *Kanlanchoe* were with the use of x-rays (Naylor 1931) on the *Bryophylum calycinum* which has the same regenerative ability as the *Kalanchoe pinnata.* These demonstrate that increasing the dose of X-ray radiation brought about a corresponding increase in regenerative ability.

Mutations in carnation after gamma irradiation were caused by alterations in a polygenic system. These mutations were propagated vegetatively up to the vM_3 generation. Ferweda (1965) experimented on potato using x-rays and EMS. He obtained mutations which could be perpetuated vegetatively. In one instance, the ivy-leaf mutant could be propagated even through seed. Van Harten et al. (1972) report that the ivy-leaf mutant is a dominant trait without pleiotropic effects.

The general effect of radiation is the inhibition of normal physiological processes such as normal growth, regeneration and fertility. However, several studies have demonstrated the stimulatory effects of radiation. Studies on the effects of CO_2 on gamma-irradiated plantlets of the *Kalanchoe* showed that irradiation had observable stimulatory effects on seedling height (Stein and Sparrow 1965). They attributed this increase to the unusual increase in the length of the first and second internodes of the seedlings. However, as the seedlings matured, there was a relative decrease in height which was attributed to the inability of

the succeeding internodes to elongate. Similar findings on initial seedling height stimulation have been demonstrated in experiments on *Pinus rigida* (Mergen and Johnson 1964) and on the Helianthus (Skok et al. 1965). It has been pointed out that the cause of the initial stimulation is the presence of pile up precursors which were not utilized for the early stages of growth, but which later contributed to the unusual growth of the seedlings (Stein and Sparrow 1965).

MATERIALS AND METHODS

The species used for this study was *Kalanchoe pinnata* (Pers.) which has several medicinal properties. It is locally called kataka-taka (Tagalog) or *siempre viva* (Spanish).

Juice from the leaves is used as an astringent, antiseptic, counterirritant against poisonous insect bites and medicine for earache and ophthalmia (Quisumbing 1951). Mixed with lard, the juice is used for diarrhea, cholera and phthisis.

Dalziel and Dymock (in Quisumbing 1951) state that the juice is used as a diuretic and as a cure for bilious diarrhea and lithiasis. Quisumbing further states that fresh leaves are pounded and applied to burns or as poultices on boils. Pounded leaves are used also as poultices on the soles of the feet to stop hemorrhages (Blanco 1878), as topicals in dislocations and callositis (Guerrero 1930). Mixed with salts, they are applied as a plaster to the abdomen to relieve enuresis (Sulit in Quisumbing 1951).

Friese (in Quisumbing 1951) states that the leaves are used as an emollient and refrigerant on swellings caused by neuralgia or toothaches. Rivera (1941) reports that the juice of the leaves is used in the tratment of acute nephritis.

Three hundred fifty leaves from 240-250 plants raised from one plant of *Kalanchoe pinnata* Pers. were selected based on age of the leaf, number of notches and leaf size. The leaves for treatment were simple leaves which were the sixth or seventh pairs of leaves of each plant.

After thouroughly washing the leaves with tap water and distilled water, the leaves were treated with gamma radiation in doses ranging from 1000 r to 6000 r. Each dose was administered to 50 leaves while a set of 50 non-irradiated leaves served as control. After irradiation, the leaves were rehydrated in distilled water for 30 min in a water bath with a constant temperature of

32°C. After rehydration, the leaves were set in pans containing washed sand and kept constantly moist with a thin layer of water over the sand surface. The leaves were sprouted in partial shade.

Thirty days after setting the leaves in the pans, they were scored for number of plants sprouting per leaf, plantlet height, number of leaves per plantlet and somatic mutations in the form of leaf abnormalities and chlorophyll mutations. The plantlets were detached from the leaves and transplanted in field plots according to a dose-to-row plan. The distance of planting was 30 cm between rows and 18 cm between plants.

Seven months after transplanting the VM (first irradiated vegetative generation) plants were scored for plant height and occurrence of induced leaf abnormalities. Fifty VM_1 leaves of each dose were grown in pans containing water and washed sand. Pans were checked continually to insure that the leaves were covered with water. After 30 days, the VM_2 leaves were scored for the number of plantlets per leaf, plantlet height, occurrence of somatic mutations in the form of leaf abnormalities and chlorophyll mutations.

RESULTS AND OBSERVATIONS

1. Percentage regeneration of plantlets. In view of VM_1, the effects of gamma radiation on regeneration are shown in Table 1. When the regeneration, expressed as a percentage, is plotted as function of gamma radiation dose, a dose-effect relationship is observed. As radiation dose was increased from 1000 r, the number of plantlets regenerated per leaf decreased. Compared with the control, relatively fewer plantlets grew from leaves irradiated with doses ranging from 2000 r (70.50%) to 6000 r (38.30%).

There was a slight increase in the number of plantlets at 1000 r (102.87%). However, when higher doses were applied, a dose-effect relationship was seen. As the radiation dose increased, there was a decrease in the number of plantlets regenerated per leaf.

In the VM_2, as shown in Table 2, there was a slight stimulation in the number of plantlets that germinated at the lowest dose of radiation (100 r). Just like the VM_1, when higher doses were applied, there was a decrease in the number of plantlets as compared with the control.

2. Height of plantlets. Table 2 shows the mean heights of plantlets measured 30 days and 210 days after treatment. Plantlet height reduction was obtained which increased more or less with dose.

Data show that 30 days after irradiation, there was a slight increase in plantlet height at 1000 r (101.98%) as compared with the control. A gradual decrease in plantlet height was seen at higher doses of radiation. After 210 days, there was an obvious dose-effect relationship seen at all doses.

3. Chlorophyll mutations in the VM_2. It is evident from the data (Table 4) that the highest mutation frequency was obtained at moderate doses of 4000 r (12.60%). The frequency dropped abruptly at 5000 r (7.56%) and finally to 6000 r (5.97%), the lowest frequency.

The chlorophyll mutations spectrum (Table 5) shows the different types of chlorophyll mutants observed: viridis (deep green), chlorina (yellow-green), xantha (yellow) and albina (white). The frequency of each type of mutation is expressed as a percentage of the total mutant types. The highest frequency was that of the chlorina type at 3000 r (68.63%). Xantha were oberved only at 3000 r (5.2%) and albina were observed only at 4000 r (2.33%). In all doses, the chlorina mutants were the most frequent while xantha and albina were very rare.

4. Morphological abnormalities. The different kinds of morphological abnormalities induced by gamma radiation were leaf abnormalities with rounded apices, lack of normal serrations and cordate leaves (Table 6).

5. Frequency or morphologial abnormalities in the VM_2. Table 7 shows that the VM_1 scored after 90 days increased in the number of leaf abnormalities with increased radiation dose. After 210 days, there was also seen an increasing frequency of abnormalities as the dose increased to 4000 r. There was a slight decrease in the frequency from 4000 r (36.54%) to 5000 r (29.41%). The highest frequency of abnormalities was obtained at the highest dose of 6000 r (39.13%).

For the VM_2 generation, the data show an obvious increase in the frequency of abnormalities as the dose of radiation increased. The lowest percentage was obtained at the lowest dose (616%) and the highest percentage at the highest dose (1766.18%).

DISCUSSION

From the foregoing results, it is evident that gamma rays affected the generative capacity of leaves, plantlet height, shape and form of leaves in *Kalanchoe pinnata* Pers. These effects are useful biological indices of the radiosensitivity of a species. Several mutation experiments have shown that results obtained may be specific for each mutagenic agent and that response varies with different biological materials, treatment conditions and post treatment techniques. Mutagenic efficiency is known also to be influenced by several biological factors such as nuclear volume, cellular stages, variety of species, the age or stage of development and the radiosensitivity of each plant material.

Leaves of *Kalanchoe pinnata* Pers. that were exposed to gamma radiation showed a significant decrease in the number of plantlets regenerated per leaf (Table 1), a distinctly notable decrease in seedling height as radiation dose increased, the appearance of chlorophyll mutations whose frequecy increased at moderate doses but which decreased as higher doses were applied, and the appearance of an increasing number of leaf abnormalities with increasing doses.

Differences in opinion as to the number of meristematic cells that give rise to young plantlets in vegetatively propagated plants make it difficult to study the effect of radiation on the regeneration of plantlets. However, the fundamental role of meristematic cells in morphogenesis and their sensitivity to radiation are well recognized. Meristematic cells are more susceptible to radiation damage than resting cells (Gunckel 1957). Gunckel further states that both chromosomal damage and mitotic inhibition of irradiated cells are the effects of irradiation. Evans (1965) pointed out the effects of radiation on meristematic cells such as mitotic cycle delay, formation of chromosomal aberrations and loss of proliferative capacity due to either premature differentiation or cell death.

It has been observed that there was a reduction in the number of plants that regenerated per leaf as radiation dose increased. Evidently, this reduction cannot be ascribed to mitotic cycle delay because no new growth occurred. Neither can it be attributed to lethal chromosomal aberrations, as limitations of the study did not include cytological examinations. However, the reduction may be explained by the loss of proliferation capacity as a result of either premature differentiation or cell death.

Using leaf development as an index of the biological effects of gamma rays, many abnormalities of leaf shape were observed. More explicitly, irradiated *Kalanchoe pinnata* leaves yielded plants with some leaves that had rounded apices, lacked normal serrations or were heart-shaped. All the plants that grew from leaves treated with different doses of gamma rays produced leaves which lacked normal serrations. Non-serrated leaves appeared in all the groups dosed with gamma rays.

Leaves with non-serrated margins were also observed in the control plants. While less non-serrated leaves were obtained from the unirradiated plants than from the irradiated plants, this indicates that spontaneous mutations were produced probably under normal growth conditions. It has been suggested that the failure of leaf blades to develop properly is to be attributed to high concentrations of phytohormones, chromosome fragmentation and normal deficiencies (Gunckel 1957). Spontaneous mutations are caused by point mutations (Gaul 1964; Konzak et al. 1965).

Abnormal leaf shapes were observed as early as the six-leaf stage (85 days after parent leaves received gamma irradiation). Irregularities in leaf shape were observed to increase in number in the first four leaf pairs of the plants, but decreased unmistakably with the appearance of new leaves as the plants grew older (up to seven months). This may be explained as being due to the capacity of the plants to recover from the effects of radiation and the inherent ability of the plant to repair damaged tissue.

The presence of heart-shaped leaves (Fig. 3) could be explained by the death of cells in the center of the meristematic regions which have specific influences on the development of leaves and leaf shape. Thus, leaf aberrations may be largely due to the application of lethal doses of radiation affecting early stages of embryonal development, thereby resulting in leaf abnormalities. Assuming that physiological activity has started in the meristematic regions toward the formation of new plants, gamma radiation may have adversely affected certain embryonal mechanisms which resulted in the non-development of leaf apices. Thus, the appearance of bifurcated or heart-shaped leaves in the progenies.

Damage to the tissues, organs and the whole plant as discussed, represent the morphological and physiological effects of irradiation. On the other hand, the genetic effects of radiation are best exemplified by the presence of chlorophyll mutations (Table 3).

Several types of chlorophyll mutations were observed: viridis (dark green), chlorina (yellow green), xantha (yellow), and albina (white). It was observed that the incidence of xantha and albina were proportionately much less as compared with the other types of chlorophyll mutations.

These studies demonstrate that one of the biological effects of ionizing radiation is the induction of chlorophyll mutations. These mutations are believed to be caused by changes in the chromosome structure -- either a break or a deletion. This would mean changes in the nucleotide sequence which would mean a change in the information coded in the DNA. Sylenga (1964) attributes dominant chlorophyll mutations to chromosomal aberrations of the 2-break type (2 hit events). Changes in the color of *Streptocarpus* (Strickberger 1969) are due to biochemical effects which can be traced to separate genes. These genes appear to produce their effects by the addition or subtraction of hydroxyl (OH) or methoxyl ($O\text{-}CH_3$) units to the sugar of the DNA molecule. Thus, any change in the genetic material could bring about changes in the production, function and specificity of an enzyme: in this case, the production of normal pigmentation. The degree to which plants respond to increasing radiation dose indicates the extent to which injury influences the production of chlorophyll pigments. Chlorophyll mutations may be attributed to proplastid damage or the deletion of one or more genes (Love 1969).

Ionizing radiation affects the genetic make-up by transferring its energy to atoms or molecules present in or near the cell nucleus. Radiation energy causes molecular breakage which provides substrates for chemical reactions in the cell. Thus, in the case of chlorophyll mutations, the DNA molecule is affected directly or indirectly by ionizing radiation.

Any change in the molecular structure of the genes would change the information coded in the DNA. A gene, which is a segment of a DNA molecule, is responsible for the synthesis of an enzyme. Any change in the DNA molecule would bring about a change in the gene, thereby inhibiting the normal synthesis of the enzyme.

Concomitant to the different stages of development of normal chloroplasts is pigment formation. From a colorless granule, yellow pigments are initially formed, followed by green pigments. The different genes controlling pigment formation and the development of chloroplasts act in series, each gene carrying on where the previous gene left off. The effect of mutation on

the different genes becomes evident in the inhibition of succeeding stages, resulting in the formation of chlorophyll mutations.

In the present study, the life span of the albina mutants was very short. This can perhaps be attributed to extensive radiation damage, which gave no chance for recovery. Studies have shown evidence of recovery and repair of induced physiological damage and of a greater part of genetic damage. These can explain the disappearance of chlorophyll mutations in the *Kalanchoe pinnata* after 30 days of growth.

Chlorophyll mutations obtained in the VM_2 generation show that the highest frequecy of abnormalities was obtained at 4000 r. A decrease in frequency of mutants was quite evident at increased doses of radiation. This decrease could be attributed to repair and survival . Of the chlorina mutants obtained at the VM_2, the most abundant were the chlorina mutants in plantlets irradiated at 3000 r. Chlorina and viridis mutants were obtained in all doses. Only one xantha and one albina were obtained at 3000 r and 4000 r, respectively.

These results indicate that at higher doses, the progress of the DNA-repairing phenomenon could be hindered possibly by the inactivation of the repair system. Increase in radiation increases the number of deletions or breaks. As a consequence, there is a change in the nucleotide sequence which brings about a change in the DNA information. These changes in the DNA code are carried over the VM_2 generation.

SUMMARY AND CONCLUSIONS

Leaves of *Kalanchoe pinnata* were exposed to gamma radiation with doses starting from 1000 r. After irradiation, they were rehydrated and sprouted in pans containing water and sand. After 30 days, plantlets which regenerated at the margins of the leaves were scored for height, number of plantlets regenerated per leaf and morphological and chlorophyll mutations. Results showed that irradiation caused biological damage shown in the reduction of the number of plants regenerated per leaf, reduction in height and occurrence of leaf abnormalities and chlorophyll mutations.

Gamma irradiation produced both somatic and chlorohpyll mutations in *Kalanchoe pinnata.* The occurrence of morphologi-

cal abnormalities was dependent on radiation dose: the higher the dose, the greater the number of leaf abnormalities.

Inhibitory effects of irradiation are indicated in the decrease in the number of plantlets regenerated per leaf and the decrease in plantlet height.

Somatic mutations included twin shoots and leaf abnormalities like lack of serrations and rounded or heart-shaped apices. These are useful indices of mutagenicity. The application of higher doses of radiation increased the frequency of leaf morphological abnormalities. However, it did not cause a proportional increase in chlorophyll mutations. Chlorophyll mutation frequency was greatest at moderate doses of 4000 r.

Occurrences of chlorophyll mutant plantlets such as viridis, chlorina, xantha and albina are good indices of genetic mutations. Chlorina mutants were the most frequent, while albina and xantha types were rare.

Gamma irradiation produced both morphological and genetic effects in *Kalanchoe pinnata*. In this study, the frequency of chlorophyll mutations was not proportional to dose of radiation. However, morphological abnormalities were shown to increase proportionately with increasing radiation doses. Radiation, as evidenced by the occurrence of morphological abnormalities, speeds up the occurrence of variations already found in nature.

Table 1. Percentage regeneration of plantlets of *Kalanchoe pinnata* leaves after gamma irradiation

Gamma ray dose	Number of plantlets per Range	VM_1 Leaf $\pm$ Mean	Percentage Regeneration
0	2 - 11	5.56 $\pm$ 3.04	100.00
1000 r	2 - 11	5.72 $\pm$ 2.07	102.97
2000 r	1 - 9	3.92 $\pm$ 1.53	70.50
3000 r	1 - 7	3.87 $\pm$ 1.85	69.60
4000 r	1 - 7	3.73 $\pm$ 1.64	67.08
5000 r	1 - 6	3.00 $\pm$ 1.43	53.95*
6000 r	1 - 4	2.13 $\pm$ 1.002	38.30*

* Significant at the 5% level

Table 2. Mean height of VM_1 plantlets of *Kalanchoe pinnata* after gamma irradiation

Gamma ray dose	Range (cm)	Mean (cm)	Percentage of control
30 days after irradiation			
0	3-37	17.71 ± .921	100.00
1000 r	8-28	18.06 ± .495	101.98
2000 r	9-34	17.67 ± .1140	99.77
3000 r	7-32	17.36 ± .848	98.02
4000 r	8-30	15.67 ± .789	88.48
5000 r	5-40	14.86 ± .872	83.91
6000 r	6-20	7.33 ± .440	41.49*
210 days after irradiation			
0	100-460	224.41 ± 72.09	100.00
1000 r	60-420	195.85 ± 28.04	87.27
2000 r	70-404	194.39 ± 25.55	86.62
3000 r	32-398	183.57 ± 44.59	81.80
4000 r	65-285	178.46 ± 74.25	79.52
5000 r	80-275	158.69 ± 43.52	70.71
6000 r	54-235	120.88 ± 48.68	53.87*

* Significant at the 5% level

Table 3. Percentage regeneration of VM_2 plantlets in *Kalanchoe pinnata* after gamma irradiation

Gamma ray dose	Range	Mean number of plantlets per leaf	Percentage regeneration
0	4-16	9.60 ± 1.97	100.00
1000 r	4-18	10.50 ± 2.75	109.37
2000 r	3-17	9.20 ± 3.63	95.83
3000 r	3-16	8.08 ± 3.93	84.17
4000 r	3-15	7.92 ± 3.76	82.50
5000 r	3-16	7.52 ± 3.56	78.33
6000 r	4-16	7.32 ± 3.06	76.25

Table 4. **Frequency of chlorophyll mutations in VM_2 plantlets of *Kalanchoe pinnata***

Gamma ray dose	Total number of plantlets scored	Number of chlorophyll mutants	Frequency %
0	473	0	0
1000 r	490	46	9.39
2000 r	505	49	9.70
3000 r	436	51	11.70
4000 r	341	43	12.60
5000 r	397	30	7.56
6000 r	385	23	5.97

Table 5. **Chlorophyll mutation spectrum in VM_2 plantlets of *Kalanchoe pinnata* induced by gamma irradiation**

Gamma ray dose	Total number of mutants	V %	Ch %	X %	A %
0					
1000 r	46	39.13	60.87		
2000 r	49	46.94	53.06		
3000 r	51	25.49	68.63	5.88	
4000 r	43	32.56	65.12		2.33
5000 r	30	40.00	60.00		
6000 r	23	34.00	65.22		

V - viridis
Ch - chlorina
X - xantha
A - albina

Table 6. Morphological abnormalities induced by gamma irradiation of the *Kalanchoe pinnata*

Plant character	Char. of variant	Irradiation dosage
1. Serrated leaves	Leaves which lack serrations	All doses including control
2. Pointed apex	Rounded leaf apices, cordate apices sometimes without normal serrations	1000 r - 6000 r
3. Single primary shoot	Plants with multiple primary shoots usually 2	All doses except at 2000 r

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Metazoan Ectoparasites of Some Cultured Fishes from Laguna Lake and Vicinities

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ABSTRACT

__Chanos chanos__ and __Oreochromis mossambicus__ were collected from fishponds in Obando, Bulacan and Malabon, Rizal, respectively, in 1982 and 1983. __Transversotrema laruei__ (Trematoda) was recovered from the skin of both fishes. __Argulus indicus__ (Crustacea) was also found on the skin of __O. mossambicus__.

Laguna Lake fishes studied in 1982 and 1983 were __Clarias batrachus, Ophicephalus striatus__ and __Oreochromis niloticus__. __Actinocleidus__ sp. (Trematoda) was recovered from the gills of __Clarias batrachus__ while __Argulus indicus__ was recovered from the skin of __O. striatus__. __T. laruei__ infested the skin of __O. niloticus__ while __Cichlidogyrus sclerosus__ (Trematoda) was recovered from the gills.

Fishes colllected from La Mesa Reservoir in 1989 and 1990 were __Arius thalassinus, Glossogobius giurus, Oreochromis niloticus, Tilapia zillii__ and __Therapon plumbeus__. __Cleidodiscus__ sp. (Trematoda) was recovered from the gills of __A. thalassinus__. __Ergasilus philippinensis__ (Crustacea) was found on the gills of __G. giurus__. __Cichlidogyrus sclerosus__ and __E. philippinensis__ were recovered from the gills of __O. niloticus__ and __Tilapia zillii__. __Cichlidogyrus tiberianus, C. tilapiae, C. longicornis gravivaginus__ were also found on the gills of __T. zillii__. The gills of __Therapon plumbeus__ were infested with __E. philippinensis__ and __Diplectanum__ sp. (Trematoda).

INTRODUCTION

The decline of fish catches from natural waters has increased the importance of aquaculture in providing food for the world's rapidly growing population. Fish production by aquaculture often involves increasing the density of the fish population under cultivation. The unnaturally high population density favors the spread of diseases and parasites. Every parasite harms its host. If few parasites are present, the effect on the host may be minimal; if large numbers are present, the fish may be killed. A knowledge of the occurrence of parasites on fishes is important for effective aquacultural management.

This paper presents the prevalence and intensity of metazoan parasite infestation on the skin and gills of some fishes from Bulacan and Malabon fishponds, Laguna Lake and La Mesa Reservoir.

MATERIALS AND METHODS

Specimens of *Chanos chanos* (milkfish) were collected from fishponds in Obando, Bulacan in December 1982 and September 1983. *Oreochromis mossambicus* (Java tilapia) specimens were collected from fisponds in Malabon, Rizal in August and September 1982 and December 1983. *Clarias batrachus* (freshwater catfish) from Laguna Lake were bought from Nepa-Q-Mart, Quezon City from February to August 1983 while the mudfish *Ophicephalus striatus* specimens from the same lake were obtained from Farmers Market, Quezon City, from September to November 1983. Collection of *Oreochromis niloticus* (Nile tilapia) was from a fishpen in Laguna Lake in Angono, Rizal. Fishes collected from La Mesa Reservoir were: *Oreochromis niloticus* in August 1990; *Tilapia zillii* in September 1989 and from March to August 1990; *Therapon plumbeus* (freshwater perch) from April to August 1990; *Arius thalassinus* (green sea catfish) from May to August 1990; and *Glossogobius giurus* (goby) from March to October 1990.

Fresh fish specimens from Bulacan, Malabon and Laguna Lake were examined for skin and gill parasites. Fishes from La Mesa Reservoir were frozen after collection and examined for parasites at a later date. Fish length in centimeters and weight in grams were recorded.

Fins, mucus scrapings, scales, skin and gills were first examined by ocular inspection and then under the stereomicroscope. Gills of small fish were compressed between two slides and examined. Gills of large fish were teased then placed in jars for washing and decanting. To dislodge monogenea from the gills of fresh specimens, magnesium sufate crystals were added to the water used for washing and decanting. The species and number of parasites recovered from each host were recorded.

Trematodes recovered were fixed in AFA (alcohol-formalin-acetic acid), stained in borax carmine or acetocarmine and mounted in balsam. Temporary mounts in glycerine were also prepared.

Crustacean parasites were fixed in 70% ethyl alcohol and mounted in glycerine. Some specimens were stained with carbol fuchsin and mounted in balsam.

Microphotographs and camera lucida drawings of the parasites were made from mounted specimens.

For each fish species, the prevalence and intensity of infestation by each parasite species were determined. The prevalence of infestation is the percentage of fish infested of the total fish examined. The intensity of the infestation or average parasite burden was determined as follows:

$$\text{average parasite burden} = \frac{n}{n_1}$$

where n = number of parasites
n_1 = number of fish infested

OBSERVATIONS AND RESULTS

Chanos chanos specimens had fork lengths ranging from 6.0 to 13.5 cm and weights from 2.2. to 30.4 g. *Oreochromis mossambicus* were 6.1 to 13.1 cm in standard lengths and weighed from 7.6 to 70.4 g. Ranges in standard lengths and weights of fishes from Laguna Lake were: *Clarias batrachus,* 12.4 to 19.0 cm, 14.5 to 79.0 g; *Ophicephalus striatus,* 16.0 to 28.8 cm, 54.2 to 325.0 g; and *Oreochromis niloticus,* 6.2 to 15.1 cm, 8.1 to 104.8 g. The total lengths and weights of the fishes collected from La Mesa Reservoir were: *Arius thalassinus,*

15.1 to 24.6 cm, 29.1 to 117.9 g; *Glossogobius giurus,* 12.1 to 24.1 cm, 9.5 to 19.0 g; *Tilapia zillii,* 7.9 to 24.1 cm, 8.1 to 218.0 g; *Oreochromis niloticus,* 9.7 to 11.1 cm, 22.5 to 24.8 g; and *Therapon plumbeus,* 6.5 to 12.3 cm, 4.0 to 29.4 g.

The ectoparasites recovered from the different fish hosts are shown in Table 1. Six of the 32 *Chanos chanos* examined were infested with the digenetic trematode, *Transversotrema laruei.* The trematodes were recovered from the skin of fish with fork lengths of more than 10.0 cm. The smaller fish were not infested. The average worm burden was 1.8.

Transversotrema laruei was also recovered from the skin of 72 of the 78 *Oreochromis mossambicus* collected from Malabon. The average parasite burden was 42.8.

Of the 114 *Clarias batrachus* examined, 5 were infested with the gill fluke, *Actinocleidus* sp. The intensity of infestation was 4.2.

Three of the 47 *Ophicephalus striatus* examined harbored *Argulus indicus* with an average burden of 2.0.

Oreochromis niloticus collected from a Laguna Lake fishpen harbored *T. laruei* and *Cichlidogyrus sclerosus.* Of the two *O. niloticus* specimens collected from La Mesa Reservoir, one was infested with *Cichlidogyrus sclerosus* while the other harbored the copepod, *Ergasilus philippinensis.*

Tilapia zillii collected from La Mesa Reservoir has 17.0% infestation with *Cichlidogyrus* spp. with an average burden of 8.1. The species recovered were *C. tiberianus, C. tilapiae, C. longicornis gravivaginus* and *C. sclerosus. Ergasilus philippinensis* was also found on 154 of the 182 fish specimens examined. The average burden was 23.2.

All of the 15 *Therapon plumbeus* collected were infested with *Ergasilus philippinensis* with average burden of 30.1. Seven fish specimens or 46.7% harbored *Diplectanum* sp. with an average burden of 13.9.

Of the 20 *Arius thalassinus* examined, 3 or 15.0% were infested with *Cleidodiscus* sp. with an average burden of 1.0.

Four of the 15 *Glossogobius giurus* examined were infested with *Ergasilus philippinensis.* One to six parasites were recovered from infested fish with an average burden of 2.2.

Descriptions of the 10 species of ectoparasites recovered from the eight fish species studied (with measurements in micra unless otherwise stated) are as follows:

Cichlidogyrus tiberianus Paperna, 1960
(Trematoda: Dactylogyridae)
Figs. 2 D-G; 4 A-B

Diagnosis (based on three specimens): Body 360.8-564.2 long, maximum width 77.7-129.5. Prohaptor with four lobes, containing two groups of head organs. Two pairs of eyes. Copulatory organ of two parts, ejaculator and accessory piece. Ejaculator 53.6- 55.5 long, consisting of an oval-mouthed funnel which continues as a long sickle-shaped tube with a sharp tip. Adjacent to funnel is accessory piece 38.8-40.7 long, with irregular complicated structure. Vagina opens on left side of body near its center; vaginal prop represented by plate and curled ducts both consisting of sclerotized substance. Opisthaptor with two pairs of anchors of unequal size; first pair longer (31.4-37.0 long), second pair (29.6-31.4 long). Anchors with curved wing attached to shaft. Basal piece of compound bar tapers gradually with tips bent slightly inwards, 37.0-46.2 long, appendages 11.1-13.0 long; V-shaped bar 42.6-55.5 long. Fourteen marginal hooklets, each with well-developed base growing thicker toward its proximal extremity; spike sickle-shaped, outer side with attached needle-like appendage directed parallel to shaft.

Host : *Tilapia zillii*
Location : gills
Host locality : La Mesa Reservoir

Cichlidogyrus tilapiae Paperna, 1960
(Trematoda: Dactylogyridae)
Figs. 1 A-D; 3 A-B

Diagnosis (based on eight specimens): Body 351.5-434.8 long, maximum width 53.6-66.6. Body slender, elongated, tapering gradually at the posterior extremity, where it is much narrower than the transversely oval opisthaptor. Prohaptor with four lobes containing head organs. Eyespots two pairs. Copulatory organ consisting of ejaculator and accessory piece. Ejaculator 18.5- 24.1 long, its funnel-shaped base continues in a narrow tube slightly bent at the tip. Accessory piece shaped as a thin plate 27.8-31.4 long, with folded rims; it originates near the funnel and terminates in a bent bifur-

cated tip. Opisthaptor with two pairs large anchors, two supporting bars and 14 marginal hooklets. Anchors of both pairs about equal in size, 27.8-37.0 long. A small thin wing on shaft of each anchor. Compound bar with basal piece 25.9-31.4 long divided into three segments of equal length by constrictions at two points. Two appendages 11.1-14.8 long join margin of basal piece at points of constrictions. V-shaped bar 42.6-48.1 long with symmetrical tooth-like projections on internal surface. Hooklets with thickened base, a tapering shaft and a sickle-shaped spike. Each hooklet with a needle-like appendage originating at base of spike and running parallel to shaft on side opposite to spike.

Host : *Tilapia zillii*
Location : gills
Host locality : La Mesa Reservoir

Cichlidogyrus longicornis gravivaginus Paperna and Thurston, 1969
(Trematoda:Dactylogyridae)
Figs. 2 A-C; 4 E-F

Diagnosis (based on three specimens): Body 392.2-573.5 long, maximum width 61.1-107.3. Eyes two pairs, only one pair compound with lenses. Copulatory organ of two parts, ejaculator and accessory piece. Ejaculator 22.2-27.8 long, its funnel-shaped base continues in a narrow tube slightly bent at the tip. Accessory piece 20.4-27.8 long, stouter than ejaculator. Basal piece of compound bar 59.2-61.1 long, its distal ends in the form of wide triangular plates; appendages very long 38.9-46.2. V-shaped bar supported by heavily sclerotized plate which follows the proximal margins of the opisthaptor. Fourteen marginal hooklets.

Host : *Tilapia zillii*
Location : gills
Host locality : La Mesa Reservoir

Cichlidogyrus sclerosus Paperna and Thurston, 1969
(Trematoda: Dactylogyridae)
Figs. 2 D-F; 4 A-C

Diagnosis (based on seven specimens): Body 662.5-841.7 long, maximum width 80.0-170.2. Prohaptor with four lobes containing head organs. Eyespots two pairs. Copulatory organ of two parts, ejaculator and accessory piece. Ejaculator begins with an oval mouthed funnel on one side of which is attached a flat basal plate, continues as a sickle-shaped tube gradually narrowing to a sharp tip; ejaculator measured on a straight line from basal plate to tip 32.5-42.5 long; accessory piece robust, sausage- shaped, 35.2-57.5 long. Opisthaptor poorly demarcated from most of body. Anchor two pairs with wings on shafts. Anchors of both pairs equal in size 31.4-33.3 long. Compound bar consists of shallow V-shaped piece with two loop-shaped appendages dividing piece into three nearly equal parts; basal piece 35.2-50.0 long, appendages 15.0-17.5 long. The second bar V-shaped, heavy, a thin shelf or ledge along inner edge, length taken as shortest distance between tips 35.2-52.5; 14 marginal hooklets, hooklets small with poorly developed base.

Host : *Oreochromis niloticus*
Tilapia zillii
Location : gills
Host Locality : Laguna Lake, La Mesa Reservoir

Diplectanum sp. Diesing, 1858
(Trematoda: Dactylogyridae)
Fig. 4 C-D

Diagnosis (based on three specimens): Body elongated, 281.2-310.8 long, maximum width 46.2-61.1. Opisthaptor well delineated from body, transversely oval, with 14 marginal hooks, 2 pairs anchors and 3 transverse connecting bars. Opisthaptor 74.0-98.1 wide. First pair of anchors 29.6-35.2 long, second pair 25.9-31.4 long. One dorsal and one ventral squamodisc present. Eyes two pairs. Intestinal caeca end blindly without posterior fusion.

Host : *Therapon plumbeus*
Location : gills
Host locality : La Mesa Reservoir

Cleidodiscus sp. Mueller, 1934
(Trematoda:Dactylogyridae)
Figs. 4 E; 5 A

Diagnosis (based on three specimens): Body 693.8-869.5 long, 74.0-99.9 wide. Eyes four, posterior pair larger. Gut bifurcate. Cirrus a simple cuticularized tube. Vitellaria of numerous small and discrete follicles in lateral bands extending from pharyngeal region into peduncle; bands confluent anteriorly and posteriorly. Haptor distinct, discoidal; with two pairs anchors and seven pairs hooklets. Anchors with superficial roots of each pair connected by transverse bar. Bars separate, non- articulate with each other.

Host : *Arius thalassinus*
Location : gills
Host locality : La Mesa Reservoir

Actinocleidus sp. Mueller, 1937
(Trematoda: Dactylogyridae)
Fig. 5 B

Diagnosis (based on four specimens): Body elongated, 325.0-437.5 by 97.5-112.5. Opisthaptor disc-shaped with two pairs of anchors and 14 marginal hooks. Two dissimilar connecting bars between anchors more or less V-shaped, articulating with each other. One bar consisting of two pieces. Eyespots present.

Host : *Clarias batrachus*
Location : gills
Host locality : Laguna Lake

Transversotrema laruei Velasquez, 1958
(Trematoda: Transversotrematidae)
Fig. 5 C

Diagnosis (based on 10 specimens): Body leaf-like, wider than long, length 324.0-402.6, width 628.5-864.0. Eyespots present. Acetabulum 78.6-88.4 in diameter. Oral sucker absent. Mouth opening directly into pharynx, 39.3-58.9 in diameter.

Esophagus narrow, bifurcating just anterior to midbody; branches uniting posteriorly forming cycloid intestine. Testes branched one on each side of acetabulum. Ovary with uneven margins, anterior to, and smaller than left testis. Uterus in testicular region. Vitellaria follicular, heavily developed, in semicircle outside intestinal loop. Egg large 49.0-117.8 long by 78.4-88.2 wide.

Host : *Oreochromis mossambicus*
Location : skin (under scales)
Host locality : Malabon, Rizal

Ergasilus philippinensis Velasquez, 1951
(Crustacea : Ergasilidae)
Fig. 5 D

Diagnosis (based on 10 specimens): Female - Total length 556.9- 885.4. Cephalothorax 285.6-357.0 long, 207.1-285.6 wide, violin- shaped including first leg-bearing segment, rounded anterior end slightly wider than truncated posterior end; eyespot near anterior margin; second to fifth leg-bearing segments well- defined, their widths decreasing posteriorly; free-thoracic segments combined length 71.4-142.8, width anterior 142.8-192.8, posterior width 64.3-142.8; genital complex subcircular, length 50.0-64.3, width 57.1-78.5; abdomen three-segmented, segments nearly equal in size, abdomen length excluding uropods 42.8-78.5, width 35.7-42.8. Egg sac length 178.5-285.6, width 71.4-92.8. First antenna six-segmented, with numerous setae. Second antenna slender as long as total body length. First four pairs of legs biramous, all rami with three segments. Fifth leg uniramous, one segmented, bearing two apical setae. Uropods rectangular about 3/4 length of abdomen. Male unknown.

Host : *Glossogobius giurus*
Tilapia zillii
Orechromis niloticus
Therapon plumbeus
Location : gills
Host locality : La Mesa Reservoir

Argulus indicus Weber, 1892
Fig. 5 E

Diagnosis (based on five specimens): Carapace ovate, considerably narrowed anteriorly with broad lateral lobes which fall slightly short of the abdomen, just reaching it or slightly overlapping it. Body of males, 3.68-5.36 mm by 2.76-4.35 mm; females 4.44-5.54 mm by 3.77-4.15 mm. Cephalic area broadly triangular, distinctly separated from the rest of the carapace and projecting a little anteriorly. Anterior respiratory area minute, posterior one very large and oblong. Knob or hook lacking on anterior surface of first antennae. Basal plate of second maxillae not lobed, tips of maxillary teeth blunt, ribs of suction cup composed of three rods. Swimming lobe of fourth appendage boot- shaped, heel pressed against end of thorax, toe extending to or beyond edge of abdomen. Whole animal golden yellow flecked with black.

Host	:	*Ophicephalus striatus* *Oreochromis mossambicus*
Location	:	skin
Host locality	:	Laguna Lake; Malabon, Rizal

DISCUSSION

Transversotrema laruei has been previously reported on *Lates calcarifer* and as progenetic cercaria on *Mollienesia latipinna, Scatophagus argus, Mugil* sp., *Megalops cyprinoides Tilapia mossambica, Anodontostoma chacunda, Hemiramphus georgii* and *Therapon argenteus* (**9**). The recovery of this trematode from the skin of *Oreochromis mossambicus* and *Chanos chanos* in Malabon and Obando fishponds, respectively, and from *Oreochromis niloticus* from Laguna Lake in the present study, indicates not only wide host specificity of the parasite but also its adaptation to a freshwater habitat. Cercaria of *T. laruei* develops in the snail, *Thiara riquetti* Grateloup (**9**). The introduction of *T. laruei*-infested tilapia to water bodies inhabited by its snail host, provided opportunities for the establishment of the parasite in this water body.

Several species of *Cichlidogyrus* have been reported in several tilapia species in Israel and Africa (**6, 7**). Duncan (1973) recovered *C. sclerosus* from the gills of cultured *Tilapia mossambica* in Sampaloc Lake and Alligator Lake, Laguna. His report is the first record of *Cichlidogyrus* outside Africa and the Middle East. The spread of the parasite to the Philippines may have been due to the introduction of infested tilapia originating from Middle East or African stocks. The present study is the first report of *Cichlidogyrus* infestation of tilapia from La Mesa Reservoir.

Diplectanum has been described as parasites of marine fish (**4**). The only *Diplectanum* reported from South East Asia is *Diplectanum* sp. on *Epinephelus tauvina* cultured in net cages in Singapore (**4**). *Diplectanum* sp. has now been recorded on a Philippine fish from a freshwater habitat. Further studies are required for specific identification of the specimens recovered.

Various species of *Cleidodiscus* have been found on many species of freshwater fishes in North America (**3**). There is no record of *Cleidodiscus* on culture fishes in South East Asia. The recovery of *Cleidodiscus* sp. from the gills of *Arius thalassinus* in the present study is the first report of this genus on Philippine fish.

Actinocleidus sp. found in the present study on the gills of *Clarias batrachus* was previously reported on a related host, *Clarias macrocephalus* from Lumbang, Laguna (**2**). In Indonesia, *Actinocleidus* sp. was reported on *C. batrachus* (**4**). Many species of *Actinocleidus* have been reported from North American freshwater fishes (**3**). Specific identification of the specimens on *Clarias batrachus* requires further study.

The genus *Ergasilus* comprises more than 80 species widespread in various marine and freshwater habitats of the world. Only four species have been recorded from South East Asia: *Ergasilus thailandensis* on the gills of *Pontius ophroides* from Thailand; *E. borneonensis* from an unidentified fish in Indonesia; *E. mugilis* from *Glossogobius giurus* in Thailand; and *E. philippinensis* from the gills of *G. giurus* in the Philippines (**4**). Kabata (1985) doubts the identification of the species found on *G. giurus* in Thailand. Prior to this study, the only published record of *E. philippinensis* on fish was from Laguna Lake from the gills of *G. giurus* (**4**) which led Kabata (1985) to surmise that this parasite is fairly host-specific. Mamaril (1986) reported the presence of a single specimen of *E. philippinensis* in a plankton sample collected from La Mesa Reservoir. The recovery of this copepod from the gills of *G. giurus, T. zillii, O. niloticus* and *Therapon plumbeus* in the present study is the first record of *E. philippinensis* on host other than *G. giurus* and indicates that the parasite has a wider host specificity than surmised earlier.

There are about 100 species of *Argulus* distributed worldwide in both marine and freshwater habitats. Only three species have been reported on South East Asian cultured fish: *A. foliaceous* (L), *A. indicus* Weber, 1892 and *A. siamensis* Wilson, 1926. In Indonesia, *A. indicus* has been recovered from several fishes including *Ophicephalus striatus* and *Clarias* sp. In Thailand, *Argulus indicus* was found on tilapia and other fishes (**4**). It appears that *Argulus indicus* has a relatively wide fish host specificity.

Table 1. Prevelance and intensity of ectoparasitic infestation on the different fish hosts

Fish species*	Locality and period of fish collection	Parasite species	Prevalence (%)	Intensity
Chanos chanos (32)	Obando, Bulacan Dec. 1982, Sept. 1983	*Transversotrema laruei*	18.8	1.8
Oreochromis mossambicus (78)	Malabon Aug. - Dec. 1982 Jan. 1983	*T. laruei*	92.3	42.8
		Argulus indicus	9.0	1.7
Clarias batrachus (114)	Laguna Lake Feb. - Aug. 1983	*Actinocleidus* sp.	4.4	4.2
Ophicephalus striatus (47)	Laguna Lake Sep. -Nov. 1983	*Argulus indicus*	6.4	2.0
Oreochromis niloticus (127)	Laguna Lake Aug. 1982 - Jan 1983	*T. laruei*	5.1	6.2
		Cichlidogyrus sclerosus	1.6	1.0
(2)	La Mesa Res. Aug. 1990	*C. sclerosus*	50	3.0
		Ergasilus philippinensis	50	1.0
Tilapia zillii (182)	La Mesa Res. Sept. 1989 - Aug. 1990	*Cichlidogyrus spp.*	17.0	8.1
		E. philippinensis	84.6	23.2
Therapon plumbeus (15)	La Mesa Res. Apr. - Oct. 1990	*E. philippinensis*	100.0	30.1
		Diplectanum sp.	46.7	13.9
Arius thalassinus (20)	La Mesa Res. May - Aug. 1990	*Cleidodiscus* sp.	15.0	1.0
Glossogobius giurus (15)	La Mesa Res. Mar. - Oct. 1990	E. philippinensis	26.7	2.2

* Sample size in parenthesis

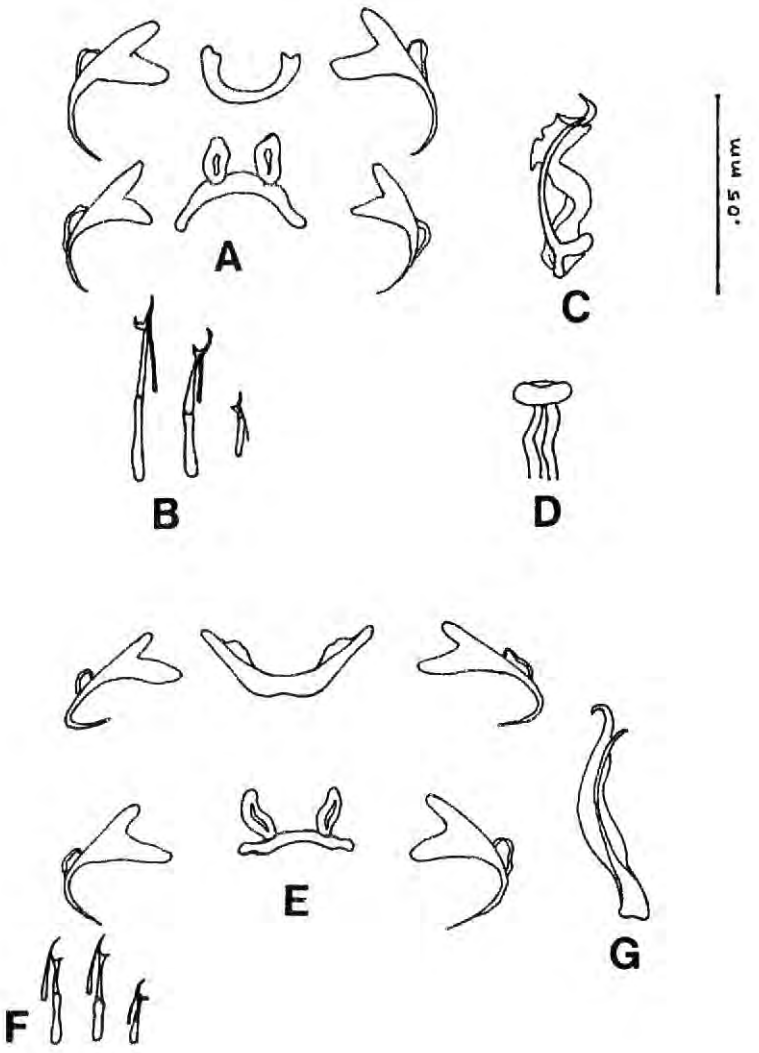

Figure 1. A-D, *Cichlidogyrus tiberianus* A- anchors and bars, B - hooklets, C - copulatory organ, D - vaginal prop; E-G *C. tilapiae* E - anchors and bars, F - hooklets, G - copulatory organ

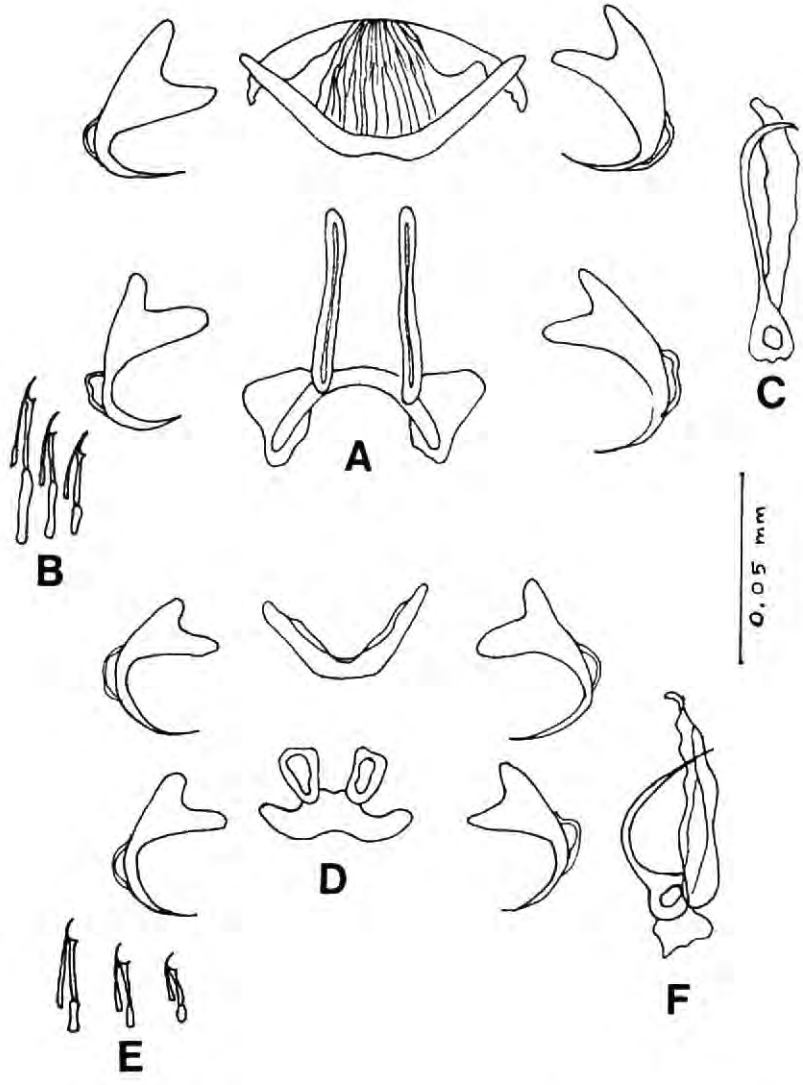

Figure 2. A-C. *C. longicornis gravivaginus* A - anchors and bars, B - hooklets, C - copulatory organ; D-G. *C. sclerosus* D - anchors and bars, E - hooklets, F - copulatory organ

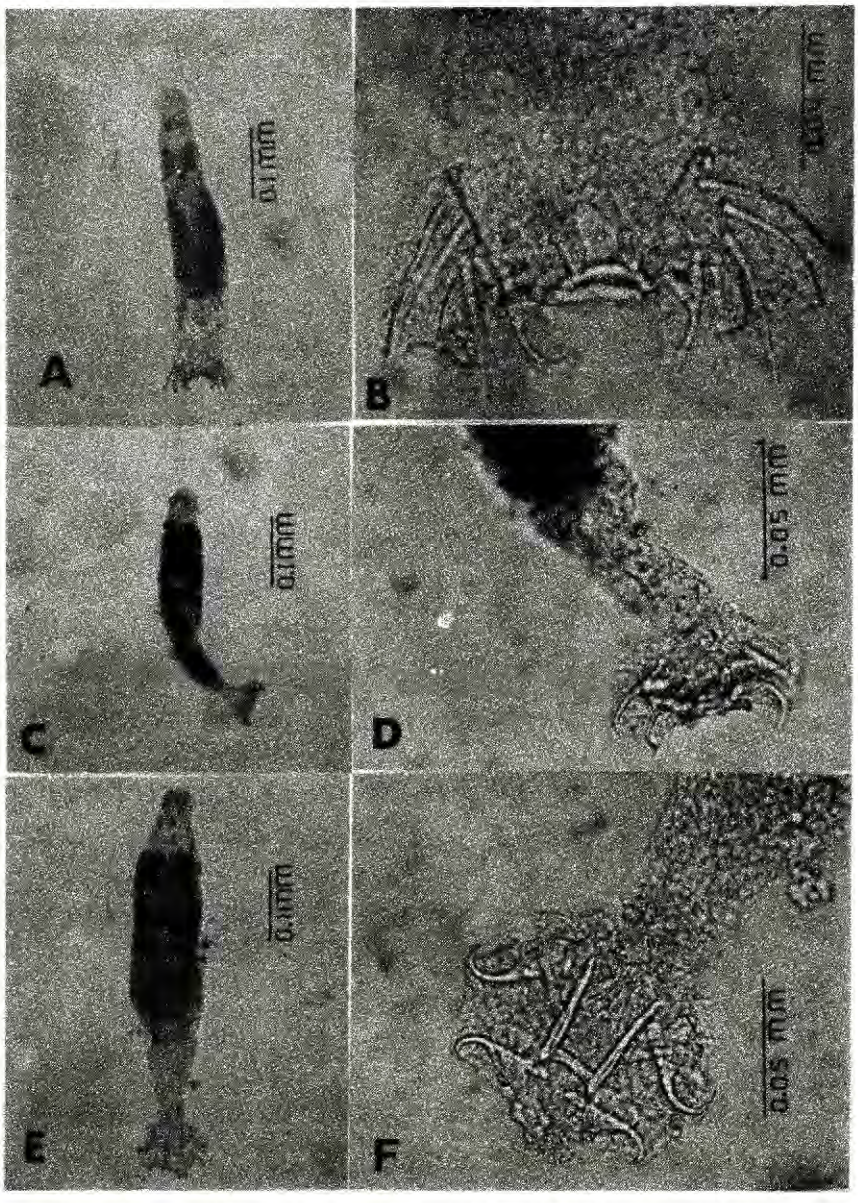

Figure 3. A - *C. tiberianus*, whole worm
B - *C. tiberianus*, opisthaptor
C - *C. tilapiae*, whole worm
D - *C. tilapiae*, opisthaptor
E - *C. longicornis gravivaginus*, whole worm
F - *C. longicornis gravivaginus*, opisthaptor

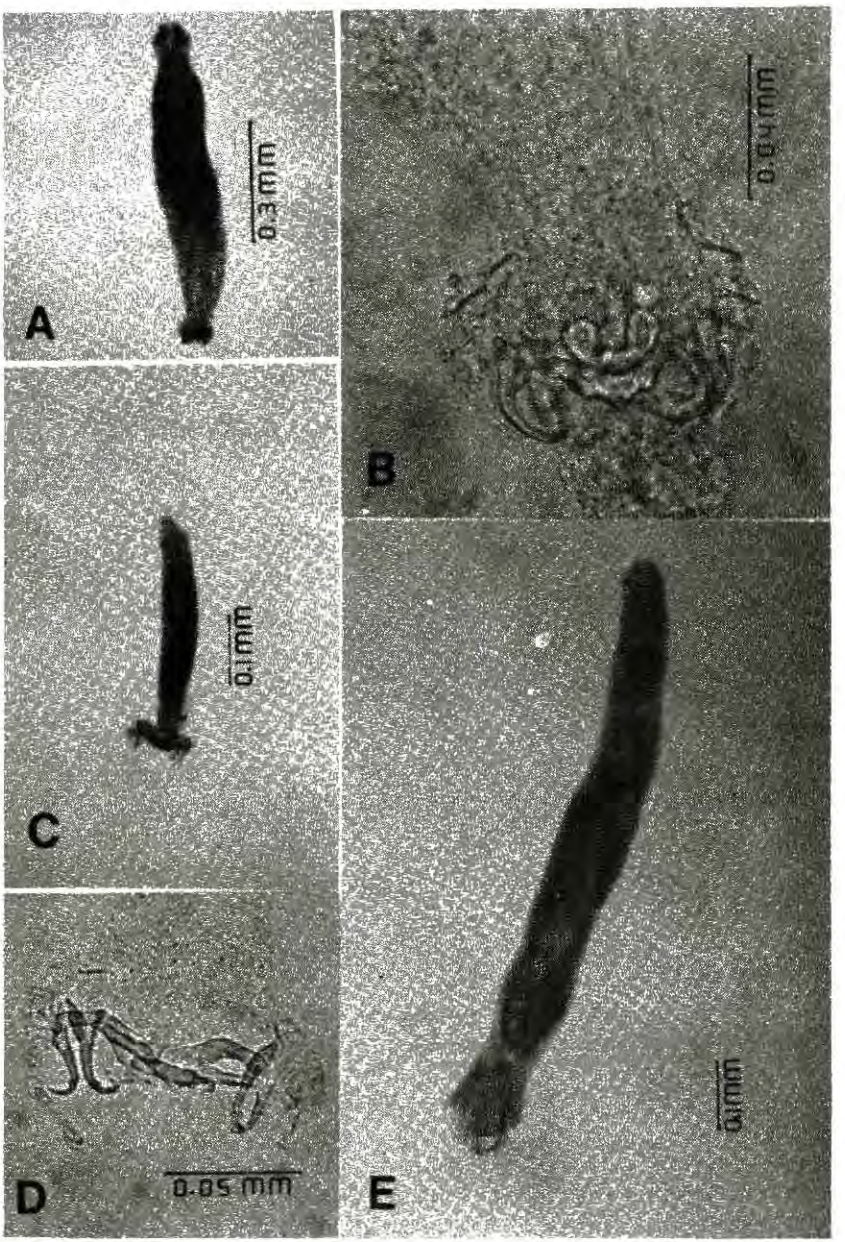

Figure 4. A - *C. sclerosus*, whole worm
B - *C. sclerosus,* opisthaptor
C - *Diplectanum* sp., whole worm
D - *Diplectanum* sp., opisthaptor
E - *Cleidodiscus* sp., whole worm

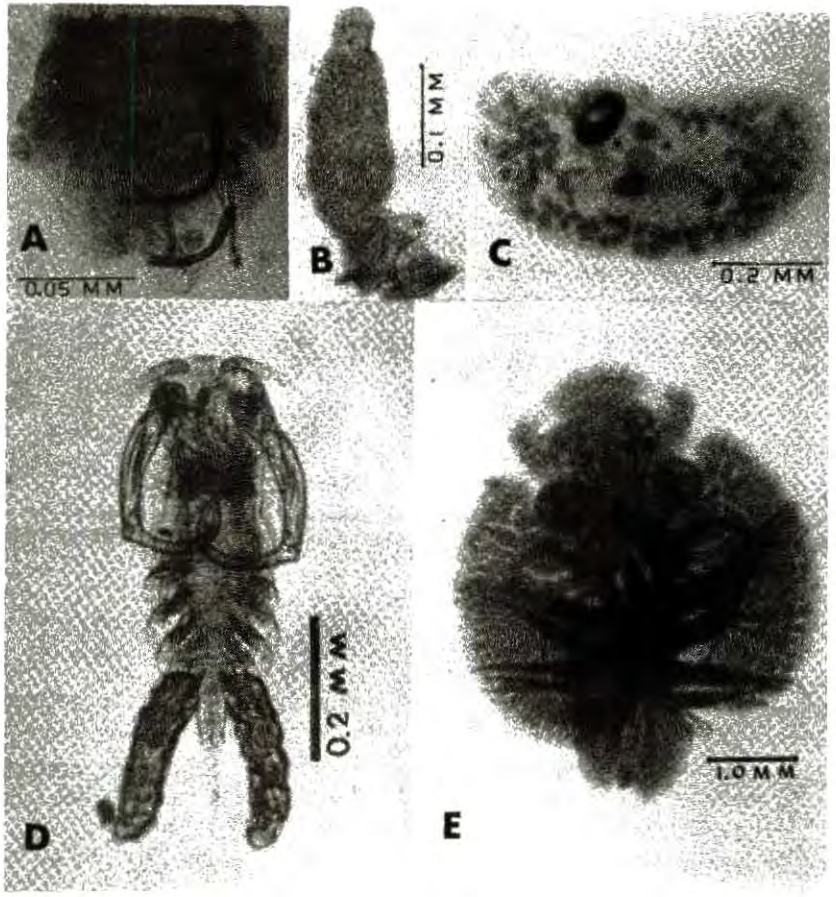

Figure 5. A - *Cleidodiscus* sp., opisthaptor
B - *Actinocleidus* sp.
C - *Transversotrema laruei*
D - *Ergasilus philippinensis*
E - *Argulus indicus*

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Heavy Toxic Metal Resistance of Selected Rhizobium Strains

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ABSTRACT

*Thirty-three **Rhizobium** strains isolated from different host plants and locations were screened for resistance to the heavy toxic metals mercury, copper and cadmium. Five strains, BJr 7 and 12 from **Vigna radiata** (mungbean) and BJL1 21, 23 and 30 from **Leucaena leucocephala** (ipil-ipil) were resistant to mercury up to 50 ppm but were sensitive to 40 ppm Cu and 10 ppm Cd. Strains from **Enterolobium saman** (acacia), BEs 10, 11, 15, 20, 24 and 30 and BJVr 2 and 4 could tolerate up to 40 ppm Cu but were sensitive to mercury. Strains tolerant to 10 ppm Cd were BL1 57, THA 201 and M5. The rest of the strains tested did not survive in 10 ppm Cd.*

The resistance of BL1 80 to mercury, copper and chromium was induced further by stepwise transfer of surviving culture grown at lower metal concentrations to culture media containing higher concentrations. By this technique, BL1 80 was able to tolerate concentrations of 50 ppm Hg, and 60 ppm Cr. Tolerance to copper was quite low. The results suggest that the resistance of microorganisms can further be developed to render a microbial culture that has a greater potential to detoxify heavy metals in the environment.

INTRODUCTION

Metals in the environment have undergone great changes in distribution and solubilization due to industrialization and modernization. Modern agriculture, mining and heavy to light industries in some way or another have introduced higher amounts of toxic metal to the environment which pose risks to the health and well-being of people, animals, plants and microorganisms. This imbalance of heavy metals imposes a burden on the regulatory systems of higher forms of organisms. The transport of metal ions through cell membranes that took billions of years to evolve is being challenged. However, microorganisms have a relatively shorter generation time and consequently their evolution rates are faster. This enables them to evolve within a short time, mechanisms to maintain low concentration of metals intracellularly.

This study deals with the survival of selected *Rhizobium* strains (isolated from different host plants and locations) in culture media containing varying concentrations of the heavy toxic metals mercury, copper and cadmium. The selection of the strains was based on their ability to produce large amounts of mucilaginous polysaccharides. The development of resistance to toxic metals by gradual exposure of a selected strain BL1 80 to increasing metal concentrations was also dealt with.

REVIEW OF LITERATURE

The ability of microorganisms to trap and concentrate metals has been reported for quite some time (**8**). Recently, Mamaril et al. (**7**) reported that tropical *Rhizobium* isolated from areas located near volcanic regions can tolerate high concentrations of lead and mercury. These *Rhizobium* strains sequester the metal on cell surfaces which are covered with a layer of mucilaginous polysaccharides.

It has also been reported that nodulated plants have lower metal content in their upper portion than non-nodulated plants. Douka and Xenoulis **(5)** have reported that soybean and other nodulated plants grown under field and laboratory conditions have significantly lowered heavy metal concentration in the upper portion of the plants. The concentrations of metallic elements such as Mo, Mn, Zn, Cu, Co and Sr in the plants were determined by X-ray fluorescence techniques. They also determined the radioactive content of a pasture field experiment after the Chernobyl accident. It was found that

the concentrations of Cs- 134, C-137 and (Ru-Rh)-105 were lower in the upper portion of the nodulated plants than in non-nodulated plants suggesting that these radioactive elements were concentrated more in the nodules or roots. These findings are important since the useful biomass of the plants are the upper portions which are eaten by man and animals.

Transmission electron micrographs of *Rhizobium* strains grown in culture media containing lead or mercury (**7**) showed that these metals were trapped on the cell surface as complexes or precipitates. Adsorption of heavy metals via extracellular traps minimizes the massive entry of these metals into the cell. A breakdown of the cell's defenses will cause an imbalance in the cell's metabolism and thus cause the death of the organism. Analyses of the supernatant after the cells were removed showed drastic reduction of lead and mercury concentrations.

The removal of heavy metals from solution by microorganisms can occur in several ways (**14**). One way is by altering the solubility of the heavy metal salts. Solubility of metal salts is affected by a number of factors such as pH, temperature, standard reduction potential, concentration of competing anions and cations and surface active substances. Surface active substances may include particulates and macromolecules (polysaccharides, proteins, etc.). The exposed negatively charged groups such as the hydroxyl, carboxyl, amino, amide, imidazole, sulfhydryl, thiol, phenolic and phosphate groups found on the cell surface are responsible for providing negatively charged surfaces to microorganisms. These charges could be neutralized or reversed by the presence of high concentrations of positively charged metallic ions (**4**). The negatively charged groups may act as extracellular ligands which can form stable complexes with the metals and thus prevent their cellular uptake.

The cell may also use the activities of surface bound enzymes for extracellular metal precipitation. The precipitation of insoluble metal complexes can occur through biosynthesis of membrane-associated sulfate reductases (**6**) or oxidizing agents (**14**). The reduction of sulfate to sulfide and the diffusion of oxygen or hydrogen peroxide can provide effective means of precipitation of metals. This metabolic activity of the cell is closely linked to the resistance of the microorganism to heavy metals.

Intracellular traps offer enzyme-mediated resistance to heavy toxic metalloids such as arsenic, antimony and cadmium (**1**). Toxic metals such as mercury and tin are removed via synthesis or organometals. Biomethylation gives certain

microorganisms advantages in eliminating heavy metals. The synthesis of less polar organometallic compounds from polar inorganic ions help regulate cellular elimination which involves diffusion controlled processes (**13**). Another means of controlling metal concentration is through the synthesis of ligands in the form of small molecules with high stability constants such as in the removal of iron by siderophores. These mechanisms require energy to pump the metal ion out of the cell. Enzyme-mediated activities are usually coded by DNA on bacterial plasmids or transposons and not by the normal chromosomal genes (**9**).

Microbial resistance to heavy metals has followed two distinct patterns. Microorganisms subjected to extreme environmental conditions as in active volcanic regions, which abound in hot springs, volcanic lakes and deep sea vents have evolved structures that enable them to adapt to high concentrations of metals (**3**). These evolutionary adaptations to the environment have been passed on to succeeding generations. The other pathway by which microorganisms acquire metal resistance is the acquisition of extachromosomal DNA called plasmids. The synthesis of these plasmids can be increased or decreased depending on the severity of environmental conditions. This mechanism requires inputs of cellular energy which involve nonequilibrium processes and are important considerations in determining rates of metal uptake by the cell.

Microorganisms which have resisted high metal concentrations due to extreme environmental condiditions or are genetically manipulated can be utilized to recover metals from industrial waste waters. Microbial biomass could effectively be used to decontaminate waste effluents from mines, refineries, nuclear fuel plants, battery and electroplating operations (**10**). Metals present in low concentrations in aqueous solutions can be concentrated up to several thousand times its concentration in the environment by a number of microbial species (**11**). The concentration of metals may be as much as 15-50% (w/w) of the cell dry weight. It has been reported that the adsorption of metals by microbial cell mass may be highly specific for one metal and exclude other metals (**2**). The adsorption capacities of some microbial biomass are found to be greater than some adsorbents available in the market (**12**).

The uptake of metal ions by microorganisms in general may occur in two stages. The first stage involves a rapid process occurring on the cell surface and does not involve metabolic processes or inputs or energy. The second stage occurs within the cell cytoplasm and is a slow process. The

processes involve cellular energy to transport ions across the cell membrane against a concentration gradient and are diffusion controlled. Transfer through membranes requires a combination of energized events with carrier molecules which may be specific small molecules or proteins.

MATERIALS AND METHODS

Rhizobium strains/isolates

Thirty-three *Rhizobium* strains which are heavy producers of mucilaginous polysaccharides were obtained from the BIOTECH Culture Collection Laboratory for screening for resistance to heavy metals.

Culture media and conditions

The *Rhizobium* strains were maintained on slopes of yeast extract mannitol agar (YEMA).

The culture medium for the growth experiments was yeast extract mannitol broth (YEMB) to which varying concentrations of metal were added. Control experiments were in YEMB without addition of metal salts. Metal concentration preparation ranged from 0 to 50 ppm Hg, 0 to 40 ppm Cu and 0 to 10 ppm Cd.

Ten ml of the prepared YEMB media were placed in 18 x 150 mm test tubes and inoculated with 0.2 ml of the precultured strain. The culture was shaken at 28°C and incubated for one week. All operations were done under aseptic conditions. A schematic diagram of the procedure is shown in Figure 1. Growth observations were made daily based on turbidity. Optical density readings were made at 570 nm after one week.

BL1 80 strain found to be tolerant to Hg in a previous study (**7**) was tested further for its tolerance to higher concentrations of Hg, Cu and Cr. Concentrations of 10, 20, 30, 40 and 50 ppm Hg, 10 and 20 ppm Cu and 30, 40, 50, 60, 70, 80 ppm Cr were prepared. Viable cell count was taken daily for four days for each of the tests. The surviving cells from a medium of lower metal concentration were again used as preculture for the test with a higher concentration. A stepwise increase of metal concentration was first cultured on low concentration of the metal and the surviving cells were used as pre-culture for the next higher concentration.

RESULTS AND DISCUSSION

The results of the screening test for the resistance of 33 selected *Rhizobium* strains to mercury, copper and cadmium are tabulated in Table 2. Based on the tabulated results, 13 strains were found to be tolerant to 40 ppm Hg. They are NGR 69, BL1 57, BL1 80, BJL1 21, 23, 30, BJVr 1, 5, 7 and 12 and TSU 357, CAI Jap 110 and CAI Tri 100. At higher concentrations of 50 ppm Hg, only five strains were resistant: BJL1 21, 23, 30, BJVr 7 and 12. These strains, however, were not resistant to 40 ppm Cu. A different set of strains was found resistant to copper. These were the strains isolated from acacia and mungbean. Strains tolerant to 15 ppm Cu were BEs 10, 15, 20, 24, 30, BJVr 1, 2, 3 and 4. The same strains were tolerant at 40 ppm Cu although growth was not as fast in this concentration. All these strains were sensitive to 10 ppm Hg except for BJVr 1 and 2 which showed moderate growth at this concentration. All the strains were able to tolerate 1 ppm Cd. Twelve strains were tolerant to 5 ppm Cd. Most of the strains tolerant to copper could withstand 5 ppm Cd. At 10 ppm Cd, only three strains survived: BL1 57, THA 201 and M5. However, these strains were sensitive to mercury except for BL1 57 which was moderately tolerant to this metal.

It is evident from these results that different *Rhizobium* strains differed in the physico-chemical nature of their cell surfaces. The cell surface may have differences in the functional groups of the proteins and polysaccharides that make up the mosaic of interspersed cationic and anionic exchange sites. The specificity of these sites plays a role in the resistance of a strain to a particular metal. For example, isolates from ipil- ipil were more resistant to mercury but quite sensitive to copper and cadmium while isolates from acacia were more resistant to copper but sensitive to mercury. Isolates from mungbean were variable in their tolerance to mercury, copper and cadmium. BJVr 7 and 12 were tolerant to mercury and 5 ppm Cd but sensitive to copper. BJVr 1 had a wider range of metal tolerance. It could tolerate 20 ppm Hg, 15 ppm Cu and 5 ppm Cd. On the other hand, BJVr 4 was sensitive to mercury but quite tolerant to copper and 5 ppm Cd. M5 was sensitive to both mercury and copper but tolerant to cadmium. The differences in the metal tolerance of these strains may be due to environmental and intrinsic factors which involve differences in cell surfaces and cellular uptake of the metals.

Resistance patterns of BL1 80 to mercury, chromium and copper

The survival and growth of BL1 80 cells in media containing increasing concentrations of metals are shown in Figures 2 and 3 for mercury, Figures 4 and 5 for chromium and Figure 6 for copper. The cell counts of BL1 80 at lower concentration of mercury showed increasing cell counts from initial inoculation up to the fourth day. The general trend showed that the cells could still survive at these lower mercury concentrations. At higher concentrations of 40 ppm Hg, the cell count after the second day of incubation was lower than the initial cell count, indicating a loss of inoculant cells which could not tolerate 40 ppm Hg. The cells that were able to survive may have evolved mechanisms to cope with the situation. Thus, growth was evident. The cell count after the fourth day was a bit higher than the initial count. At 50 ppm Hg, there was a gradual loss of surviving cells until the third day, indicating that most of the cells could not cope with 50 ppm Hg. Cell count on the fourth day increased which may mean that these surviving cells had adapted to their environment. However, the cell counts were lower than the initial cell count showing that the growth rate was very much affected by 50 ppm Hg.

Resistance to chromium was quite high. BL1 80 was very tolerant to 30 ppm Cr. At 40 and 50 ppm Cr, cell counts increased after the first day of incubation. After the second day, the surviving cells had adapted protective measures to enable them to cope with 40 and 50 ppm Cr. Cell counts after the fourth day were higher than the initial cell counts, indicating continued growth of the surviving cells. The behavior of BL1 80 cells at much higher concentration of 60, 70 and 80 ppm Cr was erratic. At these high concentrations of Cr, blue green precipitates were visible on the cell surfaces. The pre-culture made up of surviving cells in 50 ppm Cr continued to multiply in 60 ppm Cr.

Cell count of BL1 80 in 70 ppm Cr dropped on the first day but increased on the second and third day. A drop in cell counts, which was much lower than the initial count, was observed on the fourth day. Death of the cells may have been brought about by the massive entry of chromium ions into the cells, thereby breaking down some protective mechanisms. Cell count at 80 ppm Cr showed an initial increase on the second day but gradually decreased on the third and fourth days. Adaptation of the BL1 80 to 80 ppm of Cr was not adequate enough to insure growth of the surviving cells. Based on the figures, the tolerance of BL1 80 to chromium can be increased at 60 ppm. BL1 80 cells

start to become unstable at high concentrations of 70 and 80 ppm Cr.

Tolerance to BL1 80 to 10 and 20 ppm Cu was much lower than that for mercury and chromium. The trend of survival of BL1 80 cells at high concentrations of 40 and 50 ppm Hg was similar to that for Cu. Cell count dropped from the first day of incubation. More cells died at 20 ppm Cu than at 10 ppm Cu during the first and second days of incubation. On the fourth day, cell counts increased, an indication that the surviving cells evolved some strategies to cope with the high amount of copper. However, the cell count on the fourth day was still lower than the initial count, which meant that growth rates were adversely affected by the high concentration of copper.

The increased production of plasmids coding for the synthesis of proteins or enzymes responsible for detoxification of metals could be the mechanism adapted by BL1 80 cells to tolerate and resist high metal concentration.

SUMMARY AND CONCLUSION

Thirty-three *Rhizobium* strains observed to produce large amounts of mucilaginous polysaccharides were screened for their resistance or tolerance to mercury, copper and cadmium. These strains were isolated from different host plants (ipil-ipil, acacia, soybean, mungbean, peanut and clover) and places (Australia, Thailand, Japan and different provinces of the Philippines). In general, strains isolated from *Leucaena leucocephala* (ipil-ipil) were more resistant to mercury than to copper while those isolated from *Enterolobium saman* (acacia) were more tolerant to copper but quite sensitive to mercury. Isolates from *Vigna radiata* (mungbean) varied in their tolerance to mercury, copper and cadmium.

Strains resistant to 50 ppm Hg were BJV4 7 and 12 and BJL1 21, 23 and 30. These strains had lower resistance to copper. Strains resistant to 40 ppm Cu were BEs 10,11, 15, 20, 24 and 30 and BJVr 1, 2, 3 and 4. However, they were sensitive to mercury except for BJVr 1 which could tolerate 20 ppm Hg. All the strains could survive in 1 ppm Cd except for BJVr 3. At 5 ppm Cd, the tolerant strains were BL1 57, THA 2 and 201, CB 756, M5, BEs 10, 15, 20, 24 and 30 and BJVr 1, 2, 4 and 12. Moderately resistant to 5 ppm Cd were TSJ 357, A 702 KO2,

CAI Jap 110 and CAI Tri 100. Except for BL1 57, THA 201 and M5, no other strain survived at 10 ppm Cd. Some specificity by the strains to particular metals was evident in this study.

Resistance of BL1 80 to mercury, chromium and copper may be increased by stepwise transfer of surviving cells in a culture containing lower concentrations of the metal to a culture medium with higher concentrations of the metal. However, this acquired resistance can be increased to a certain degree only, after which the cells become unstable.

When the cells were transferred to a medium with higher concentrations of metal, there was a gradual loss of viable cells during the first and second day of incubation. On the third or fourth day, the surviving cells began to increase their growth rate. Maximum concentration of chromium that BL1 80 could tolerate under the conditions of this study was about 60 ppm; that for mercury may be increased above 50 ppm. Tolerance to 10 and 20 ppm Cu could not be determined since cell count on the fourth day was still below initial cell count. This may indicate that BL1 80 cells do not have adequate mechanisms for the detoxification of copper. However, this may be reinforced by this technique.

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Table 1. List of *Rhizobium* strains/isolates used in heavy metal tolerance tests

Accession No.	Strain Designation	Host Plant
1079	BL1 57	*Leucaena leucocephala* (ipil-ipil)
1086	TAL 600	*Leucaena leucocephala* (ipil-ipil)
1150	THA 2	Soybean (Bangkok)
1157	THA 201	Peanut
1159	NC 92	Peanut
1164	NGR 69	Ipil-ipil
1273	CB 756	(Australia)
1275	M5	Mungbean
1347	BL1 80	Ipil-ipil
1392	BEs 50	*Enterolobium saman* (*Samanea saman*, acacia)
1383	BEs 11	" " "
1395	BEs 15	" " "
1396	BEs 20	" " "
1397	BEs 24	" " "
1403	BEs 30	" " "
1431	BJL1 4	Ipil-ipil (Los Baños, Laguna)
1435	BJL1 14	Ipil-ipil (Taal, Batangas)
1437	BJL1 19	Ipil-ipil (Malolos, Bulacan)
1439	BJL1 27	Ipil-ipil (Mabalacat, Pampanga)
1442	BJVr 2	Mungbean (Calaca, Batangas)
1444	BJV4 4	Mungbean (Calaca, Batangas)
1445	BJVr 7	Mungbean (Calaca, Batangas)
1440	BJL1 5	Ipil-ipil (Los Baños, Laguna)
1456	BJL1 21	Ipil-ipil (Calumpit, Bulacan)
1458	BJL1 23	Ipil-ipil
1461	BJL1 30	Ipil-ipil
1462	BJVr 1	Mungbean (Calaca, Batangas)
1464	BJVr 3	Mungbean (Calaca, Batangas)
1464	BJVr 12	Mungbean (Davao City)
1638	TSJ 357	Mungbean
1639	A 70 3 KO2	
1641	CAI Jap 110	*R. trifolii*
1642	CAI Tri 100	*R. trifolii*

Table 2. Screening for tolerance/resistance of selected *Rhizobium* strains to mercury, copper, and cadmium

Strain	Metal Concentration, ppm							
	Mercury			Copper		Cadmium		
	30	40	50	15	40	1	5	10
BL1 57	+ +	+ +	-	+	-	+ + +	+ +	+
TA1 600	-	-	-	+	-	+ +	-	-
THA 2	-	-	-	+	-	+ +	+ +	-
THA 201	-	-	-	+	-	+ +	+ +	-
NC 92	-	-	-	+	-	+ +	-	-
NGR 69	+ + +	+ +	-	+	-	+ + +	-	-
CB 756	+ +	+	-	+	-	+	+	-
M5	+	+	-	+	-	+ +	+	+
BL1 80	+ +	+ +	-	+ +	-	+ +	-	-
BE2 10	+	-	-	+ +	+	+ +	+ +	-
BEs 11	-	-	-	+ +	+	+ +	-	-
BEs 15	-	-	-	+	+	+ +	+ +	-
BEs 20	-	-	-	+ +	+	+ +	+ +	-
BEs 24	-	-	-	+ +	+	+ +	+ +	-
BEs 30	-	-	-	+ +	+	+ +	+ +	-
BJL1 4	-	-	-	-	-	+ + +	-	-
BJL1 14	-	-	-	-	-	+ +	-	-
BJL1 19	+ +	-	-	-	-	+ +	-	-
BJL1 27	+ +	+	-	+	-	+	-	-
BJVr 2	+	-	-	+ +	+	+ +	+	-
BJVr 4	-	-	-	+ +	+	+ +	+	-
BJVr 7	+ +	+ +	+ +	+	-	+ +	+ +	-
BJL1 5	+ + +	+ +	-	-	-	+ +	+ +	-
BJL1 21	+ +	+ +	+ +	+	-	+ +	+	-
BJL1 23	+ + +	+ +	+ +	+	-	+ +	-	-
BJL1 30	+ +	+ +	+ +	+	-	+ +	+ +	-
BJVr 1	+ +	+ +	-	+ +	+	+ +	+ +	-
BJVr 3	-	-	-	+ +	+	-	-	
BJVr 12	+ +	+ +	+ +	+	-	+ +	+ +	-
TSJ 357	+ +	+ +	-	-	-	+	+	-
A702 KO2	+ +	-	-	-	-	+ +	+	-
CAI JAP 110	+ +	+ +	-	-	-	+ +	+	-

Legend:

O.D.	
0.00 - 0.10	negligible tolerance (-)
0.11 - 0.35	fair tolerance (+)
0.36 - 0.70	good tolerance (+ +)
0.71 - 1.00	high tolerance (+ + +)

Optical density was measured at 570 nm

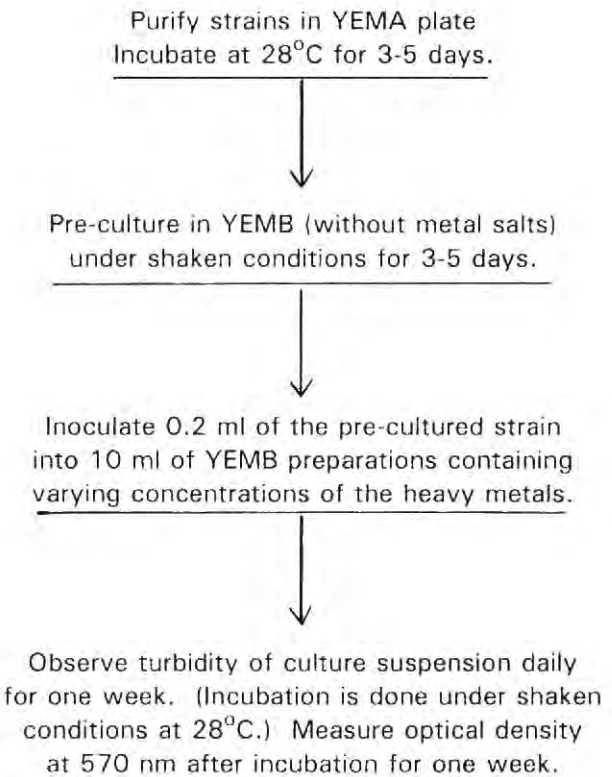

Figure 1. Schematic diagram for the determination of metal resistance by selected Rhizobium strains

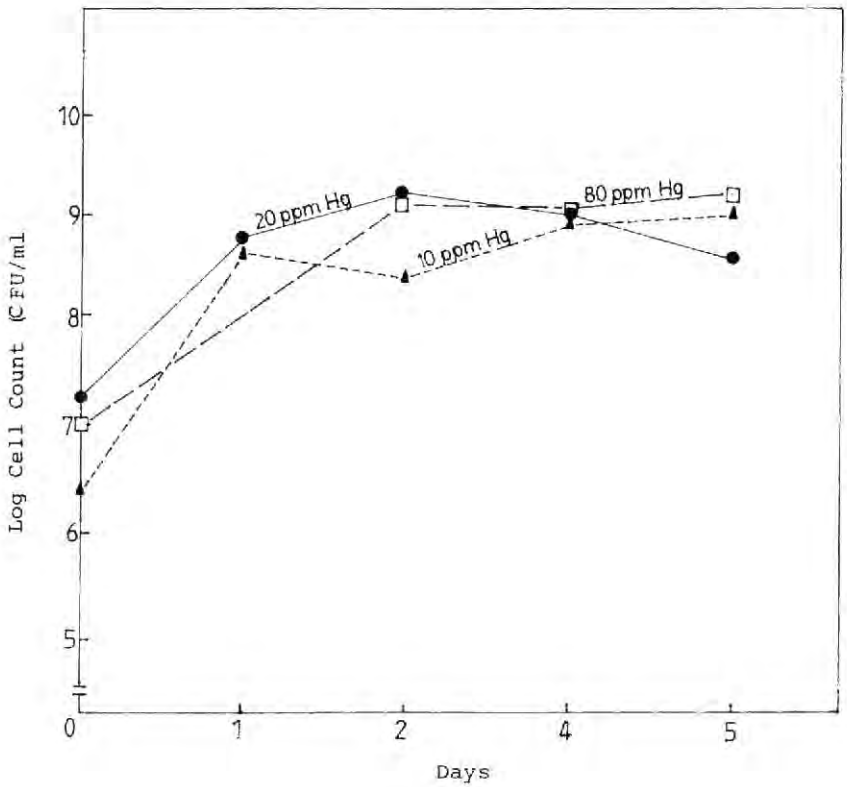

Figure 2. Growth of BL1 80 in YEM broth containing different concentrations of Hg^{2+}

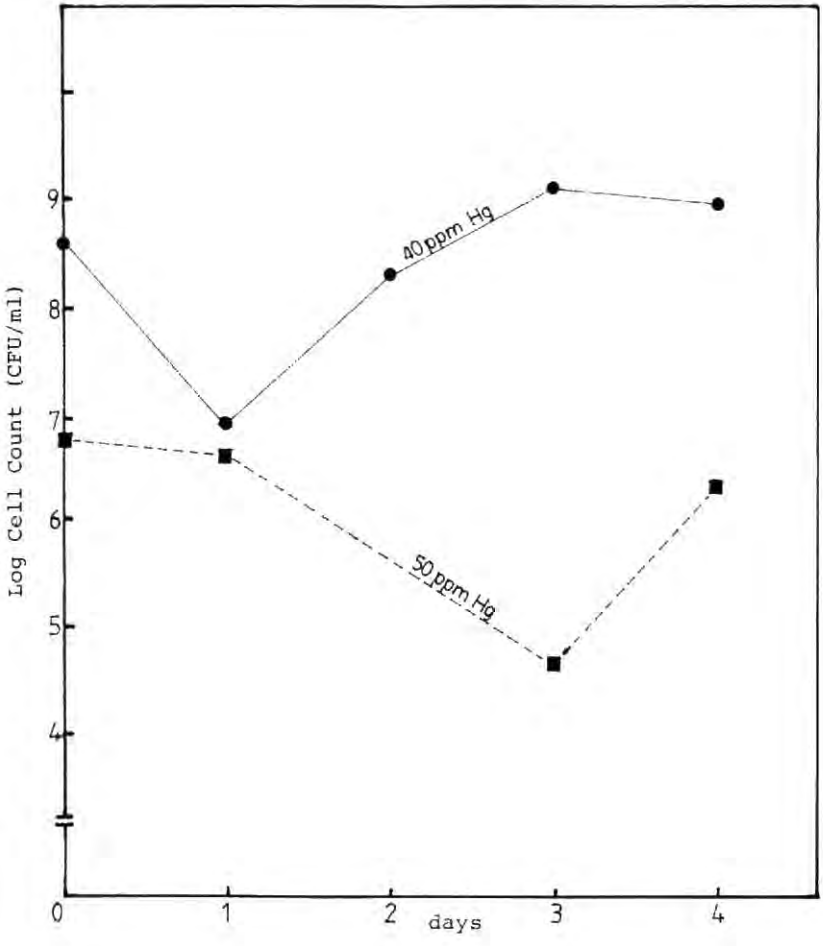

Figure 3. Growth of BL1 80 in YEM broth containing different concentrations of Hg^{2+}

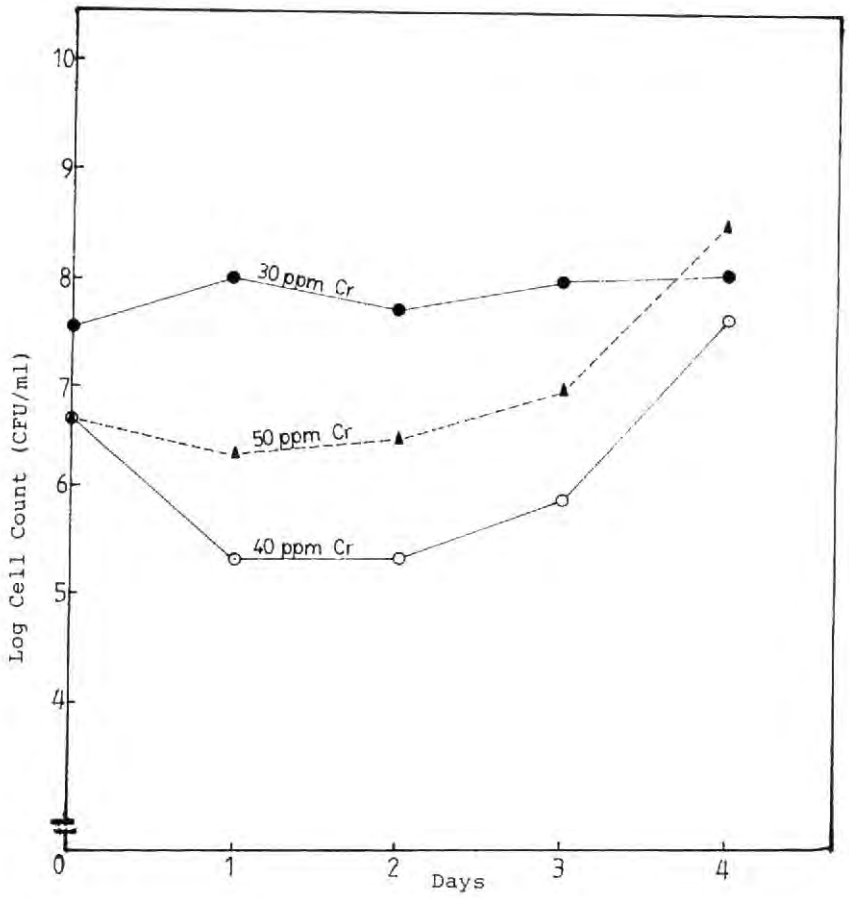

Figure 4. Growth of BL1 80 in YEM broth containing different concentrations of Cr^{2+}

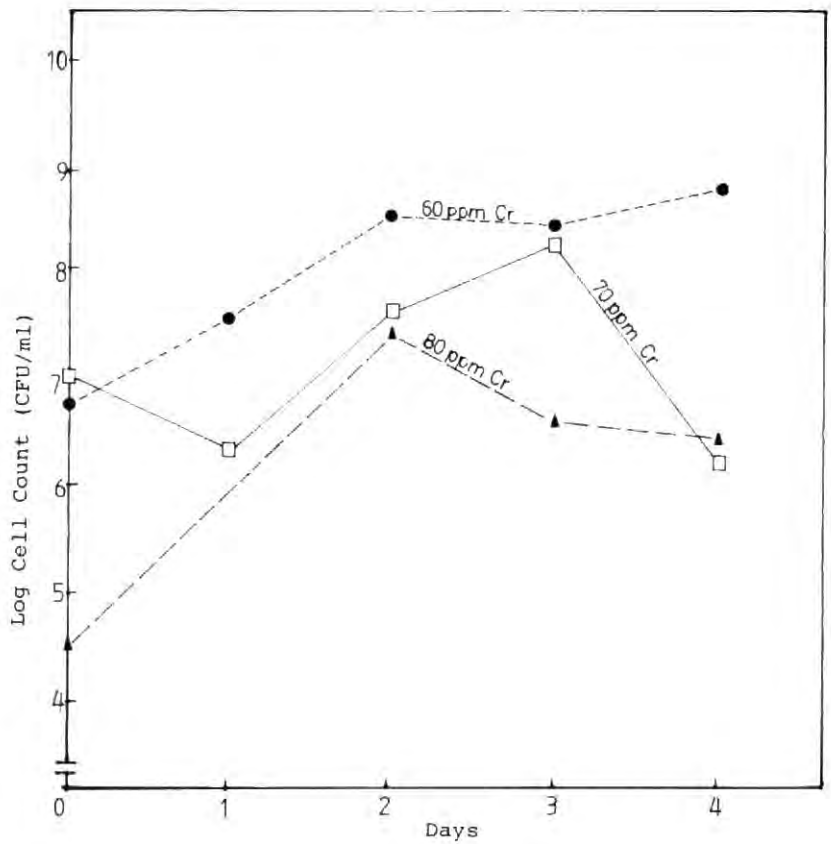

Figure 5. Growth of BL1 80 in YEM broth containing different concentrations of Cr^{2+}

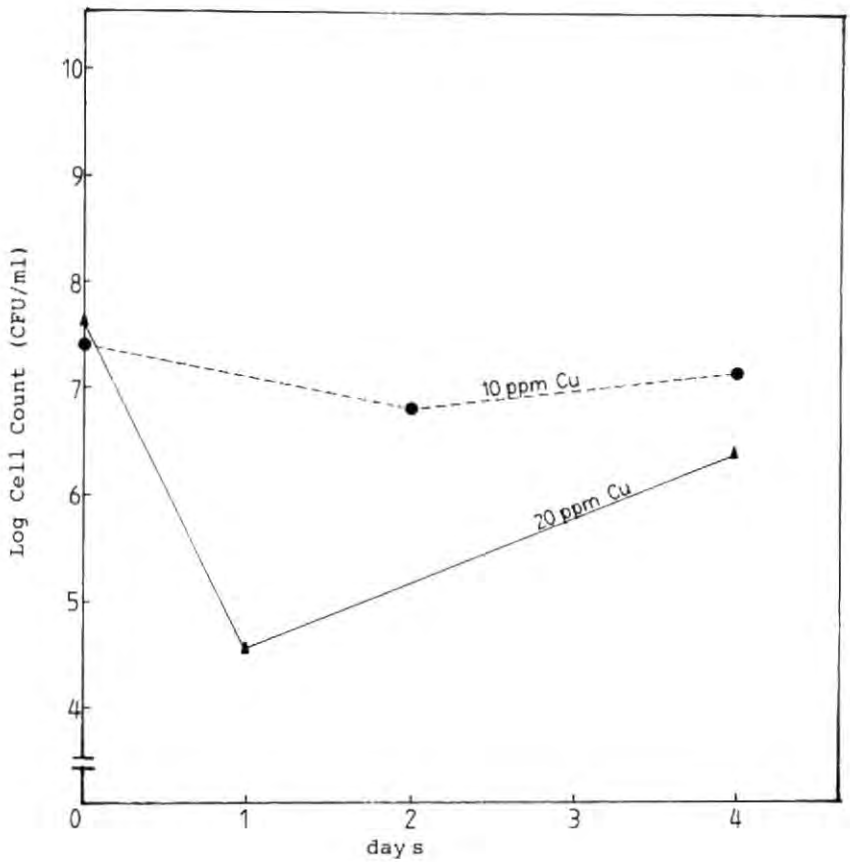

Figure 6. Growth of BL1 80 in YEM broth containing different concentrations of Cu^{2+}

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The Reproductive Biology and Laboratory Maintenance of *Vivipara angularis* Muller (Prosobranchia: Viviparidae)

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ABSTRACT

*Monthly sampling of **Vivipara angularis** Muller in the UP lagoon was done to obtain a year-long trend. Physical conditions of the collection site during sampling were noted. Sex was determined from the appearance of the curve of "hooked" right tentacle (the copulatory organ of the male), as opposed to the fine, pointed one of the female. Biometric data (shell length and width) of the monthly samples were obtained by the use of a Vernier caliper and were correlated to life cycle and sexual dimorphism. The reproductive systems of the male and female **Vivipara** were studied by gross dissection and histological sections. This sensitive species of mollusks was tested for its food preference to develop feeding strategies for its maintenance in the laboratory. Protein content of laboratory -reared snails was assayed. This could serve as a means to come up with an economical protein alternative for human and animal consumption.*

INTRODUCTION

In the field of malacology (the study of mollusks), a great number of researchers have indulged in the various aspects of reproduction among oviparous gastropods. The viviparous species, however, is often "ignored." This is probably because it is difficult to rear under laboratory conditions. As a consequence of its mode of reproduction, which, is viviparity, its fecundity is low.

Variations in the shell features of viviparids impose a problem in nomenclature. However, in the Philippines, only *Vivipara angularis* (Fig. 2) and *Bellamya philippinensis* have been reported to be snails of medical importance belonging to Family Viviparidae (**4, 15**). The earliest report found on the species was done by Alonte in 1930. He studied the biology of *V. angularis* Muller, a common duck-feed snail in Laguna de Bay (**1**) which is known as one of the edible freshwater mollusks in Manila (**10**). Different local names given to this snail in the various regions of the Phiippines (susong, pangpang, egue in Tag.; ege in Bis.; leddeg in Iloc.; and betocol in Zam.), reflect its wide distribution in the country. It is usually found inhabiting shallow stagnant or still waters such as lakes, ponds, streams, lagoons, ricefields, canals and ditches (**8**). However, the problem of low fecundity aggravated by collection for both human and animal consumption contributes to its extinction. Environmental disturbances comprise a secondary threat to its existence.

Apart from the studies mentioned above, no other local reports have been encountered, most especially on the reproductive biology, ecological distribution and life cycle of *Vivipara angularis*.

This investigation aimed to: (a) obtain biometric data (shell length and width, body weight) which may be related to its life cycle and sexual dimorphism; (b) study some aspects of its reproductive biology through sampling from selected natural populations; (c) study its reproductive anatomy through gross dissection and histological sections; (d) study its feeding habits in the field and develop feeding strategies for *Vivipara* maintained in the laboratory; and (e) identify food types preferred by the snail and food types that improve its protein content. It is hoped that the data generated by this study will add to the scant existing knowledge on this subject.

METHODOLOGY

I. Field Collection

Monthly sampling of *Vivipara angularis* Muller (Fig. 2) in the UP Lagoon (Fig. 1) began in March 1990 and continued until a year- long sample had been obtained. Physical conditions like air, water, and substrate temperature of the collection site during sampling were noted.

II. Culture Method

All collected samples were sieved and washed systematically to remove mud. Free-living young were separated from adults. All snails were reared in glass aquaria (Fig. 3) provided with continuous aeration. Adult snails were fed with lettuce leaves and dacaying leaves. Cultures were cleaned and replaced with decholorinated water twice a week.

III. Laboratory Rearing Experiments

Young vivipara (Fig. 4) expelled by laboratory-reared gravid females were collected daily and placed in separate basins. The basins were washed twice a week to remove excreta. For three weeks, the snails were fed with lettuce leaves. They served as the stock cultures for growth, reproduction and protein content determination experiments. Snails that survived after three weeks were grouped as follows: one pair, two pairs, three pairs and four pairs. They were placed separately in basins with designated food types: mud, lettuce, dacaying leaves and algae, respectively. Biometric data (shell length and width, weight and number of whorls) at the start of pairing, mating stage, first and last gestation and death were noted.

Immature and mature vivipara are being tested for their food preference since these snails are known to shift their nutrition at certain stages of their life cycle. The same procedure as above has been followed. However, this stage of the project is ongoing and the initial results are still subject to retesting.

IV. Sex Determination and Biometric Data

Sex determination was based on the appearance of the truncated right tentacle in males (Fig. 5) as opposed to the fine, pointed left tentacle of the male, and left and right tentacles of

the females (Fig. 6). Shell length and width were measured with a Vernier caliper. Shell height was the maximum distance from the apex to the outer edge of the aperture while shell width was the measurement of the biggest whorl (**3**). Snails were weighed on a mettler balance. Female snails were counted and classified as immature, mature (with ova), gravid (with uterine young) or barren (with empty uteri). Dissection was done to obtain a direct count of uterine young. This permitted increased accuracy in the assessment of female reproductive output.

V. Gross Dissection and Histological Sections

Specimens were relaxed in water with menthol crystals. This relaxation technique took time since the species are operculated. The specimen is pinned to a paraffin block and dissection is carried out with a Will stereomicroscope and stainless steel fine-point forceps and scalpels. Gross dissection revealed most features. The study of the reproductive structures was carried out on both fresh and preserved specimens. Specimens were preserved with 70% ethyl alcohol and a few drops of glycerol to prevent "dryness or stiffness" of tissues. Photographs were taken using a stereomicroscope with camera attachment.

Preparation of histological sections to observe cellular composition and organization of reproductive organs involved fixation in Zenker's fluid, dehydration in ethyl alcohol, clearing in xylene, paraffin embedding, sectioning at 7u thickness and staining with the standard hematocylineosin method.

VI. Determination of Protein Content

Snails from the July 1990 collection were fed with mud and lettuce leaves for nine months while snails collected in May 1990 were fed with lettuce leaves for 11 months. Samples taken from these laboratory-reared snails were subjected to protein content analysis by the Bradford method. Shell length and width as well as weight, were noted before and after the snails were deshelled. A physiological buffer (Carriker's solution) was added to the tissue and homogenized in a glass homogenizer. Protein content was determined using the Bradford method since it is more accurate than the Biuret method.

RESULTS

I. Biometric Data

Biometric data obtained from the nine sampling periods showed that generally, females were longer (Fig. 7), wider and heavier than males. Sub-adult and adult snails mostly had four whorls. In very few instances, snails with shell length and width as low as 14.8 mm and 14.3 mm, respectively, when dissected for presence of uterine young or ova were classified as mature while those below these measurements were classified as immature. Most of the snails with a shell length of 18.0 mm and shell width of 16.0 mm were gravid. Dissection of gravid females revealed that more developed young (with shell) were located anterior to midportion of the pallial oviduct while embryos/ova enclosed in white transluscent capsules occupied the midportion to posterior region of the pallial oviduct. Uterine young were found to be wider than they were long. They were usually three-whorled.

In the course of the present investigation, it was discovered that there is marked sexual dimorphism of size in *Vivipara angularis* as observed by Van Cleave (1932) in *Viviparus contectoides (***14).** A single female snail with a shell length of 31 mm was found in the collection used for this study. Male specimens of the species encountered in the collection never exceeded 26 mm. Van Cleave (1932) also noted that the close correlation between height and diameter for the entire population curve shows no basis for sex recognition **(14).** Thus, in all the later studies, the single measurement of height was taken into consideration.

A. Female Biometric Data

In December 1990, and January and February 1991 the bar graphs (Fig. 9), as well as the line graphs (Figs. 15- 17), showed an average shell length of around 18 - 19 mm. During these months, the snails had the highest % of uterine young and a very low % of uterine ova (Fig. 9). This indicates that these months cover the period of gestation, when most snails give birth to their young. In March, April and June 1990 (Figs. 8 and 9), the % of uterine young gradually decreased until it reached its lowest in June. However, a markedly high percentage of uterine young

was obtained in May 1990. This was not expected considering the general trend of the sample. Such a deviation may be accounted for by the physical conditions of the collection site. Four days before the May sampling period a sudden downpour filled the drying lagoon. This might have affected the activities of the snails.

Biometric data obtained in July 1990 (Fig. 10) indicate that many *Vivipara* have shell length of 14-16 mm. These were the growing snails delivered during the gestation period. As stated earlier, at this size, most of the snails were immature. This is consistent with the low % of uterine young versus a high % of uterine ova (Fig. 8) during this month.

In August 1990 (Fig. 11), there was a reversal in ratio of uterine young and ova, the % of uterine young being higher than the % of uterine ova. This was a result of the maturation of snails from the previous month (Figs. 10 and 11), as shown by the graph peaking at points when shell length was longer. This was also true for the September and October biometric data (Figs. 12 and 13).

In Fig. 7 the bar graph shows that females are biggest in November 1990, when they attain their oldest stage and then die the next month (December 1990). Post gravid snails of the November collection mostly had a shell length of 22 mm and shell width of 18.5 mm. They contained less number of uterine young as shown by a slightly lower % of uterine young in this month compared to December (Fig. 8). This may be accounted for by the fact that post gravid snails had expelled their young in the earlier months.

B. Male Biometric Data

In April 1990, most mature male snails had died but left some immature ones (Fig. 9). A sudden increase in the number of snails with shell length characteristic of the immature and maturing snails was observed in the July biometric data (Fig. 10). This may be similar to earlier observations of Van Cleave (1932) in *V. contectoides* that there is marked growth increment in the first few months after birth and a slow growth increase thereafter. Since male vivipara are generally smaller in size than females, it is therefore, safe to say, that males reach sexual maturation when they are smaller in size than mature females.

In August to December 1990 (Figs. 11-15), most snails had reached sexual maturity with a shell length of 15-18 mm. Some of the snails attained sizes characteristic of old ones and later died. This is shown by the steadily decreasing number of snails with shell length of 20 mm.

II. Laboratory Rearing Experiments

Initial results of young vivipara paired and fed separately with lettuce leaves and mud showed a high survival rate in contrast to those fed with algae and decaying leaves. Moreover, the optimum number of snails with normal growth was four per basin.

III. Gross Dissection

A. Female Reproductive Organ

The birth pore of the female reproductive system found on the anterior surface of the vagina exhibits bright orange flecks above a speckled gray background of melanin (Figs. 18 and 20). The elliptical vagina is situated in the distal end of the pallial oviduct and terminates at the edge of the mantle. It exhibits a grayish appearance. Originating at the posterior end of the mantle cavity and proceeding anteriorly to the narrower vagina, the pallial oviduct lies parallel to the rectum at the extreme right edge of the mantle cavity and exhibits moderate to heavy concentrations of melanin in the epidermal covering.

In non-gravid females it is a large, comparatively thick-walled, white duct while in gravid females the organ is expanded and enlarged in diameter to form the brood pouch. It is relatively thin-walled and easily identified by the presence of developing young.

The white pink seminal receptacle is located anterior to the albumen gland. It is externally distinguishable from the pallial oviduct in gravid females by its narrower diameter. It is externally indistinguishable in non-gravid females. The V-shaped oviduct is located anteroventrally to the albumen gland and receives the ovary at its proximal end. Its distal end enters the posterior part of the seminal receptacle. The oviduct appears white for most of its length, and frequently pink to rose in the distal region. The faintly translucent, white ovary (often difficult to discern in living animals) is located in the apical whorl along the digestive gland. The ovary is a long, narrow duct which sometimes possesses a variable number of short lateral branches or small protrusions **(2)**.

B. Male Reproductive Organ

Gross dissection revealed features of the male reproductive structure (Figs. 19 and 21). The curved, modified right tentacle of the male vivipara serves as its copulatory organ. It is mainly black with transverse bands of orange in an irregular pattern. The bean-shaped testis is rust orange, long and fusiform organ located along the right edge of the mantle **(2)**. The vas deferens, a short, nearly transparent structure, leaves the testis at about the middle to posterior portion. It curves slightly at the mantle wall, passes through the posterior end of the food groove and then enters the prostate gland. The large creamy yellow prostate gland receives the vas deferens and passes forward beneath the groove towards the right tentacle. It has a smooth, thickly muscular appearance in contrast to the grainy appearance of the testis. It spans nearly the entire length of the mantle cavity then narrows into a terminal vas deferens that passes through the right tentacle and opens at its tip.

IV. Histological Sections

Histological sections for both male and female reproductive organs of *Vivipara angularis* were done using the Paraffin method.

A. Female Reproductive Organ

The ovary (Fig. 18) is very small, translucent to orange-brown delicate structure lying on the digestive gland. It is often very hard to discern in live specimens and researchers have not yet been successful with its histology.

The seminal receptacle of spermathecae (Figs. 22 and 23) where sperm received during copulation is strored, is lined by tall columnar epithelium and surrounded by circular muscles.

The pallial oviduct (Figs. 22 and 24) is lined by mucosal cells thrown into folds, perhaps for greater distension capacity when eggs, embryo, and young snails are contained in this structure. It is lined by simple columnar ciliated cells with light staining cytoplasm and deeply staining basal nuclei.

The vagina (Fig. 25), located at the distal end of the pallial oviduct, exhibits irregular folds in its mucosa. The surface epithelium is transitional in type. This ephithelium is stratified and composed of several layers of generally similar cells which are usually cuboidal with round nuclei.

B. Male Reproductive Organ

The right copulatory tentacle's surface contains flecks of bright fuchsia to red over a solid sub-epidermal concentration of melanin (Figs. 26 and 27). It is lined by columnar cells with basally located nuclei. The seminal duct included within the tentacle is composed mostly of circular muscles with some interspersed connective tissues. The muscular tentacle can be accounted for by the need for a mechanism to effectively ejaculate the sperm during copulation.

The light staining prostate gland (Fig. 28) is lined by simple cuboidal cells. A thin muscular coating of an external circular muscle layer and an internal longitudinal muscle layer around the entire tissue can be observed.

The vas deferens (Fig. 29) exhibits a narrow, irregular lumen and a thick muscular layer. The epithelium is pseudostratified columnar.

The testis (Figs. 30 and 31) is composed of seminiferous tubules, each surrounded by an outer layer of connective tissue and an inner basement membrane. The basement membrane encloses the specialized germinal epithelium of the seminiferous tubules which consists of two cell types: the supporting cell of Sertoli cells and the spermatogenic cells.

The Sertoli cells are triangular and tapering with very irregular outlines extending from the basement membrane to the free luminal surface. The very distinct ovoid or angular nucleus is outlined with fine, sparse chromatin.

The spermatogenic cells are arranged in rows, mostly superimposed on Sertoli cells, obscuring their cytoplasm. The most primitive or immature spermatogenic cells, the spermatogonia, are situated adjacent to the basement membrane of the seminiferous tubules. Spermatogonia divide mitotically to produce several generations of cells.

The primary spermatocytes, the largest germ cells in the seminiferous tubules, occupy the middle of the germinal epithelium. The primary spermatocytes give rise to secondary spermatocyte by meiotic division.

The secondary spermatocytes are distinctly smaller than the primary spermatocytes. The nuclear chromatin is less dense.

The spermatids are smaller cells with small nuclei containing both fine and larger chromatin granules. The cells become closely

associated with the surface of Sertoli cells, and in this environment differentiate into spermatozoa by the spermiogenesis process.

The small, deep-staining heads of spermatozoa are embedded in the cytoplasm of Sertoli cells while their tails extend into the lumen of the seminiferous tubule. Most of the findings of this study are in accordance with the descriptions of Di Fiore (1989)[5].

V. Protein Content

Preliminary results of protein concentration analysis of snails fed with different food types (sample 1 - lettuce only; samle 2 - lettuce and mud) indicate that feeding with lettuce and mud could improve the protein content of *V. angularis* twofold (Fig. 32).

DISCUSSION

Vivipara angularis inhabiting the slow moving waters of the UP lagoon were subjected to field and laboratory analyses of population samples taken at monthly intervals over a period of one year. Gross dissection and histological sections elucidated the existence of sexual dimorphism, disputing the claim of Alonte (1930) that this snail is hermaphroditic **(1).** Although shell features offered no means of identifying sexes, sex determination was based on the appearance of curved or "hooked" right tentacle in males as opposed to fine left tentacle of males and right and left tentacles of females. THe right tentacle serves as its intromittent copulatory organ. Sexual dimorphism was also observable in size, females being generally larger than males. These findings further establish that Vivipara angularis is indeed sexually dimorphic.

Biometric data obtained through a scheme of monthly sampling covering a period of one year, described the life cycle of *V. angularis.* Gestation period was observed to cover a span of seven months, starting in August, and peaking in February of the next year (Fig. 8). Most of the female snails in these months are gravid, having a shell length of around 18-19 mm and containing uterine young in a graded series of development. The percentage of uterine young gradually decreased and reached its lowest in June, the time when gravid snails have expelled all their uterine young. In March to June, snails delivered during the

gestation period developed into immature snails and became sexually mature in June. Since the growth period was from March to June, and the start of the gestation period was in August, a short mating period was in early June.

A very high percentage of uterine ova in June indicated that the snails had just undergone copulation. Growth of uterine ova into uterine young started in July as evidenced by the slight increase in the percentage of uterine young from June to July. More developed young were observed in August, when the gestation period commences.

The initial results in the laboratory rearing experiments show that there was an observably high survival rate in snails fed with mud and lettuce. It is interesting to note that they were also found to have higher protein content than snails fed with lettuce only. However, the results are not conclusive as the data obtained were from preliminary expreiments only.

Figure 1. **The collection site: U.P. lagoon**

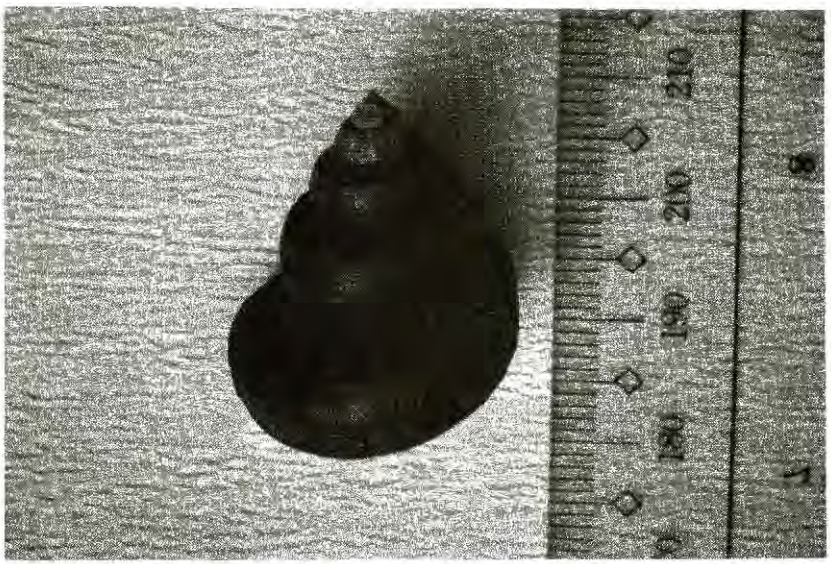

Figure 2. The specimen: *Vivipara angularis*

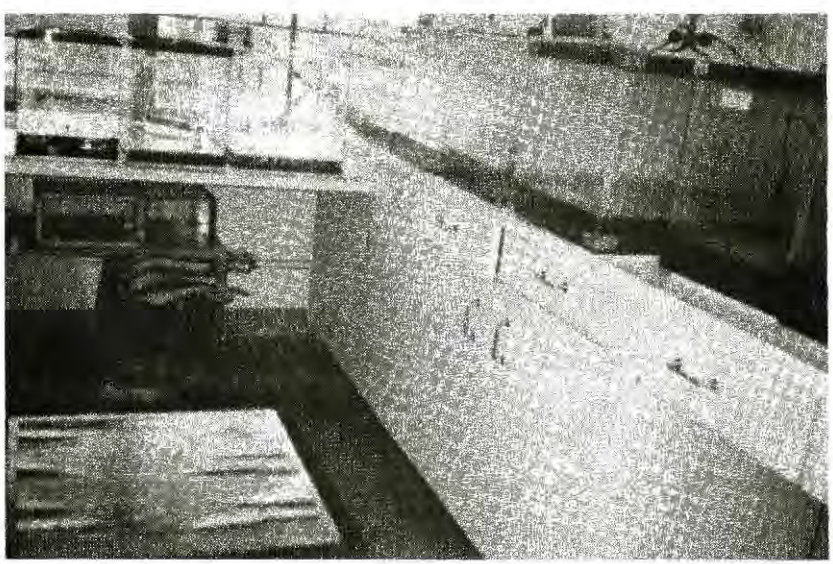

Figure 3. Snails were reared in glass aquaria provided with continuous aeration.

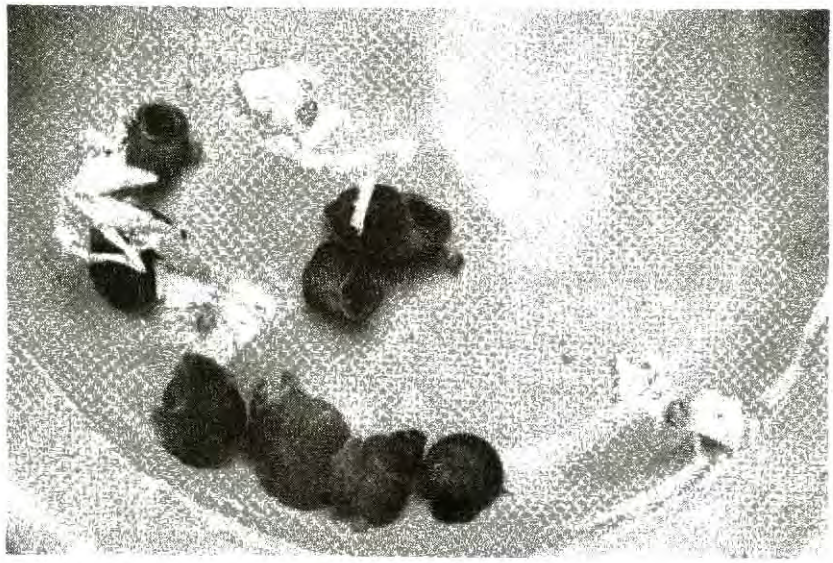

Figure 4. Gravid female snails with newly-expelled young. They were fed with lettuce leaves.

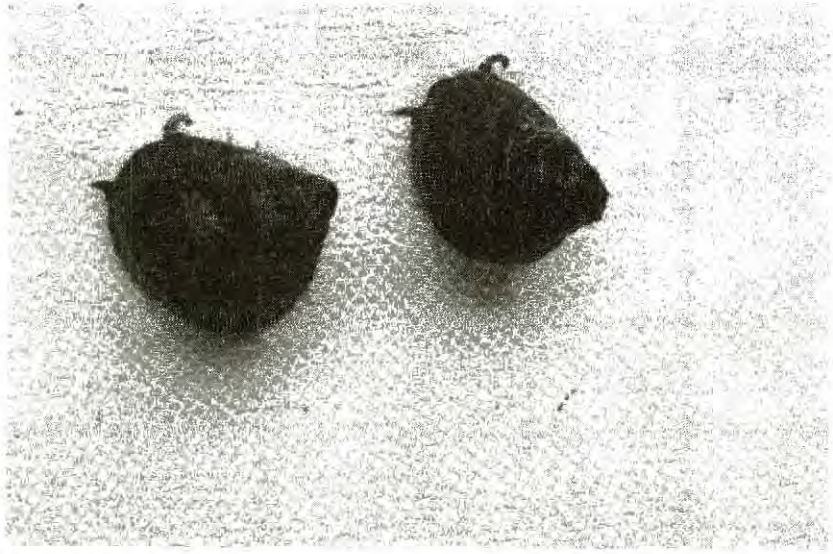

Figure 5. Male *Vivipara angularis* showing their "hooked" right copulatory tentacles

Figure 6. Female *Vivipara angularis* showing their fine, pointed left and right tentacles.

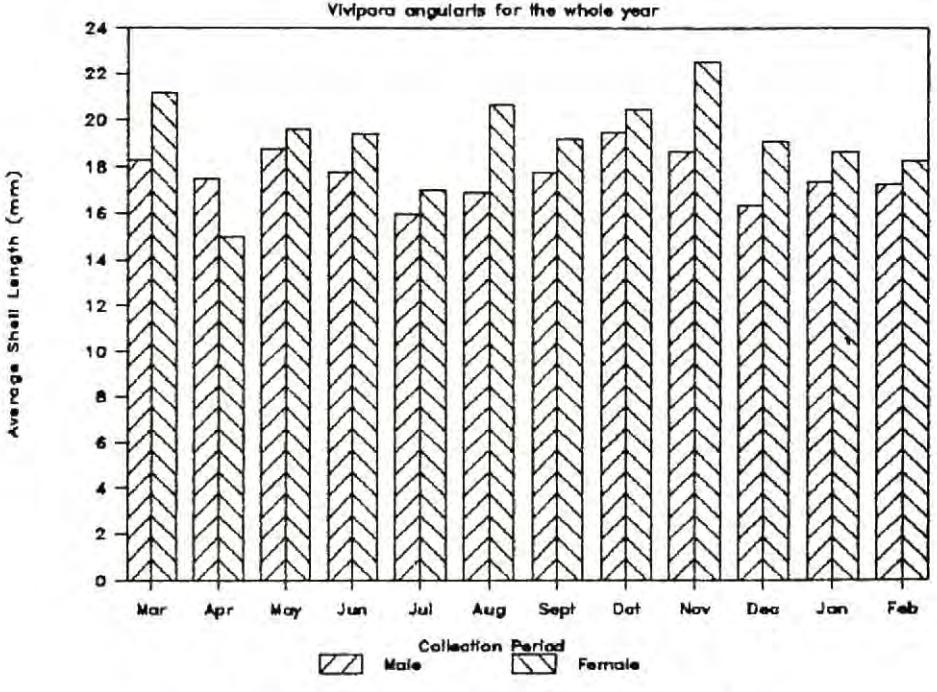

Figure 7. Average Shell Length of Male and Female

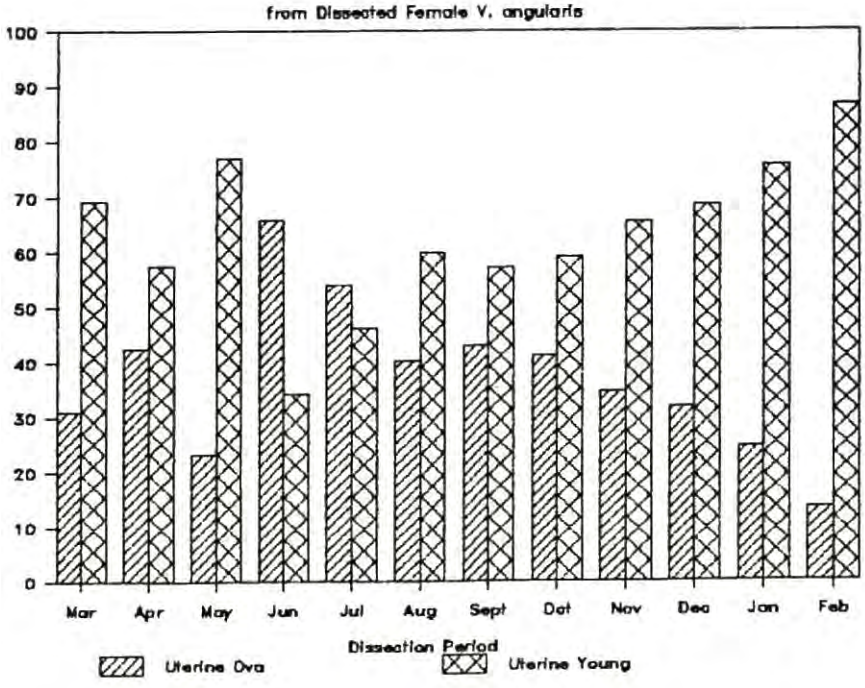

Figure 8. Percentage of Uterine Ova and Young

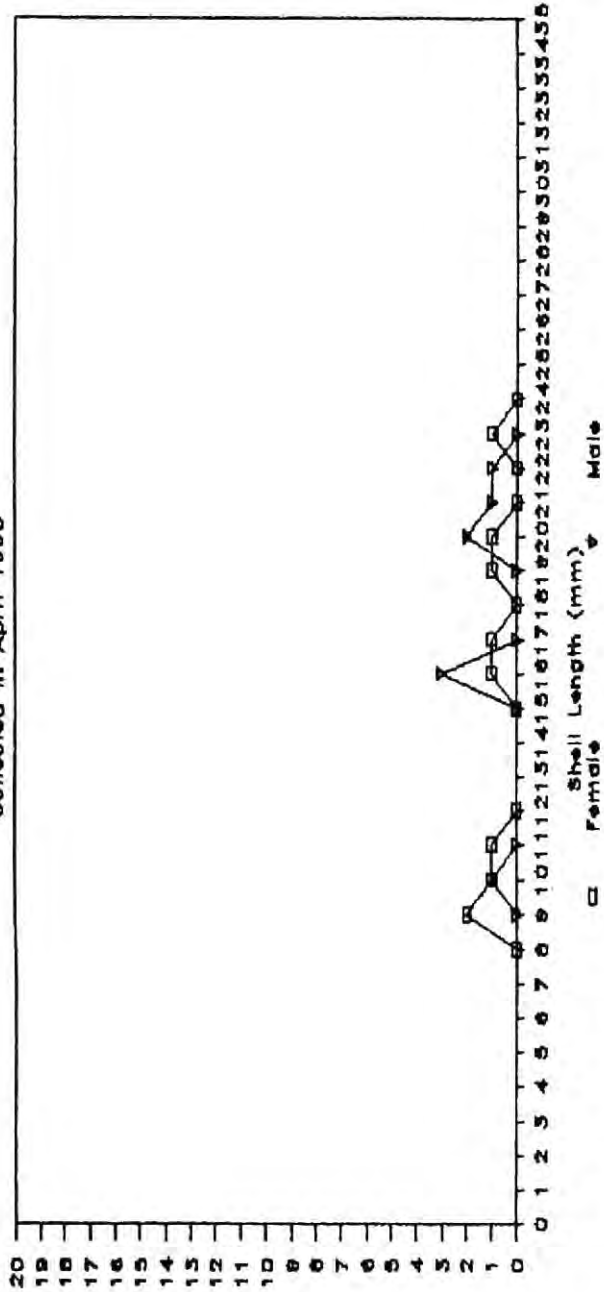

Figure 9. Biometric Data of *V. angularis*

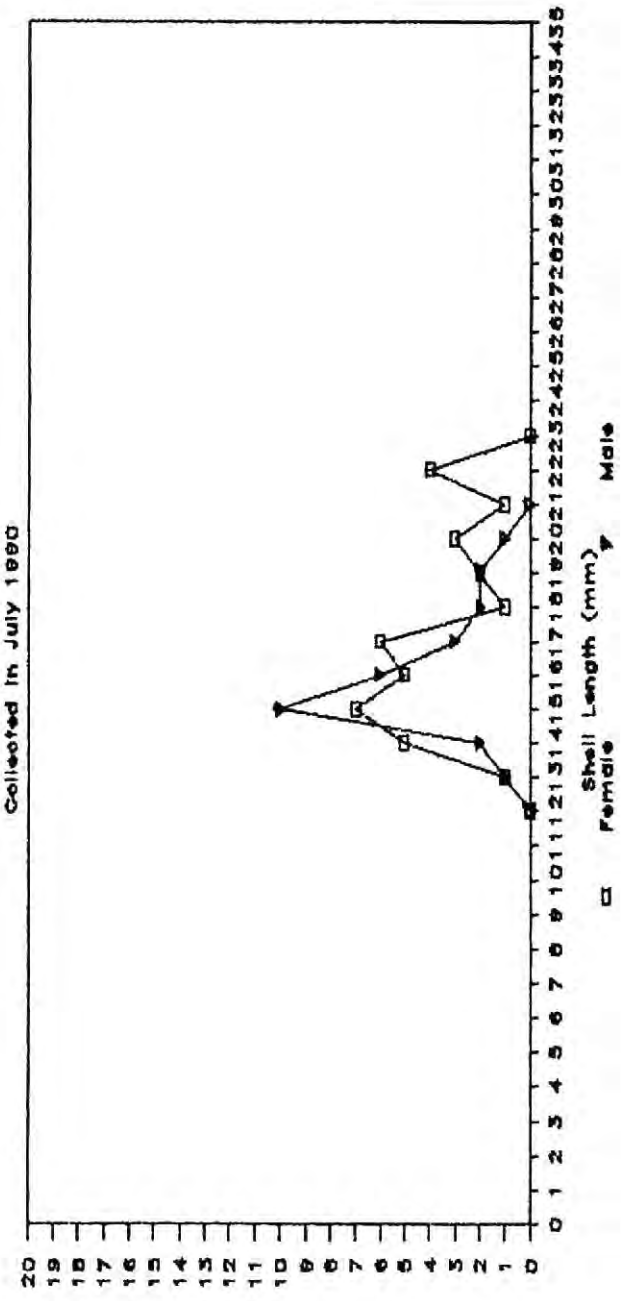

Figure 10. Biometric Data of *V. angularis*

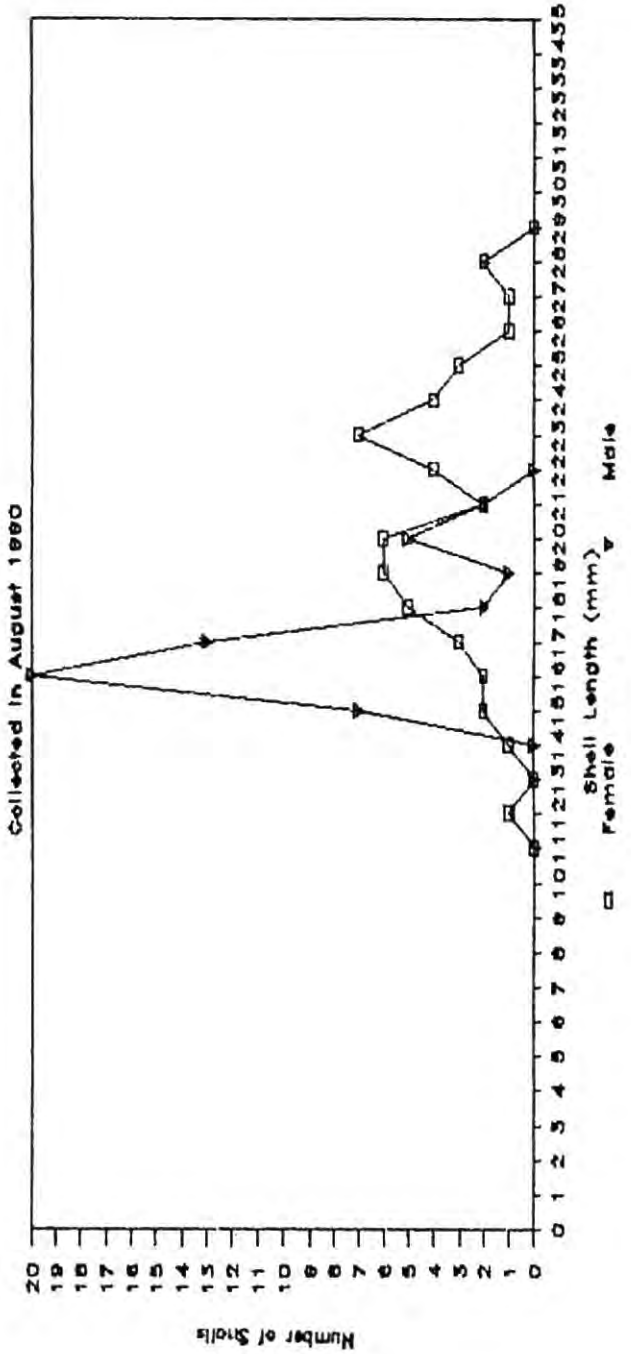

Figure 11. Biometric Data of *V. angularis*

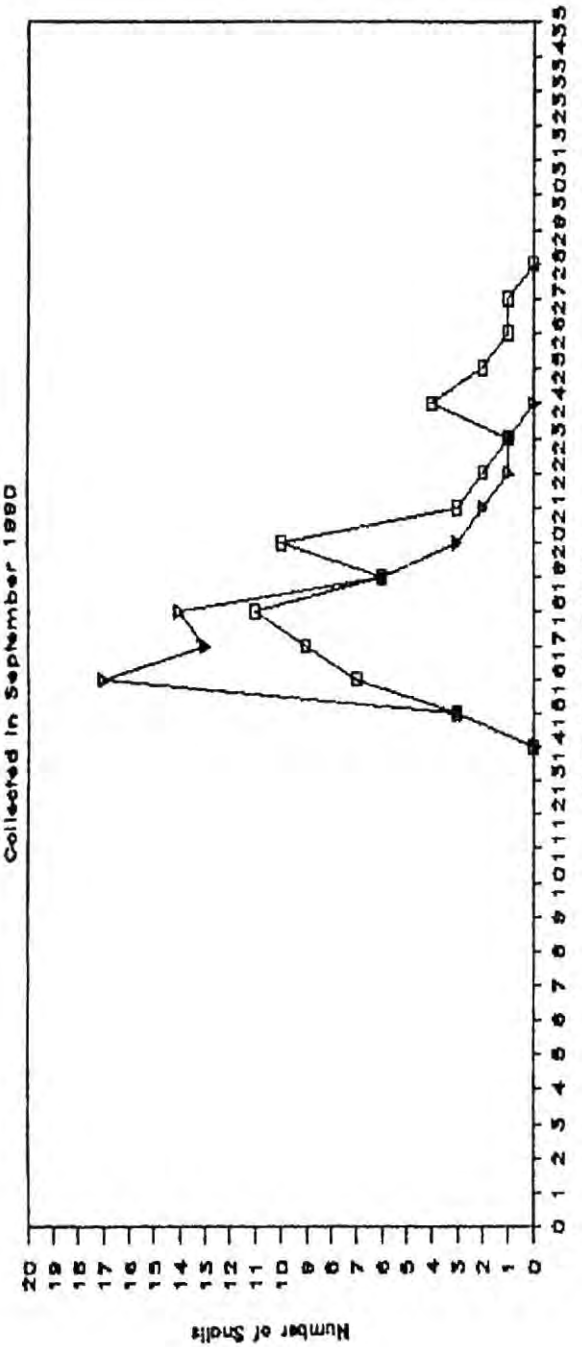

Figure 12. Biometric Data of *V. angularis*

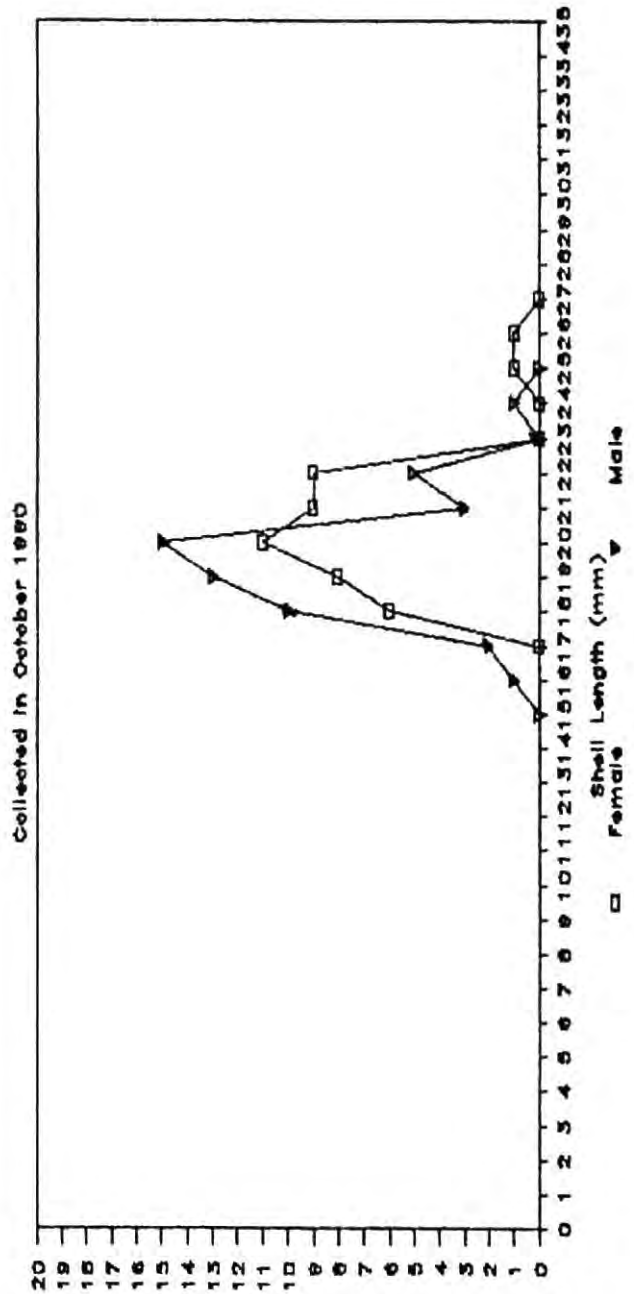

Figure 13. Biometric Data of *V. angularis*

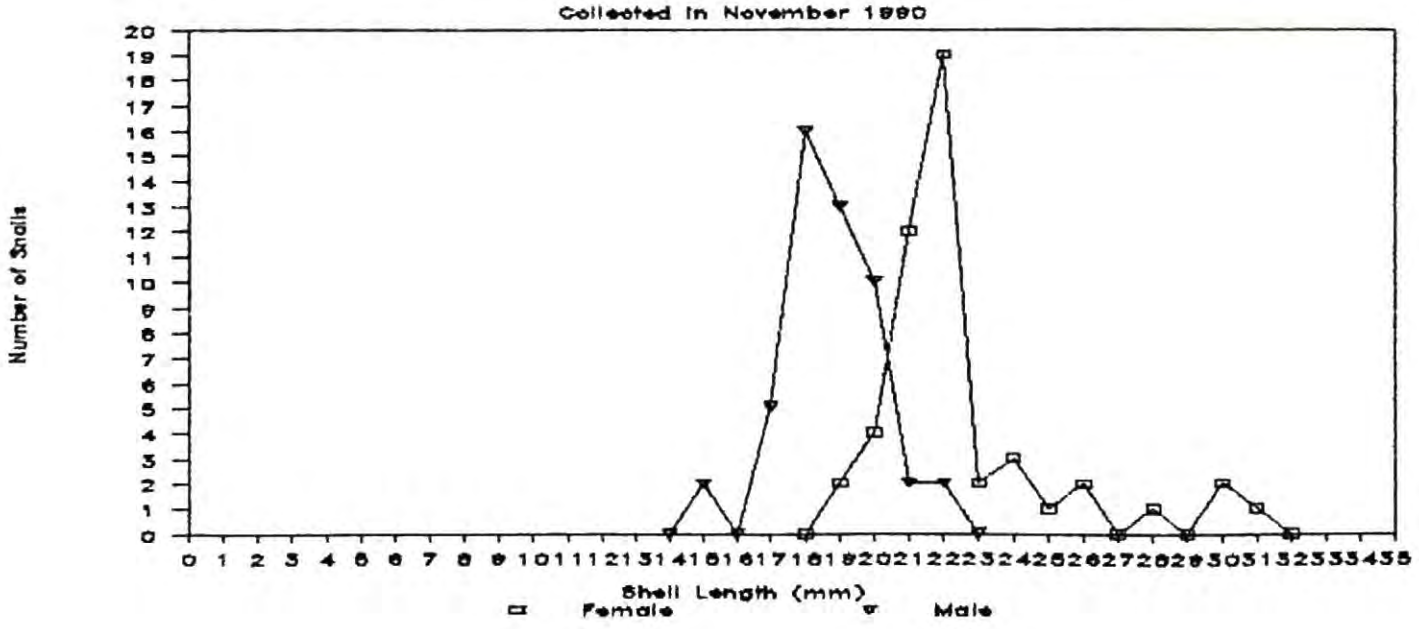

Figure 14. Biometric Data of *V. angularis*

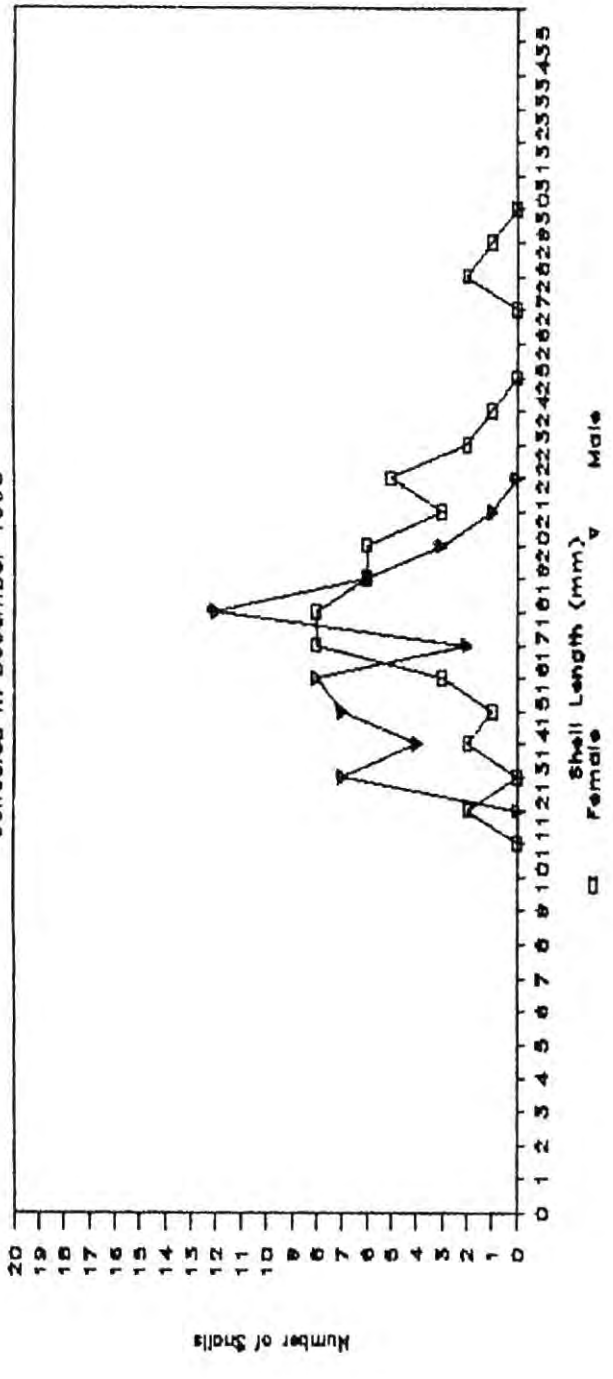

Figure 15. Biometric Data of *V. angularis*

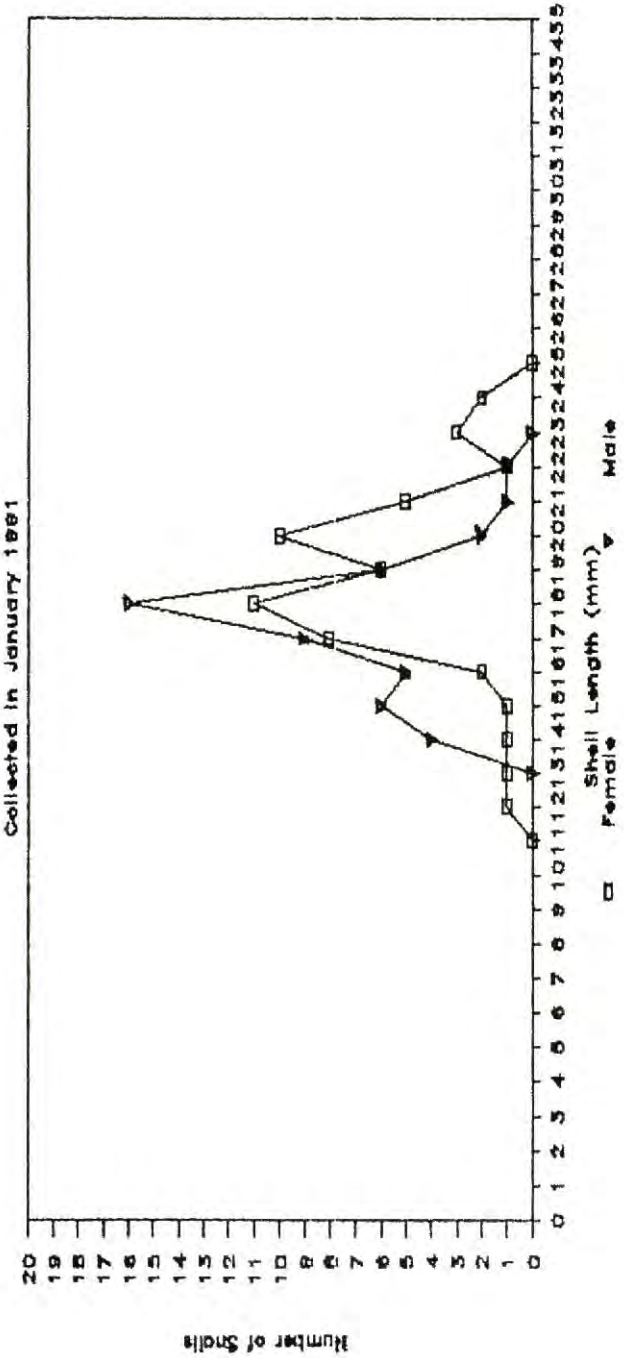

Figure 16. Biometric Data of *V. angularis*

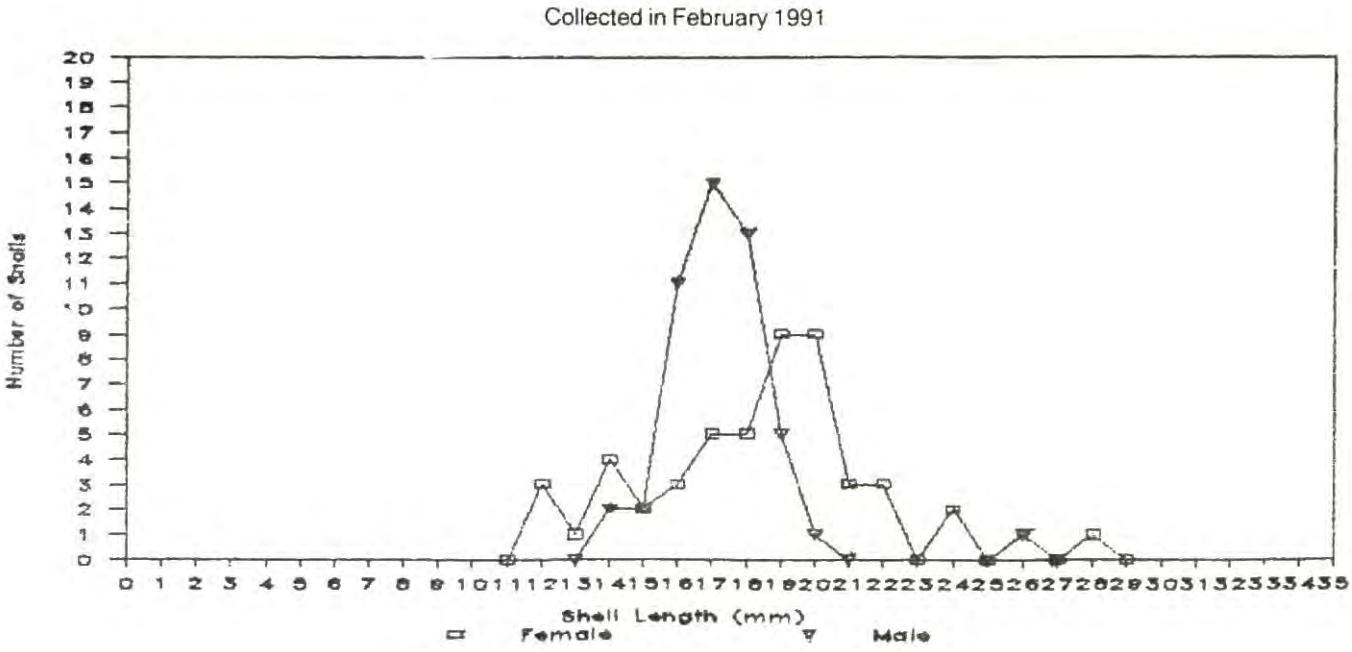

Figure 17. Biometric Data of *V. angularis*

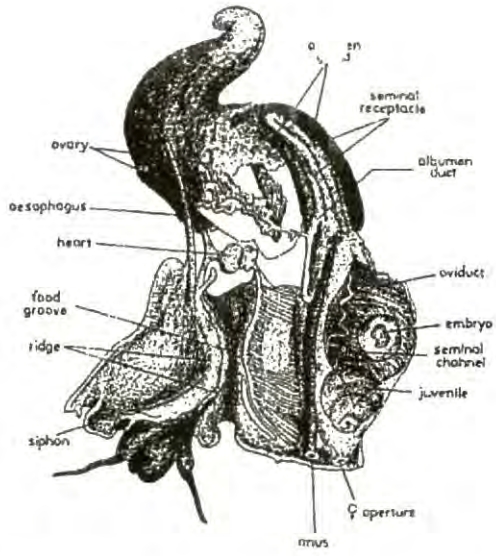

Figure 18. *Vivipara angularis*, female, with mantle cut open along right edge and deflected to animal's left; Oviducal pouch cut open revealing juvenile and embryo

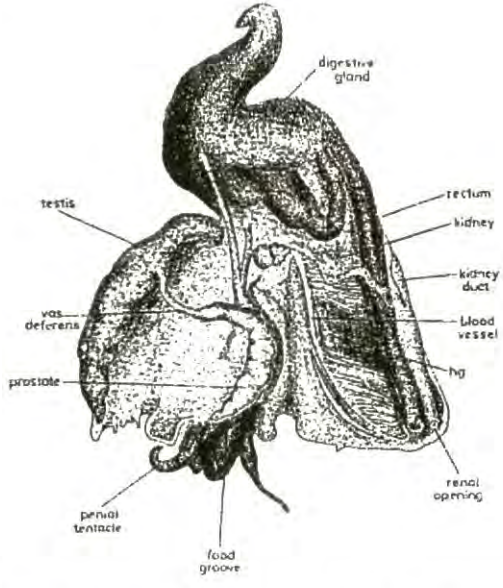

Figure 19. *Vivipara angularis*, male, with mantle cut open along right edge and deflected to animal's left (so deflecting rectum from right to left of animal)

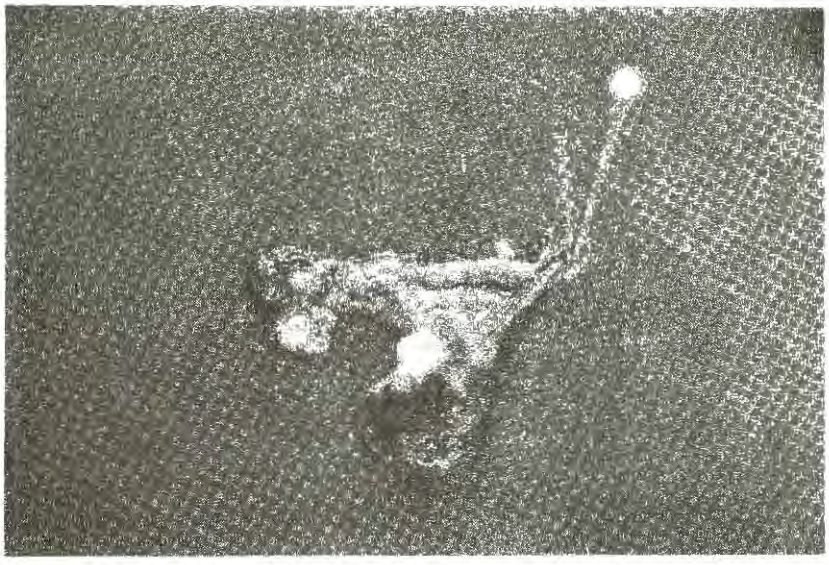

Figure 20. Dissected female *Viviparа angularis* revealing:

a. birth pore
b. vagina
c. pallial oviduct
d. seminal receptacle
e. uterine young
f. uterine ova

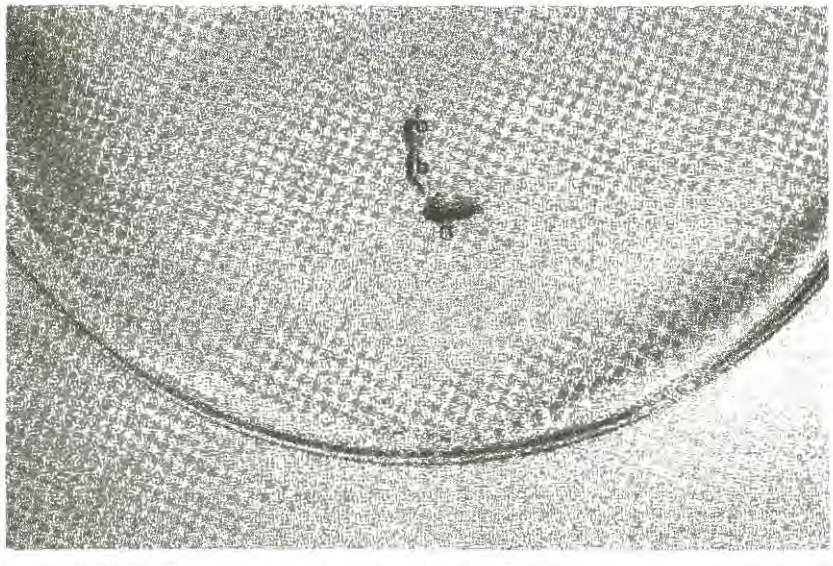

Figure 21. Dissected male *Viviparа angularis*:

a. right copulatory tentacle
b. prostate gland
c. vas deferens
d. testis

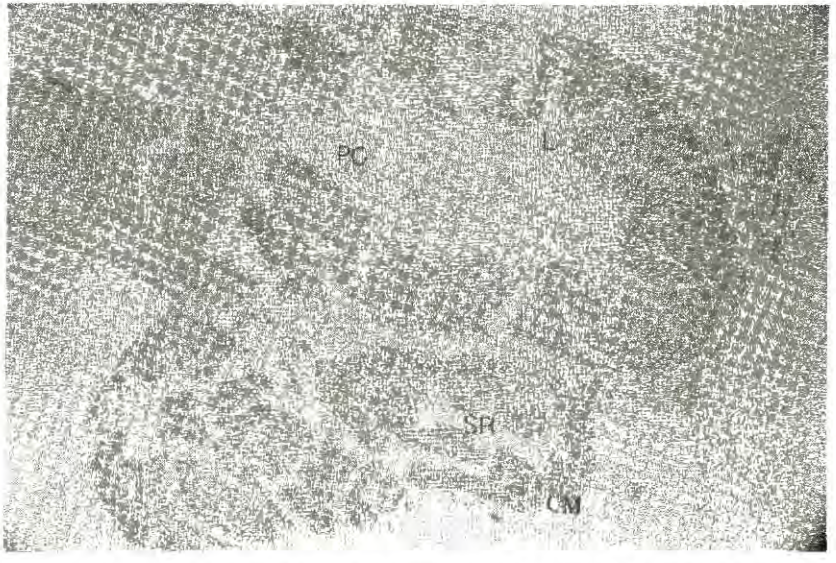

Figure 22. The smaller seminal receptacle (SR) is medial to the bigger pallial oviduct (PO). Both structures are surrounded by circular muscles (CM). Note that the pallial oviduct has a bigger lumen (L).

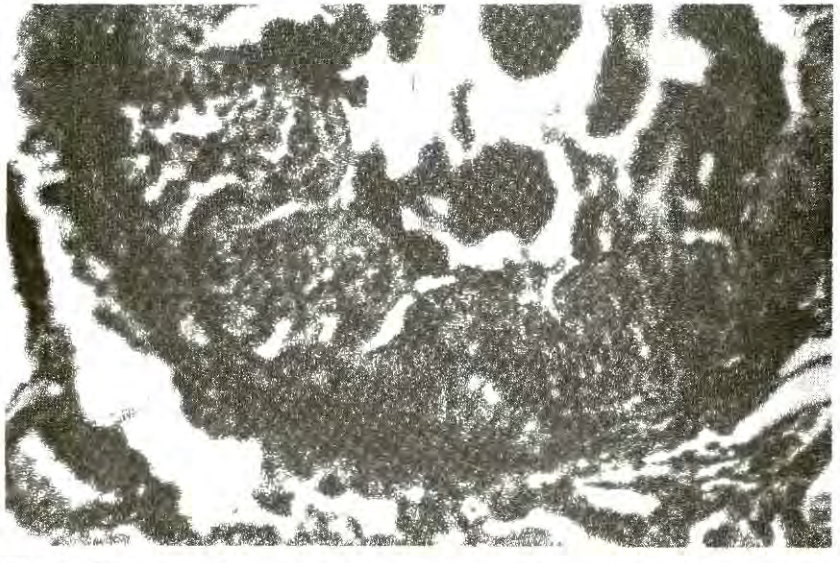

Figure 23. Higher magnification of seminal receptacle showing tall columnar epithelium (E) surrounded by circular muscles (CM).

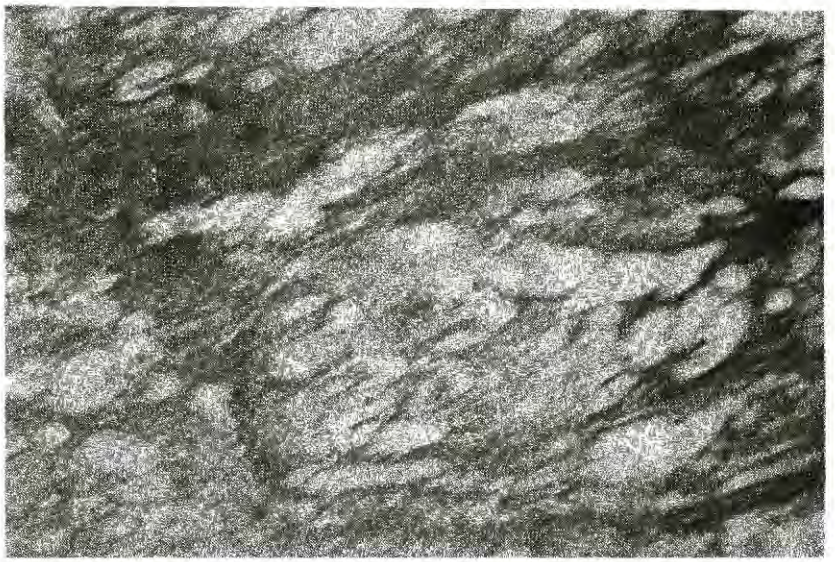

Figure 24. Cross section of the mucosal folds of the pallial oviduct (100X) lined by simple columnar ciliated epithelium (E) with darker staining basally located nucleus (N)

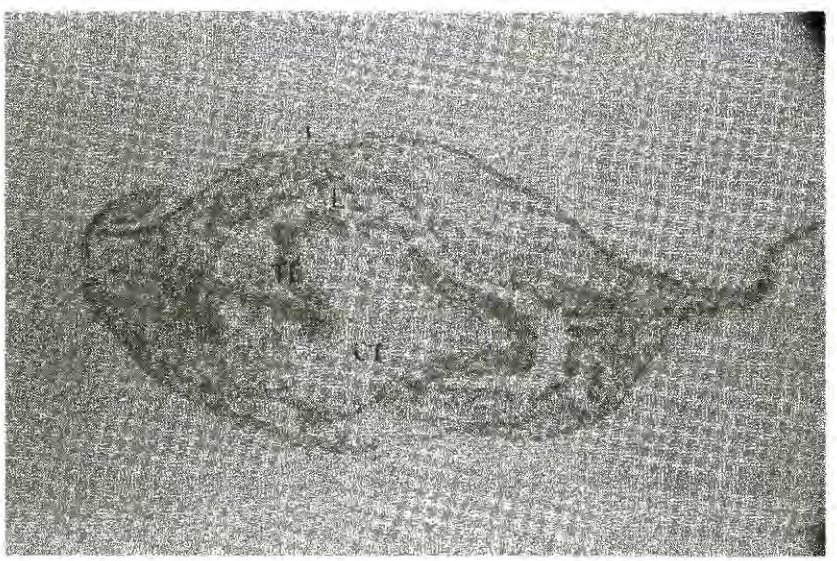

Figure 25. Transverse section of vagina (10X) reveals its irregular lumen (L), transitional type of epithelium (TE) and connective tissue (CT).

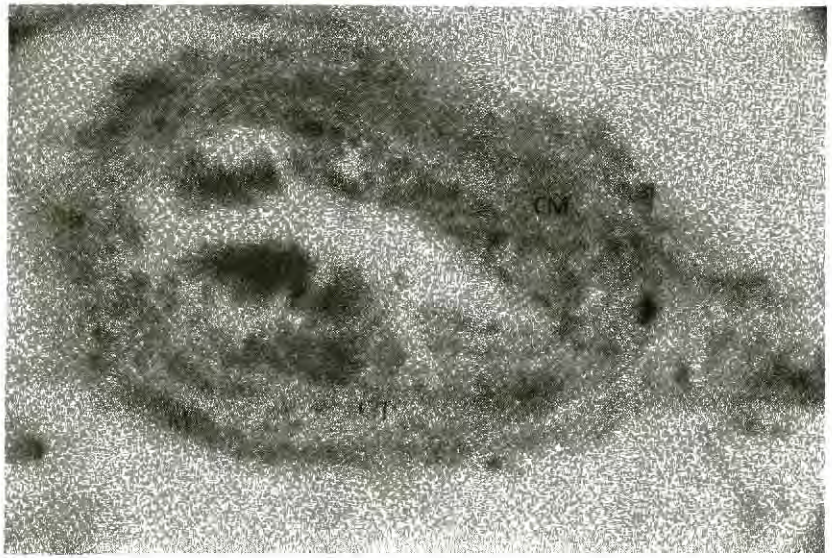

Figure 26. Cross section of the right copulatory tentacle (10X). The seminal duct (SD) is surrounded by a thick layer of circular muscles (CM) and connective tissue (CT). Black pigments called melanin (M) are found in the sub-epidermal layer.

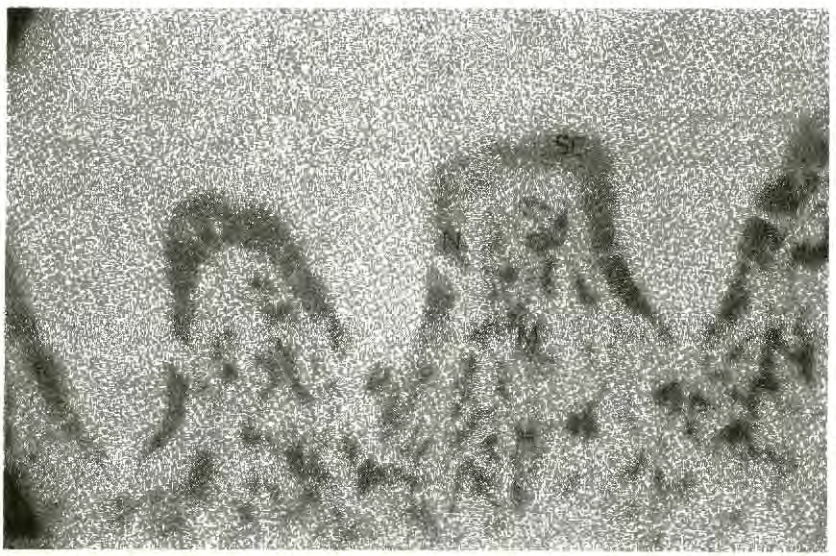

Figure 27. Higher magnification of the surface epithelium (SE) of the right tentacle showing brightly pigmented columnar cells with basal nucleus (N) and melanin (M).

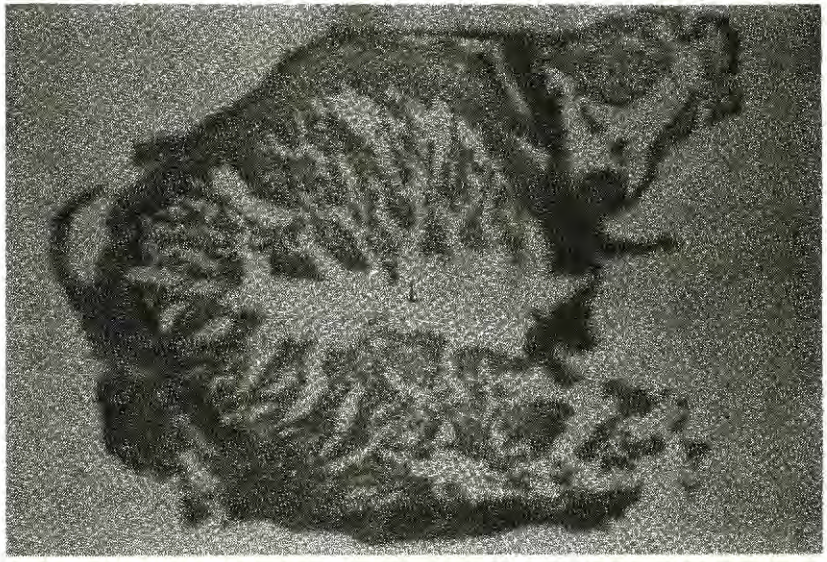

Figure 28. Transverse section of prostate gland with thin muscle layer (M) and circular lumen (L). It is lined by simple cuboidal cells.

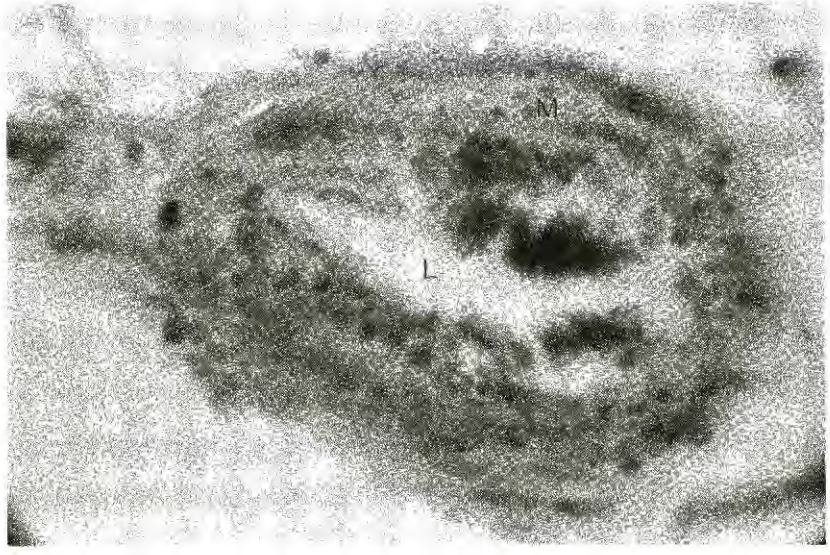

Figure 29. Vas deferens (40X) exhibiting a pseudostratified columnar epithelium (E), irregular lumen (L), and thick muscular layer (M)

Figure 30. Seminiferous tubules of testis illustrating the Sertoli cells (S) and spermatozoa (SP) with deep staining nuclei (N) and faintly staining tails (T). The smaller spermatids (SD) are also shown in this picture.

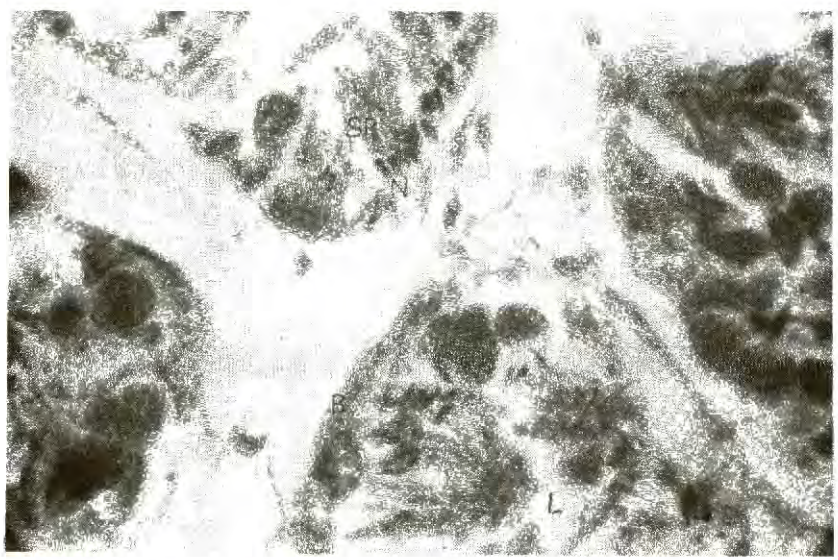

Figure 31. Another cross section of testis showing seminiferous tubules enveloped by basement membrane (B), spermatozoa (SP) with deep staining heads or nuclei (N) and the triangular Sertoli cells (S) closely associated with the spermatozoa.

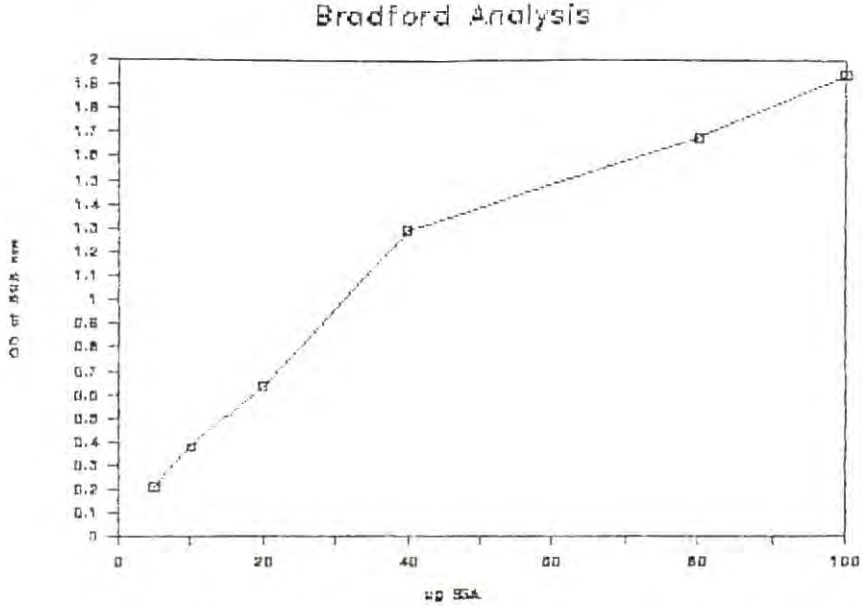

Figure 32. Protein determination of soluble extracts of *V. angularis* fed with two different food types

STANDARD

x:ug BSA	y: Ave. OD at 595 nm
5	0.2093
10	0.3777
20	0.6363
40	1.2867
80	1.6730
100	1.9366

Regression Output:

Constant	0.265661
Std Err of Y Est	0.182565
R Squared	0.947813
No. of Observations	6
Degrees of Freedom	4
X Coefficient (s)	0.017747
Std Err of Coef.	0.002082

SAMPLES

DILUTION	ABS 595	AVERAGE ABS 595	ug Extract	ug x DIL. Factor (ug/4 ul sup.)	ug/ul SUP
Sample 1 (fed with lettuce; wt. = .218 g. deshelled)					
1:25	0.408 0.403	0.41	7.89	197.25	49.31
Sample 2 (fed with lettuce and mud; wt. = .391 g, deshelled)					
1:25	0.554 0.587 0.545	0.56	16.34	408.50	102.13

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Microbial Production of L-Methionine Using Carbohydrates of Agricultural and Industrial By-Products

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ABSTRACT

Methionine is an essential amino acid which cannot be synthesized internally by mammalian cells. It is also a limiting amino acid in vegetables. The incorporation of methionine in food and feed materials improves the nutritional value of these diets. Bioconversion of carbohydrates into amino acid could be used to uplift the socio-economic level of the country. This study aimed to optimize the fermentation conditions of methionine production by ***Corynebacterium glutamicum*** *using agricultural and industrial by-products.*

The effects of the composition of fermentation medium, process parameters and different types of bioreactor were carried out in a batch fermentation. A yield of 7 g/l methionine was obtained when a 25° Brix molasses-coconut water medium was used together with 2% nitrogen from $(NH_4)_2SO_4$ as the nitrogen source under optimized process conditions.

The k_La-volumetric oxygen transfer coefficient was determined in the fermentation medium for scaling up to pilot scale. Based on the economic feasibility studies, an internal rate of return (IRR) of 15-20% can be projected for the product of purified L- methionine.

INTRODUCTION

Methionine, an alpha-L-amino-gamma-methylthio-n-butyric acid is nutritionally essential for mammals and fowls since it furnishes not only a unique skeletal structure but also serves as an important methylating agent and facilitates the synthesis of choline in the body. This particular amino acid cannot be synthesized internally from food and feed materials in the diet. However, as a limiting amino acid in vegetables, methionine may be added to these food and feed materials to imrpove the protein quality of these products.

The amino acid requirement of the Philippines is met through importation. Methionine could be produced locally by microbiological methods using available raw materials.

Coconut and sugarcane are the major industrial crops in the Philippines. About 2,700 million liters of coconut water are discarded annually by the desiccated coconut factories. This water contains sugars and nutrients and may be utilized as a fermentation medium. Sugarcane and molasses (by-product of sugar industries) are the cheapest sources of sugar. The bioconversion of these agricultural products or by-products to methionine may boost the economic importance of the Philippines in the world market.

During the past four decades, much research activity has been focused on the production of amino acids by fermentation methods, and many processes for various amino acids have been developed. L-methionine was produced by *Escherichia coli* (Wijesundera and Woods 1962), *Salmonella typhimurium* (Smith 1961), *Bacillus megaterium* (Roy et al. 1986) and *Corynebacterium acetophilum* (Muroota et al. 1979).

This study was conducted to determine the effects of the composition of fermentation medium, process parameters, different types of bioreactors and scaling up for methionine production.

THEORETICAL CONSIDERATION ON GASSING-OUT METHOD FOR DETERMINATION OF k_La

The schematic diagram of the gassing-out method is shown in Figure 1. Nitrogen gas is fed into the bottom of the vessel at a constant volumetric flow rate to deoxygenate the medium in a fermenter. Then the aeration is started and changes in dissolved oxygen concentrations are monitored over time by a recorder.

The oxygen mass balance of the non-biological system is given by an equation (1).

$$\frac{dC}{dt} = k_La(C^* - C) \qquad (1)$$

Where k_La = volumetric oxygen transfer coefficient (min^{-1} or h^{-1})

C^* = saturated dissolved oxygen concentration in equilibrium with the gas phase (mg/l or mole/l)

C = dissolved oxygen concentration at time, t (mg/l or mole/l)

If the equation is integrated with the limits: $C = C_o$ at $t = 0$ and $C = C$ at $t = t$. An equation (1) becomes an equation (2).

$$\ln [(C^* - C)/(C^* - C_o)] = -k_Lat \qquad (2)$$

Hence, by plotting $\ln[(C^* - C)/(C^* - C_o)]$ in the y-axis against time in the x-axis, the numerical value of k_La is obtained by calculating the slope of the curve.

If the DO probe is connected to a millivolt recorder, the absolute value of dissolved oxygen concentration (C), need not be known in order to determine k_La.

Let E = recorder response at C
E_o = recorder response at C_o
E^* = recorder response at C^*

If the recorder response was directly proportional to the dis- solved oxygen concentration, then $E = C$, $E_o = C_o$ and $E^* = C^*$. Thus, to determine k_La in the gassing-out system, only the recorder response and time data are required. The equation (2) would then become:

$$\ln [(E^* - E)/(E^* - E_o)] = -k_Lat \qquad (3)$$

MATERIALS AND METHODS

Microorganism

Corynebacterium glutamicum ATCC 21608 was found to be the most appropriate L-methionine-producing organism (Pham et al. 1991). It was maintained on nutrient agar (Difco) slants at refrigeration temperature and was used for the succeeding experiments.

Cassava, banana and coconut water were obtained from the Junction market at Los Baños, Laguna, Philippines. Coconut water was kept in the freezer until use. Sugarcane was purchased from a nearby farm at BIOTECH, Laguna and the juice was extracted with a crusher fabricated at the BIOTECH Pilot Plant.

Fresh cassava and banana were peeled and cut into 1 cm^3 The samples were ground with 1:1 (w/w) water in a blender. Starch was gelatinized by heating samples at 100°C for 30 min., then saccharified by glucoamylase (2 ml/kg d.w. of substrate) at 55°C for 48 hours. The final concentration of reducing sugars was 280 g/l.

Molasses was bought from a sugar factory in Calamba, Laguna. Fungal glucoamylases (AMG 150 L) were donated by Novo Industri, A/S, Denmark. Yeast extract and agar were obtained from Difco. All other reagents were either from Merk or were of analytical grade.

Inoculum Preparation

A loopful of 24 h slant culture of the organism was transferred asceptically to a 250-ml Erlenmeyer flask containing 50 ml seed medium with the following composition: 50 g/l sugar from molasses; 20 g/l $(NH_4)_2SO_4$; 10 g/l yeast extract; 2% (v/v) corn steep liquor; 0.4 g/l KH_2PO_4; 0.5 g/l K_2HPO_4 and distilled water added to 1:1. The pH was adjusted to 7.0 with 2N NaOH.

Fermentation Experiments

The composition of the fermentation medium was: 100 g/l sugar; 20 g/l $(NH_4)_2SO_4$; 2% (v/v) corn steep liquor; 1 g/l KH_2PO_4; 3 g/l K_2HPO_4; 1 g/l $MgSO_4 \cdot 7\ H_2O$ coconut water as diluent added to 1:1. The pH was adjusted to 7.0 with 2N NaOH.

The fermentation was carried out in: (a) 250-ml Erlenmeyer flasks containing 50 ml medium inoculated at the rate of 2% (v/v). The flasks were mechanically shaken on a rotary

shaker operated at 150 rpm at 30°C for 96 h; (b) five 1-standard commercial stirred tank fermenter, B.E. Marubishi, containing 3-1 fermentation medium; (c) 20-1 fabricated stirred tank fermenter; and (d) 20-1 fabricated air lift fermenter.

k_La- Studies for Scaling Up

The oxygen volumetric mass transfer coefficient, k_La was measured using the adopted method of Wise (1951). The nitrogen (oxygen-free gas) is initially introduced into the system to deoxygenate the liquid phase.

Analytical Methods

pH determination. The pH of the fermentation broth was measured using an Orion Research Model 701 A Ionalyser.

Biomass determination. Microbial cells were estimated by centrifuging a 10-ml fermentation broth at 3000 rpm for 20 min. The cells were washed twice with distilled water and dried in an oven drier to constant weight at 70°C.

Methionine determination. Methionine in the supernatant separated from microbial cells was determined using sodium nitroprusside followed by acidification of the reaction mixture with the phosphoric acid method proposed by del Amo et al. (1952).

Sugar determination. A mixture of 1-ml supernatant and 4 ml of 1N HCl was placed in a test tube and heated in a water bath at 60°C for 30 min. Sugar as glucose concentration was determined by the colorimetric DNS method (Borel et al. 1962).

RESULTS AND DISCUSSION

Effect of Composition of Fermentation Medium

The methionine concentration in the fermentation broth prepared from four different sugar sources after 96 h is shown in Figure 2. Molasses proved to be the best sugar substrate, yielding 0.95 g/l methionine by *C. glutamicum*. This yield was five times more than that obtained in the sugarcane juice medium and 10 times more than that of the banana and cassava media. Besides a high sugar content, molasses also contains vitamins (e.g. biotin and minerals) which could have contributed to the high methionine production when this was used as the sugar source.

Among the substrates tested, molasses was also the richest and the cheapest source of sugar, containing 60% total sugars and 20% reducing sugars.

The nitrogen is also required for the microorganism synthesis of amino acids. The effect of different nitrogen sources on methionine production may be seen in Table 1. The addition of 30% (v/v) ipil-ipil juice would give the best yield of methionine. Increasing further the ipil-ipil juice content of the medium in combination with $(NH_4)_2SO_4$ decreased the methionine yield. This is probably due to the decrease of sulphur concentration in ammonium sulphate. Methionine is a sulphur- containing amino acid and it can be expected that more synthesis occurred as the supply of elemental sulphur to the fermentation medium was increased. It is more evident in the medium with 100% ipil-ipil juice that gave a very low methionine yield (0.9 g/l) and high sugar concentration.

Azolla juice, another nitrogen source, was also evaluated. For this test, however, $(NH_4)_2SO_4$ was not used and only a 10% (v/v) azolla juice was used. The time of addition of azolla juice was varied at 4-h intervals. Results are shown in Table 2. It can be seen that azolla juice produced very little methionine, yielding only about 25% when $(NH_4)_2SO_4$ was used. It can also be seen that from 0-12 h, addition of azolla juice had no effect on methionine production. However, addition of azolla juice beyond 12 h tended to decrease methionine production.

Effects of Process Parameters

The process parameters such as pH, temperature and agitation rate were carried out in a shake flask culture. Results of the effect of initial pH on the methionine yield are shown in Table 3. Optimum initial pH of fermentation medium was 6.50. It can be seen that the growth of the organism was favored in a slightly acidic medium, illustrated by the biomass content at harvest which decreased with increasing pH. A pH of 6.50 was chosen as an optimum pH since highest methionine yield was obtained at this pH value and this is still in the slightly acidic range where growth of the organism was favored.

Adequate oxygen supply to aerobic cultures is very important in the optimization of fermentation processes. Agitation provides a uniform supply and distribution of oxygen throughout the fermentation medium.

The effect of agitation rate on the methionine production is shown in Table 4. The yield decreased with increasing agitation rate. The methionine yield at 100 rpm is about two times that produced at 150 rpm and five times that produced at 200 rpm. Also, it can be seen that the growth of organism and not the synthesis of methionine was favored at higher agitation rates. Thus, agitation rate of 100 rpm was chosen as optimum and this agitation rate yielded 3.63 g/l methionine.

The effect of temperature on methionine production was also studied by subjecting the inoculated fermentation media to different incubation temperatures ranging from 20°C to 40°C. Results indicate that methionine production was optimum at 30°C (Table 5). However, it was observed that better cell growth occurred at temperatures lower than this (20°, 25°C) as indicated by higher values obtained for biomass at these temperatures. Therefore, the process temperature affects differently the growth and product synthesis rates.

Effects of Different Types of Bioreactors

The process parameters optimized in a shake flask culture were applied in producing methionine in bioreactors. Results shown in Table 6 were obtained with a stirred tank fermenter. There is no significance in the methionine yield between four days and five days of fermentation, though a high growth of organisms was obtained in the fermentation process. The process temperature and pH did not vary much throughout the run. The 2.54 g/l of methionine produced was comparable to that produced in a shake flask culture.

Molasses was found to obtain high methionine synthesis. Thus, data on methionine production using molasses in a 5-1 stirred tank fermenter are presented in Table 7. The optimum methionine yield of 6.95 g/l was attained in less time when nitrogen was applied at the start of fermentation. It can also be noted that methionine production decreased when fermentation was extended after the second day. Biomass concentration reached 42.82 g/l after 72 h. This concentration was maintained up to 96 h of fermentation, while an almost 50% reduction in sugar content of the broth was obtained after 48 h of fermentation.

Methionine production using the three types of fermenters used in methionine production are shown in Figure 3. The maximum yield obtained in the fabricated stirred tank fermenter was comparable to that obtained from standard commercial

stirred tank fermenter. The fabricated air lift (tower) fermenter produced the lowest yield. It may be concluded that the stirred tank fermenter was most suitable for use in the production of methionine to obtain high yield. Perhaps the adequate oxygen supply available to the fermentation medium enhanced the growth and yield of methionine.

Studies on k_La for Scaling Up

Methionine production by microogranisms always requires oxygen for their growth and metabolism. Therefore, the efficiency of fabricated bioreactors was determined by the overall volumetric oxygen transfer coefficient (k_La) in the liquid phase of the fermentation process.

The effect of air flow rate on the k_La value for the 20-l fabricated stirred tank fermenter is illustrated in Figure 4. The k_La exhibited a maximum value as aeration rate was increased. Using agitation speeds of 100 rpm and 400 rpm, the maximum k_La occurred at 1 vvm and 1.5 vvm, respectively. As shown also in the figure, operating the fermenter at a very high air flow rate (20 l/min) and agitation speed (400 rpm), resulted in a lower oxygen transfer rate. This phenomenon is known as "flooding effect". According to Wang (1979), flooding is a phenomenon which occurs when air flow rate is so high that the impeller is rotating in a gas phase and therefore cannot assist in the transfer of gas into a solution.

Compared with aeration rate, the degree of agitation had a profound effect on the oxygen transfer efficiency (Fig. 5). The k_La value increases with increasing agitation speed. The reasons for the greater effect of agitation are the following: (a) it disperses air in smaller bubbles thus increasing the available area for oxygen transfer; (b) it delays the escape of air bubbles from the liquid; and (c) it prevents the coalescence of air bubbles. However, the increase in agitation rate results in a corresponding increase in power consumption which affects the cost of operating the fermenter.

The effect of aeration and agitation on the k_La values of three bioreactors is illustrated in Figure 6. Using the same aeration and agitation rates, the 20 l fabricated fermenter had a lower oxygen transfer efficiency than the 5 l Marubishi fermenter. The differences were more pronounced at low agitation rates (100 rpm and 200 rpm). The 20 l airlift fermenter had a higher oxygen transfer coefficient than the two stirred tank reactors which were operated at low agitation rates (100 rpm and 200 rpm). This means that at a lower power input,

the airlift fermenter had a higher oxygen transfer efficiency compared with the stirred tank reactor. However, the use of the airlift fermenter is limited to a small range of k_La values. Thus, its lesser adaptability among the different fermentation processes.

The k_La values for the three fermenters operated under different fermentation parameters are presented in Tables 8-10. Using the multiple log-linear regression analysis, the following equations were determined:

a) 20-l fabricated stirred tank fermenter

$$k_La = 3.85 \times (P_g/V)^{0.40} \times (V_s)^{0.55} \quad (4)$$
$$r^2 = 0.953$$

b) 5-l Marubishi stirred tank fermenter

$$k_La = 12.58 \times (P_g/V)^{0.39} \times (V_s)^{0.69} \quad (5)$$
$$r^2 = 0.942$$

c) 20-l fabricated airlift fermenter

$$k_La = 41.6 \times (V_s)^{0.9229} \quad (6)$$
$$r^2 = 0.995$$

These correlation equations (4-6) for k_La are important not only in scaling up a fermentation process but also in estimating the amount of power that a system will consume under certain conditions. However, it should be pointed out that these correlations were empirically derived and hence, may strictly apply only to the equipment on and process through which they were developed.

Economic Feasibility Studies

The process of microbial production of methionine using molasses is given in Figure 7. It was assumed that the production plant had been established and production cost was based on raw materials, chemicals, supplies, electricity and labor. The cost evaluation for methionine production was calculated using 200-l and 1000-l fermenters and was based on 1987 prices.

The methionine product is chemical grade and costs ₱3.90/g at wholesale price.

Table 11 shows the cost evaluation for methionine production using two fermenter sizes based on 160 kg sugar from molasses. The profits are ₱12,267.80 and ₱13,517.20 for 200-l and 100-l fermenter capacities, respectively.

SUMMARY AND CONCLUSIONS

Batch fermentation of methionine production was carried out on the effect of composition of fermentation medium using *C. glutamicum* in a shake flask culture. Among the sugar substrates tested (molasses, sugarcane juice, banana and cassava), molasses diluted by coconut water to give a 25° Brix total sugars proved to be optimum for methionine production, producing 3.6 g/l after four days of fermentation.

A 2% $(NH_4)_2SO_4$ was found to be the optimum for methionine production due to the presence of sulphur which was necessary for the synthesis of this amino acid. Organism growth was favored in a slightly acidic medium with a pH of 6.50 before sterilization. However, while the organism growth was obtained at higher agitation rate, methionine synthesis at higher agitation rate was greatly reduced.

The effect of temperature on methionine production was also studied. It was found that methionine production was favored at lower temperatures up to 30° - 34°C, after which the synthesis of methionine decreased.

A higher methionine yield of 7 g/l at a much shorter time was produced when $(NH_4)_2SO_4$ was used in the medium. Methionine content of the broth decreased when the fermentation was prolonged after 48 h. A stirred tank fermenter was also found most suitable for the cultivation of methionine.

Scaling up to pilot plant was studied using the k_La coefficient which was determined by the gassing-out method. The empirical equations of correlation between superficial velocity and gassed power were determined for use in scaling up for different fermenter types.

The economic feasibility studies showed that the scale fermenter is an important factor in the profitability of methionine production. It was assumed that the depreciation and preparation costs of equipment were not included. If the industry established 1000 one-scale fermenters, the profit obtained would be ₱675,860 for one year.

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Table 1. Effect of ipil-ipil juice on methionine production after 72 h fermentation

	Biomass (g. dry cells/l)	Final pH	Reducing sugar remained (%)	Methionine concentration (g/l)
10% ipil-ipil + 20% corn oil + 70% distilled water	7.60	3.75	11.9	2.00
30% ipil-ipil + 20% corn oil + 50% distilled water	6.00	4.13	10.6	2.70
50% ipil-ipil + 20% corn oil + 30% distilled water	6.30	4.33	11.2	1.50
80% ipil-ipil + 20% corn oil	6.00	4.24	9.1	1.80
100% ipil-ipil	6.50	4.29	9.1	0.30

Table 2. Effect of the time of addition of azolla juice on methionine production after 72 h fermentation

Time of addition (h)	Biomass (g. dry cells/g)	Final pH	Reducing sugar remained (%)	Methionine concentration (g/l)
0	12.50	5.59	13.1	0.66
4	11.20	5.58	14.5	0.64
8	11.20	5.66	16.2	0.64
12	11.10	5.64	15.7	0.64
16	14.60	5.59	15.5	0.27
20	9.90	5.55	14.8	0.27
24	12.60	5.57	16.0	0.05
28	13.70	5.73	15.3	0.14

Table 3. Effect of pH on methionine production after 72 h fermentation

Initial pH	Biomass (g. dry cells/g)	Final pH	Reducing sugar remained (%)	Methionine concentration (g/l)
5.50	4.60	4.77	14.7	0.40
6.00	3.70	5.04	13.8	0.45
6.50	2.92	5.18	15.6	0.50
7.00	2.58	5.34	14.1	0.15
7.50	2.66	5.38	17.8	0.37

Table 4. Effect of agitation rate on methionine production after 96 h fermentation

Agitation rate (rpm)	Biomass (g. dry cells/l)	Final pH	Reducing sugar remained (%)	Methionine concentration (g/l)
100	5.7	5.49	20.4	3.63
150	6.4	4.89	18.2	1.63
200	7.2	5.07	16.7	0.35

Table 5. Effect of temperature on methionine production after 96 h fermentation

Process temperature (^{o}C)	Biomass (g. dry cells/l)	Final pH	Reducing sugar remained (%)	Methionine concentration (g/l)
20	8.7	5.25	15.2	1.80
25	7.9	5.25	15.3	2.20
30	5.7	5.49	14.0	3.63
35	1.1	5.64	12.1	1.16
40	1.2	5.45	12.1	1.48

Table 6. Methionine production in stirred tank fermenter with glucose as substrate

	Fermentation time		
	0 h	96 h	120 h
pH	5.25	4.83	4.07
Temperature (°C)	29	28	28
D.O. (ppm)	1.6	1.8	2.15
Agitation (rpm)	350	520	530
Biomass (g/l)	15.6	54.4	61.1
Reducing sugars (%)	18.6	16.4	14.4
Methionine (g/l)	0.05	2.36	2.54

Table 7. Methionine production in 5-l stirred tank fermenter using molasses as substrate

	Fermentation time			
	0 h	48 h	72 h	96 h
pH	5.68	5.45	5.38	5.28
Temperature (°C)	30	34	36	36
D.O. (ppm)	5	5.5	5.4	2.3
Agitation (rpm)	200	500	500	500
Biomass (g/l)	18	39.9	42.8	43.2
Reducing sugar (g/l)	20.2	11.4	10.6	10.4
Methionine (g/l)	0.04	6.95	3.01	1.87

Table 8. k_La values in 20-l fabricated stirred tank fermenter using different combinations of superficial velocity and gassed power

P_g/V (dyne sec. cm2)	V_s (cm/sec)	k_La (h^{-1})
52.1	0.3139	13.24
52.1	0.6278	25.50
52.1	0.9417	28.73
416.5	0.6278	45.91
416.5	0.9417	70.50
514.6	0.9417	184.30
554.6	0.6278	194.80
642.6	0.3139	37.21
666.4	0.3139	183.00
833.0	0.1569	28.08

Table 9. k_La values in 5-l Marubishi fermenter using different combinations of superficial velocity and gassed power

P_g/V (dyne sec. cm2)	V_s (cm/sec)	k_La (h^{-1})
175.89	0.2256	43.2
1894.04	0.2256	73.8
7305.81	0.2256	132.6
18183.6	0.2256	198.6
236.77	0.1128	22.2
2272.85	0.1128	43.2
8219.05	0.1128	96.0
21214.25	0.1128	178.2

Table 10. k_La values in airlift fermenter using different combinations of superficial velocity

Air flow rate (l/min)	Aeration rate (vvm)	V_s (cm/sec)	k_La (h^{-1})
5	0.33	0.5413	24.00
10	0.67	1.0827	42.00
15	1	1.6240	69.00
30	2	3.2481	122.00

Table 11. Cost evaluation for methionine production using different fermenter sizes and based on the prices in 1987

	Cost Evaluation	
	Fermenter Capacity	
	200 l	1000 l
Molasses	₱ 250.8	₱ 250.8
Fermentation	5117.1	5257.8
Separation	1451.5	3787.5
Crystallization	593.4	682.8
Drying	14.0	1.7
Labor	5656.0	2453.0
Total product cost	₱ 13182.8	₱ 12433.4
Value of methionine produced	₱ 25950.6	₱ 25950.6
Profit	₱ 12767.8	₱ 13517.2

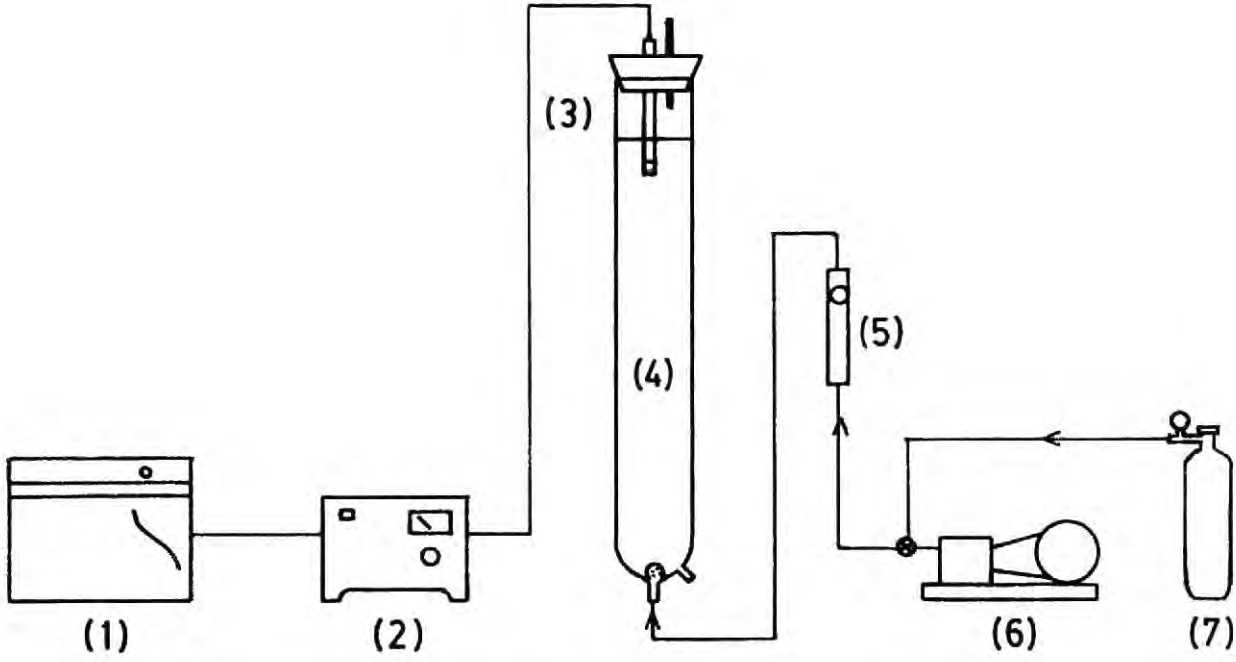

Figure 1. Schematic diagram of K_La determination by gassing out method in bubble column fermentor

(1) recorder, (2) D.O. meter, (3) D.O. probe, (4) bubble column, (5) air flowmeter, (6) compressor, (7) N_2 tank

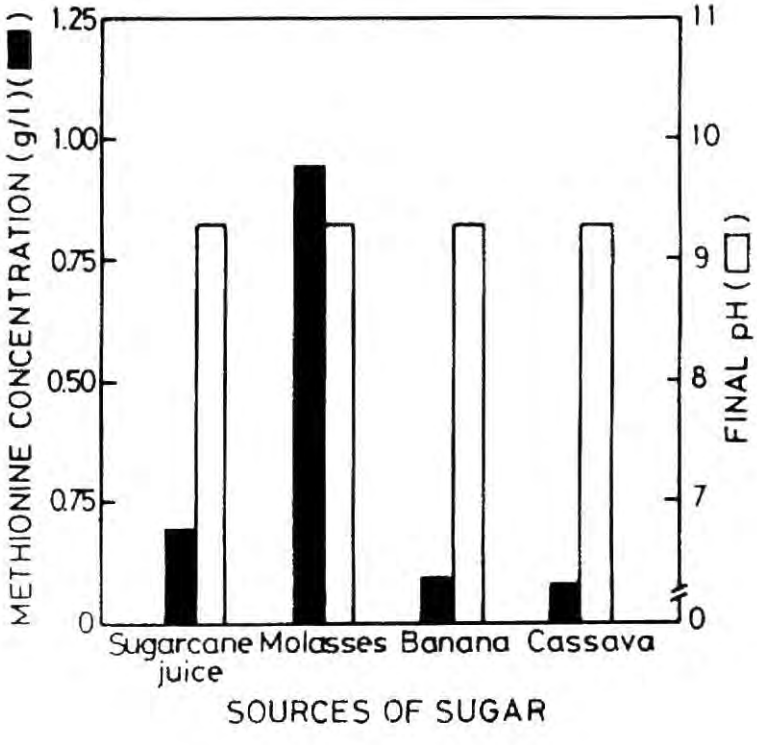

Figure 2. **Effect of sources of sugar on methionine production at 30°, initial pH of 7.0, 150 rpm and for 96 h fermentation**

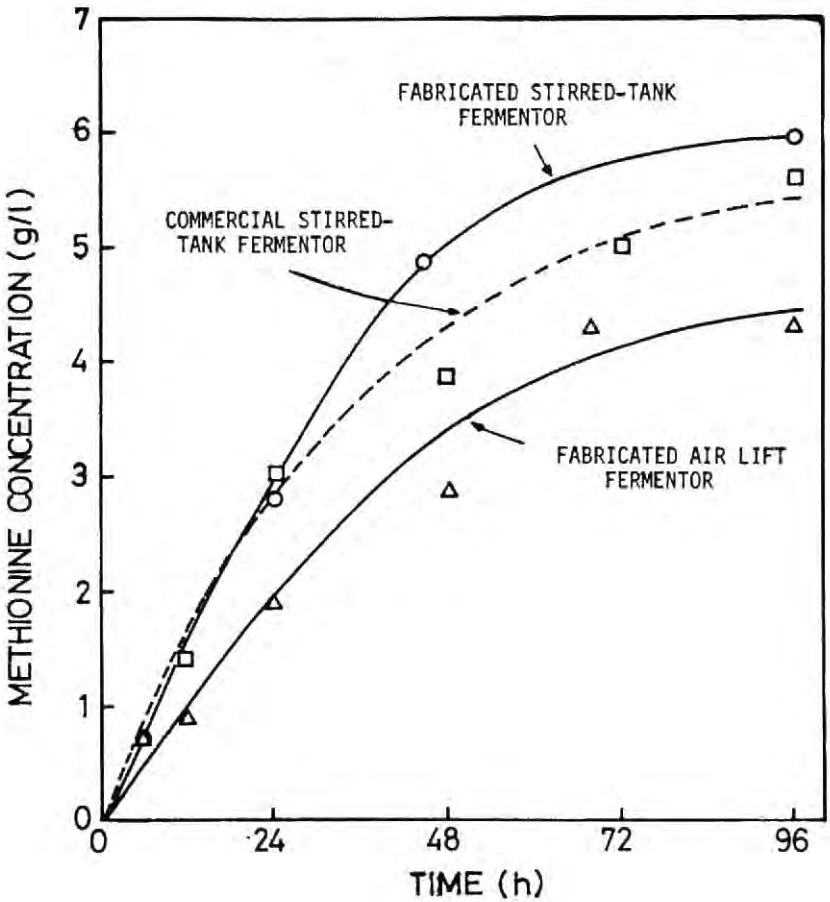

Figure 3. Comparative methionine production using three types of fermentors

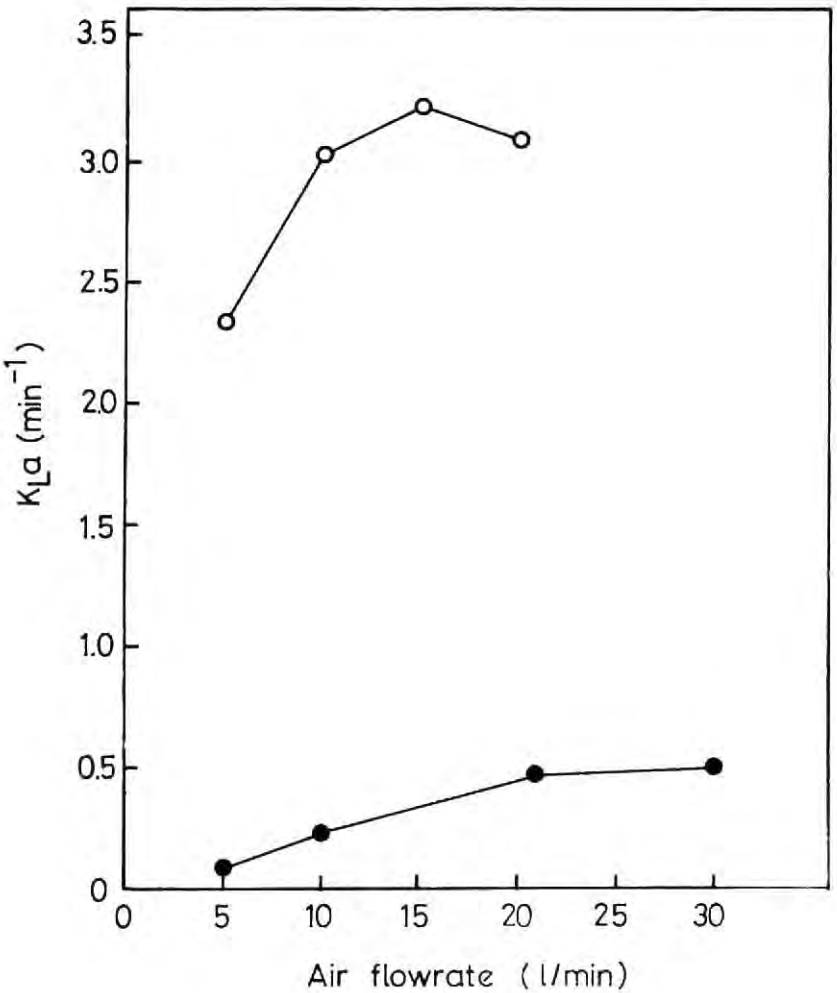

Figure 4. Effect of airflow rate on K_La Agitation rate (rpm): o-o 400; ●-● 100

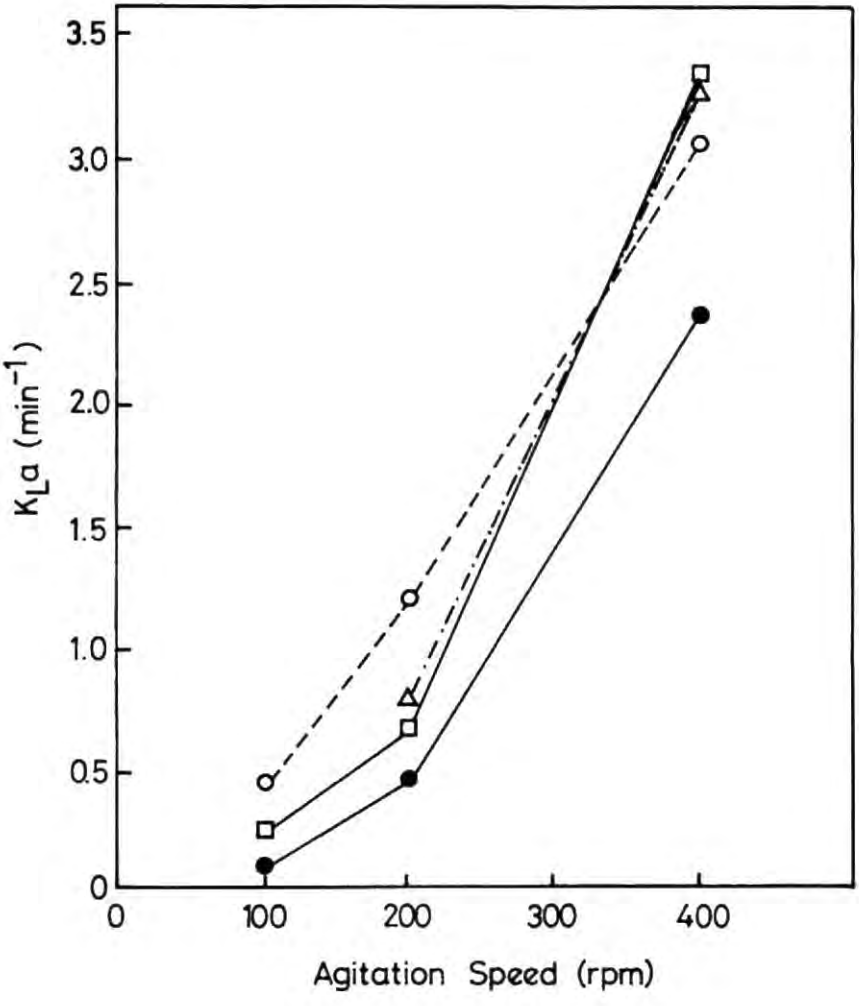

Figure 5. Effect of agitation rate on K_La Aeration rate (1/min): o-o 20; Δ–Δ 15; □-□ 10; ●-● 5.

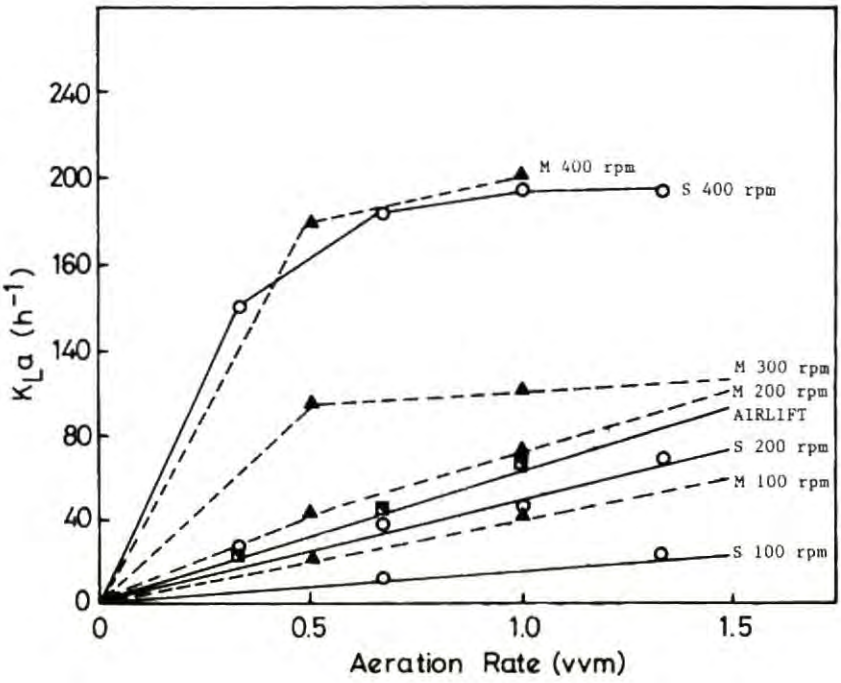

Figure 6. Effect of aeration-agitation on K_La using different bioreactors

(M = Marubishi 5L fermentor, S = 20L fabricated stirred tank fermentor)

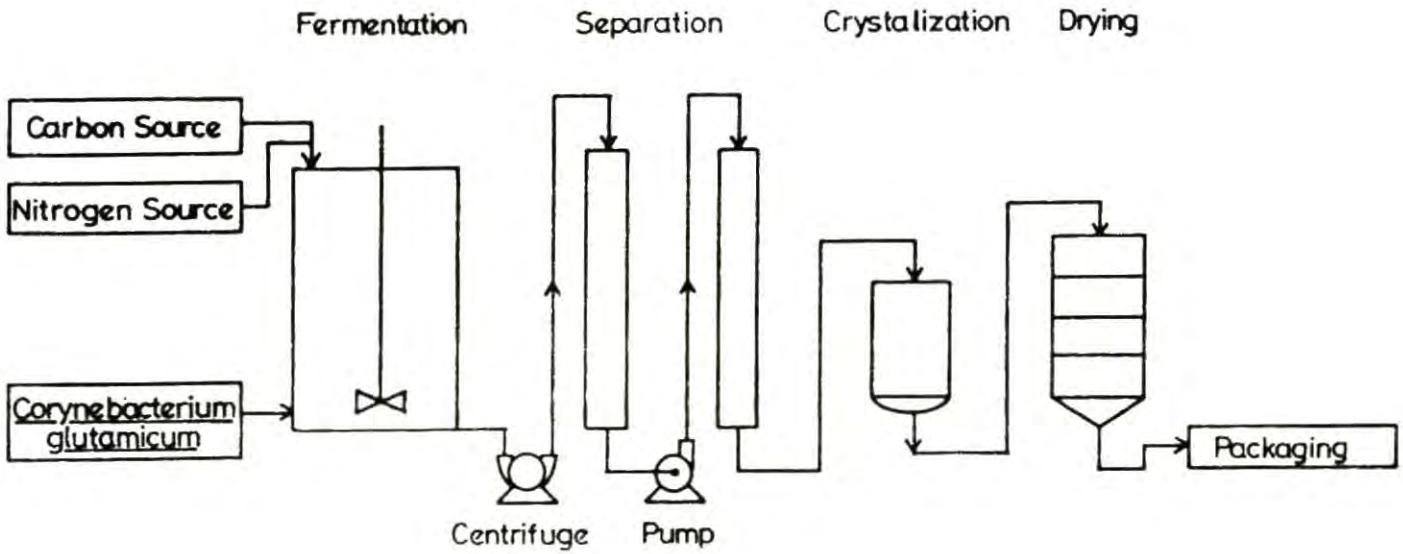

Figure 7. Schematic diagram of L-methionine production

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Toxicity and Mutagenicity Testing of Selected Herbal Drugs

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ABSTRACT

Three of the 10 herbal drugs tested showed toxicity based on chicken embryo assay of water and oil extracts. These herbal drugs (golden seal root, black walnut and herbal tea) taken to improve memory, exhibited fatal effects on the chicken embryo four days past inoculation. This finding does not support the superfluous therapeutic claims that go with these drugs.

Mutagenicity test using the Ames-Salmonella bacterial test system employing rat liver homogenate (S9) as mammalian metabolic activator revealed that eight of the herbal drugs were not mutagenic. The possibility, however, of mutagenicity with higher doses cannot be ruled out. The mutagenicity potential of golden seal root and black walnut was not revealed at 20% water extract concentration used due to the direct toxic effect on the tester strain. Such determination was shown to be possible at 2% water extract concentration for black walnut. Nevertheless, this shows the validity of the method when used in conjunction with S9 which could be prepared locally from Sprague-Dawley rats. This further confirms the necessity of using S9 in assessing mutagenicity potential.

INTRODUCTION

Herbal drugs are processed dosage forms usually from plants used traditionally in the treatment of diseases. These products have active ingredients that may be acting synergistically or antagonistically in producing the activity necessary in the treatment of diseases (**8**). In preparing drugs from plants, primary consideration is no doubt given to effectiveness in treating or curing health problems. However, as with any pharmaceutical production , evaluation of toxicity and hazards should always be linked to the efficacy that enables consumers to weigh the benefits versus risks. Although these constitute the standard operating procedure in developing a plant into a drug, such information is not available despite the fact that herbal medicine is now gaining wide acceptance.

Toxicological testing aims to establish the safety of a drug sample (i.e., lack of toxic and mutagenic components). Chemical contaminants may find their way into the drug preparations through contamination of raw materials during processing or throught infection by a variety of pests. When taken in small doses over a period of time with the drug, they may become toxic (**15**). It is also of interest to note that toxic compounds can be formed during processing. Residues from compounds used in growing the plants, such as pesticides and fertilizers, could also be considered as contaminants. Fungal contamination may also produce toxic effects due to production of mycotoxin. Moreover, plants have been known to contain specific substances that are considered toxic or may be mutagenic (**7**). In fact, studies have already shown that plant extracts contain inactive substances which, when metabolized by specific enzymes, are activated and converted into mutagens. There has also been considerable evidence that most mutagens are carcinogens and vice versa. Hence, the products proven to be effective as drugs for certain illness may still pose potential hazards.

In the face of growing concern for safety and lack of safety information on herbal drugs now locally being sold in the local market, it would be of interest to evaluate these products by conducting toxicity and mutagenicity tests. Hence, this study was conducted at the Antibiotics Laboratory of the National Institutes of Biotechnology and Applied Microbiology (BIOTECH), UPLB, College, Laguna from October 1989 to July 1990.

MATERIALS AND METHODS

Herbal Drug Samples

Herbal drugs for internal use in humans were evaluated in this study (Figs. 1-3). They were selected and obtained from the different drug outlets (Table 1). Prescribed uses and actual costs were also indicated. For each sample, approximately 100 g was placed inside a plastic bag, sealed and stored at 4°C until tested.

Toxicity Test

The chicken embryo assay method (**5, 9**) was used to determine the presence or absence of toxicity in the drug samples. Both the water and oil extracts were tested. A 20% water extract was prepared by homogenizing the mixture of drug and distilled water in Waring blendor and collecting the aqueous extract by filtration and centrifugation at 14,000 rpm at 4°C for 15 minutes. The oil extract was prepared according to standard procedure (**5**) using soya oil.

The experiment was conducted at the Bureau of Animal Industry (BAI) Poultry Station in Alabang, Muntinlupa, Metro Manila. One- day-old fertile eggs were incubated at 37-38°C at 65% relative humidity for five days. After incubation, the eggs were candled to select only those with live embryo and to outline the location of the air sac. After surface sterilization of selected eggs with 95% ethanol, they were aseptically and slowly bored with a flamed-sterilized dissecting needle within the air sac area. The test material was aseptically injected into each egg through the hole using 1.0 ml tuberculin syringe. The hole was immediately sealed with sterile melted paraffin. Immediately, the eggs were incubated at 37-38°C (65% RH) for four days. After incubation, the inoculated eggs were candled and examined for viability of embryo. The number of surviving embryo was determined for each treatment. After 21 days incubation, percent hatchability was also determined. The Chi square test of goodness of fit was done to determine if the number of surviving embryo was equally distributed among the treatments. Differences between treatments were assessed using the Z-test for proportions against the positive control. Two trials were conducted and the results were combined as average of the two after analysis of the repeated experiment showed no significant differences between the two trials.

Mutagenicity Test

The Ames-Salmonella test (**16**) was used as a measure of the mutagenicity of the herbal drug samples. This was conducted both in the absence and presence of a mammalian metabolic activation system using rat liver homogenate (S9).

Bacterial strain and media used. *Salmonella typhimurium* TA98 BIOTECH 1326 was used and was maintained on a plate of Minimal Glucose Agar (MGA) containing histidine, biotin and ampicillin. For the assay, the organism was inoculated into L-broth and was shaken overnight until highly turbid growth occurred.

The following media and reagents were prepared before the experiment: 0.5mM histidine and biotin solutions, top agar (0.6% agar) and Minimal Glucose Agar which served as the base agar. S9 Mix was prepared just prior to use and was maintained on ice. The S9 Mix is a NADPH-generating system produced by the addition of NADP and glucose 6-phosphate to the S9 fraction (**2**). This was done to simulate NADPH-dependent mammalian metabolism.

Preparation of rat liver homogenate (S9). Two Sprague-Dawley male rats weighing approximately 200 g each were used. Five days before sacrifice, the rats were induced to activate microsomal system by 0.1% sodium phenobarbital given through their drinking water. The rats were fed ad libitum until the 5th day. On the 5th day of induction, they were killed by cervical dislocation.

To remove the liver from a rat (for liver homogenates preparation), all procedures were carried out under aseptic conditions using sterile glassware and surgical tools. The animal was placed on its back on a styrofoam board, its feet secured with pins, its abdominal fur removed with scissors and its skin thoroughly swabbed with 95% ethanol. A cut was made through the skin using a sterile scalpel. The skin flaps were folded back and pinned onto the board. The muscle layer was cut through carefully with a fresh pair of sterile scissors and the liver was excised.

To prepare the liver S9 fraction, the procedure of Garner et al. (**12**) was followed. All steps were carried out at -4°C using cold, sterile solutions and glasswares. The freshly excised livers were placed in a preweighed beaker containing approxi-

mately 1 ml of chilled 0.15M KCl/g of wet liver. The livers were washed in fresh chilled KCl to ensure a sterile preparation and to remove hemoglobin which could be inhibitory to the activity of Cytochrome P-450 enzymes. The washed livers were transferred to a beaker with 3 ml 0.15M KCl/g wet liver, minced with sterile scissors and homogenized in a Waring blendor. The homogenate was centrifuged for 10 min at 9000 xg and the supernatant was decanted and saved. The preparation was distributed in 1 ml portions in Eppendori tubes, frozen quickly on a bed of crushed ice and stored immediately at -70°C. The sterility of the preparation was determined prior to use by a small test streak on a nutrient agar plate.

Plate incorporation test. Water extracts prepared as previously described were used as test material. The experiment employed a completely randomized design (CRD) with 14 treatments (10 drug samples + 4 controls) and 3 replicates/treatment. The experimental unit was an MGA plate. The four controls were for sterility (no organism added), for spontaneous reversion (solvent added in place of test material), for positive response (a standard mutagen added in place of test material) and for positive response using a locally available medicinal plant previously reported as mutagenic.

The test was done by combining 0.1 ml of the bacterial tester strain, 0.1 ml of the test material and 0.5 ml of the phosphate buffer in a top agar. This mixture was poured on to an MGA plate. To treatments requiring metabolic activation, 0.5 ml of the S9 Mix was added in place of the phosphate buffer before pouring the top agar. The poured plate was quickly tilted, rotated and allowed to harden on a level surface to achieve uniform distribution of the top agar. Within an hour, the plates were inverted and placed in a 37°C incubator for 48 h. Following incubation, revertant colonies against a background lawn were counted and recorded. A sample was considered mutagenic if the average number of revertants/plate was greater than two times the spontaneous level (**13, 22**). Colonies appearing on a plate without background lawn were not revertants and were not counted. Two trials were conducted to determine replicability of results. Results reported are the averages of the two trials.

RESULTS AND DISCUSSION

Toxicity Potentials of the Herbal Drug Samples

Effects of Water Extract on the Chicken Embryo

The possible toxic effects of water extracts were revealed by viability and hatchability of chicken embryo (Table 2). Tests for significance of differences in percentage of surviving embryo four days after inoculation showed that seven of the samples elicited the same effect on the chicken embryo as in the controls. This showed that these samples did not contain water soluble toxic material constituents and were not contaminated with any toxic chemical.

The other three samples (herbal tea for memory, golden seal root and black walnut) each resulted in 62.5% live embryo which were significantly lower than the control. Hence, water extracts of these samples contained toxic constituents which might be inherent in the preparation or present as a result of chemical contamination or biodegradation attributable to presence of microbial contaminants.

Percentage of eggs hatched after 21 days of incubation showed the same statistical significance. However, the value for the uninoculated control was lower compared to that of the uninoculated control of the live embryo. Nevertheless, this was greater than the normal value of 80% by commercial hatching eggs (**6**). Based on the percentage of eggs hatched, herbal tea for improved memory, golden seal root and black walnut again exhibited the same toxic effects as revealed by 25% to 30% eggs hatched. This was significantly lower than the percentage of eggs hatched in the controls. This confirmed the presence of water soluble toxic components in these samples.

Effects of Oil Extract on the Chicken Embryo

The percentage of live embryo four days after inoculation with oil extracts (Table 3) showed a relatively lower survival rate than those inoculated with water extracts. It appeared that oil effected the survival of the embryo. This was shown in the significantly lower percentages of live embryo elicited by benostrum, benergen, lagundi syrup, herbal tea for longevity, turgor bran and alfalfa tablet. This showed that the effects on the

embryo by the samples were due to presence of oil and not due to presence of toxic material inherent or present in the sample. The other samples (balaya, herbal tea for memory and golden seal root) elicited 7.5% to 15.0% live embryo only, which was significantly lower than for both controls. None of the 20 embryos inoculated with black walnut survived four days after. This shows the presence of toxicity in its oil extract.

The percentage of eggs hatched 21 days after inoculation with oil extracts (Table 3) was lower than the percentage of live embryo. All the samples (including sterile oil) exhibited significantly lower percentage of eggs hatched than the uninoculated control. However, the percentages of eggs hatched by balaya, benostrum, benergen, lagundi syrup, herbal tea for longevity, turgor bran and alfalfa tablet were comparable with that by sterile oil. Hence, the low hatchability by these samples was due to the effect of oil on the development of the embryo and not due to the presence of toxic metabolites in the sample oil extract. Balaya oil extract was shown to be toxic on the embryo but its effect on the percentage of eggs hatched was not significant. Thus, it was not considered toxic. A component of balaya oil extract might have affected the embryo significantly but that effect was not significant when determined by the percentage of eggs hatched. The very low hatchability, however, of 5.0% by herbal tea for memory and golden seal root, and the absence of eggs hatched by black walnut, implies a clear presence of toxic metabolite in these samples.

Toxicity Assessment

Based on the results of the chicken embryo assay of water and oil extracts of herbal drug samples, herbal tea for memory, golden seal root and black walnut were toxic using 20% and 10% (w/v) water and oil extracts, respectively. These smaples had exhibited fatal effects against the embryo, resulting in lower embryo survival and eggs hatched. The toxicity of herbal tea for memory does not support the claim of the manufacturer that it improves the inellectual capacity of a person (Table 1). Likewise, the toxicity of golden seal root and black walnut does not support the claim that they can cure almost all diseases and correct bodily disorders (Fig. 3).

Toxicity of herbal preparations has been previously reported (**3, 10, 18, 19, 21**). Majority of these reports point to herbal tea as a health hazard and not as the herbal remedy to various diseases and disorders claimed by numerous users. Consumers should be wary about the therapeutic claims of these products. For instance, Paraguay tea is very popular in Brazil and in the United States, but it was implicated in a case of veno-occlusive disease of the liver in Britain (**18**). Another herbal tea of proven toxicity is the camomile tea which has been well documented as a cause of contact dermatitis (**4**). In the Philippines, many kinds of herbal teas are currently available. Among the popular ones, the Taheebo herbal tea, which claims to be approved by the FDA and recognized by the governments of Brazil, Canada and the United States (**24**), has been promoted extensively through brochures and leaflets which extol the healing properties of this product. Many Filipinos, however, have now expressed questions or doubts regarding the curative properties and safeness of this product.

No herbal drug is registered with the Bureau of Foods and Drugs (**1**). Aside from the herbal products endorsed by the Department of Health (DOH) for use in government hospitals and drugstores, no other herbal products have been submitted for registration with the BFAD. Moreover, herbal drugs are far from being approved by the regulatory body if manufacturers could not satisfy requirements, among which are the identification and isolation of the active constituent responsible for the claimed therapeutic effect. Toxicity studies, therefore, of products generally regarded as safe (GRAS) should be one of the priorities of institutions engaged in health related researches.

Mutagenicity Potentials of the Herbal Drug Samples

Mutagenicity was measured in terms of the number of histidine revertants/plate induced by the test material (**2**). This is based on the premise that mutagenic material will induce the reversion to the prototrophic state of histidine auxotrophic strains deficient in its repair system mechanisms. To test the validity of the bacterial test system, several controls were included in the assay. Control treatments using solvent only in place of test material, assessed the spontaneous reversion property of *Salmonella typhimurium* TA 98. Using dis-

tilled water, the solvent used for extraction of herbal samples, the strain elicited an average of 38.0 revertants/plate in the absence of S9 (Table 4). This was well within the acceptable range of 30-50 revertants/plate in the absence of S9 (**16**). Using dimethyl sulfoxide (DMSO), the solvent for 2-aminofluorene, the value obtained was 32.0 revertants/plate in the absence of S9. A value lower than that obtained from distilled water was similarly reported by Shahin (**22**) and Shibuya et al. (**23**).

Another equally necessary control treatment is a positive control employing a diagnostic mutagen to confirm the reversion property of the strain in the presence of the mutagen. The arylamine and a potent hepatocarcinogen (**20**), 2-aminofluorene (2-AF) was used as a positive control. Its selection facilitated the validation since 2-AF requires metabolic activation for the expression of its genotoxic potential (**17**). Hence, the effectivity of using S9 could also be determined. Dissolved in DMSO and used at a concentration of 20 ug/plate, 2-aminofluorene elicited an average of 44.0 and 126.0 revertants/plate in the absence and presence of S9, respectively (Fig. 4). The value was within the acceptable range, both in the absence and presence of S9. Thus, 2-AF was considered mutagenic only in the presence of S9 based on the accepted basis of mutagenic activity which is greater than two-fold increase of revertants over the spontaneous level. Hence, the S9 Mix prepared from liver homogenate of phenobarbital-induced Sprague-Dawley rats was effective as a mammalian metabolic activation system.

A further check on the test system was the inclusion of a medicinal plant reported to be mutagenic. Raw garlic bulb water extract at a concentration of 20% (w/v) was used. In the absence of S9, garlic aqueous extract elicited an average of 84.0 revertants/plate; whereas in the presence of S9, a lower average count of 64.0 revertants/plate was obtained. Previous mutagenicity screening tests on this medicinal plant revealed that raw garlic bulb was mutagenic in the Ames-Salmonella test without S9 (**14**). However, the mutagenicity was lost upon metabolic activation when measured by the host-mediated assay. Host-mediated assay as an alternative to using S9 as a metabolic activation system has a major weakness. The conversion to active mutagenic forms may have taken place in the mouse upon injection of the test ma-

terial. However, the concentration of these active forms reacting with the test strain present in the peritonium of the mouse could be lower (possibly too low) to produce a significant number of mutations (**11**). The lower concentration stems from the fact that not all of the active forms reach the peritonium where mutagenicity reaction takes place. Nevertheless, the use of the Ames-Salmonella test with and without S9 confirmed the elimination of the mutagenic effect of raw garlic bulb upon metabolic activation. This was shown in the greater than twofold increase in the number of revertants in the absence of S9 and the lower count obtained in the presence of S9. This result, therefore, agreed with the reported nonmutagenicity of this medicinal plant upon metabolic activation.

Among the samples, only lagundi syrup and herbal tea for memory elicited revertant counts comparable with the value in spontaneous reversion count. These two samples, therefore, could be considered nonmutagenic as well. The rest of the samples, balaya, benostrum, benergen, herbal tea for longevity, turgor bran and alfalfa tablet, elicited counts higher than the spontaneous reversion count. However, these counts failed to reach the greater than twofold increase over the spontaneous level criterion in assessing mutagenicity. Thus, the samples could still be considered nonmutagenic. It seemed that the herbal drug samples did not contain water-soluble components that could be activated to mutagenicity. High reversion rates, however, may imply that the samples might be mutagenic at a higher dose, but not too high as to exert toxic effect to the tester strain. Furthermore, other extracting solvents and strains prescribed in the Ames-Salmonella test should be tried with S9 to determine if components extractable with a solvent could be activated to mutagenicity. Hence, the use of S9 as a metabolic activation system is indispensable in assessing mutagenicity employing the Ames-Salmonella test.

Examination of mutagenicity plates of golden seal root and black walnut showed no background lawn. Therefore, no counts were done on these plates. Apparently, the concentration of 20% water extract was toxic to the tester strain. Hence, no growth, and no background lawn occurred. Few small colonies appeared on the plates, having survived on the trace of histidine present on the medium. This condition has

been repeatedly observed by Ames et al. (**2**) and Maron and Ames (**16**) on highly toxic compounds. To prevent this, the originators have prescribed that the concentration or dose of samples to be assayed should be taken from the linear portion of a dose-response curve. If no toxicity determination is done, a 2.5-fold increase over the spontaneous level will also be considered an indication of mutagenicity if found in repeated experiments (**22**). A 2% concentration was tried for black walnut and the normal background lawn appeared with revertant colonies. Therefore, at this concentration, black walnut lent itself to the detection of mutagenicity potential but at 20% water extract concentration, such determination was not possible due to the direct toxic effect on the tester strain.

Table 1. **Source, manufacturer, prescribed uses and actual cost of herbal drugs studied toxicologically**

SAMPLE	SOURCE AND MANUFACTURER	PRESCRIBED USES[a]	COST (₱)
Balaya	Ben Cruz Herbal Clinic, Taft Avenue, Manila and Ben Cruz Herbal Clinic, Taft Avenue, Manila	As a remedy for high blood pressure intestinal and respiratory tract infections	0.30/ tablet
Benostrum	Ben Cruz Herbal Clinic, Taft Avenue, Manila and Ben Cruz Herbal Clinic, Taft Avenue, Manila	Medicine for diarrhea (LBM), food poisoning, cough, allergy and colds.	0.30/ tablet
Benergen	Ben Cruz Herbal Clinic, Taft Avenue, Manila and Ben Cruz Herbal Clinic, Taft Avenue, Manila	For improvement of digestion and metabolism and prevention of premature graying of hair; also as energy food	30.00/ 250 mL

continued next page

Table 1. continued . . .

SAMPLE	SOURCE AND MANUFACTURER	PRESCRIBED USES[a]	COST (₱)
Lagundi Syrup	Medicinal Plant Section, Ethnobotany Lab. IBS, UPLB College, Laguna and Medicinal Plant Section, Ethnobotany Lab. IBS, UPLB Coolege, Laguna	As analgesic, antipyretic, bronchodilator and expectorant	9.00/ 60 mL
Herbal tea for longevity (Fo-ti-tieng)	Sto. Niño Botanical Center Sta. Cruz, Manila and Unknown	Rejuvenates internal organs; revitalizes glands; adds years to life; gives vigor and virility, also sharpens memory.	50.00/ 25 g
Herbal tea for memory	Sto. Niño Botanical Center Sta. Cruz, Manila and Unknown	Helps maintain and improve memory as antidote to mental fatigue and forgetfulness; as remedy for bringing agility to intellect	50.00/ 25 g
Turgor bran	Ben Cruz Herbal Clinic, Taft Avenue, Manila and Ben Cruz Herbal Clinic, Taft Avenue, Manila	As brain and nerve food; for diabetes, constipation hemorroid; anti-anemia, vacular; respiratory, height and weight problems; effective for pimples, high blood pressure, varicose veins; prevents fat tumors.	10.00/ 500 g

continued next page

Table 1. continued . . .

SAMPLE	SOURCE AND MANUFACTURER	PRESCRIBED USES[a]	COST (₱)
Alfalfa tablet	Bio-Synergy Inc. Agrix Supermarket Los Baños, Laguna and International Vitamin Corp. USA	As multi-vitamin supplement rich in chloropyll, calcium and phosphorus; scrubs out cholesterol deposit in the arteries	1.00/ tablet
Golden seal root	Herbix Enterprises, Los Baños, Laguna and Wachter's Organic Sea Products Corp. California, USA	As remedy for a wide range of diseases and disorders (Fig. 3)	8.00/ tablet
Black walnut	Herbix Enterprises, Los Baños, Laguna and Wachter's Organic Sea Products Corp. California, USA	As remedy for a wide range of diseases and disorders (Fig. 3)	5.00/ tablet

[a] Taken from product label and/or brochure obtained from the drug outlet

Table 2. Effects of water extract of herbal drug samples on viability and hatchability of chicken embryo[a]

TREATMENTS	LIVE EMBRYO %	EGGS HATCHED %
Control: Uninoculated	95.0[a]	85.0[a]
Control: Sterile water	90.0[a]	62.5[a]
Balaya	85.0[a]	62.5[a]
Benostrum	95.0[a]	80.0[a]
Benergen	95.0[a]	60.0[a]
Lagundi Syrup	80.0[a]	70.0[a]
Herbal tea for longevity	85.0[a]	62.5[a]
Herbal tea for memory	62.5[b]	30.0[b]
Turgor bran	90.0[a]	65.0[a]
Alfalfa tablet	85.0[a]	60.0[a]
Golden seal root	62.5[b]	30.0[b]
Black walnut	62.5[b]	25.0[b]

[a] Based on two trials each using 20 eggs/treatment. Chi-square test and Z test for proportion against the positive control were conducted to determine significance of the differences in percentages between treatments. Percentages followed by same letter are not significantly different.

Table 3. Effects of oil extract of herbal drug samples on viability and hatchability of chicken embryo[a]

TREATMENTS	LIVE EMBRYO %	EGGS HATCHED %
Control: Uninoculated	90.0[a]	90.0[a]
Control: Sterile oil	47.5[b]	37.5[b]
Balaya	15.0[c]	15.0[b]
Benostrum	72.5[ab]	60.0[b]
Benergen	52.5[ab]	50.0[b]
Lagundi Syrup	72.5[ab]	62.5[b]
Herbal tea for longevity	70.0[ab]	60.0[b]
Herbal tea for memory	7.5[c]	5.0[c]
Turgor bran	65.0[ab]	62.5[b]
Alfalfa tablet	52.5[b]	47.5[b]
Golden seal root	7.5[c]	5.0[c]
Black walnut	0[c]	0[c]

[a] Based on two trials each using 20 eggs/treatment. Chi-square test and Z test for proportion against the positive control were conducted to determine significance of the differences in percentages between treatments. Percentages followed by same letter are not significantly different.

Table 4. Number of histidine revertants/plate induced by water extracts of herbal drug samples on *Salmonella typhimurium* TA98 with and without S9 mix

SAMPLES	REVERTANTS/PLATE[a]	
	-S9 Mix	+S9 Mix[b]
Control, distilled water	38.0	61.0
Control, Dimethyl sulfoxide (DMSO); 100 u1	32.0	55.0
Control, 2-Aminofluorene; 20 ug/plate	44.0	126.0
Control, garlic bulb aqueous extract	84.0	64 0
Samples:		
Balaya	73.0	82.0
Benostrum	62.0	68.0
Benergen	50.0	54.0
Lagundi Syrup	32.0	32.0
Herbal tea for longevity	53.0	53.0
Herbal tea for memory	34.0	42.0
Turgor bran	48.0	55.0
Alfalfa tablet	44.0	48.0
Golden seal root	0[c]	0
Black walnut	0	0

[a] Average of two trials. The number of spontaneous revertants has not been subtracted from the totals.

[b] 0.5 mL per plate used contained 0.02 ml S9 or 4% S9.

[c] No background lawn observed. This might be due to toxicity of the extract to the tester strain. Hence, no counts were done.

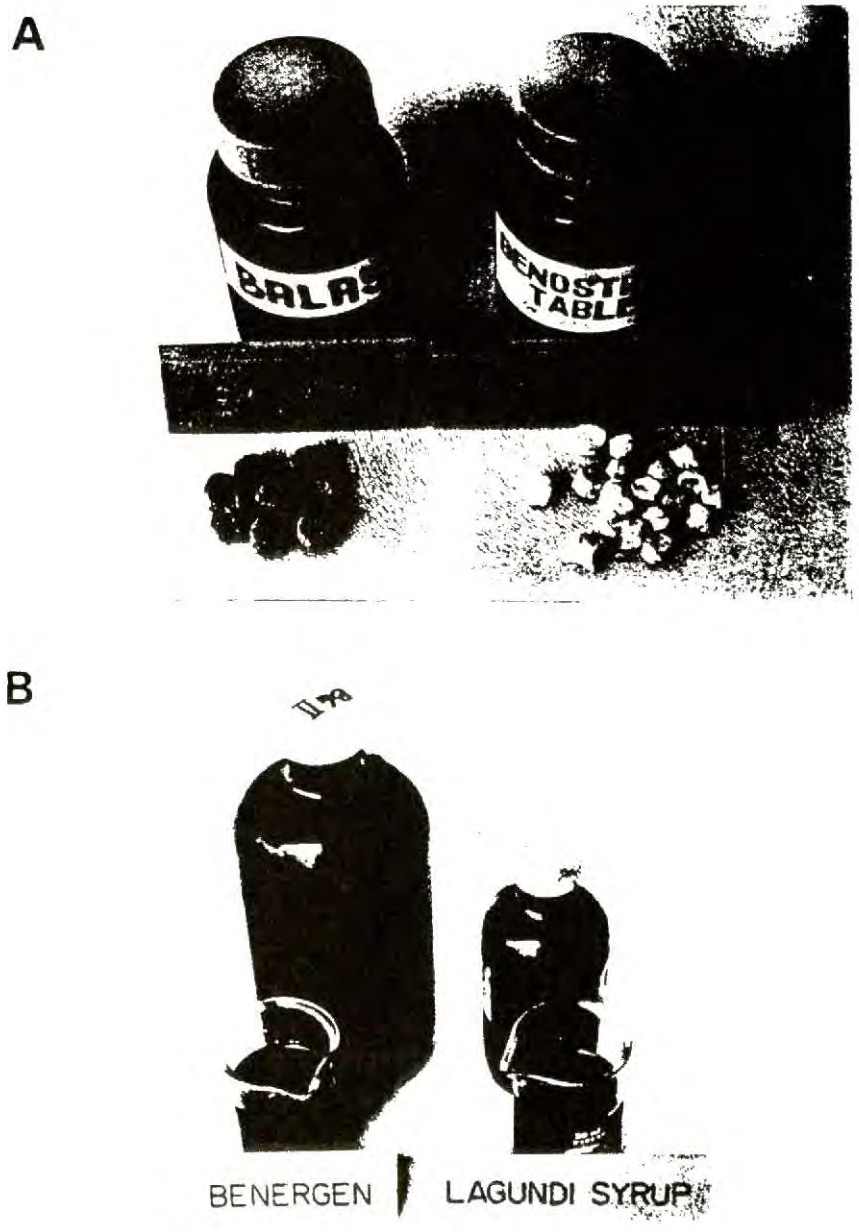

Figure 1. Formulation and appearance of packed herbal drug samples balaya and benostrum (A) and benergen and lagundi syrup (B).

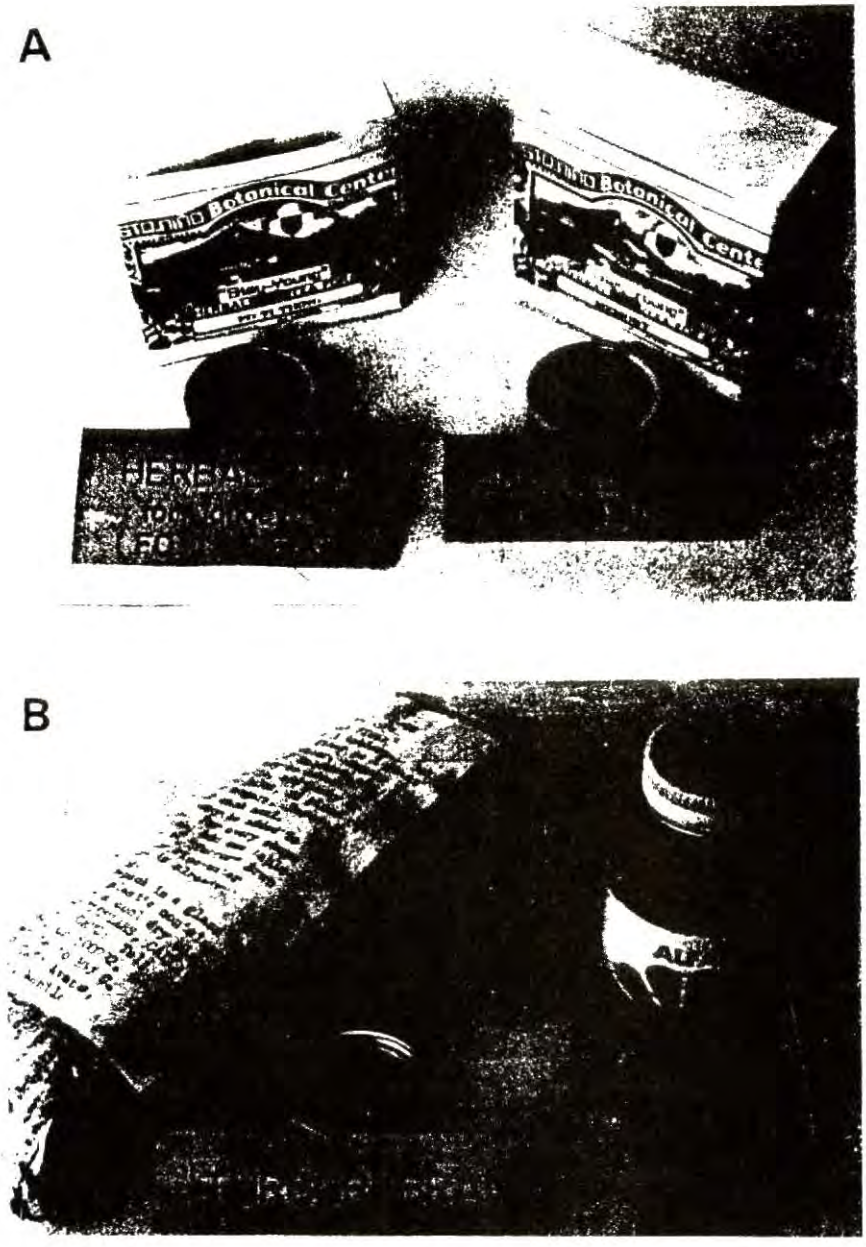

Figure 2. Formulation and appearance of packed herbal drug samples herbal teas for longevity and memory (A) and turgor bran and alfalfa tablet (B)

Has been used for the following:
Allergies, antibiotic, appetite, asthma, bladder infections, bleeding bowel, bronchitis, burns, cankers, catarrh, chicken pox, circulation, colds, colitis, constipation, coughs, diabetes, digestion, earache, eyewash, eczema, flu, gall bladder, gonorrhea, sore gums, hay fever, heart, hemor rhages, hemorrhoids, infections, inflammation, kidney, liver, measles, menstruation, morning sickness, mouth sores, nasal passages, nausea, nerves, pancreas, prostate gland, psorlasis, respiratory, ringworm, skin, skin cancer, small pox, syphillis, sore throat, ulcers, urethra, weight loss, wounds.

Has been used for the following:
Antiseptic purposes, athlete's foot, boils, cancer, colitis, dandruff diarrhea, eczema, electrocution, antidote, hair, hemorrhoids, hoarseness, infections, inflammations, impetigo, leucorrhea, malaria parasite, mouth sores, nails, ringworm, skin rash, ulcerated sores, syphillis, tape worm, sore throat, thyroid, tooth enamel, tuberculosis, tumors, ulcers, prolapsed uterus, vaginal discharge, varicose veins, expulsion of worms and parasites

Figure 3. **Formulation and appearance of packed product and the prescribed uses of herbal drug samples golden seal root (A) and black walnut (B)**

Figure 4. **Histidine revertants on Minimal Glucose Agar (MGA) of standard mutagen 2- aminofluorene in the absence (-S9) and presence (+S9) of rat liver homogenate (S9) mix**

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Diniconazole Effects on Water Relations, Photosynthesis and Alpha-tocopherol Levels of Peanut Plants[1]

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ABSTRACT

It is widely known that triazole alcohols increase plant stress tolerance but specific physiological mechanisms are unknown. Peanut seedlings treated foliarly with the fungicide diniconazole (DINI) exhibited increased net photosynthesis (twofold) and decreased stomatal resistance. Leaf water potential of DINI-treated plants was consistently higher than controls during a 28-hr drying cycle. Alpha-tocopherol levels were 30% lower in DINI-treated plants. Thus, increased photosynthetic and water use efficiency in DINI-treated plants may be more important in stress tolerance than elevated levels of antioxidants.

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INTRODUCTION

Triazole alcohols have been reported to show plant growth regulatory activity in several plant species. Effects include increases in leaf thickness, epicuticular wax, chloroplast size, photosynthetic pigments, nucleic acids and proteins, growth retardation, delay in leaf senescence and decreased stomatal aperture (**1, 4, 5, 7, 9, 11**). Triazoles also protect plants from injury due to temperature extremes, drought and air pollutants (**6, 11, 13, 14**). Specific physiological or biochemical mechanisms involved in stress tolerance are unknown. The present study investigated the effects of diniconazole (DINI) (Fig. 1), (a triazole fungicide), on net photosynthesis, leaf water potential, diffusive resistance, photosynthetic pigments and alpha-tocopherol (an antioxidant) in peanut plants.

MATERIALS AND METHODS

Plant material

Peanut (*Arachis hypogea* L. var. Argentine) seedlings were grown under continuous light (392 uE $m^{-2}sec^{-1}$) and controlled temperature ($28 \pm 2^{o}C$). Diniconazole was applied foliarly at the rate of 4 mg per plant (recommended field rate) every 2 weeks for 8 weeks commencing when the seedlings were 14 days old and in the pre-flowering stage. Diniconazole was given to 20 plants while another 20 were sprayed with distilled water to serve as control. These replicates were arranged in a randomized complete block design. The data were statistically analyzed for variance.

CO_2 assimilation, diffusive resistance and water potential

Net photosynthesis (mg $CO_2dm^{-2}hr^{-1}$) was measured on all 20 replications using an infrared gas analyzer configured as an open system (Model ADC-225 MK 3, Analytical Development Company) when the plants were 41 days old. At 49 and 59 days of age, stomatal resistance and water potential, respectively, were determined during a 28-hr. drying cycle, utilizing five replicates per treatment randomly chosen for each of the four

periods within the drying cycle. Stomatal resistance (sec cm^{-1}) was determined using a diffusive resistance meter (Model LI-COR LI-60, Li-Cor Inc.), while leaf water potential (bars) was determined using a J-14 Model hydraulic press. At day 63, the shoot height was recorded and the plants were harvested, frozen in liquid nitrogen, freeze dried for 72 hr and stored at -23°C.

Alpha-tocopherol extraction

Alpha-tocopherol was extracted from 200 mg dry ground leaf tissue with 3 volumes (5 ml) of cold 80% ethanol (v/v) followed by 3 partitionings with 1% BHT in hexane (w/v) following modified procedures of Cort et al. (**2**) and Grumbach (**8**). The combined washings were evaporated to dryness, dissolved in hexane and analyzed by an HPLC (Model HP 1090 Liquid Chromatograph, Hewlett Packard). The samples were run through a silica column (5 um, 200 x 4.6 mm). The HPLC was equipped with a fluorescence detector (Model HP 1046, Hewlett Packard; excitation = 294 nm; emission = 325 nm) (**3**).

Pigment extraction

Pigments were extracted from 50 mg of dry ground leaf tissue using three volumes (2.5 ml) of ethyl acetate-acetone (60:40 v/v) and analyzed by an HPLC (C-18 reverse phase [5 um, 200 x 4.5 mm]) equipped with a photodiode array UV/visible detector programmed to monitor wavelengths of 410, 430, 445, 450 and 455 nm (**10**).

RESULTS AND DISCUSSION

Shoot height and dry weights

Plants treated with diniconazole were shorter and more compact with thicker, greener leaves (Fig. 2) than the control plants (Fig. 3). Diniconazole significantly reduced shoot height of peanut plants by 46% and shoot and leaf dry weights by 20% (Table 1). The reduction in shoot height and dry weight reported here parallels that reported for *Phaseolus vulgaris* L . treated with various trizole derivatives (**7**).

Net photosynthesis

Peanut plants treated with diniconazole exhibited a twofold increase in net photosynthesis (Table 2). Increase in carbon dioxide assimilation has been reported in rape plants treated with triapenthenol (**12**). However, this reflected only a 10-20% increase in carbon dioxide uptake in treated plants over the control. Increased photosynthetic rates in diniconazole-treated peanuts may be attributed to a 43% decrease in stomatal resistance observed during a 28-hr drying cycle (Table 3). These findings are in contrast to those reported for triadimefon-treated tomato, soybean, pea, wheat and radish plants (**5, 6**). Triadimefon reportedly increased stomatal resistance with increasing concentrations, reduced transpiration rates and increased yield in these plants (**5,** 6).

Analysis of pigments revealed a significant increase in the relative proportion of chlorophyll a in diniconazole-treated plants while phaeophytin levels declined (Table 4). The increased levels of chlorophyll could account for the greener leaves in treated peanut plants. Stimulation of total chlorophyll by triazoles in other plants has been reported (**4, 7**). However, these studies reported on total chlorophyll (a + b) levels. This study has shown specifically that it is chlorophyll a which increased significantly in diniconazole-treated plants. The combined observations in increased chlorophyll levels and reduced stomatal resistance in treated plants may contribute to the twofold increase in net photosynthesis.

Leaf water potential

Table 5 reflects that diniconazole-treated plants had significantly higher (17%) leaf water potential values during a similar drying cycle. Diniconazole may thus cause a reduction in water consumption through a mechanism other than closure of stomata. The observed reductions in leaf area and increased root biomass could be part of the underlying mechanism of drought tolerance in peanuts as was the case in winter barley treated with triapenthenol and its enantiomers (**12**).

Antioxidant metabolism

Alpha-tocopherol levels were significantly lowered by 30% in diniconazole-treated peanut plants. Mackay et al. (**13**) reported

an increase in antioxidants of triazole (S-3307)-treated wheat plants exposed to ozone. However, their analysis was made on the total antioxidant potential of the lipid fraction of microsomal membranes, based on the ability of the lipid extract to inhibit the *in vitro* oxidation of exogenous linoleic acid. The oxidation reaction they observed was similar to that seen of alpha-tocopherol and thus, the antioxidant capacities of their samples were expressed as alpha-tocopherol equivalent (**13**). In this study, alpha-tocopherol was measured directly by HPLC.

SUMMARY AND CONCLUSIONS

The observed changes in morphological characteristics (i.e., leaf area, shoot height), stomatal resistance, photosynthetic rates and pigment composition, could all contribute positively to increasing drought resistance and productivity in diniconazole-treated peanut plants. Alpha-tocopherol production appears to be reduced in diniconazole-treated plants and may not be a factor in drought resistance. However, further studies are needed on other antioxidants (ascorbic acid and glutathione), which are known to interact with alpha-tocopherol to provide a mechanism of oxidant protection in plants.

Table 1. Shoot height and shoot/leaf dry weights of DINI-treated and control peanut (*Arachis hypogea* L.) plants

TRT	HT (cm)	SHOOT (mg)	LEAF (mg)
Control	37.2	1230.9	745.0
Treated	20.1	804.9	595.5

Table 2. **Net photosynthesis of DINI-treated and control peanut (*Arachis hypogea* L.) plants**

TREATMENT	NET PHOTOSYNTHESIS (mg CO_2 dm-2 hr-1)
Control	2.44
Treated	4.97

Table 3. **Stomatal resistance of DINI-treated and control peanut (*Arachis hypogea* L.) plants over a 28-hr drying cycle**

HOURS POST WATERING	DIFFUSIVE RESISTANCE (asc cm^{-1}) CONTROL	TREATED
4	13.3	7.4
8	11.4	5.5
24	17.2	10.0
28	11.7	7.0

Table 4. **Relative % of photosynthetic pigments in DINI-treated and control peanut (*Arachis hypogea* L.) plants**

PIGMENTS	CONTROL (relative %)	TREATED
Xanthrophyll	30.5	25.5
Chlorophyll b	13.6	13.8
Chlorophyll a	23.1	38.9
Pheophytin	22.8	13.5
Beta-carotene	10.1	16.5

Table 5. Leaf water potential of DINI-treated and control peanut (*Arachis hypogea* L.) plants over a 28-hr drying cycle

HOURS POST WATERING	WATER POTENTIAL (bsrs)	
	CONTROL	TREATED
4	-7.7	-6.4
8	-8.4	-6.9
24	-8.7	-7.4
28	-9.3	-7.6

Table 6. Alpha-tocopherol levels in DINI-treated and control peanut (*Arachis hypogea* L.) plants

TREATMENT	ALPHA-TOCOPHEROL (% dry weight)
Control	0.030
Treated	0.021

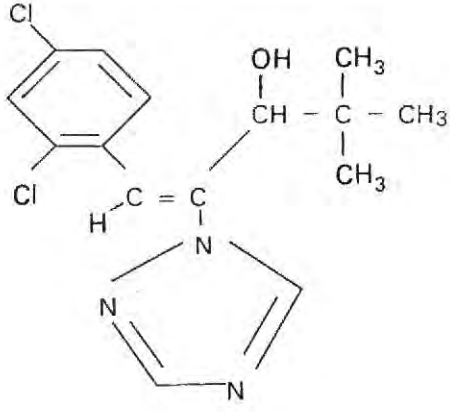

Figure 1. Chemical structure of DINICONAZOLE [(E)-1-(2,4- Dichlorophenyl)-4, 4-dimethyl-2-(1,2, 4-triazol-1-yl)-1-penten-3-ol]

Figure 2. Diniconazole-treated peanut (*Arachis hypogea* L.) plants prior to harvest at day 63. Plants are more compact with shorter internodes.

Figure 3. Control peanut (*Arachis hypogea* L.) at day 63. Plants are taller than treated ones.

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Intraspecific Hybridization Between *Penicillium Aurantio-brunneum* by Fusion of Somatic Protoplast

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ABSTRACT

Three biochemical mutants were obtained from ***Penicillium aurantio-brunneum*** *following UV irradiation. For mutant identification, the Holliday technique was found to be convenient for characterizing auxotrophic mutants.*

The number of protoplasts liberated from this fungus was influenced by several factors like enzyme concentration, osmotic stabilizer, mycelial weight, culture age and pH of the lytic mixture. The release of protoplast described in this work was similar to that reported in other filamentous fungi. Protoplasts retain the capability to synthesize a new cell wall, and re- establish apical growth. This regenerative process has been observed under suitable conditions in agar media. The protoplasts regenerated in different hypertonic agar media, but the addition of carbon and nitrogen supplement accelerated the speed of regeneration.

INTRODUCTION

Hyphal anastomosis that lead to heterokaryon are important phases in the life cycle of most fungi. In some fungi, heterokaryosis is readily accomplished by migration of the nuclear components into the cytoplasm. This makes the entire colony heterokaryotic (Burnett 1975). In these groups of fungi, a single fusion could result in heterokaryotization and prototrophic growth of the whole colony. But there have been

reports that in some groups, heterokaryon is limited to the cells directly involved in the fusion process (Roberts 1976). These fungi could only form prototrophic colonies if enough heterokaryotic cells that allow movement of complementary nutrients to neighboring cells were established. This could in turn sustain the prototrophic growth. The successful establishment of a heterokaryon is a complex process that is influenced by environmental and genetic factors as explained by Garen (1968). The genetic barrier to hyphal fusion and heterokaryon formation is due to the presence of rigid cell walls, which inhibit close contact and fusion. Therefore, the first condition for membrane fusion is the removal of the cell wall, resulting in the formation and fusion of protoplasts.

The first published reports of protoplast isolation from *Saccharomyces cerevisiae* and *Neurospora crassa* appeared in the late 1950s (Eddy and Williamson 1957; Emerson and Emerson 1958; Bachmann and Bonner 1959). The Brno group at their research center in Czechoslovakia concentrated on yeast protoplasts in the study of cell cycle and cell division phenomena (Necas 1971). The Salamanca workers in Spain focused their attention on the search for mycolytic enzymes particularly active against filamentous fungi (Villanueva and Garcia Acha 1971). Their combined works laid an important foundation for the expansion of research activities on microbial protoplasts.

The isolation of protoplasts is now a routine procedure used in several areas of fungi research, in the study of enzyme localization (Trevithick and Metzenberg 1961; Andres and Peberdy 1974), for the preparation of cell membranes (Rodriguez Aguirre et al. 1964), organelles (Garcia Acha et al. 1966), ultrastructure (Gibson and Peberdy 1972; Gibson 1973; Isaac 1978) and cell wall regeneration (Lopez-Belmonte et al. 1966; de Vries and Wessels 1975). More recently, the study of protoplast fusion led to a new approach in genetic modification of fungi (Peberdy 1979; Morgan 1983; Peberdy 1989).

Protoplast fusion has been used successfully to produce heterokaryons between fungal strains that are incompatible by using conventional methods (Dales and Croft 1977). Successful crosses have been achieved with many species, including some of industrial importance like *Schizosaccharomyces* (Sipiczki and Ferenczy 1977), *Penicillium* species (Anne and Peberdy 1976), *Cephalosporium acremonium* (Hamlyn

and Ball 1979) and *Candida tropicales* (Fournier et al. 1977), using the protoplast fusion technique.

A comprehensive investigation was therefore undertaken to test the formation of heterokaryon by fusion of somatic protoplasts intraspecific hybridization between different mutant strains of *P. aurantio-brunneum*. Additional information derived from this work may enhance the possibility of improving and establishing new desirable combinations of nuclei from different strains. This would undoubtedly open the way to a breeding program that could be of value to *P. aurantio-brunneum* and related species.

MATERIALS AND METHODS

Mutagenesis. Spores taken from a 4-day-old culture were suspended in phosphate buffer. The method adopted for ultraviolet light (UV) treatment was similar to that described by Pontecorvo and Sermonti (1953). The spores were exposed to UV light while being agitated constantly using a magnetic stirrer. Exposure time was terminated at varying times and the suspension was suitably diluted before plating on a complete medium.

Mutant isolation. Mutagenized spores were plated on a complete medium and incubated at 27°C. All colonies or sectors appearing to be auxotrophic were transferred to the complete medium and retested by replication on a minimal medium. Colonies which did not grow were tested for their specific nutritional requirements. The method desbribed by Holliday (1956) as using 12 solutions, each containing six different nutritional requirements, was used for the characterization of auxotrophic mutants.

Lytic enzyme and osmotic stabilizers. Novozym, a commercial enzyme preparation, was used in protoplast production at 2% concentration. Two buffer/stabilizer systems were used: $MgSO_4$ and KCl.

Protoplast isolation. Mycelium from the exponential growth phase was used at 20 mg fresh weight per ml of the lytic mixture. The protoplast was separated from mycelial debris by filtration through a sintered glass filter of porosity 1. Protoplast yield was based on haemocytometer count on the filtrates following lytic digestion.

Protoplast regeneration and reversion. Appropriate dilutions vere made of the protoplast suspensions and inoculated into different osmotically stabilized media. A colony count was made to assess the reversion frequency of the protoplasts after incubation at 27°C for several hours.

Protoplast fusion. Protoplasts of two nutritionally complementary strains of *P. aurantio-brunneum* were mixed and suspended in a pre-warmed solution of 30% (w/v) PEG. The suspension was incubated for 10 min, diluted with hypertonic minimal medium and centrifuged. It was washed and resuspended with NaCl before plating in hypertonic minimal and production medium to select the heterokaryons and to count the total number of viable protoplasts.

RESULTS AND DISCUSSION

Auxotrophic Mutants

Over a thousand colonies derived from strains of *P. aurantio- brunneum* were screened after UV irradiation. Three auxotro-phic mutants were isolated. Designated as M_1, one was a mutant nutritionally deficient in adenine, thiamine and PABA. Two methionine-requiring strains were coded as M_2 and M_3, respectively. There were also several more leaky mutant strains recovered but these could not be fully characterized, due perhaps to the effect of multiple mutations (Table 1). These leaky mutants, later discarded, produced thin mycelial growths on MM in the absence of the appropriate supplement (Fig. 1). On CM or a properly supplemented MM, their growth was similar to their prototrophic parent.

UV irradiation has been reported to be an effective mutagen in several taxonomic groups of fungi (Cooke and Jones 1970). Exposure of *P. aurantio-brunneum* spores to this treatment to a level of 5% survival produced the auxotrophic mutants used in this work. UV, however, is a strong physical mutagen and is directly absorbed by the DNA bases (Fincham et al . 1979).

It was also shown to cause structural changes in the chromosome and may cause chromosomal translocations (Kaper 1975). The use of other mutagenic treatments as good alter-

natives to UV irradiation in the routine mutagenesis of this fungus will be pursued in future works.

Protoplast Release by Lytic Digestion

The release of protoplast from *P. aurantio-brunneum* using Novozym, a commercial lytic enzyme preparation, followed a similar pattern of events reported in several filamentous fungi (Santiago 1982). The swelling of hyphal tips prior to the emergence of protoplast is very distinct in the first hour of lytic digestion. A gradual degradation of the hyphal wall follows, allowing the liberation of a big number of protoplasts in the succeeding hour. The release of protoplasts followed a sigmoidal pattern.

Light Microscopy of Protoplasts

After 10 min incubation in the lytic mixture, some hyphal tips became swollen and protoplasts subsequently emerged by an extrusion of cytoplasm at the digested tips or the immediate subapical region. Older parts of the mycelium were digested and protoplasts appeared through pores along the whole length of the hyphae. In some instances, a single large protoplast comprising the whole contents of the terminal hyphal segment was released. Generally, the cytoplasm moved through a pore and split into two or more spherical bodies of unequal size. After 5 h incubation, the mycelium was completely transformed into protoplasts. Left behind were mycelial debris of undigested compounds made up of empty hyphae and walls.

Protoplast size was variable and morphologically distinct. Early protoplasts released in the first hour of incubation in the KCl-stabilized system were small spherical bodies, dense and non-vacuolated. After 2 h digestion, the protoplasts were bigger in size and often contained a small vacuole. Similar differences in size between early and late protoplasts were observed in the $MgSO_4$-stabilized system. In the first hour of incubation, small and non-vacuolated protoplasts were released. However, these were bigger compared to those released in KCl. Using this osmotic stabilizer, the mycelium became completely fragmented during digestion and protoplasts were released from the open ends of older hyphae. After more than 2 h incubation, large protoplasts were found with cytoplasm displaced to one side by a

large vacuole. Release of protoplasts from *P. aurantio-brunneum* was similar to that of most reported filamentous fungi (Gabriel 1968; Peberdy and Gibson 1971; De Vries & Wessels 1972; Santiago 1985).

Optimal Conditions for Protoplast Production

Effect of Enzyme Concentration–

The optimum enzyme concentration at which maximum release of protoplasts occurs is 30 mg/ml. Higher concentrations cause lysis of the protoplasts, probably due to high levels or proteinase in the digestion mixture.

Osmotic Stabilizer

Several compounds were tested for their effectiveness in promoting protoplast release. The relative density of the protoplasts varied depending on the stabilizer system used. Incubation with KCl or $MgSO_4$ resulted in more than 35% increase in yield compared to results from other stabilizers. In mannitol, sorbitol and NH_4Cl, small non-vacuolated protoplasts were produced in the early hour of digestion, but vacuolated protoplasts appeared later.

Stabilizers are essential to provide osmotic support after removal of the cell wall. The effectiveness of inorganic salt over the organic compound was demonstrated in this work. Inorganic salt has advantage over sugar solutions because bacterial growth is prevented during lytic incubation (Gaston and Villanueva 1965).

Effect of Mycelium Concentration

Fresh weights of mycelium from exponential cultures of this fungus were used at varying concentrations of 10-50 mg/ml of the digestion mixtures. Increasing the mycelial concentration had a marked effect on protoplast production. Within the range tested, the final yield of protoplasts was highest with 30 mg/ml^{-1} fresh weight of mycelium. Addition of more mycelia beyond this concentration shows reduction in the number of protoplasts liberated. This is probably due to the limiting effect of lytic enzyme in the mixture at high mycelial concentration.

Effect of Culture Age

The age of the mycelium had a marked effect on protoplast release. Large numbers of protoplasts were obtained from the 16 h culture, within the exponential growth phase of this fungus. Older mycelia beyond this age were less susceptible to lysis, probably due to the changed composition of the cell walls during the aging process.

It had been reported that older mycelium used in the isolation of protoplast from *Aspergillus niger* produced very low yield (Musilkona and Fencl 1968). Anne (1977) explained that changes in the effectiveness of the protoplast inducing systems on *Penicillium* mycelium of different ages could be caused by alterations in the ratio and/or texture of the cell wall components, thereby influencing susceptibility to lytic enzymes. The reduction in protoplast yield with increasing age of *Schizophyllum commune* mycelium was reported to be correlated with a decrease in the susceptibility of L-1, 3-glucans in the hyphal wall to enzymatic digestion. Zonneveld (1972) explained that the deposition of L-1, 3-glucans, an outer wall layer in older hyphal, may be an important factor in degradative resistance in some filamentous fungi.

pH of the Lytic Digestion Mixture

With KCl as stabilizer in 0.6M phosphate buffer, the pH of the digestion mixture was tested at different pH values. Protoplasts could be obtained at a wide range of pH values between 4.0 and 8.0. The maximum number of protoplasts liberated was observed between ph 5.5 and 6.0. Below pH 4.0, mycelium was not digested and above 8.0 protoplasts lysed soon after emergence. For that finding, the lytic systems were adjusted to pH 5.6-5.8, pH values similar to those reported for *Trichoderma harzianum* enzyme used by De Vries and Wessels (1972), Anne et al. (1974) and Santiago (1982).

Regeneration and Reversion of Protoplasts

Effect of Different Media

The ability of protoplasts to regenerate a new cell wall and undergo hyphal reversion was tested in different hypertonic agar

media. Though the size of protoplasts from older mycelium was significantly smaller than that from the younger mycelium, the ability to revert to colonies was comparable in all cases. The reversion frequency, calculated for protoplasts in complex media, is slightly higher than that for defined media. The most important finding in reversion frequency was observed in protoplasts isolated and grown in agar media stabilized with KCl.

The reversion frequency obtained in *A. nidulans* (Isaac 1978) was lower at 10-30% than that reported for *Neurospora crassa* (Bachmann and Bonner 1959), *Fusarium culmorum* (Garcia Acha et al. 1966) and *S. Commune* (de Vries and Wessels 1975).

Protoplast Fusion

Fusion between protoplasts of nutritionally complementary strains was detected by the growth of heterokaryotic colonies on agar MM. Non-fused protoplasts did not grow even when high density protoplasts from two auxotrophic strains were plated. PEG-induced fusants between protoplasts from auxotrophic strains of *P. aurantio-brunneum* are presented in Table 2.

The results demostrate that heterokaryons on MM were formed after fusion of protoplasts and were not due to cross-feeding and subsequent anastomosis between reverting protoplasts. The fusion frequency for this fungus was the same for some species like *A. nidulans* which readily formed heterokaryons and *C. acremonium* in which the heterokaryotic condition was not easily attained by conventional methods (Niiesch et al. 1973). It further demonstrated that cytoplasmic fusion was due to complementation shown by the results obtained in these experiments with mono- auxotrophic strains and di-auxotrophic strains.

SUMMARY AND CONCLUSIONS

The basic step in strain improvement work dealing with industrial microorganisms involves the isolation of mutants that can be readily traced by genetic analysis. Three biochemical mutants were obtained following UV irradiation. UV light, however, was reported to cause chromosomal translocation (Kafer 1975) and therefore the use of other mutagens is recommended. For mutant identification, the Holliday technique (Holliday 1954)

was found to be convenient for characterizing auxotrophic mutants.

Through the lytic action of Novozym, a commercial enzyme preparation, a large number of protoplasts was released from *P. aurantio-brunneum* mycelia. The number of protoplasts liberated was influenced by several factors like enzyme concentration, osmotic stabilizers, mycelial weight, culture age and pH of the lytic mixture. The release of protoplasts described in this work was similar to that reported for other filamentous fungi.

Protoplasts retain the capability to synthesize a new cell wall and re-establish apical growth. This regenerative process has been observed under suitable conditions in agar media. In this study, protoplasts regenerated in different hypertonic agar media but addition of carbon and nitrogen supplements accelarated the speed of regeneration.

Solutions containing 6,000 molecular weight polyethylene glycol (PEG) were found effective in fusion of protoplasts from *P. aurantio-brunneum.*

RECOMMENDATIONS

The results of the present study show the development of an efficient method for protoplast isolation, fusion and subsequent reversion within the same species of *P. aurantio-brunneum.* This offers bright propects for genetic improvement of other industrially important endemic fungal strains which could contribute to the advancement of basic research in genetics.

In spite of this contribution, however, somatic fusion leading to hybrid formation between species of different taxonomic groups of fungi essential to a better understanding of heterokaryon, diploid or aneuploid behavior has not yet been done. Further exploratory work to include an interspecific hybridization is therefore highly recommended. Such information as may be derived from this work could be of considerable importance, in terms of its contribution to the further understanding of the life cycle of this fungus. Furthermore, fusants from an interspecific hybridization between species of *Penicillia* could produce penicillin before they had rebuilt a cell wall, therefore offering a good procedure for the formation of a cell free system for penicillin production.

Table 1. Auxotrophic mutants of *Penicillium aurantio-brunneum*

MUTANT	GENETIC MARKERS	MUTAGENIC AGENT
M_1	Ade = Adenine Thi = Thiamine Paba = p-amino benzoic acid moderate growing	UV
M_2	Met = Methionine fast growing	UV
M_3	met = Methionine slow growing	UV

Table 2. Frequencies of heterokaryon formation for different auxotrophic strains of *P. aurantio-brunneum* before and after fusion treatment

Fusion was done by suspending the protoplasts in solution containing 30% (w/v) PEG, 0.01 M $CaCl_2$ and 0.05 M glycine, pH 7.5; dilutions were plated into hypertonic MM and CM.

PROTOPLAST MIXTURES	Heterokaryons (x10-3)/ml developed on MM		Protoplasts (x10-6)/ml reverted on CM		Fusion Frequency
	Before PEG	After PEG	Before PEG	After PEG	
$M_1 \times M_2$	0	0.17	13.0	0.14	0.12
$M_1 \times M_3$	0	4.0	16.0	3.30	0.12

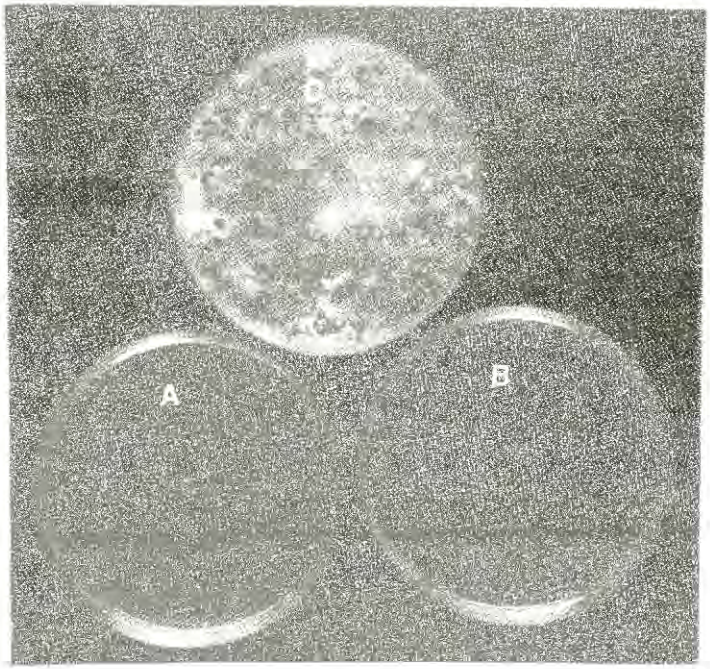

Figure 1. Morphological growth of leaky mutant of *Penicillium aurantio-brunneum* on MM (A & B) and CM (C).

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AGRICULTURAL SCIENCES

Control of Flowering, Seed Germination and Progeny Evaluation of Taro *Colocasia esculenta* (L.) Schott

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ABSTRACT

Breeding work in gabi ***Colocasia esculenta*** *(L.) Schott has lagged behind breeding work in other root crops. This is due to flowering and seed germination problems. This research was conducted to evaluate the potential of producing hybrids.*

Four experiments using gibberellic acid (GA_3) as flower promoter, two experiments in seed germination and a series of evaluation trials of progenies produced were done.

Flowering using GA_3 in 28-day-old plants was better compared to that in 60-day-old plants. Genotypic differences were significant in terms of plant response to GA_3 application. Addition of nitrogen (N), phosphorus (P) and potassium (K) in GA_3-treated plants affected flowering competence, number of flowers per plant and proportion of flowers with pollens.

Germination of seeds in sterilized soil medium was comparable to germination of those sown in agar media. Surface sterilization of seeds with $Ca(OCl)_2$ was effective in controlling fungal growth.

Some clones among the 2000 genotypes evaluated were identified to possess qualities comparable to VG-1 (standard check). Problems regarding progeny evaluation, particularly on dry matter content and acridity determination, were identified.

INTRODUCTION

Colocasia esculenta (L.) Schott, popularly known as taro (or locally as gabi) is probably the oldest cultivated crop in the Asian-Pacific countries (**20**). It is a staple in many Pacific Island nations and a supplement to rice and corn in the Philippines.

In the Philippines, people cultivate taro on a small-scale to augment cash derived from selling the leaves for vegetables and corms for carbohydrates. However, the crop ranks lower than sweet potato and cassava in crop production and hectarage of planting. Moreover, the breeding program for the production of improved varieties has lagged behind.

One major constraint in taro breeding is the sporadic occurrence of flowers in very few taro plants growing in natural conditions. It is believed to be a non-flowering plant (**14, 24**) although materials were found to produce flowers naturally (**13, 17**). Earlier findings have shown that a great proportion of different genotypes failed to flower (**16,17**) under a two-year observation period. Flowering is synchronous and not all plants may flower in an accession.

Sometimes taro flowering leads to fruit setting but seed germination under natural conditions has not been observed. It is believed that taro seeds may lose viability before they are dispersed onto the ground. Likewise, the small seeds (which are usually 1 mm long and 0.5 mm in diameter) are easily lost after the fruit decays due to fungal attack. Sometimes, unripened fruits are eaten by grasshoppers and other chewing insects.

This paper presents results of the methodology adopted for progeny production and evaluation -- simple experiments on the promotion of flowers and seed germination on taro done from 1988 - 1990.

MATERIALS AND METHODS

A. Experiments on Flowering

1. Effect of timing and frequency of GA_3 application

The effects of time of initial application (28 and 60 days after planting) and frequency of application (once, twice, thrice and four times) on the flowering of local taro clones (PRG 078

and PRG 092) were evaluated. Both clones were observed to rarely flower under natural conditions.

There were five experimental units per treatment combination per replication. The experiment was laid out in RCBD with three replications.

A single headsett of each clone with three defoliated petioles was planted in a clay pot (32 cm top diameter) filled with a soil and rice hull compost mixture (5500 cm^3 at 1:1 by volume). The soil-compost mixture had 0.34% total N, 71.42 ppm extractable P and 1,415 ppm exchangeable K.

All opened leaves were sprayed with 250 mg GA_3/L. In each liter of solution, 1.5 ml of chemical sticker was added. Spraying was done using a mist sprayer at a rate of about 100 ml of solutions per plant or until dripping point.

Data gathered for this experiment were: (1) flowering competence as expressed by the percent of treated plants that flowered; (2) number of days to first normal inflorescence emergence; (3) number of flowers per plant; and (4) per cent of flowers with pollen per plant. THe anova with subsampling was used when appropriate. Similar data were gathered for the succeeding experiments.

2. Effects of genotype and strategy of application

Six accessions of gabi were selected based on their propensity to flower under natural conditions. Headsetts were prepared from PRG-066 and PRG-092 (rarely flowering), PRG-068 and PRG-062 (moderately flowering) and PRG-686 and PRG-687 (highly flowering) genotypes. The same planting procedure as in Experiment 1 was adopted.

The strategies of GA_3 application were: (1) 500 mg/L applied at 30, 45 and 60 DAP; (2) 750 mg/L applied at 30 and 45 DAP; and (3) 1500 mg/L applied at 30 DAP only. FIve potted plants were assigned to each treatment per replication. The RCBD was used with three replications.

3. Effect of fertilization and GA_3 on flowering

The genotypes PRG-068, PRG-694, PRG-066 and PRG-687 were used in this experiment. Planting material preparation was similar to Experiment 1.

Nursery beds of 2m x 2m were prepared and planted to four plants spaced at 1m x 1m. The soil was clay loam with 0.21% total N, 13.94 ppm extractable P and 176 ppm exchangeable K.

The treatments were the following: (1) plants sprayed with water only; (2) plants sprayed with 1000 mg GA_3/L; (3) plants sprayed with water and fertilized at the rate of 6.75 g N, 4.5 g P_2O_5 and 4.5 g K_2O per plant; and (4) plants sprayed with 1000 mg GA_3/L and fertilized similarly as in treatment 3. Spraying of GA_3 or water was done at 28 DAP.

The RCBD was used with two replications.

4. Effect of N, P, K

Fertilization affected flowering based on the results of Experiment 3 and a pot experiment was conducted to determine the effect of N, P and K. There were eight (2^3) treatment combinations. Five pots were assigned to a treatment combination. The RCBD with three replications was used.

The N, P and K were applied at the rate of 2.25 g per pot.

B. Experiments on Seed Germination

1. Effect of media on germination

Fruits of PRG-689 were collected one month after pollination from self-pollinated plants. To extract the seeds, the fruits were squashed on a 2-mm wire mesh under slowly running tap water and the extracted seeds were air dried for three days prior to sowing.

The media used in the experiment were: (a) 1% agar; (b) 2% agar; (c) soil extract agar; (d) soil + 1% agar; (e) soil; (f) rice hull compost; and (g) tissue paper. All the media were autoclaved at 250^0C (15 psi) for 20 min.

Sterilization of seeds was done by soaking them in 5% NaOCl for 10 mins, then rinsing with three changes of sterile distilled water.

The RCBD was used with three replications. The experiment lasted for only 60 days.

2. Effect of seed source and $Ca(OCl)_2$ on seed germination

Seeds from 10 genotypically different sources were extracted, air dried for three days and soaked either in 5% $Ca(OCl)_2$ or distilled water for 10 minutes. One hundred seeds were sown in a petri dish lined with tissue paper moistened with distilled water. The experiment was laid out in RCBD with three replications. Daily scoring for germination was done for 100 days. A seed was considered to have germinated when the green cotyledon had emerged from the seed coat. The percent germination 22 was obtained and the coefficient of velocity was computed using the formula:

$$\text{Coefficient of Velocity} = \frac{\text{total number of seedlings}}{A_1 T_1 + A_2 T_2 + \ldots + A_n T_n}$$

where: A = number of seedlings emerging on a particular number of days (T)

C. Progeny Evaluation and Selection

After four months of seedling growth (from petridishes to seed boxes), the materials were transferred to a nursery bed for two-month acclimatization before the field trials were started. A schematic diagram of the flow of progeny evaluation is shown in Figure 1.

In each phase of the evaluation, selection was done based on either the dry matter content, palatability or yield. Dry matter evaluation was based on fresh weight while palatability and general acceptability scoring (Hedonic scale) were adopted on some materials presumed to have high dry matter content based on the "flotation method" of uncooked corms. Raw clean corms were dropped in a plastic pail filled with 3 l of water. Materials that floated were considered as having low dry matter content (less than 38%).

During the single plot evaluation, 12 plants were grown. Plant heights of 10 sample plants were measured from the base to the tip of the first fully expanded leaf. This was done on some samples of progenies only of the trial done in 1989. Corm yield (kg/plant) and corresponding dry matter content (%) were gathered at the single plot trial stage, which had two rows of 12 plants. Out of the 20 plants in a plot, 5 sample corms were chipped, sun dried for a day and transferred to an oven set at 50^0C for two days. The materials were weighed obtaining dry matter content as:

$$\text{DMC (\%)} = \frac{\text{Dry Weight}}{\text{Fresh Weight}} \times 100$$

A replicated trial, consisting of 21 genotypes selected from various evaluation trials, was conducted to examine the possibility of obtaining an outstanding genotype. There were six replications and the experiment was laid in RCBD.

RESULTS AND DISCUSSION

Effect of Plant Age on Flowering

A higher percentage of plants flowered when those were sprayed once at 28 DAP(Table 1), indicating an increase in flowering competence of the plants. Likewise, early emergence of flowers was observed in about 14 weeks after planting or about 10 weeks after initial spraying (Table 2). Further GA_3 application seems to retard the flowering competence (**12**) and delay floral emergence.

With a single application of GA3 lesser plants sprayed at 60 DAP flowered. Late floral emergence was observed, but further application enhanced early floral emergence, about seven weeks from initial application.

These results show that application of GA_3 at 250 mg/L may be sufficient when applied only once, specially in younger plants. Secondly, it could be inferred that timing of GA_3 application is important in synchronizing flower emergence.

It was observed in other crops that GA_3 played a role in assimilate distribution (**25**). Under natural conditions, the dry matter tends to be distributed toward the storage organs as the plant matures. But with GA_3 application, the partitioning of dry matter is disturbed, leading to dry matter being distributed toward the shoot apex. Gibberellic A_3 induces mitotic activities (**4**) in the apical meristem. Both photo-induction and GA_3 enhanced floral initiation. This could possibly be supported by the increase of nutrients (**22, 6, 7**) and energy brought about by the action of GA_3 (**4, 19**) in the breakdown of starch.

In younger taro plants, though these had less stored starch, the translocation of assimilates toward the shoot from the leaves after a period from GA_3 application may have encouraged flowering. Nevertheless, further application may lead to ineffectivity since taro also has to translocate starch to the corms.

For the older plants, the re-routing of the assimilate toward the shoot may have been delayed (**25, 22**), resulting in a reduction in flowering competence and delay of floral emergence. Subsequently, further spraying increased GA_3 concentration resulting in the mobilization of stored starch (**7**) for use in the cell division (**5**) at the apical meristems.

Effect of Genotype and Strategy of Spraying on Flowering

In Experiment 1, both rarely flowering and highly flowering genotypes had similar reactions. However, in Experiment 2, strong genotypic differences were observed. In the highly flowering genotypes, 100% flowering was obtained even if different strategies of applying GA_3 were used (Table 3). Moderately flowering types had an enhanced flowering competence (80-90%); and the rarely flowering genotypes had only 60-80% plants with flowers. This shows that even if GA_3 was applied, flowering competence was still genotypically dependent.

In rarely flowering types, the flowering competence may not be perfected by either applying GA_3 at 1500 mg/L once or at 500 mg/L three times or 750 mg/L two times. Staggered application may be efficient as observed in the response of PRG-066 and PRG-062.

Likewise, genotypic differences were observed in the date of floral emergence. The date of flowering varied continuously depending on the propensity of the genotype to flower (Table 4). This suggests that even if GA_3 is applied, emergence of flowers may not be warranted to be uniform and perfectly synchronized. Hence, staggered planting may solve the problem of synchrony of flower emergence. The number of flowers produced per plant was a function of the genotype. The strategy of GA_3 application did not affect the date of emergence and the number of flowers per plant.

Effect of Fertilizer Application on Flowering

Flowering competence of GA_3-treated plants seemed to be affected by the addition of phosphorus (P) and potassium (K) (Table 5). This could be related to the function of both P and K on the energy accumulation and assimilate distribution toward the reproductive organs. Flowering is an energy-requiring process; the addition of both P and K is necessary. A high nitrogen (N) nutrition (**15**) failed to significantly affect flowering competence.

The application of fertilizer failed to affect the date of flower emergence. However, combined application of N, P and K affected the number of flowers produced in GA_3-treated plants (Table 6). An interaction between genotype and fertilizer addition was apparent. This implies that fertilizer can affect responses to GA_3.

Of the three nutrients, P and K significantly affected the number of flowers (Table 7). This indicates that both elements should be added to enhance flowering. It is possible that the apical meristem may have been further stimulated to flower as one more leaf axil was found to bear flowers. Normally, only a single leaf axil produces two or three flowers.

Both P and K affected the number of flowers with pollen (Table 8). It seems that P was important in pollen production, though K was equally likely to affect the presence of pollen. Without N, combined application of P and K resulted in a significant increase in the proportion of flowers with pollen. Without adding K, combined application of N and P was able to enhance pollen production. A similar effect was obtained when P was left out; N and K warranted sufficient pollen produced. Nonetheless, N alone was not sufficient for pollen production.

Inducing plants to flower leads to the doubling of the activity rate of glucose-6-phosphate dehydrogenase in the short apex (**12**), indicating an active breakdown of carbohydrates. SInce the energy requirement increases, supplemental nutrition becomes imperative (**6**). In taro, both P and K must be readily absorbed for use in the energy-requiring process of floral development. The role of P is related to ATP production while K aids in the translocation of assimilates. Likewise, the number of flowers and number of flowers with pollen were subsequently affected.

Seed Germination: Effect of Medium

Germination of seeds was enhanced by using agar media (Table 9). However, this encouraged fungal growth which infected the germinating seeds. The speed of seed germination (coefficient of velocity) was faster in agar medium than in compost or moistened paper. Because of the favorable growth condition afforded by the agar media (**13**), the rapid multiplication of the fungus overcame seed germination (**16, 17**).

Like the agar media, compost medium harbored some fungi and even encouraged the growth of some bacteria. It seems that the compost medium was not effectively sterilized by the method used. Probably compost medium may have germination inhibiting substances affecting germination of taro seeds.

The pure soil medium had an advantage over the media with agar. It did not encourage fungal growth. Also, the germination was comparable to media with 1% agar and soil + 1% agar.

This shows that pure soil could be used to reduce cost of seed germination.

Seeds sown in tissue paper resulted in low germination but the emerging cotyledon was easily observed. This makes paper an effective medium in testing germination. Fungal infection was low, hence effective sterilization techniques should be sought to eliminate possible seed decaying agents.

Effect of Seed Source and Pre-germination Treatment

Genetically, all seeds gathered from seed source #028-4 to 169-68 (Table 10) were different. Although they were gathered from naturally self-pollinated plants, all of these seeds were collected simultaneously, from plants growing under similar conditions.

The use of $Ca(OCl)_2$ was intended to test its effectivity and its use as a possible substitute of NaOCl. It was observed that pre- germination sterilization with $Ca(OCl)_2$ resulted in insignificant reduction of germination. Hence, $Ca(COl)_2$ was effective as a surface sterilant for taro seeds. A comparable percent germination was observed with $Ca(OCl)_2$ and distilled water although fungal growth was observed in seeds soaked with distilled water.

The role of NaOCl and $Ca(OCl)_2$ in the surface sterilization of seeds had been found to be effective in seeds of several plant species. It was effective in overcoming thermo-inhibition of germination (**8, 9, 10** and **11**). It is the chlorine component that weakened the pericarp. Although both NaOCl and $Ca(OCl)_2$ were recommended for surface sterilization, they both failed to achieve uniform germination. It is highly probable that non- uniformity in taro seed germination was not controlled by thermo- inihibition. Other causes may be inherent in the seed, which led to slow rate of germination.

Progeny Evaluation

The flow of evaluation from single plant to replicated trials was consistent with the general norm of evaluating clonally propagated plants. Nonetheless, from the seedling stage to the single plant stage (acclimatization period), losses were incurred due to changes in the environmental conditions particularly the heat of the sun at noon time.

At the single plant evaluation, variation in plant height, petiole coloration and corm flesh color were observed. But at this stage, natural selection was allowed to act, thus adapted genotypes were obtained.

Wide variation among progenies obtained from self-pollinated plants was more pronounced than among cross-pollinated progenies (Table 11). Selection of materials was based on the dry matter content and corm yield and compared with the performance of the standard check (VG-1).

Evaluation of systematically produced progenies was carried out until the single plot trial. The summary data from this batch is shown in Table 11. It is shown that yield and dry matter content varied largely among families. Again, progenies of self- pollinated plants had larger variabilities than those from cross-pollinated plants. This suggests that both selfing and crossing could enhance variant production. Variabilities, in terms of chemical compostion and morphological characters (**23**) were also observed in seedlings from selfed plants. Variability through hybridization therefore can be increased, to be able to select improved genotypes.

At single plot, selection was done. After evaluating 2000 seedlings from five separate batches, twenty-four progenies were identified (Table 12). However, selection based on yield resulted in low quality and less acceptability. Consumers considerably favor taro with high dry matter content (more than 38%) and no acrid taste. With such criteria, most progenies that could have been selected would possess low yield potential. This indicates that further hybridization needs to be done to assemble all desirable genes in one plant.

Further evaluation of materials at the replicated trial stage (Table 13) showed that identification of an improved variety could be done.

Correlation (for a given character trait) between different stages of evaluation shows that corm yield was affected by the environmental condition prevailing in each trial (Table 14). Even within a family, i.e., PRG-686 x PRG-068 and PRG-094 progenies, negative correlation coefficients were obtained. However, the dry matter content seemed to hold a positive correlation between stages of evaluation. This implies that dry matter content should basically be a major criterion in the early selection of progenies.

One major problem in evaluating progenies is the acridity factor. Although Ca oxalate has been thought to affect acridity, other substances associated with Ca oxalate is believed to

control it (**3, 24, 27**). There is no easy method for *en masse* selection of progenies with non-acid character. At present, evaluation on acridity uses human panelists. However, once a panelist had tasted an acrid sample, further evaluation was effected, rendering later results inaccurate.

At present, it seems likely that selection in taro must first concentrate on dry matter content, taking yield only at a later stage. At the single plant to single row trial, dry matter evaluation using the flotation method could be done. Quantitative analyses of dry matter should be done either at single plot or during the replicated trials. At the more advanced stage of evaluation yield, starch content and other properties (**18**) may be used as criteria in the ultimate selection of a new variety.

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Table 1. Effect of frequency and initial date of GA_3 application on flowering competence (% plant that flowered)

Frequency	Date of Initial Spraying	
	28 DAP	60 DAP
Once	93	47
Twice	87	47
Thrice	87	60
Four Times	67	80
cv (%) = 26.67	Sy = 14.15	$LSD_{0.05}$ = 28.3

Table 2. Number of days to emergence (DAP) of first normal inflorescence as affected by date of initial spraying and frequency of GA_3 application

Frequency	Date of Initial Spraying	
	28 DAP	60 DAP
Once	98	146
Twice	108	142
Thrice	115	126
Four Times	127	112
cv (%) = 8.96	Sy = 5.41	$LSD_{0.05}$ = 11.53

Table 3. **Effect of genotypes and strategy of GA_3 application on flowering competence (% plants that flowered)**

Strategy	Genotype					
	PRG 066	PRG 092	PRG 062	PRG 068	PRG 686	PRG 687
Water	NF	NF	NF	NF	27	27
500 mg/L GA3 applied 30, 45 60 DAP	80-	67	93	80	100	100
750 mg/L GA3 applied at 30 and 43 DAP	60	60	93	93	100	100
1500 mg/L GA3 applied at 30 DAP	67	67	80	93	100	100
cv (%) = 27. 08		Sy = 14.17			LSD0.05 = 14.17	

Table 4. **Number of days to emergence (DAP) of first normal inflorescence and average number of flowers per plant as influenced by genotypes**

Genotypes	Days of Emergence	No. of Flowers per Plant
PRG 066	160	2
PRG 092	156	2
PRG 068	153	2
PRG 062	134	3
PRG 686	132	5
PRG 687	115	5
cv (%)	11.62	0.73
Sy	20.62	13.86
LSD0.05	23.76	1.5

Table 5. Effect of P and K interaction on the proportion of plants that flowered on GA_3-treated *C. esculenta* (cv. Kalpao)

Treatment Combination	Flowering Plants (%)
P_0 K_0	100
P_0 K_1	86
P_1 K_0	93
P_1 K_1	100
cv (%) = 11.82 Sy = 5.48	$LSD_{0.05}$ = 13.9

Table 6. Effect of genotype and fertilizer on number of flowers per plant treated with 1000 ppm GA_3

Genotype	With Fertilizer	Without Fertilizer
PRG 066	9	4
PRG 068	6	2
PRG 687	8	4
PRG 694	4	4
cv (%) = 11.82	Sy = 5.48	$LSD_{0.05}$ = 13.9

Table 7. Average number of flowers per plant as affected by N, P and K addition in GA_3-treated *C. esculenta* (cv. Kalpao)

	Without N		With N	
	W/O P	With P	W/O P	With P
Without K	4	3	2	4
With K	4	4	4	5
cv (%) = 14.39	Sy = 0.331		$LSD_{0.05}$ = 0.71	

Table 8. Effect of N, P and K fertilization on proportion (%) of flowers with pollens of plants treated with GA_3

	Without N		With N	
	W/O P	With P	W/O P	With P
Without K	21	22	13	31
With K	13	44	32	31
cv (%) = 61.91	Sy = 1.38		$LSD_{0.05}$ = 3.01	

Table 9. Effect of medium on germination of C. esculenta and number of replications with fungal growth (observed for 60 days)

Medium	Germination Percentage	Coefficient of Velocity	Number of replication w/ fungal Growth'
1% agar	71	0.070	3
2% agar	59	0.068	3
Soil extract agar	61	0.073	3
Soil + 1% agar	71	0.076	3
Soil + Distilled Water	70	0.077	0
Compost + Distilled Water	30	0.042	3
Tissue paper + Distilled Wataer	63	0.054	1
cv (%)	14.05	8.43	
Sy	6.82	0.004	
LSD0.05	14.05	0.01	

Table 10. **Effect of seed sources and soaking with 5% CaOCl on seed germination of C. esculenta (observed after 10 days)**

Seed Source (Genotypes)	Germination Percentage		Coefficient of Velocity	
	CaOCl	Distilled H_2O	CaOCl	Distilled H_2O
028-4	73	78	0.032	0.026
169-3_2	96	97	0.068	0.024
169-21	95	82	0.030	0.043
169-22	97	94	0.040	0.048
169-6	90	85	0.052	0.031
169-171	96	96	0.057	0.042
169-172	98	94	0.065	0.044
169-173	97	99	0.051	0.107
169-174	91	87	0.055	0.059
169-68	85	80	0.038	0.041
cv (%)		10.78		32.89
Sy		5.62		0.09
$LSD_{0.05}$		11.36		0.018

Table 11. Plant height (cm), yield (kg/plant) and corm dry matter content (%) of some progenies of either self or cross-pollinated plants

Parents[1] (PRG)	Number of Progenies (n)	Mean	S.D.	C.V. (%)
		Plant Height[2]		
688 x 686	94	162.18	40.38	24.9
688 x 105	28	168.89	32.15	19.04
688 x 006	16	147.25	36.24	24.61
244 x 218	21	166.81	25.37	15.21
690	13	108.31	19.89	27.6
		Corm Yield[3]		
686 x 068	50	0.21	0.07	33.81
374 x 100	8	0.23	0.04	16.43
265 x 068	8	0.24	0.09	36.13
263 x 213	12	0.24	0.07	29.9
094	28	0.26	0.16	61.54
687	22	0.22	0.11	50.60
169	40	0.32	0.40	31.25
		Dry Matter Content[4]		
686 x 068	50	28.26	5.24	18.56
374 x 100	8	23.83	16.17	67.85
265 x 068	8	32.11	6.36	19.82
263 x 213	12	29.48	8.19	27.78
094	28	30.10	8.94	29.70
687	22	24.37	4.0	16.41
169	40	37.38	6.87	18.39

1 Number refers to accession numbers based on the records of PRCRTC, ViSCA, Baybay, Leyte

2 Plant height measured from 10 sample plants at the single row trial evaluation

3 Yield measured as an average of 20 sample plants grown during the single plot trial

4 Dry matter content measured based from five sample plants at the single plot trial

Table 12. Some characteristics of "elite" progenies selected from 2000 seedlings based on single plot trials

Progeny Code[1]	Parental Source	Yield kg/plant)	Dry Matter Content (% FW)	Acridity[2] Scores
GO 049	PRG 094	0.39	39.83	6.9
GO 245	PRG 068	0.80	22.42	6.3
GO 236	PRG 068	0.30	24.23	6.66
GO 227	PRG 068	0.42	30.83	6.4
GO 186	PRG 028	0.61	24.00	6.1
GO 243	PRG 142	0.45	28.24	3.8
GO 250	PRG 142	0.47	22.50	5.7
GO 201	PRG 142	0.55	24.73	.nd
GO 211	PRG 142	0.44	25.73	.nd
GO 212	PRG 142	0.45	28.00	.nd
GO 214	PRG 142	0.44	15.50	.nd
GO 151	PRG 169	0.53	34.00	5.57
GO 153	PRG 169	0.48	40.33	7.48
GO 164	PRG 169	0.41	36.67	6.83
GO 217	PRG 169	0.44	21.07	.nd
GO 219	PRG 169	0.46	28.63	.nd
GO 134	PRG 687	0.43	20.00	7.3
GO 137	PRG 687	0.47	21.67	8.4
GO 140	PRG 687	0.41	31.07	6.92
GC 001	PRG 686 x 068	0.40	34.33	7.33
GC 221	PRG 687 x 006	0.50	28.57	.nd
GC 235	PRG 687 x 006	0.88	21.42	.nd
GC 241	PRG 686 x 006	0.58	20.20	.nd
GC 139	PRG 213 x 642	0.47	21.77	.nd
VG.1	check	0.39	39.87	6.6

[1] GO : progenies from naturally poliinated flowers
GS : progenies from artificially self-pollinated
GC : progenies from crosses

[2] Acridity scores based on the Hedonic scale;1, disliked very much; 9, liked very much; means computed from scores of 20 independent panelists; nd means not determined.

Table 13. Yield (t/ha) and dry matter content (%) of selected progenies evaluated under replicated trial

Progenies[1]	Yield (t/ha)	Dry Matter Content (%)	Acridity	Rating[2] Acceptability
GC 127	4.77	27.97	nd	nd
GC 119	4.77	25.41	nd	nd
GC 120	4.51	40.61	6.9	7.17
GC 117	4.48	37.43	nd	nd
GC 121	5.20	29.79	6.4	6.69
GC 125	4.32	38.66	nd	nd
GS 141	5.23	24.24	nd	nd
GC 122	4.77	27.45	nd	nd
GC 138	2.67	27.97	nd	nd
GS 137	4.17	28.88	nd	nd
GS 123	2.24	28.61	nd	nd
GC 134	1.95	32.28	nd	nd
GC 142	6.32*	32.87	6.4	7.08
GC 139	5.68*	28.08	nd	nd
GC 133	5.96*	32.20	nd	nd
GC 131	3.47	38.0	nd	nd
GS 140	3.95	36.48	nd	nd
Iniito local check	6.08	48.41	7.6	7.79
VG-1 standard check	5.12	38.94	7.4	7.74
Sy	0.0153	2.28		
HSD	0.078	12.38		
C.V. (%)	22.27	10.33		

[1] GC - progenies from crosses
GS - progenies from selfs

[2] Acceptability rating based on Hedonic scale: 1, dislike very much; 9, liked very much

Table 14. **Correlation coefficient values between stages of evaluation, in terms of yield and dry matter content of some taro progenies**

Stage of Evaluation	Single Plant	Single Row	Single Plot	Replicated Plot
		Yield		
		G-686 x 068 Progenies		
Single Plant	-	.(51)	(51)	(21)
Single Row	0.403	-	(51)	(21)
Single Plot	0.308^{*}	0.07^{ns}	-	(21)
Replicated Plot	0.334^{**}	0.656^{**}	0.142^{ns}	-
		PRG-094 Progenies		
Single Plant	-	.(33)	(33)	(20)
Single Row	0.277^{ns}	-	(31)	(20)
Single Plot	0.192^{ns}	0.057^{ns}	-	(20)
Replicated Plot	0.133^{ns}	0.622^{**}	0.261^{ns}	-
		Dry Matter Content		
		PRG-686 x 068 Progenies		
Single Plant	-	-	50	19
Single Plot	-	0.41^{**}	-	20
Replicated Plot	-	0.64^{**}	0.67^{**}	
		PRG-094 Progenies		
Single Plant	-	-	30	20
Single Plot	-	0.44^{*}	-	20
Replicated Plot	-	0.48^{*}	0.67^{*}	-
		General Computation		
		Yield		
Single Plant	-	.(115)	(114)	(59)
Single Row	0.314^{**}	-	(114)	(59)
Single Plot	0.019^{ns}	0.11^{ns}	-	(59)
Replicated Plot	0.192^{ns}	0.59^{**}	0.129^{ns}	-
		Dry Matter Content (%)		
Single Row	-	-	(110)	50
Single Plot	-	0.49^{**}	-	50
Replicated Trial	-	0.63^{**}	0.61^{**}	-

** highly significant; * significant; ns, not significant

Values enclosed in parenthesis refer to number of observation pairs.

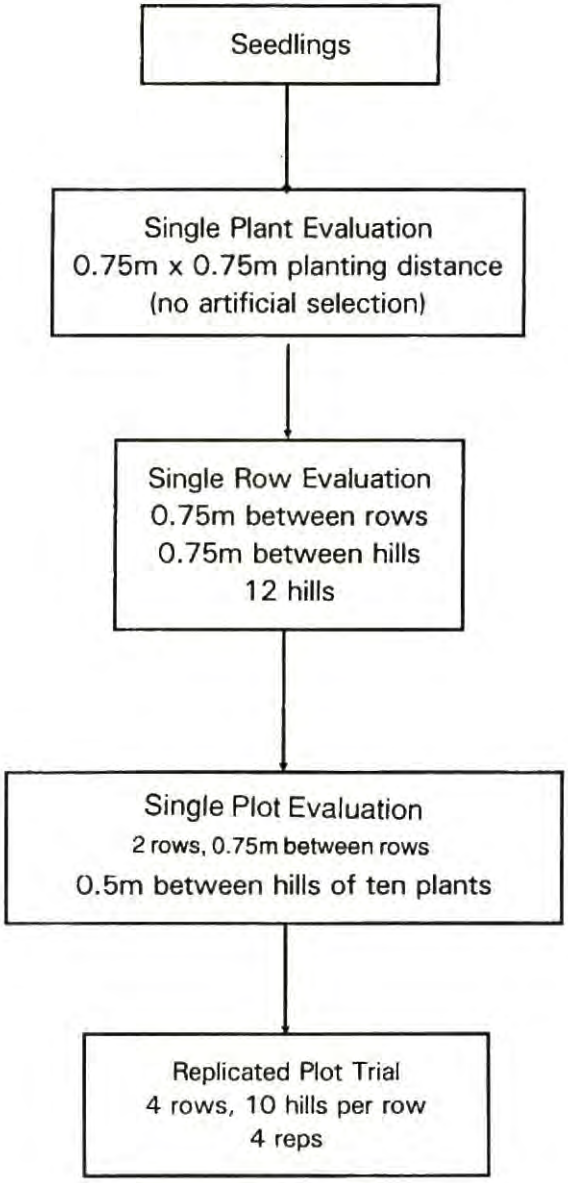

Figure 1. **Schematic diagram of the evaluational phase and flow of materials in a taro genotypic selection**

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Efficacy of *Trichoderma Aureoviride* Rifai as Biocontrol Agent for *Sclerotium Rolfsii* Sacc. Causing Stem Rot in Peanut (*Arachis Hypogea* L.)

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ABSTRACT

A study was conducted in the Department of Plant Protection, ViSCA, Baybay, Leyte to: (1) evaluate the antogonistic effects of different ***Trichoderma*** *isolates in vitro; (2) determine the effects of chemical pesticides on* ***Trichoderma*** *and* ***Sclerotium rolfsii in vitro;*** *(3) determine the most effective delivery system of* ***Trichoderma;*** *and (4) evaluate the efficacy of* ***Trichoderma*** *as biocontrol agent for* ***S. rolfsii*** *and compare it with a fungicide in the greenhouse and in the field.*

All isolates of Trichoderma except ***T. harzianum*** *IRRI were found effective as biocontrol agents for* ***S. rolfsii*** *under laboratory conditions, based on their ability to suppress the growth of the pathogen and their capacity to colonize the area where the pathogen had established.*

Generally, ***in vitro*** *evaluation of different chemical pesticides showed fungitoxic effect on* ***T. aureoviride*** *and* ***S. rolfsii,*** *although the sporulation of the antagonist was stimulated by Furadan 3G at 1000 ppm concentration level.*

Broadcasting T. aureoviride controlled stem-rot by Brassicol applied as soil treatment under greenhouse and field conditions. Furthermore, increase in yield of peanut was obtained when rice bran preparation of the antagonist was applied by broadcast method.

INTRODUCTION

The groundnut or peanut (*Arachis hypogea* L.) is one of the most important leguminous crops grown in the country today. It is high in calories due to its fat (47-50%) and protein (20%) content. Peanut kernel is also an excellent source of riboflavin, thiamine, nicotinic acid and vitamin E. In 1983, the Philippines produced 35 metric tons of unshelled peanuts equivalent to ₱175.4 million (**13**).

One of the major constraints to peanut production is the presence of diseases. The most common soil-borne pathogen attacking peanut is *Sclerotium rolfsii,* which causes pre- and post-emergence damping-off disease. This disease can economically reduce the yield of peanut by attacking the plant near the soil line, causing wilting and, eventually, death of the plant (**14**). A single infection can cause severe plant damage because this pathogen has extensive ectotrophic mycelial growth (**2**).

The use of pesticides in the control of *S. rolfsii* is widely employed and has been proven to be effective. Brassicol 75% WP, Dithane D-14 37.5% EC, Dithane Z78 75% WP, Benlate 75% WP, Vitavax 75% WP and Captan effectively control the disease when applied by seed dressing and soil drench method (**7, 16, 20**). However, due to the adverse effects of pesticides in the environment, it is imperative to use biological control as an integral part of the crop protection program.

One of the most promising groups of microorganisms which has antagonistic relationships to a number of soil-borne plant pathogens is *Trichoderma*. Species under this genus are active suppresants to some pathogenic fungi and their effect have been comparable to that of mercurial fungicides (**5**). *Trichoderma* was also found to be highly antagonistic to *S. rolfsii in vitro* and in greenhouse tests (**9**). One study found that under greenhouse conditions, one to three applications of *T. harzianum* effectively controlled *S. rolfsii* on blue lupines, tomatoes and peanuts (**19**).

Various methods of applying *Trichoderma* for the control of various soil-borne pathogens have been employed. The use

of diatomaceous earth granules for growth and delivery of *T. harzianum* significantly controlled *S. rolfsii* of peanuts when 140 kg/ha of the granules was applied to the soil surface. This is equivalent to that achieved using 10% PNCB granules at 112 kg/ha (**1**). Likewise, another study (**3**) revealed that fermentor biomass preparation of *T. viride* applied as dust to seed potatoes infested with sclerotia of *Rhizoctonia solani* before planting reduced the disease incidence by 50%.

Treating seeds with the biocontrol agent is also an effective method of delivery. Treating pea or raddish seeds with conidia of *T. hamatum* protected seed and seedlings from *Phythium sp.* or *R. solani,* respectively. This was nearly as effective as fungicide seed treatment using Captan or PNCB (**10**). Wheat-bran plus peat mixture preparation of *T. harzianum* efficiently controlled damping-off induced by *P. aphanidermatum* in peas, cucumbers, tomatoes and peppers. Furthermore, *T. harzianum* in a seed coating mixture containing 5 x 10^9 conidia per milliliter of the antagonist was as effective in sandy soil as the broadcast application of wheat-bran/peat preparation (**17**).

Some factors affecting the efficiency of *Trichoderma* in suppressing the population of the pathogens, like the use of pesticides applied to the soil, should be considered. Sprays used to control *Cercospora* leafspot of peanut reduced the natural population of *T. viride*, resulting in an increase in stem blight caused by *S. rolfsii* (**1**). Herbicide EPTC also reduced the biocontrol potential of *T. viride* on *S. rolfsii* (**15**).

Adding *Trichoderma* to the soil to control *S. rolfsii* is a potential non-chemical means of controlling plant diseases. Yet, efficiency of the biocontrol agent in pest control can not be obtained under all conditions, thus, integration with chemical pesticides may be deemed necessary. However, its compatibility with chemical pesticides in an integrated approach and delivery of the biocontrol agent using indigenous materials are still unreported, hence this work.

This study was conducted with the following objectives: (a) evaluate the antagonistic effect of different *Trichoderma* isolates *in vitro;* (b) determine the effect of chemical pesticides on *Trichoderma* and *S. rolfsii in vitro*; (c) determine the most effective delivery system for *Trichoderma*; and (d) evaluate the most pathogenic isolate of *Trichoderma* and compare its efficacy with a fungicide in the greenhouse and in the field and evaluate its effect on the yield of peanut.

MATERIALS AND METHODS

Isolation of *Sclerotium rolfsii* and *Trichoderma* sp.

Fifty-gram soil samples infested with *S. rolfsii* were collected. The soil was suspended and stirred in 1.0 L sterile water, poured in a 150-mm mesh screen and washed under running water until the water coming out from the sieve was clear. Particles remaining in the sieve were collected and were floated in a 200-ml beaker of water. Floating sclerotial bodies were placed in the petri plate containing solidified potato dextrose agar (PDA) and were incubated in the laboratory. When growth was observed, a mycelial disc was removed from the edge of an expanding colony and aseptically transferred to another petri plate. This was then incubated in the laboratory at room temperature as pure culture. Pure culture of *S. rolfsii* was maintained in PDA test tube slants.

For *Trichoderma* isolation, diseased sclerotia of *S. rolfsii* were collected from the soil planted with peanut previously described in the isolation of *S. rolfsii*. The diseased sclerotia were placed aseptically in the petri plate containing solidified PDA. The plate was incubated in the laboratory at room temperature. Once growth was observed in the medium, a colony of the fungus was transferred to another petri plate containing solidified PDA. Pure cultures of the different isolates were maintained in PDA slants and were sent to the Department of Plant Pathology, International Rice Research Institute (IRRI), College, Laguna for identification.

Effects of Different *Trichoderma* Isolates on the Growth and Development of *S. rolfsii in Vitro*

Three species of *Trichoderma,* namely: *T. harzianum, T. aureoviride* collected from Samar and Leyte and pure cultures of *T. harzianum* and *T. glaucum* from the International Rice Research Institute (IRRI), were evaluated *in vitro* for their potential as biocontrol agents against *S. rolfsii*. The experiment was arranged in complete randomized design (CRD) with five replications, with each replication having five sub-samples. The following were the treatments used: T0 - *S. rolfsii* alone; T1 - *T. glaucum* IRRI isolate + *S. rolfsii*; T2 - *T. harzianum* IRRI isolate + *S. rolfsii*; T3 - *T. aureoviride* Leyte isolate + *S. rolfsii*; T4 - *T aureoviride* ViSCA isolate + *S. rolfsii*; T5 - *T. aureoviride* Samar isolate + *S. rolfsii;* T6 - *T. harzianum* Samar isolate + *S. rolfsii;* and T7 - *T. harzianum* Leyte isolate + *S. rolfsii.*

Five-millimeter diameter mycelial discs were removed from the edge of expanding colonies of *Trichoderma* and *S. rolfsii* grown separately on plated PDA. The paired isolates of *Trichoderma* and *S. rolfsii* were placed aseptically on opposite sides of a 100-mm petri plate containing 15 ml PDA. These were incubated in the laboratory at room temperature. Growth of both organisms was oberved daily and the degree of antagonism was scored on a scale of 1-5 (**4**), as follows:

1 - *Trichoderma* completely overgrew the pathogen and covered the entire medium.

2 - *Trichoderma* overgrew at least two-thirds of the medium surface.

3 - *Trichoderma* and *S. rolfsii* each colonized approximately one-half of the medium surface (more than one-third and less than two-thirds).

4 - *S. rolfsii* colonized at least two-thirds of the medium surface and appeared to withstand encroachment by *Trichoderma.*

5 - *S. rolfsii* completely overgrew *Trichoderma* and occupied the entire medium surface.

Fourteen days after inoculation, the sclerotial bodies of the pathogen that developed were aseptically dislodged from the agar surface using sterile forceps and were placed in the petri dish containing solidified PDA to determine their viability.

Effects of Chemical Pesticides on the Growth and Development of *T. aureoviride* and *S. rolfsii in Vitro*

T. aureoviride Samar isolate and *S. rolfsii* were the organisms used as antagonist and pathogen, respectively, in this experiment. Six chemical pesticides, namely: Brassicol; Benlate; Furadan 3G; Nemacur 10G; Atrazine; and 2-4D Ester were used to determine their effects on the growth and development of *T. aureoviride* and *S. rolfsii.* Each chemical was mixed in autoclaved melted PDA in three different concentration levels, namely, 500 ppm, 1000 ppm and 1500 ppm. The pesticide-mixed PDA was poured onto a sterile petri dish and was allowed to solidify. A five- millimeter mycelial disc of *T. aureoviride* was removed from a five-day old culture and was aseptically transferred to the petri dish with poison-mixed PDA. Similar procedure was also followed in determining the effect

of chemical pesticides on the growth of *S. rolfsii, T. aureoviride* or *S. rolfsii* grown in PDA alone which served as the control.

All the treatments were incubated at room temperature, arranged in completely randomized design (CRD) and replicated five times. Growth of both organisms was observed daily. The number of conidia of *T. aureoviride* was determined 14 days after inoculation using a hemacytometer.

Evaluation of the Efficacy of the Antagonist in the Greenhouse

One hundred forty-four 20-cm pots were filled with sterilized soil and infested with mycelial suspension with 10 sclerotial bodies of *S. rolfsii* per pot. Five days after infestation, two peanut seeds were sown in each pot. The following were the treatments: T0 = *S. rolfsii* alone; T1 = seed dress with *T. aureoviride* + substrate; T2 = seed dress with Brassicol at manufacturer's recommended rate of 4g/kg seeds; T3 = seed dress with Brassicol and *T. aureoviride* + substrate at the rate of 10 g/200 grams peanut seeds; T4 = broadcast method with *T. aureoviride* + substrate; T5 = broadcast method + seed dress with *T. aureoviride* + substrate; T6 = broadcast method with *T. aureoviride* + substrate + fungicide seed treatment; T7 = broadcast method and seed dress method with *T. aureoviride* + substrate + Brassicol soil treatment; and T8 = Brassicol soil treatment.

Seed dress method with *T. aureoviride* was done by coating the seeds of peanut with a rice bran substrate containing approximately 3×10^9 conidia/ml applied at the rate of 10 g substrate/200 grams seeds. Then the treated seeds were air-dried and sown in potted soil.

Application by broadcast method with *T. aureoviride* was done by spreading 30 g of rice bran substrate containing approximately 3×10^9 conidia/ml in each pot before sowing the peanut seeds.

Seed treatment with Brassicol was done by placing the peanut seeds in plastic bags containing Brassicol powder. Then, the bag was shaken for 20 seconds until the seeds were fully coated with the fungicide. On the other hand, soil treatment with the fungicide was done by diluting the chemical in water using manufacturer's recommended rate of 50g/liter, then sprayed onto the potted soil. The concentration level of 500 ppm was used in fungicide treated treatments combined with *T. aureoviride*, either by seed dress or broadcast method. Plants in pots inoculated with *S. rolfsii* alone served as control.

Seventy-five days after sowing, disease incidence was taken. Developed sclerotial bodies of *S. rolfsii* were removed from the pots using the sieve-floatation method as previously described. The experiment was arranged in completely randomized design (CRD) in the greenhouse, and treatments were replicated four times with four sub-samples in each replicate.

Evaluation of the Efficacy of the Antagonist in the Field

The experiment was conducted in the experimental area of the Department of Plant Protection using the same treatments used in the greenhouse, except for broadcast method which was done by spreading 0.3 kg of the substrates in every row of the plot. Two weeks before planting, the field was artificially infested with *S. rolfsii* by pouring a four-liter suspension of the pathogen containing mycelia and 50 sclerotial bodies in each row of the plot with an area of 4 x 5 square meters.

The experiment was laid out in an area of 400 square meters using randomized complete block design (RCBD) with nine treatments each and replicated three times. Peanut seeds were sown at a distance of 25 cm per hill and 50 cm per row in an area of 4 x 5 square meters per treatment.

Data Gathered

I. Screenhouse Experiment

a) Number of diseased plants - All plants that showed symptoms such as neck rot, damping-off and fungal growth in the stem were classified as diseased.

b) Number of sclerotial bodies

II. Field Experiment

a) Number of infected plants - Any plant that showed symptoms in the stem or pods or with fungal growth of S. rolfsii was considered as infected plant.

b) Number of marketable and non-marketable pods - Pods without or with half-filled seeds were considered as non-marketable pods.

c) Weekly meteorological data on temperature, relative humidity and rainfall were also gathered.

RESULTS AND DISCUSSION

Description of Different *Trichoderma* Isolates

Among the five isolates collected, two were identified as *T. harzianum* Rifai while the other three were identified as *T. aureoviride* Rifai. The five *Trichoderma* isolates were designated based on their origin as follows: *T. harzianum* Samar; *T. aureoviride* Leyte; *T. harzianum* Leyte; *T. aureoviride* Samar; and *T. aureoviride* ViSCA. *T. harzianum* Samar and *T. aureoviride* Samar were isolated from diseased sclerotia of peanuts obtained from the Gandara Seed Farm (GSF), Western Samar while *T. harzianum* Leyte and *T. aureoviride* Leyte were isolated from soil infested with *S. rolfsii* and planted with peanut and were obtained from the Romualdez Experiment Station (RES), Babatngon, Leyte and Abuyog Experiment Station (AES), Abuyog, Leyte, respectively. *T. aureoviride* ViSCA, on the other hand, was obtained from the experimental area of the Farming Systems Development Project (FSDP-EV), ViSCA, Leyte.

All isolates obtained had ovoid conidia. The average daily growth of each isolate ranged from 13.7 mm - 19.7 mm. *T. aureoviride* Leyte showed the highest average daily radial growth of 19.7 mm, followed by *T. aureoviride* Samar at 17.7 mm. *T. aureoviride* ViSCA, *T. harzianum* Samar and *T. harzianum* Leyte had an average daily radial growth of 16.6, 17.4 and 13.7 mm, respectively.

Twenty-four hours after inoculation in petri plates with solidified PDA, all the isolates were observed to have developed mycelia. All *T. harzianum* isolates showed white, smooth-surfaced mycelial growth initially. As the culture matured, green pigments forming a narrow round band were observed. The medium where *T. harzianum* isolates grew changed its color from white to light reddish brown as the days progressed. On the other hand, all isolates of *T. aureoviride* showed white, cottony mycelial growth. Formation of green to dark green, wide and round bands occurred in all isolates as the culture matured. The reddish brown coloration of the medium took place ultimately in all *T. aureoviride* isolates.

Effects of *Trichoderma* Isolates on the Growth and Development of *S. rolfsii in Vitro*

Generally, 36 hours after inoculation, the growth of both the antagonist and the pathogen met at a common point in the petri plate in all treatments, with the former occupying a larger

space than the latter. Eight hours later, a zone of inhibition that ranged from 4-6 mm, appeared between the antagonist and the pathogen in treatments where *S. rolfsii* was grown together with *T. glaucum* (T1), *T. aureoviride* Leyte (T3), *T. aureoviride* Samar (T5), *T. harzianum* Samar (T6) or *T. harzianum* Leyte (T7) (Fig. 1). In contrast, no inhibition zone was observed in treatments where the pathogen was grown in combination with *T. harzianum* IRRI (T2) or *T. aureoviride* ViSCA (T4). Rather, an overlapping of growth between the two organisms occurred. The results suggest that treatments where a formation of zone of inhibition occurred had some biochemical reactions that arrested or interfered with the growth of the pathogen. Such biochemical reactions may be due to some enzymes or antibiotics secreted by the antagonist which are detrimental to the growth and development of the pathogen. *T. hamatum* produces the cell wall degrading enzyme B (1-3) glucanase and chitinase and may explain its ability to control diseases induced by *R. solani* and *S. rolfsii* (**6**).

Radial growth of the pathogen grown alone or in combination with each of the different *Trichoderma* isolates ranged from 8.92 - 41.0 mm 4 days after inoculation (Table 1). The highest radial growth of *S. rolfsii* was observed when it was grown alone, but this was reduced when these were cultured together with different *Trichoderma* isolates. There were significant differences among treatments. The shortest radial growth of *S. rolfsii* was observed when the pathogen was cultured together with *T. aureoviride* Samar (T5) but this was not significantly different when it was grown with *T. harzianum* Samar (T6), *T. aureoviride* Leyte (T3), or *T. glaucum* (T1). The difference in the growth of the pathogen when grown together with the different *Trichoderma* species was attributed to the inhibitory effects induced by some *Trichoderma* isolates and the fast growing ability of the antagonist that arrests or overruns the growth of the pathogen. Another study (**19**) similarly showed that growth of *S. rolfsii* ceased when edges of the pathogen and the antagonist met and the colonies of *S. rolfsii* were soon overrun with *T. harzianum* when grown together in a petri plate with V8 agar. Moreover, they described the findings as an adequate proof that the antagonist is capable of attacking and killing *S. rolfsii* in culture.

The development of sclerotial bodies by *S. rolfsii* was also affected by each of the *Trichoderma* isolates grown together with the pathogen.

There were also significant differences on the number of sclerotia produced by the pathogen among treatments (Table 1). The highest number of sclerotia was obtained when the pathogen was cultured alone. Among treatments where *S. rolfsii* was cultured in association with each of the different Trichoderma isolates, *S. rolfsii* paired with *T. harzianum* IRRI (T2) produced the highest number of sclerotia. The lowest sclerotial number was obtained when the pathogen was grown together with *T. harzianum* Leyte (T7), but not significantly different in treatments where the pathogen was grown together with *T. harzianum* Samar (T6), *T. aureoviride* Samar or *T. aureoviride* Leyte (T3).

Sclerotial bodies that developed from treatments where *S. rolfsii* was cultured together with each of the different *Trichoderma* isolates, did not germinate when seeded in petri plates with solidified PDA; instead, growth of *Trichoderma* dominated in the medium. However, 75% germination of sclerotia was obtained in treatment where the pathogen was grown together with *T. harzianum* IRRI (T2); but this was still lower than when *S. rolfsii* was grown alone where the germination was 100% (Table 1). On the other hand, *T. aureoviride* ViSCA showed a different reaction toward *S. rolfsii*. No zone of inhibition was formed when the antagonist was cultured together with the pathogen. However, eventually the antagonist outgrew the pathogen and consequently colonized the area previously occupied by the pathogen and its sclerotial bodies. Apparently, *T. aureoviride* ViSCA does not produce any antibiotic or enzyme that would inhibit the growth of *S. rolfsii*, but could colonize it by direct penetration of either mycelia or sclerotia. This observation conformed with the findings of Henis and Papavizas (1983) that hyphae of Trichoderma degraded sclerotia by directly penetrating the rind and cortex of sclerotia. Furthermore, Elad et. al. (1980) revealed that *T. harzianum* directly invaded the mycelia and sclerotia of *S. rolfsii*.

The degree of antagonism of the different *Trichoderma* isolates to *S. rolfsii* showed that all the *Trichoderma* isolates except *T. harzianum* IRRI colonized the area where the pathogen had established 14 days after seeding (Table 2). The result suggests that the six isolates of *Trichoderma* tested are effective biocontrol agents for *S. rolfsii* under laboratory conditions.

Effects of Chemical Pesticides on the Growth and Development of *T. aureoviride* and *S. rolfsii*

Among the six chemicals tested, only fungicides Benlate and Brassicol inhibited the growth of *T. aureoviride* and *S. rolfsii*, respectively, at 500 ppm, 1000 ppm and 1500 ppm con-

centration levels (Table 2 and Fig. 5). The effects of the different concentration levels of Brassicol and Benlate on the growth of *S. rolfsii* and *T. aureoviride* and the sporulation of the antagonist are also presented in Table 2. The fungicide Brassicol significantly reduced the growth and sporulation of *T. aureoviride* at a higher concentration level. Similar effect was also observed on the growth of *S. rolfsii* as the concentration levels of Benlate increased (Figs. 6 and 7). Backman and Kabana (1975) observed that Benlate controlled Cercospora leafspot of peanut but reduced the natural population of *T. viride*, resulting in an increase of stem blight caused by *S. rolfsii.*

The difference in growth and sporulation of *T. aureoviride*, as affected by the different levels of Brassicol, may be attributed to the fungicidal effect of the chemical. Similar finding was observed by Villanueva (1983) on the effect of Brassicol on the growth and sporulation of *Paecilomyces lilacinus* isolates, where higher concentration levels of the chemical (500 ppm-1000 ppm) reduced the sporulation and dry weight of the fungus. However, the fungicide can still be integrated with the antagonist for the control of *S. rolfsii* since Brassicol can effectively inhibit the growth of the pathogen and a considerable number of conidia of *T. aureoviride* can still be obtained at 500 ppm (Table 2). Peshney and Moghe (1980) observed that Brassicol inhibited the growth of *S. rolfsii in vitro.* Moreover, Dhannikar and Peshney (1982) reported that Brassicol effectively controlled *Sclerotium* wilt of groundnut by seed dressing and soil drench method.

The effects of the different concentration levels of nematicides Furadan 3G and Nemacur 10G on the growth of *T. aureoviride* and *S. rolfsii* and the sporulation of the antagonist are shown in Table 2. The different concentration levels of Furadan 3G did not significantly affect the growth of the antagonist but significantly influenced the sporulation of *T. aureoviride* and the growth of *S. rolfsii.* A significant increase in the sporulation of the antagonist was observed with 1000 ppm but it was lower with 1500 ppm of Furadan 3G (Table 2). The result suggests that at a 1000-ppm concentration level, the sporulation of the fungus was stimulated but was adversely affected when the concentration level was increased. This difference could be due to the carbon present in the nematicide that could be utilized by the fungus at certain concentration levels which may stimulate its sporulation. Certain predaceous fungi can utilize low concentrations of nematicides as carbon source and show an increased growth at certain concentrations under laboratory conditions (**12**).

The decreasing growth of *S. rolfsii* at higher concentration levels of Furadan 3G can probably be attributed to the pesticidal effect of the chemical, which slightly reduced the growth of the pathogen.

On the other hand, Nemacur 10G reduced the growth and sporulation of the antagonist compared with the control (untreated), although no significant differences were observed among the concentration levels used. Nonetheless, Nemacur 10G did not influence the growth of *S. rolfsii* at all concentration levels.

Herbicides 2-4D Ester and Atrazine significantly affected the growth of *S. rolfsii* and *T. aureoviride* as well as sporulation of *T. aureoviride* (Table 2). The trend was toward the reduction of growth and sporulation at a higher level of concentration except in Atrazine where the different concentration levels did not influence the growth of *T. aureoviride.* The differences observed in all treatments may be attributed to the toxic effect of the herbicides tested which resulted in a slight reduction of growth of *S. rolfsii* and sporulation of *T. aureoviride.* Herbicides Afalon and Sencor were shown to be fungistatic to *P. lilacinus* isolates only at higher concentration levels used (**18**).

Test for Efficacy of *T. aureoviride* Applied Alone or in Combination With Brassicol by Different Methods of Application Under Greenhouse and Field Conditions

Seeds that germinated 5-7 days after sowing showed vigor after one month in all treatments in both greenhouse and field conditions, since there was enough moisture for the growth and development of the plant. However, about two months after sowing, when plants started to flower, white mycelial growth was observed at the base of the stem close to the soil surface in the untreated control plants. This was followed by darkening of the stem and, consequently, wilting as days progressed.

Trichoderma aureoviride, applied in the soil by broadcast method and by seed dress method alone or in combination with Brassicol, provided 100% protection against stem rot of peanut caused by *S. rolfsii* similar to the control provided by Brassicol when applied as soil treatment (T_1, T_4, T_5, T_6, T_7 and T_8). However, when Brassicol was applied as seed treatment, 37.5% infection occurred. The disease incidence decreased to

15.6% when the fungicide was applied with *T. aureoviride* together as seed treatment (T_3) (Table 3). The high disease incidence when Brassicol was applied as seed treatment alone may be attributed to the consequent effect of daily watering of the plant which may have washed out or diluted the fungicide, thus reducing its concentration. The 15.6% infection obtained in treatment where the antagonist and Brassicol were applied together as seed treatment (T_3) was probably due to the fungicidal action of Brassicol which lessened the activity of the antagonist in controlling *S. rolfsii.*

Sclerotial bodies of *S. rolfsii* recovered from potted soil in each treatment in the greenhouse ranged from 10.00 - 142.75 (Table 3). The high number of sclerotial bodies recovered in treatments where the antagonist or the Brassicol was applied as seed treatment can probably be attributed to the distance between the seed where the chemical or the antagonist was placed and the initial source of *S. rolfsii*. This may be because the sclerotial bodies had already developed before the effect of the treatment took place. Meanwhile, the lowest number of sclerotia obtained in treatments where the antagonist was applied using the broadcast method without Brassicol (T_4 and T_5), was attributed to the considerable amount of the antagonist applied and its capacity to attack the initial and developed sclerotia of *S. rolfsii* thus, hindering their development. However, sclerotial bodies were slightly higher when broadcast method with *T. aureoviride* was combined with Brassicol either as seed or soil treatment and were not significantly different when Brassicol was applied to the soil (T8). The result suggests that the activity of *T. aureoviride* was reduced with the presence of Brassicol. This finding is supported by the effect of Brassicol on *T. aureoviride* in the laboratory experiment where it lessened the growth and sporulation of the antagonist.

All sclerotial bodies recovered from all treatments germinated in the planted PDA. However, 14 days after seeding, green pigments dominated the culture, indicating the colonization of *T. aureoviride* in all treatments where the antagonist was applied using seed dress or broadcast method.

Under field conditions, disease incidence ranged from 12.57% - 55.67%. Brassicol applied as soil treatment alone (T_8) or in combination with *T. aureoviride* (T_7) obtained the lowest percentage infection of 12.57% and 12.92%, respectively. This was, however, not significantly different in treatment where the antagonist was either seed dressed (T_2) or seed dressed plus broadcasted (T_5) or broadcasted alone (T_4), with respective diesase ratings of 14.92, 13.43 and 14.20% (Table 4). Slight in-

crease of infection was observed when the antagonist was applied in combination with the fungicide. The increase in the disease incidence when the antagonist was applied together with the fungicide may be attributed to the fungitoxic effect of Brassicol on *Trichoderma*.

Application of the antagonist by broadcast method has been tested and has been proven effective as a biocontrol agent. *T. harzianum* grown in diatomaceous earth granule impregnated with 10% molasses provided similar control with PNCB under wet conditions (**1**). Furthermore, a wheat-bran plus peat mixture (1:1) preparation of *T. harzianum* was found to efficiently control damping-off induced by *P. aphanidermatum* (**17**). Further explanation is that broadcast application of the antagonist is superior to seed coating application.

Peanut yield was significantly affected by the different treatments tested. Marketable yield of peanut ranged from 2.20 - 3.52 kg per plot and the different treatments were significantly different from one another (Table 4). The highest yield was obtained in treatments where the antagonist was applied by broadcast method alone or in combination with the fungicide and these were significantly different from the treatment where the Brassicol was applied alone by seed dress or soil treatment alone. The lowest marketable yield was obtained in the control (T9) and this could be attributed mainly to the detrimental effects of the disease on the growth of the plant as evidenced by a higher rate of infection. The disease also resulted in the production of more non-marketable pods in the untreated control plots (Table 4).

Significant increase in marketable yield in treatments where *T. aureoviride* was applied by broadcast method was probably due to the control provided by *T. aureoviride* against *S. rolfsii* and the nutrients derived from rice-bran necessary for the growth and pod production of the plant. Moreover, *T. aureoviride* was reported to be a good organic decomposer, which could have hastened the decomposition of organic materials in the field and consequently provided additional nutrients for the plant. Similar finding was also observed by Elad, Chet and Katan (1980) when wheat-bran preparation of *T. harzianum* increased growth of bean plant and effectively controlled *S. rolfsii*.

Table 1. The effect of different *Trichoderma* isolates on the growth and development of *Sclerotium rolfsii in vitro*

Treatment	Radial Growth (mm)[1]	Number of Sclerotia[2]	% Germinating of Sclerotia[3]	Degree of Antagonism[4]
Control (S. *rolfsii* alone)	41.00 a	90.52 a	100%	
T. glaucum /RRI + *S. rolfsii*	11.14 e	6.84 d	0	1.05 b
T. harzianum /RRI + *S. rolfsii*	32.72 b	56.40 b	75%	5.00 a
T. aureoviride Leyte + *S. rolfsii*	10.52 ef	3.32 e	0	1.05 b
T. aureoviride ViSCA + *S. rolfsii*	19.76 c	11.72 c	0	1.05 b
T. aureoviride Samar + *S. rolfsii*	8.92 f	2.40 e	0	1.00 c
T. harzianum Samar + *S. rolfsii*	10.48 ef	2.36 e	0	1.00 c
T. harzianum Leyte + *S. rolfsii*	14.08 d	1.46 e	0	1.05 b

[1] Measured 4 days after seeding; Means followed by the same letters are not significantly different at 5% level using Duncan's Multiple Range Test (DMRT).

[2] Counted 14 days after seeding

[3] Taken 21 days after seeding

[4] Degree of antagonism scored in a scale of 1-5 classes 14 days after seeding

Table 2. Suppression of *Sclerotium rolfsii* and *Trichoderma aureoviride* 14 days after treatment with different pesticides *in vitro*

Concentration Levels/ Pesticide	Radial Growth (mm)[1]		Conidial Count[2]
	S. rolfsii	*T. aureoviride*	*T. aureoviride*
Brassicol			
Control (untreated)	8.29 a	16.30 a	3.68×10^8 a
500 ppm	0.00 b	5.74 b	7.32×10^6 b
1000 ppm	0.00 b	5.50 c	2.75×10^6 c
1500 ppm	0.00 b	5.32 d	2.45×10^6 c
Benlate			
Control (untreated)	8.50 a	15.50 a	4.30×10^8 a
500 ppm	3.45 b	0.00 b	0.00 b
1000 ppm	3.13 c	0.00 b	0.00 b
1500 ppm	2.84	0.00 b	0.00 b
Furadan 3G			
Control (untreated)	8.36 a	15.08 a	5.76×10^8 b
500 ppm	8.27 b	14.99 a	5.69×10^8 b
1000 ppm	8.15 bc	14.98 a	6.65×10^8 a
1500 ppm	8.15 bc	14.95 a	5.37×10^8 c
Nemacur 10G			
Control (untreated)	8.52	15.88 a	7.58×10^7
500 ppm	8.27	13.19 b	6.61×10^7
1000 ppm	8.15	12.86 b	6.45×10^7
1500 ppm	8.12	12.83 b	6.01×10^7
2-4D Ester			
Control (untreated)	8.49 a	16.47 a	3.73×10^8 a
500 ppm	7.48 b	12.99 b	3.63×10^8 b
1000 ppm	7.28 b	12.42 b	3.16×10^8 c
1500 ppm	4.85 c	10.37 c	3.07×10^8 c
Atrazine			
Control (untreated)	8.45 a	15.42 a	7.98×10^8 a
500 ppm	6.57 b	15.18 a	6.90×10^8 b
1000 ppm	5.30 c	15.02 a	5.87×10^8 c
1500 ppm	4.72 c	14.93 a	4.52×10^8 d

[1] Average daily growth

[2] Mean number of conidia per ml of pesticide suspension after 14 days

Means followed by the same letters are not significantly different at 5% level using Duncan's Multiple Range Test (DMRT).

Table 3. Effects of the different treatments on percent infection by *S. rolfsii* and number of sclerotial bodies recovered from pots planted to peanut under greenhouse condition 75 days after sowing

Treatment	% Infection	No. of Sclerotial Bodies
S. rolfsii alone	100.0	142.75 a
Seed dress with Brassicol	37.5	34.25 b
Seed dress with Brassicol and *T. aureoviride* + substrate	15.6	28.25 bc
Seed dress with *T. Aureoviride* + substrate	0.0	33.25 b
Broadcast method + seed dress with *T. aureoviride* + substrate + Brassicol seed treatment	0.0	20.00 bcd
Broadcast method + seed dress with *T. aureoviride* + substrate + Brassicol soil treatment	0.0	19.25 bcd
Brassicol soil treatment	0.0	19.25 bcd
Broadcast method with *T. aureoviride* + substrate	0.0	14.25 cd
Broadcast method + seed dress with *T. aureoviride* + substrate	0.0	10.00 d

[1] Means followed by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test (DMRT).

Table 4. Effects of the different treatments on percent infection by *S. rolfsii* and yield of peanut under field condition

Treatment	% infection	Yield (kg /12 m2)	
		Marketable	Non-Mktbl.
S. rolfsii alone	55.67 a	2.20 c	0.44 a
Seed dress with Brassicol and *T. aureoviride* + substrate	21.33 b	2.72 b	0.14 d
Broadcast method + seed dress with *T. aureoviride* + substrate + Brassicol seed treatment	18.76 bc	3.17 a	0.13 d
Seed dress with *T. aureoviride* + substrate	18.76 bc	3.24 a	0.13 d
Seed dress with brassicol	14.92 cd	2.91 b	0.17 b
Broadcast method with *T. aureoviride* + substrate	14.20 cd	3.20 a	0.16 cd
Broadcast method + seed dress with *T. aureoviride* + substrate	13.43 cd	3.52 a	0.14 d
Broadcast method + seed dress with *T. aureoviride* + substrate + Brassicol soil treatment	12.92 cd	3.32 a	0.14 d
Brassicol soil treatment	12.57 cd	2.88 b	0.17 b

[1] Means followed by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test (DMRT).

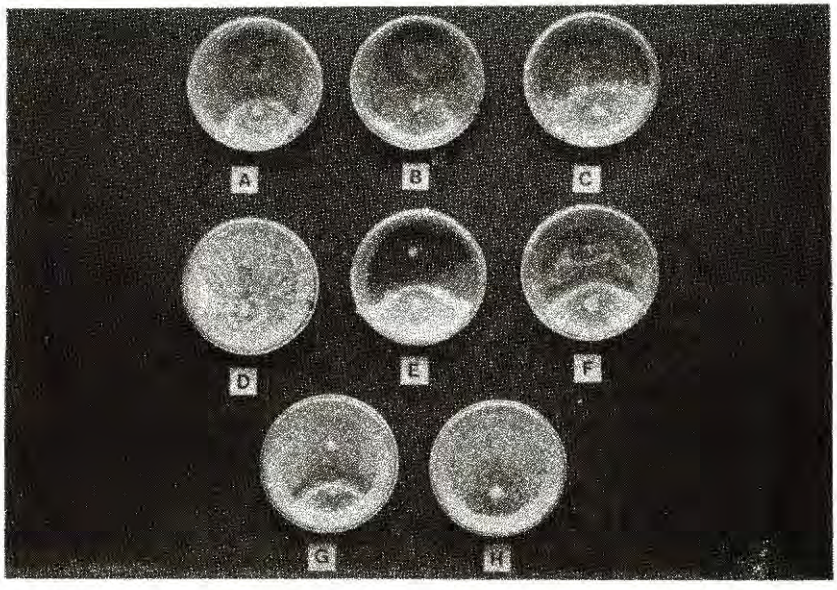
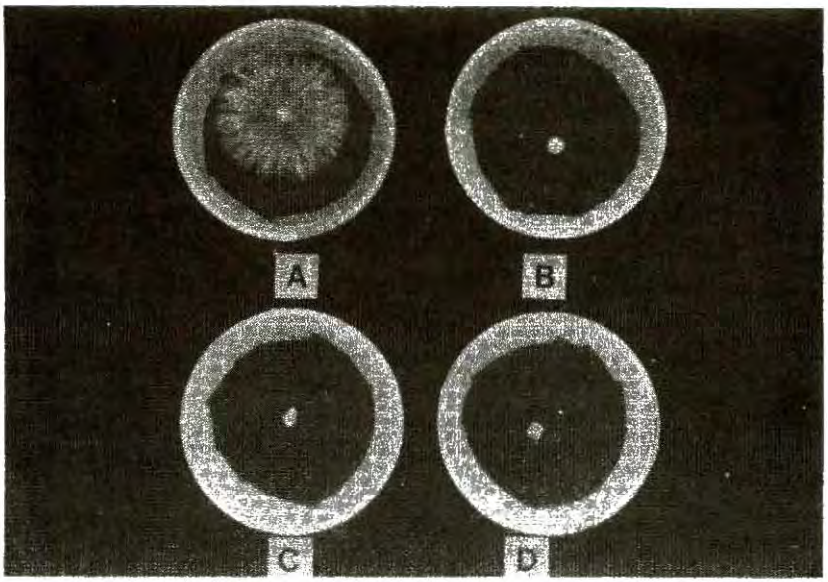
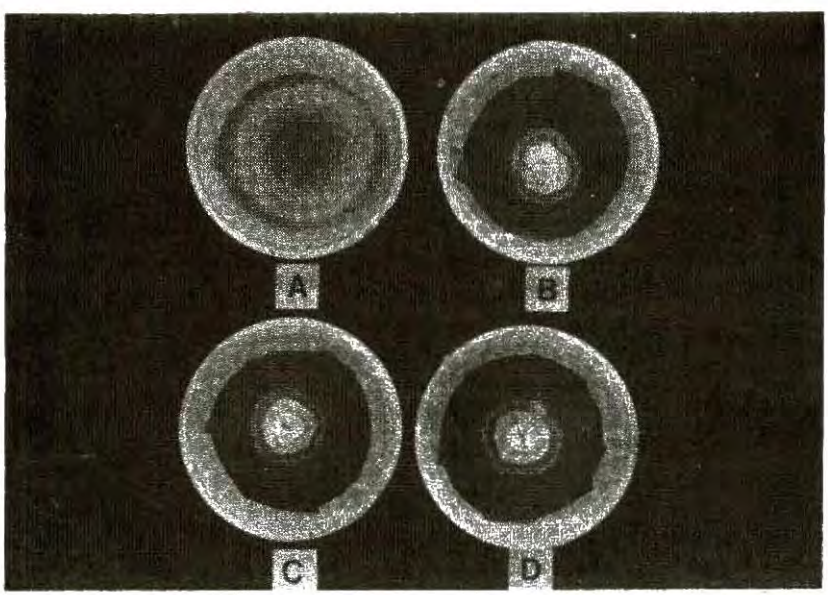


Figure 1. Growth pattern of *S. rolfsii* and different *Trichoderma* isolates 4 days (upper) and 22 days (lower) after seeding. Note the formation of inhibition zone between *S. rolfsii* and *Trichoderma* and colonization of the antagonist in the area. (A) *T. auroeviride* Samar + *S. rolfsii* , (B) *T. aureoviride* Leyte + *S. rolfsii,* (C) *T. aureoviride* ViSCA + *S. rolfsii,* (D) *T. harzianum* IRRI + *S. rolfsii,* (E) *T. harzianum* Leyte + *S. rolfsii,* (F) *T. harzianum* Samar + *S. rolfsii,* (G) *T. glaucum* + *S. rolfsii,* (H) *S. rolfsii alone*



Brassicol



Benlate

Figure 2. Fungitoxic effect of Brassicol and Benlate on the growth of *S. rolfsii* and *T. aureoviride* 3 days after seeding. (A) control (untreated), (B) 500 ppm, (C) 1000 ppm, (D) 1500 ppm.

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Evaluation of Local Ingredients (Fish, Shrimp, Snail and Leaf Meals and Ricebran) for Feeding Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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Abstract

Seven experimental diets using locally available ingredients (various combinations of fish, shrimp, snail, copra and leaf meals and rice bran) were tested in 21 aquaria measuring 30cm x 75cm x 30cm for 120 days. Treatments were randomly assigned to three aquaria, each stocked with 10 fish. Fish were fed 7 days a week at the rate of 5%of the fish's body weight and their daily feed allowances were adjusted at bi-weekly intervals on the basis of weight gain. Growth, survival rate, feed conversion ratios and protein digestibilities for each diet were compared. Statistical analysis indicated that of the diets tested, the greatest growth took place in those fish receiving feed II (23.2g) (15% shrimp meal, 60% rice bran, 14% cassava leaf meal), followed by those receiving feed VII (19.3g) (commercial chick starter mash). Lowest growth was observed in those receiving feed I (9.8g) (15% fish meal, 60% rice bran, 10% copra meal and 14% cassava leaf meal). Analysis of variance of the mean weight gain in all treatments yielded significant differences (P>0.05). Feed conversion ratios in all treatments were relatively poor, ranging from 4.9 to 6.9 as a result of poor digestibilities. They were comparatively low with the highest for feed II (62.7%) followed by feed VII (56.5%).

INTRODUCTION

Supplementary feeding plays a vital role in enhancing fish production. However, it constitutes a major item in the cost of production. The cost of feed in aquaculture can often exceed 50% of the total variable costs of production, rising to as high as 75% (Shang 1981). Feed costs could even increase considering that a lot of feedstuffs used for the purpose are imported. For locally produced materials only low quality items which cannot be used for human consumption are usually available. Hence, the need for optimizing the nutritional value and cost aspects of fish feeds is a promising area of research (Pillay 1979).

Jauncey and Ross (1982) have summarized the available information on tilapia feeds. The qualitative and quantitative aspects of supplemental feeding are well described by Hepher (1975) and Chervenski, Hepher and Tagari (1968).

The Philippines, being an agricultural-based country, has a number of agricultural by-products that can be used in animal feeds to reduce the price of well-balanced feeds. Though the kind of supplemental feed given depends on what is available and economical for the country, locally available ingredients should be screened as sources of feedstuffs, especially agricultural by- products and wastes. Cruz and Laudencia (1978) tested 14 rations containing ricebran, fish meal and various leaf meals in different combinations in aquaria-reared Nile tilapia fingerlings. Studies along this line were also done by Guerrero (1980), Pantastico and Baldia (1980) and Santiago et al. (1985) using different conventional and non-conventional feedstuffs as feed components.

This experiment attempts to compare the performance of different supplementary feeds from selected indigenous materials for Nile tilapia (*Oreochromis niloticus*) fingerlings. All materials used in the experiment are locally available in Albay Province, Philippines, but are little used in present tilapia culture activities. Moreover, their use in tilapia feeds has not been adequately investigated.

MATERIALS AND METHODS

Twenty-one aquaria, each measuring 30cm x 75cm x 30cm were filled with 25 cm of tap water each and left to

stand overnight. Each was stocked with 10 mixed-sex tilapia fingerlings. Initial weights at stocking were not significantly different ($P < 0.05$).

Aeration mechanisms were installed in all aquaria. Daily cleaning and water changing were done to prevent accumulation of fecal materials and to reduce algal growth.

Prior to feed formulation, proximate analysis was done on each of the selected indigenous feeding materials following the standard methods of AOAC (1975). However, the formulations were based purely on the percentage proportion of ingredients as practiced by local farmers in the region; the experimental diet therefore differed in protein content.

The aquaria were randomly arranged for seven feeding treatments with three replications each (Table 1).

The feeds were prepared and presented in dry mashed form. The fish were fed twice daily, seven days a week at the rate of five % of the fish body weight throughout the experimental period, adjusted at bi-weekly intervals by bulk weighing all the fish from each aquaria. Dead fish were removed but were not replaced by new individuals.

The final weight of each individual fish in each aquarium was measured after 120 days. The feed conversion for treatment groups was calculated as the ratio of the amount of feed given to the amount of weight gained. Mortalities were expressed as the total fish days per treatment. Average weight gain, survival rate and feed conversion ratio for each of the treatments were estimated and compared statistically using Completely Randomized Design (CRD) as outlined by Steel and Torrie (1960).

For determining the digestibility, the fish feed contained 1.0% chromic oxide. The fecal materials were collected 5 to 6 hours after feeding using a siphon and sieve. These were then freeze-dried and then oven-dried for 24 hours at 60 - 80°C. Chromic oxide determinations were performed after two weeks at the Institute of Fisheries Development and Research (IFDR), Diliman, Quezon City, Philippines.

RESULTS AND DISCUSSION

Average weight gain, feed conversion rates and survival rates are presented in Table 2. Figure 1 gives the growth

curves for all treatments and Table 3, the results of proximate analysis of the feed ingredients.

The greatest weight gain (23.2g) took place in those fish receiving feed containing 15% shrimp meal followed by those fish fed with chick starter mash (19.3g). Data obtained in feed III (15% Giant African snail meal, 60% rice bran, 10% copra meal and 14% cassava leaf meal) were discarded due to poor quality of water used for replacement on one occasion following a typhoon which caused high mortality. However, the results seem to be promising.

The relatively higher growth in ration II can be attributed to the higher protein content of the diet. This result suggests that at 30% dietary crude protein level, growth proved to be better. Similar observations were reported by Cruz and Laudencia (1978) on Nile tilapia fingerlings, which indicated the need for 20-30% protein in the ration for optimum growth of *Oreochromis mossambicus*. Juveniles increased with dietary protein levels up to 38% and 40%, respectively. In contrast, Santiago et al. (1986) showed that diets with higher protein contents did not necessarily result in better growth. This view is further supported by the findings of Chotiyarnwong et al. (1978) that growth of Nile tilapia fed with dietary levels ranging from 25%-45% was not significantly different. On the basis of protein source, Oke et al.'s (1977) investigation showed that shrimp wastes contained 78.7% digestible protein, were rich in lysine and methionine and therefore promoted good growth. Fowler and Bank (1976) suggest that 5% inclusion of shrimp meal is known to promote growth and inhibit the same at 20% level. In another experiment using ipil-ipil leaf meal and copra meal as the major protein sources, results showed a relatively poor growth response (Santiago et al. 1986).

The poor weight gain obtained in some groups may be due to poor feed acceptability and/or poor feed conversion. This can be substantiated by the presence of unconsumed feed particles during water change. However, no correlations were made on the unconsumed feed.

Feed conversion ratios were relatively poor as presented in Table 2. This can be expected since plant products are high in fiber that is relatively indigestible for fish. Fiber also affects

protein utilization (Lovell 1981). In the experiment, it is apparent that although protein ranges from 25%-34.7%, a substantial portion of protein supplied was conceivably used for energy rather than for growth. High percentages of indigestible components have been cited as causes for poor digestibilities and lowered availability of the energy content of plant feeds, as well as reduction in fish growth, feed conversion and efficiency, net protein utilization, condition and carcass fat (Buddington 1979; Appler and Jauncey 1983; Anderson et al. 1984) as cited by Hastings (1979). Further, considering that the stocks were of mixed-sexes, social interaction was observed especially in those fish ready for spawning. Their aggressive behavior i.e., fighting and chasing any fish swimming around them, was the usual cause of injury and subsequent mortalities. This also caused others to feed less or even totally not to feed, hence some feeds were wasted, leading to poor feed conversion and growth. A similar observation was reported by Wee and Tuan (1987).

Table 4 gives the crude protein content and protein digestibilities determined for seven feeds.

Results showed that feed II (30.8% C.P.) gave the highest growth and feed I (25.1% C.P.) the lowest, irrespective of protein source. Similarly, Cruz and Laudencia (1978), Santiago et al. (1986) also found that Nile tilapia diets with the highest protein did not necessarily produce the best growth. In addition, Jauncey and Ross (1982) stressed that tilapia was able to utilize protein below the optimum and still produce good growth.

Protein digestibility was highest for feed II (62.7%) and lowest for feed IV (10.9%). Again, it appears that high fiber content of these experimental diets incurred high digestive and metabolic costs. Austreng and Refstie (1979) and Cowey and Luquet (1983) as cited by Ash (1985) reported that protein was only useful to the animal if it could be digested and the degradation products (peptides and amino acids) absorbed. Therefore, if the true level of indigestible fibrous meterials is indeed very high, then this could adversely affect utilization and growth.

The cost per kilogram of feed ingredients are given in Tables 5 and 6, respectively.

Feed VII (Chick starter mash) had the lowest cost at ₱6.15 ($US1.00 = ₱20.00) followed by feed I (with 15% fish meal) at ₱7.20/kg. On the basis of its efficiency as feed in relation to cost, it would be worthwhile to replicate this work in the field to further justify its economic viability. Jauncey and Ross (1982) reported that the optimum dietary protein level in terms of growth was not necessarily optimum in economic terms. If the cost of feeds is beyond the reach of local farmers, they tend to use what is available and economical for the locality even if it would increase the time taken for the fish to reach marketable size. A recent survey instigated by PCARRD and ICLARM showed that most farmers, especially the poorer ones, buy their fish as and when they can afford (Smith 1985).

Despite the low digestibilities and poor feed acceptance in some groups as indicated by the presence of unconsumed feed, the study showed that locally available feed ingredients could be important alternative feed sources for tilapia. The present experiment also provides evidence that the formulation can still be improved by using two or more ingredient combinations with higher protein digestibility values. The study also showed that diets with 15% shrimp meal (feed II improved growth, however, cost proved to be limiting. Giant African snail meal (*Achatina fulica*), on the other hand, is a promising animal protein substitute considering that snails which can grow to over 30cm long are abundant in many parts of the world. Pantastico and Baldia (1976) reported a 25% increase in growth using chopped snails (*Stenomelania sp* and *Melanoidea sp*) and rice bran (30:70) in floating cages.

Rice bran (*Oryza sativa*) and copra meal (*Cocos nucifera*) are cheap and abundant agricultural by-products. These are, therefore, suggested to be some of the major ingredients for tilapia feed. Leaf meals, such as cassava, kangkong and gabi, are likewise recommended to be important alternative feed sources that may alleviate the feed shortage problem of the aquaculture industry. However, cost, ready availability, ease in preparation and maintenance of quality should also be considered. In general, all the tested feedstuffs are suitable ingredients for formulating tilapia feeds.

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Table 1. Percentage composition of seven experimental diets (Treatments I-VII) fed to Nile tilapia (*Oreochromis niloticus*) fingerlings in triplicate aquaria

	TREATMENT						
INGREDIENTS	I	II	III	IV	V	VI	VII*
Local fish meal (mixed species)	15			10			
Shrimp meal (local species)		15			7		
Giant African snail meal (*Achatina fulica*)			15	10	5	20	
Rice bran, D_1 (*Oryza sativa*)	60	60	60	50	50	50	
Copra meal (*Cocos nucifera*)	10	10	10	15	12	10	
Gabi leaf meal (*Colocasia esculenta*)				14	10	10	
Kangkong trimmings (*Ipomea aquatica*)					15	9	
Cassava leaf meal (*Manihot esculenta*)	14	14	14			5	
Vitamin mix, V-22 (Belman)	0.5	0.5	0.5	0.5	0.5	0.5	
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	

* Chick starter mash (AFSFA Feeds)

Table 2. **Average weight gain, feed conversion ratio and survival rate of Nile Tilapia (*Oreochromis niloticus)* fingerlings fed with experimental diets (Treatments I-VII) for 120 days in aquaria (For details see Table 1 and text.)**

Treatment	Average weight gain per fish (g)	Feed Conversion (feed/gain ratios)	Suvival[1] (actual numbers)
I	9.8[d]	6:6	7
II	23.2	6:7	9
IV	16.8[abc]	6:8	9
V	12.0[cde]	4:9	8
VI	16.6[be]	6:9	9
VII	19.3[a]	5:0	8

Treatment means with the same superscript letter are not significantly different ($P<0.5$; ANOVA and Duncan's Multiple Range Test).

[1] Deaths were due to aggressive territorial behavior particularly in Treatment III. Poor quality of water for replacement on one occasion ((following a typhoon) also caused several mortalities.

Table 3. **Proximate composition (%) of selected local feed ingredients used to formulate experimental diets for Nile tilapia (*Oreochromis niloticus*) fingerlings in the Philippines**

Feed Ingredient	Crude Protein	Crude Fat	Moisture	Ash
Local fish meal (mixed spp.)	23.1	9.3	19.8	14.5
Shrimp meal (local species)	47.9	5.0	14.8	20.9
Giant African snail meal (*Achatina fulica*)	27.9	7.8	15.7	11.1
Rice bran, D_1 (*Oryza sativa*)	5.7	2.4	13.0	15.0
Copra meal (*Cocos nucifera*)	18.2	6.6	9.1	7.3
Gabi leaf meal (*Colocasia esculenta*)	16.7	5.2	15.0	9.4
Kangkong trimmings (*Ipomea aquatica*)	13.5	4.4	13.2	14.6
Cassava leaf meal (*Mahinot esculenta*)	8.2	7.5	11.3	11.8

Table 4. **Percent crude protein content and determined protein digestibilities for seven experimental feeds given to Nile tilapia (*Oreochromis niloticus)* fingerlings in aquaria (For details of feed composition see Tables 1, 3 and text.)**

Feed	% Crude Protein	Protein digestibility
I	25.1	18.5
II	30.8	62.7
IV	32.1	10.9
V	33.1	28.1
VI	34.7	24.7
VII	36.5	56.5

Table 5. **Cost/kg in Philippine peso (₱) of various local ingredients used to make feed for Nile tilapia (*Oreochromis niloticus)* fingerlings: 1987 price when US $1.00 = ₱20.00 (as of September 1987)**

Feed Ingredient	Cost/kg (₱)
Local fish meal	3.50
Local shrimp meal	45.00
Dried ground Giant African snail flesh	4.50
Commercial rice bran, D_1	2.50
Commercial copra meal	3.50
Dried Gabi leaf meal	3.50
Dried whole kangkong	2.00
Dried cassava leaf meal	1.50
Vitamin mix, V-22 (Belman)	15.00/200g
Dicalcium phosphate	234.00/500g
Chick starter mash (AFSFA)	6.15

Table 6. Cost/kg in Philippine peso (₱) for seven feeds (I-VII) prepared from local ingredients for Nile tilapia (*Oreochromis niloticus*) fingerlings: 1987 price when US $1.00 = ₱20.00

	Cost/Item	Feed					
		I	II	IV	V	VI	VII*
1.	Ingredient cost	5.30	11.50	5.80	8.40	5.60	
2.	Miscellaneous cost**	0.10	0.10	0.10	0.10	0.10	
3.	Labor cost	0.05	0.05	0.05	0.05	0.05	
4.	Transportation cost	1.75	1.75	1.75	1.75	1.75	
	Total Feed Cost	7.20	13.40	7.70	10.30	7.50	6.15

* AFSFA Chick starter mash

** Refers to the cost of rubber bands, plastic bags, etc.

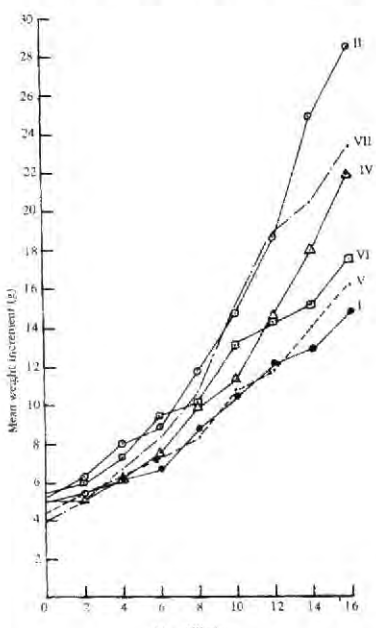

Figure 1. Growth curve for Nile Tilapia (*Oreochromis niloticus*) fingerlings fed with seven different feeds (I-VII) based on local ingredients in Albay Province, Philippines. The plotted points are means of triplicate bulk weights of groups of 10 fingerlings from separate aquaria. The bars around the final weights are 95% confidence limit from individual weights of all survivors.

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Exploitation Rate, Yield-per-Recruit and Virtual Population of Sinarapan *(Mistichthys luzonensis Smith)* in Lake Manapao, Buhi, Camarines Sur

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ABSTRACT

*Growth parameters of the von Bertalanffy function and mortality coefficients of the exponential decay model were estimated for Sinarapan (**Mistichthys luzonensis** Smith) in Lake Manapao, the only natural habitat where the minute goby is abundantly thriving. These parameters were used to estimate exploitation rate, relative yield-per-recruit, and together with catch data, virtual population of the freshwater goby.*

These biological information have not yet been reported for Sinarapan, the "world's smallest commercial fish", which is threatened with extinction. For an overall assessment of these results, the Sinarapan stock investigated can be said to be still viable for Lake Manapao sanctuary. The implications of these quantitative inputs as scientific bases for the rational management of Sinarapan in the lakelet were discussed. This gains greater significance in view of the near-total collapse of Sinarapan stocks in Lakes Buhi and Bato where the species once exceedingly abounded.

INTRODUCTION

Knowledge of the vital population parameters of a finfish resource is essential to its rational management. Such data become especially needed if the fishery has experienced disadvantageous complexities. The sad case of Sinarapan (*Mistichthys luzonensis* Smith), the "world's smallest commercial fish in Lake Buhi and Bato, Bicol Region, is a vivid example. The minute fish was amazingly abundant in both lakes in the 1930s to the 1960s but almost disappeared from 1979 to the present.

The exact mechanism of its disappearance is not known. Two major hypotheses have been offered by Gindelberger (1981b) for the near-extinction of Sinarapan population in Lake Buhi, namely: (1) the overfishing by motorized Sakag; (2) the collapsible Y-shaped push net; and (3) the predation by *Oreochromis mossambicus*. Reliable data (i.e., size-frequency and catch and effort data from well-planned surveys) to substantiate these reasons are, however, not available. This is coupled with the general lack of published information on the species.

During Sinarapan's heyday no one ever thought of the ruinous turn of events its fishery would take a decade hence. The motorization of Sakag, employees of the then Bureau of Fisheries and Aquatic Resources (BFAR) and artisanal fishermen agree, overfished both lakes and destroyed the breeding, feeding and refuge areas of the tiny goby. Today, there is only one remaining natural freshwater body where the wondrous goby is abundantly thriving; this is the site of the present work.

Lake Manapao (3.75 - hectare area and 7.6 - meters mean water depth) is a tarn or mountain lakelet in San Ramon, a barangay in the hinterlands of Buhi, Camarines Sur. It is 102 m above sea level and lies approximately $13^{0}26'$N, $132^{0}29'$E. It was probably formed, together with Lake Katugday, during Mt. Iriga's last eruption in 1641. Despite being declared as a sanctuary for Sinarapan by virtue of a municipal ordinance in 1982, Manapao is still being fished of the endangered species by fishermen residing along the mountain slopes near the lakelet. Tilapia, common carp, mudfish and catfish constitute the bulk of the catch. Sergistid shrimps, aside from tilapia and mudfish, which are suspected to prey on the minute goby, can also be found. Another lakelet, which is probably inhabited by Sinarapan, is May Danao (4 - hectare area aprroximately) lo-

cated between the northwest of Malinao, Albay and Buhi. Katugday (2.66 - hectare area), another tarn about a kilometer southwest of Manapao, was also declared a reserve area for goby in 1982 but was disastrously deplated of the fish six years later. This near-total collapse of the stock was attributed by the residents living near the lakelet to the inorganic pesticide applied by a villager who wanted an easy catch of the tilapia and common carp. Repeated sampling in the site by this author confirmed the virtual absence of Sinarapan.

The urgency to preserve Manapao is quite clear. Towards this end, assessing the condition of the endangered fish in the sanctuary will be an important step in formulating an effective management scheme for Sinarapan in its natural abode. Introduction of the goby to Lakes Buhi, Bato and Katugday for possible repopulation will make Manapao the only viable source. Two other lakelets at the foot of the now dormant Mt. Iriga, namely Pigiriron and Makuwaw, are suspected to be inhabited by this goby. Since early 1989, in Lake Bato, the goby has been showing signs of positive recovery from its grim condition a decade ago. The quantitative inputs which were obtained can also serve as scientific bases for managing other Sinarapan stocks in the future. The preliminary nature of the estimates here is recognized in view of the absence of probable validating data (eg. results from aging of hard parts) and the lack of published works on the fish.

As the world's "smallest commercial fish", some resulting parameters can represent extreme values in fishery biology work. The test of the applicability of length-based methods to a fish so minute that many larval features are still manifested in the adults will also be a significant attempt. It is also interesting to evaluate the possible impact of these results on some empirical equations, mostly based on medium and large fishes, being used today in stock assessment studies. And since all fish stock assessment works to date have dealt with sizes ranging from 10^0 - 6 grams, the present work hopes to fill a significant gap in the picture.

REVIEW OF LITERATURE

There are very few published studies on Sinarapan. The monograph by its discoverer, H.M. Smith (1901), presents a description of the external morphology of the goby and a gen-

eral discussion about its habitat. Pauly (1982b) demonstrated, assuming a condition factor of 0.6 and 1.18 as the total length/standard length ratio as applied to data by Te Winkel (1935, Table 1), the limiting role of gill size on the growth of Sinarapan. The reduced size of its organs was found to be correlated to its small body (Te Winkel, 1935). Its elongated body is covered with deciduous ctenoid scales extending from the first dorsal to the posterior margin of the gill cover (Herre 1927). It can be distinguished from other similar looking freshwater goby by its three-spined first dorsal fin. Sinarapan is a carnivore evidenced by its teeth on the jaws and the pharynx and its exceptionally short digestive tract (Te Winkel 1935). Gindelberger (1981a) reported that the minute fish is not endemic to Lakes Buhi and Bato but can also be found along the Bicol River system. In a 10- month study by Traichaiyaporn (1985) in Lake Katugday, she identified 53 phytoplankton species which constitute 30-50% of the stomach content of the goby. But she suggested that the ingestion of the phytoplakton was incidental to the swallowing of the "zooplankton which they selectively feed on.

Sinarapan is a delicacy and of utmost economic value to people residing along the lakeshore areas of Lakes Buhi and Bato, who according to Gindelberger (1981b), eat this minute fish for breakfast, lunch and dinner. The collapse of the commercial Sinarapan fishery of Lake Buhi in early 1979 displaced some 200 subsistence fishermen from their major source of livelihood (Gindelberger 1982).

MATERIALS AND METHODS

The data used in the various analyses consisted of the following: (1) 10-month length-frequency data collected from May 1988 to April 1989; (2) 12 months total catch data (in kg) of Sinarapan collected through recall interviews during the same period (Table 1); and (3) length-weight data of 151 representative samples during the 12-month period (Table 2).

A Sakag was used to collect Sinarapan samples from Manapao. The small catchment area of the lakelet (c.3 - hectare) allowed the reliable execution of stratified random sampling procedure. Two strata were designated based on a preliminary sampling in March 1988. Allocation of hauls in the strata was done such that more hauls were obtained in high fish density stratum.

Selectivity caused by length of the Sakag can be safely assumed to be very minimal owing to its small mesh (1 mm square mesh). The sampling gear was operated by thrusting 2/3 of the total length of its two-pole supports into the water; the push net, positioned in the boat's bow, was pushed forward while the boat was paddled. Hours it took per haul were counted and managed to be kept constant. Organisms other than Sinarapan (eg. tilapia, shrimps, other gobies) were sorted out of the catch. The team was unable to use the motor to propel the boat because the sampling site is terribly inaccessible to transport facilities. The speed of the boat was, theoretically, not kept constant.

There were 9 to 10 hauls per month, all taken during one sampling date at 0800-1100 hours. Absence of length-frequency samples in August and September 1988 was due to the heavy rains which deeply flooded the road to the site. This necessitated the direct interpolation of length frequencies for these months based on length frequencies for July and October 1988, as complete length-frequency data are required for Virtual Population Analyses (VPA).

Length measurements ($\pm$ 0.5 mm) were done under a magnifier, using an improvised fish-measuring sheet calibrated with a vernier caliper. During the analyses, however, the length-frequency data were grouped into 1.0 mm class interval forming 20 classes. The same class interval was used for the length-weight relationships analysis. Length (Li) and weight (Wi) data were fitted to a power regression model; the coefficient of determination and the computed t-value were assessed using the criterion by Sachs (1974, as cited by Pauly 1984). Berried female fish was not included for the length-weight data because such condition can overestimate its fresh weight. Samples were weighed on an analytical electronic balance to the nearest 10^{-6}g.

The length frequency data for 10 sampling months were filed and stored in a format accessible to other ELEFAN programs (ELEFAN I, II, and III) through a routine in ELEFAN 0 of Compleat ELEFAN (Gayanilo et al., 1988).

The von Bertalanffy Growth Formula (1938; to be referred as VBGF) parameters L^{∞} (asymptotic length) and K (growth constant) were estimated using ELEFAN I (Pauly et al., 1983 a) with t-sub-zero taken as equal to zero. K indicates the pace by which the difference of its maximum attainable size L^{∞} or

W_∞) and size at a given time (Lt or Wt) narrows. The instantaneous rates of natural (M) and total (Z) mortalities were estimated from the empirical equation of Pauly (1980b) and through catch curve analysis, respectively, both via ELEFAN II (Pauly et al. 1983b). Preliminary estimates of VBGF parameters were obtained through the modified (Pauly 1986a) Wetherall method (1986). Exploitation rate (E) of Beverton and Holt (1966) was computed as $E = F/Z$, with the fishing mortality $F = Z - M$. An $E = 0.5$ taken as an optimum exploitation level (Eopt) assuming that maximum Y'/R is achieved when F (i.e., Fopt) is approximately equal to M and that no stock-recruitment relationship exists (Gulland 1971; Pauly 1980a).

Two versions of VPA were used,namely: (1) the functional equivalent of Length Cohort Analysis (Pauly 1984) by Jones (1981); and (2) and the Length-Structured VPA (Pope et al., MS), also termed as VPA II and III, respectively, in the Compleat ELEFAN (Gayanilo et al. 1988).

The estimation of relative yield/biomass-per-recruit (Y'/B'-PR) was done using the model of Beverton and Holt (1966) as modified by Pauly and Soriano (1986) to account for wide selection exhibited by the investigated species.

RESULTS AND DISCUSSION

The results of the regression via power fit of total length to total fresh weight for mixed sexes are summarized in Table 3. The tabulated t-statistics for 149 degrees of freedom at 1% error level is 2.576. The computed "b" (= 3.499) significantly differed from 3, indicating allometric growth.

Table 4 gives the values of L_∞ and K initially estimated through the Modified Wetherall plot, optimized via ELEFAN I and adjusted using the equation of Munro and Pauly (1983). The relatively high K value obtained is typical of most short-lived tropical fish (Pauly 1978).

Total mortality coefficient obtained through catch-curve analysis was 9.234/year. Annual M and F estimated were 6.201 and 3.033, respectively. It must be emphasized that the estimate of M is an extrapolated (guessed) value from the method of Pauly (1980b) in terms of L_∞ used. Exploitation ratio is equal to 0.328 indicating moderate underexploitation of

the Manapao stock. The current fishing level of Sinarapan, due mostly to concealed operations, did not adversely affect its population as evidenced by the relatively low E. This condition, together with the possible growth compensation due to high M, may have resulted in the favorable growth of the goby. It indicates fast turnover rate which can sustain similarly high F levels. However, this does not suggest that fishing of Sinarapan in the sanctuary should be encouraged except at a strictly very minimum level for experimental purposes. An additional theoretical consideration is that the assumption of Eopt = 0.5 may possibly overestimate potential yields by a factor of 3-4 in small, short-lived tropical fishes (see Beddington and Cooke 1983). Based on the latter, the Sinarapan stock studied is overexploited. Similar to some findings (Beverton and Holt 1959), the high M corresponded to a high growth (K = 2.25/year).

The high M may be due to predation. Sinarapan does not guard its eggs (Gindelberger 1981a). After the female extrudes ripe eggs into the water (some may be attached to the roots of vegetation) for the male to fertilize, the fertilized eggs are left to hatch. This makes the eggs very vulnerable to predators and the adverse effects of environmental factors.

Inputs for Y'-PR versus E curve analysis are $L\infty$ = 24.6 mm, K = 2.25/yr, M = 6.201/yr and approximate length at first capture (Lc') equal to 15.849 mm. The B'-PR corresponding to Emax and E0.1 (exploitation rates corresponding to maximum Y'-PR, and to the point at which marginal increase of Y'-PR from an additional unit of effort is 0.1 of its value at E = 0, respectively) are given in Table 5. The increasing E (hence F) from the present level to Emax can result in a corresponding increase of Y'-PR recruit by about 15%. This is not a substantial increment if the potential dangers of increasing fishing pressure are to be considered. Y'-PR at current E is lower than Y'-PR at E0.1. B'-PR at current E is greater than B'-PR at Emax. These are desirable considerations to maximize yield from an open fishery but it is not strictly the case for the Manapao sanctuary. Furthermore, the models which derived these indices can also be used to evaluate the effects of varying F to M using empirical data which are absent to date. The use of empirical data in all these analyses should be preferred over projections based on con-

ventional assumptions for a basic reason. Sinarapan is a virtually unstudied species and relevant information on stocks of this goby in other areas is very limited. The matter of biological interaction (eg. predation by tilapia, mudfish and shrimp of Sinarapan), which is ignored in the length-based models used, needs equally important attention.

Tables 6a and 6b present summary results of VPA II analysis. All values were smoothed over an average of three. These outputs reflect a rather wide length range over which F increases (11.5-24.5 mm) which is more an effect of recruitment rather than gear-related (ie., improved efficiency). The vessel and gear, including the persons conducting the sampling, had been the same from the preliminary survey to the end of collection period. The bulk of catch (in number), but of lesser weight, is composed of small fishes (4-7 mm) with which the major portion of the population's length classes toward this extreme value partly coincides. Peak of stock biomass in September to November matches well with stable peak of F and such period was also the rainiest in the area. More and larger fish constitute this catch. Smaller ones seem to comprise the catch immediately prior to this period. It has to be emphasized that the low proportion of the catch data relative to the total standing stock is a major constraint to making conclusive statements on the virtual population estimates. As a sanctuary, this data requirement can hardly be met.

CONCLUSION AND RECOMMENDATIONS

Lake Manapao is a Sinarapan sanctuary in the sense that the famed goby, now virtually depleted in Lakes Buhi, Bato and Katugday, is still abundant here. The relatively low exploitation rate and its consistency with the mortality parameters are logical indicators of the stability of the stock. Yield/biomass-per-recruit analyses also point to this favorable condition. Results of the Virtual Population Analyses are very preliminary due to the inherent shortcoming of the data mostly due to the nature of the management scheme being adopted in the reserve area (i.e. fishing ban for Sinarapan). However, useful insights have been gained. The assumptions with

which the validity of the methods used here are tied-up, are well understood.

With the almost total disappearance of the Lakes Bato, Buhi and Katugday stocks, Lake Manapao is the only viable source of Sinarapan. This has been the concern of authorities, specifically the Fisheries and Aquaculture Divisions of the Department of Agriculture which has caused them to act. The biological inputs obtained can serve to guide the technicians of these agencies to rationally manage and protect the sanctuary. Sad to say, the case of the extinction of Sinarapan draws attention only proportionate to its diminutive size.

Additionally, the following specific studies/activities are very relevant for Manapao's rational management: (1) monthly monitoring of Sinarapan population size and biomass; (2) stomach analyses of tilapia, mudfish, catfish, shrimp and common carp caught; (3) observation of the feeding and food habits of Sinarapan; (4) indoor experiments on predator-prey relationships between Sinarapan and its suspected predators; (5) study of the life cycle of the goby; and (6) periodic monitoring of environmental parameters of the sanctuary.

Social factors vis-a-vis the biological aspects considered above must be looked into as their importance was demonstrated in the case of Lake Katugday. This can include the conduct of an information campaign concerning the dire need to maintain Manapao sanctuary and preserve Sinarapan for the 12-15 families residing near the lakelet. Positive results may not be immediately apparent but it is through this process that a clearer perspective of the problem involving socioeconomics of the people, on one hand, and the need for conservation for future benefits which may not be obvious to them at present, on the other, can be visualized.

Table 1. **Total monthly catches of Sinarapan from Lake Manapao (Data were gathered through interview of residents near the lakelet.)**

Date (mo/yr)	Catch (kg)
5/88	160
6/88	60
7/88	60
8/88	60
9/88	60
10/88	360
11/88	60
12/88	60
1/89	60
2/89	120
3/89	60
4/89	60

Table 2. Data for establishing the length-weight relationship of Sinarapan from Lake Manapao

Total Length (mm)	Fresh Weight (g)		n
	Range	Mean	
8.0	.00318	.00318	1
9.0	.00417 - .00516	.00458	4
9.5	.00582	.00582	1
10.0	.00561 - .00692	.00628	4
11.0	.00743 - .00882	.007865	4
11.5	.00869 - .00979	.00924	2
12.0	.00854 - .01266	.010256	10
12.5	.00985	.00985	1
13.0	.01207 - .01520	.013438	5
15.0	.02367 - .03410	.029416	3
16.0	.02466 - .04743	.035729	12
16.5	.03481 - .04141	.037423	3
17.0	.03433 - .07414	.044198	23
17.5	.02849 - .05235	.042604	15
18.0	.02043 - .06828	.049230	32
18.5	.04034 - .06275	.050177	8
19.0	.04312 - .07589	.055039	16
19.5	.05510 - .06163	.056616	3
20.0	.04005 - .05432	.047076	3
20.5	.05880	.05880	1

Table 3. **Results of length-weight power regression analysis (weighted by class sample n_i) for *M. luzonensis* from Lake Manapao**

Formula	$Y = a \bullet xb$
X -variable	Total length (mm)
Y- variable	Fresh Weight (g)
a	1.947×10^{-6}
b	3.499
r^2	0.9797
t_c	12.097
$t^*_{oo1 \bullet 01}$	2.756

Table 4. **Asymptotic length (L^∞, mm) and growth constant (K,yr-1) estimated and used in the various analyses**

METHOD	L_{oo} (mm)	K (yr^{-1})
Modified Wetherall Plot	22.658	2.599
ELEFAN I		
i) using uncorrected length frequencies	24.7	2.2
ii) using length frequencies corrected for incomplete recruitment and/or gear selection	24.6	2.25
ELEFAN III	25.5	2.094

Table 5. Results of relative yield per recruit and relative biomass per recruit analyses of *M. luzonensis* in Lake Manapao using the probabilities of capture of Sakag for December 1988; Optima are E_{max} = 0.5143, $E_{0.1}$ = 0.4613 and $E_{0.5}$ = 0.2550 (Parameters used were L_{oo} = 24.6 mm and M/K = 2.756.)

E	Y' /R	B' /R	E	Y' /R	B' /R
0.05	.0025779	.902433	0.55	.0136198	.205170
0.10	.0048834	.809785	0.60	.0133333	.163607
0.15	.0069164	.722125	0.65	.0128191	.126990
0.20	.0086774	.639510	0.70	.0120820	.095199
0.25	.0101676	.561991	0.75	.0111207	.068087
0.30	.0113894	.489608	0.80	.0099220	.045495
0.35	.0123450	.422391	0.85	.0084447	.027271
0.40	.0130143	.360350	0.90	.0065845	.013337
0.45	.0134811	.303486	0.95	.0040784	.003883
0.50	.0136715	.251773	1.00	.0000057	.000000

Table 6a. Catch (in number), population size and fishing mortality by length class for *M. luzonensis* outputted through VPA II

LENGTH CLASS	CATCHES (N)	POPULATION (N * 10^1)	F. MORTALITY
4.00 - 5.00	1,851,959.06	6,226,026.00	1.4245
5.00 - 6.00	1,219,389.63	5,234,634.00	1.0624
6.00 - 7.00	1,083,034.31	4,400,977.00	1.0711
7.00 - 8.00	760,169.62	3,665,646.80	0.8569
8.00 - 9.00	485,563.89	3,039,534.50	0.6248
9.00 - 10.00	463,637.85	2,509,047.00	0.6847
10.00 - 11.00	483,005.46	2,042,791.25	0.8286
11.00 - 12.00	446,828.93	1,633,023.00	0.9029
12.00 - 13.00	433,177.75	1,281,469.88	1.0477
13.00 - 14.00	391,868.13	981,764.19	1.1564
14.00 - 15.00	343,563.86	732,442.81	1.2642
15.00 - 16.00	292,230.59	529,570.12	1.3762
16.00 - 17.00	298,551.09	368,672.97	1.8749
17.00 - 18.00	272,762.54	240,074.50	2.4333
18.00 - 19.00	192,362.45	143,288.19	2.6153
19.00 - 20.00	139,175.19	78,442.55	3.1564
20.00 - 21.00	90,979.59	37,183.13	4.0017
21.00 - 22.00	14,485.14	13,986.90	1.3094
22.00 - 23.00	5,886.11	5,678.82	1.0954

Table 6a. (continued) . . .

21.00 - 22.00	14,485.14	13,986.90	1.3094
22.00 - 23.00	5,886.11	5,678.82	1.0954
23.00 - 24.00	3,171.43	1,758.26	1.6625
24.00 - 25.00	1,291.07 (Ct)	258.21 (Nt)	6.2010 (Ft)
Total Catch :	9,273,094.00	Natural mort. :	6.201
Mean E :	0.149		

Table 6b. Catch (in weight) and population biomass of *M. luzonensis* in Lake Manapao outputted through VPA II

ML (mm)	DELTA T (years)	CATCH (tons)	STEADY-STATE BIOMASS	CUMULATIVE BIOMASS
4.50	0.023	1202.11	1757101.12	1757101.12
5.50	0.024	1533.55	2743033.00	4500134.00
6.50	0.025	2365.35	3844695.00	8344829.00
7.50	0.027	2665.55	4760615.00	13105444.00
8.50	0.028	2577.14	5761578.00	18867022.00
9.50	0.030	3557.49	6417303.00	25284324.00
10.50	0.032	5164.40	6825616.50	32109940.00
11.50	0.034	6460.06	6943987.50	39053928.00
12.50	0.037	8258.25	6602807.00	45656736.00
13.50	0.040	9644.07	6040426.00	51697160.00
14.50	0.043	10718.01	5313884.50	57011044.00
15.50	0.048	11375.22	4294538.00	61305584.00
16.50	0.053	14301.39	3332152.50	64637736.00
17.50	0.060	15884.44	2330139.00	66967876.00
18.50	0.068	13471.80	1475730.25	68443608.00
19.50	0.080	11608.37	817845.19	69261456.00
20.50	0.096	8959.10	381412.69	69642872.00
21.50	0.120	1670.79	134443.64	69777312.00
22.50	0.161	789.56	47314.15	69824624.00
23.50	0.244	491.50	11167.61	69835792.00
24.50	0.525	229.78	875.36	69836664.00
TOTAL	-	132927.95	69836664.00	

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Inoculation of VA Mycorrhiza for the Improvement of Growth and Yield of Agricultural Crops, Fruit Trees and Forest Tree Species in Grassland Soil

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ABSTRACT

Eighteen different crops were screened for their response to inoculation with three different VA mycorrhizal species and/or applied with complete fertilizer, grown in grassland soil.

Growth responses of plants inoculated with mycorrhiza and applied with 60-60-60 kg NPK/ha were always better than the uninoculated - unfertilized control and the plants that were treated with fertilizer alone. Inoculated plants were taller, had more shoots, roots, had higher cob/pod yield and grew more vigorously than the control and the seedlings that were fertilized alone. Fertilizer application alone did not improve the growth of the plants. The uninoculated and unfertilized control plants had consistently the poorest growth performance compared with the other treatments.

Plants highly responsive to mycorrhizal inoculation were the following: mungbean, eggplant, guava, papaya, ***A. manqium, P. falcataria, A. auriculiformis,***

kariskis, corn, peanut, soybean, citrus and raintree. Guyabano and mahogany were intermediately dependent on mycorrhiza whereas upland rice, cacao and lanka were not dependent on mycorrhiza.

It is concluded that growth of plants in infertile areas can be improved with mycorrhizal inoculation at planting and amendment with small amounts of fertilizer.

BACKGROUND

In the Philippines, the practice of indiscriminate logging, "kaingin", over grazing and other improper land use practices have helped bring about marginal uplands or acidic uplands. These comprise about 9.3 million hectares or 31% of the total land area of 30 million hectares (IRRI 1986). These soils are generally deep and permeable with good drainage and favorable structure, hence are pontentially arable. However, under local conditions, leaching, surface run off, crop removal of bases and continuous use of acid-forming fertilizers contribute to the build up of soil acidity. These often lead to poor plant survival and growth.

Application of high rates of fertilizers and liming have been done to amend the unfavorable soil conditions. However, these require large financial inputs. Acid soils usually have high buffering capacities such that they require large amounts of lime to neutralize the pH. The predominant sloping areas of these uplands make fertilizer and lime application impractical. Therefore, alternate fertilizer sources which are cheap and indigenous should be tapped. One such alternative is the use of beneficial microorganisms which form associations with the host plant, i.e. mycorrhiza.

A. Definition

The term mycorrhiza came from two German words "mykes" for fungus and "rhiza" for plant roots, hence mycorrhiza. This fungus-root relationship is a symbiotic association whereby the fungus invades and parasitises roots of the host plant but unlike other harmful parasites, it does not damage or kill the host but instead provides many physical and physiological benefits to the latter. In return, the fungus obtains its food and other growth requirements from the host plant.

B. Types of Mycorrhiza

Harley and Smith (1983) described about six types of mycorrhiza, namely: (1) Ectomycorrhiza; (2) Vesicular-arbuscular mycorrhiza (VA) or endomycorrhiza; (3) Ectendomycorrhiza; (4) Ericoid mycorrhiza; (5) Arbutoid and monotropoid mycorrhiza; and (6) orchid mycorrhiza. The six types of mycorrhiza differ in the kind of host plant they associate with, fungi involved and the manner of association. The first two types are the most common and will be discussed further.

1. Ectomycorrhiza

In ectomycorrhiza, the infected roots are usually enlarged, the outer surface covered with a compact fungal mantle, with fungal mycelia radiating outwards into the soil and with the fungus invading the cortical tissues but confined in between the cell walls. The fungi are basidiomycetes producing typical fruiting bodies such as mushrooms or puffballs. This kind of symbiotic association is usually found in forest tree species particularly those in the family Pinaceae, Fagaceae, Betulaceae, Myrtaceae and Dipterocarpaceae.

2. Endomycorrhiza

In endomycorrhiza or VA mycorrhiza, infected roots are not enlarged. The roots have to be examined under a microscope to detect infection. The fungus forms a loose network of hyphae on the root surface and may infect roots through root hairs or directly through epidermal cells. The fungi do not only invade the cortical tissues but may also penetrate cortical cells where they develop a complex hyphal branching system like small bushes called *arbuscules*. The arbuscule is the significant structure on the VAM complex because it is the preferential site for fungus/plant metabolite (food and nutrient) exchanges. Another common feature in the endomycorrhiza is the production of thin walled spherical to ovate structures called *vesicles*, which are caused by terminal swellings of a hypha of the VAM fungus. Vesicles are found in the inner and outer layers of the cortical parenchyma. The cytological organization of the vesicles (mostly rich in lipids) and the fact that their number frequently increase in old or dead roots suggest that they are mainly resting organs. Due to the presence of vesicles and arbuscules, this group of fungi is commonly called ***vesicular-arbuscular*** mycorrhiza or ***VA*** Mycorrhiza (VAM).

VA mycorrhiza are the most widely distributed type of mycorrhiza throughout the world. They are generally abundant in grasslands and savannas, shrubs, open woodlands, dense rainforests, semi- deserts and sand dunes (Hayman 1982). VA mycorrhizal associations occur in almost all photoautotropic green vascular plants. Exceptions are plants growing in water-logged conditions like lowland rice and plants belonging to the family Cruciferaseae (Mikola 1982). Most of our important agricultural, horticultural and forest tree species form mycorrhizal associations.

C. Benefits of Mycorrhizal Association

Mycorrhiza is able to improve plant growth because of the following:

1. **Increased absorption of nutrients.** The hyphae of these fungi are able to extend into the soil, considerably beyond the area explored by root hairs, thus effectively extending the zone of nutrient absorption for poorly mobile elements such as phosphorus (P), copper (Cu) and zinc (Zn) and other elements such as nitrogen (N), potassium (K), calcium (Ca) and other micronutrients.
2. **Increased drought resistance of the host plant because mycorrhiza aids in water absorption.** The presence of the fungal mycelia in the root promotes the absorption not only of nutrients but also of water.
3. **Biological control of pathogenic root infections** as in pathogenic fungi in pines, nematodes in tomato, nematodes in tobacco and in soybeans
4. **Enhanced activity of other microorganisms** such as phosphate- solubilizing bacteria, *Rhizobium* and *Azospirillum*. In the case of *Rhizobium* and *Azospirillum*, mycorrhiza provides the phosphorus required by nitrogen-fixing microorganisms thus promoting nodulation and nitrogen fixation.
5. **Production of growth promoting hormones** such as auxins and gibberellins and other growth promoting substances such as vitamins
6. **Accelerated mineral cycling** by enhancing the uptake and translocation of nutrients from decomposing leaves and other organic litter in the rhizosphere. Mycorrhiza may directly extract nutrients bound in organic matter and convert these to organic compounds within their tissues during metabolism. The organic to organic transfer of nutrients by

mycorrhiza is significant because it bypasses such processes as decomposition and mineralization. Therefore, mineral cycling will occur at a faster rate in the presence of mycorrhiza.

7. **Improved soil aggregation** by secreting mucilagenous substances which can serve as agents in soil aggregate formation. This leads to improved soil structure and, in effect, the water holding and nutrient holding capacity of the soil.

MATERIALS AND METHODS

A. Soil preparation

An infertile, phosphorus-deficient soil sample was collected from the grasslands of Carranglan, Nueva Ecija. The soil belonged to the Annam series whose predominant characteristics are soil acidity and reddish color due to the predominance of iron and aluminum oxides. The soil was passed through a 2-mm sieve to remove large stones, placed in size 8 pots (8 inches top diameter) and fumigated with methyl bromide for 48 hours in a fumigation chamber.

Initial soil chemical and mycorrhizal analyses are presented in Table 1. Analysis showed that the soil was weakly acidic but very low in organic matter, CEC and total nitrogen.

B. Treatments and Hosts

Eighteen separate hosts were used to determine the response to inoculation with three VA mycorrhizal species and/or application of complete fertilizers. The experiments were laid out in a completely randomized design (CRD) with eight replicates per treatment. The treatments were as follows:

1. Uninoculated and unfertilized control;
2. Fertilized with 60-60-60 kg N, P_2O_5 and K_2O/ha;
3. Inoculated with *Glomus etunicatum* + 60-60-60 kg NPK/ha;
4. Inoculated with *Glomus macrocarpum* + 60-60-60 kg NPK/ha; and
5. Inoculated with *Gigaspora margarita* + 60-60-60 kg NPK/ha.

The plant hosts used were classified accordingly as agronomic crops, fruit trees and forest tree species.The different crops were selected based on their importance for food, reforestation and other beneficial attributes they may offer.

Agronomic Crops

1. Upland rice (*Oryza Sativa*)
2. Corn (*Zea mays*)
3. Mungbean *(Vigna radiata)*
4. Peanut (*Arachis hyponaea*)
5. Soybean (*Glycine max*)
6. Eggplant (*Solanum melongena*)

Fruit trees

7. Guava *(Psidium guajava)*
8. Cacao (*Theobroma cacao*)
9. Langka (*artocarpus heterophylla*)
10. Citrus (*Citrus microcarpa*)
11. Papaya (*Carica papaya*)
12. Guyabano (*Annona muricata*)

Forest tree species

13. *Acacia mangium*
14. Mollucan sau *(Paraserianthes falcataria)*
15. Raintree (*Samanea saman)*
16. *Acacia auriculiformis*
17. Mahogany *(Swietenia marophylla)*
18. Kariskis

Split fertilizer application was done. At planting, 30-30-30 kg NPK/ha (computed in: grams fertilizer/kg soil/pot) was mixed with the soil prior to inoculation and seed planting. After one month, a second dose of 30-30-30 kg NPK/ha was applied in solution form after dissolving the fertilizer in water.

The mycorrhizal spores used in the experiment came from pot cultures of Pensacola Bahia grass. Initially, spores of *Glomus etunicatum, Glomus macrocarpum* and *Gigaspora margarita* were inoculated to seedlings of Bahia grass grown in small cups. After four months, the roots of the Bahia grass and soil were recovered and the above-ground biomass discarded. The infected roots were cut into small pieces and incorporated with the soil. The mycorrhizal soil inoculant for the experiment was calibrated to contain 200 spores per plant which also contained hyphae and infected roots. Inoculation was done by layering the soil inoculant 2-3 cm below the seed or seedling at planting.

Upland rice, corn, mungbean, soybean and peanut were directly seeded into pots. Seeds of the other host plants were first pregerminated in a seed box and then the seedlings were transplanted to pots when they were 2-3 cm tall.

Host plants were maintained for two months in the screenhouse, except for some agricultural crops (corn, peanut, mungbean ang soybean) which were harvested after the complete crop cycle. Watering was done every day and spraying of pesticide was done whenever necessary. Parameters measured for the duration of the experiments are presented in Table 2. Data gathered were statistically analyzed using the Analysis of Variance (ANOVA) and treatment means compared using Tukey's W-procedure.

RESULTS AND DISCUSSION

A. Total Biomass, Height, Cob/Pod Yield or Stem Diameter

Tables 3, 4 and 5 present the response of the 18 hosts to inoculation with different VA mycorrhizal species and/or applied with complete fertilizer. The uninoculated-unfertilized control consistently gave the poorest growth performance in terms of height, total biomass, pod/cob yield and stem diameter for all hosts screened. Plants fertilized with complete fertilizer alone were most often comparable with or slightly better than the uninoculated-unfertilized control plants. However, when plants were inoculated with either of the three VA mycorrhizal species and applied with fertilizer, growth rate was highest. Exceptions to these are upland rice, cacao, langka and guyabano. In these plants, growth of the plants treated with fertilizer alone was comparable to that of the inoculated plants or there was no significant difference among the five treatments.

The three VA mycorrhizal species were equally effective in promoting growth of the plants. Although *G. margarita* almost always gave a higher value in some parameters monitored, they were not statistically different.

Significant positive correlation between mycorrhizal infection and total biomass, height and cob/pod yield or stem diameter was observed on almost all plants. This means that the good height growth, heavy total biomass and good cob/pod yield were probably due to the high mycorrhizal infection in the inoculated plants. A not-significant correlation, on the other hand, signifies that mycorrhizal infection was not related to the height, total biomass and stem diameter growth observed in these plants.

B. Mycorrhizal Dependency

Tables 6,7 and 8 present the summary of the responses of the 18 hosts to inoculation with the three VA mycorrhizal species, their dependency on mycorrhiza and presence of host specificity. Total biomass, height and cob/pod yield and stem diameter of inoculated plants were compared with those of plants treated with fertilizer alone. The percentage increase was graded based on the classification of Ferguson (1984). In his discussion on mycorrhizal dependency, growth increases greater than 40% in a given soil fertility, means that these plants are highly dependent on mycorrhiza. A 10 - 40% increase means plants are intermediately dependent on mycorrhiza and less than 10% means the plants are not dependent on mycorrhiza.

Based on this classification, the 18 hosts were grouped into three. Plants highly responsive to mycorrhizal inoculation were the following: mungbean, eggplant, guava, papaya. *A. mangium, P. falcataria, A. auriculiformis* and kariskis. These plants were highly responsive to mycorrhizal inoculation and were observed to have high positive correlations between mycorrhizal infection and their total biomass. In all these crops, total biomass, height, nitrogen and phosphorus uptake were significantly improved with mycorrhizal inoculation.

Corn, peanut, soybean, citrus and raintree were also classified as highly dependent on mycorrhiza. Although their total biomass and cob/pod yield, nitrogen and phosphorus uptake were highly responsive to mycorrhizal inoculation, height of these plants, total biomass of citrus and stem diameter of raintree were only intermediately affected by mycorrhizal inoculation.

Guyabano and mahogany were found to be intermediately dependent on mycorrhiza. These plants have intermediate to no response in total biomass and/or height and stem diameter when inoculated with either of the three VA mycorrhizal species. Correlation analysis between growth parameters of these two crops and mycorrhizal infection showed lower positive correlation values than in the crops which were highly responsive to mycorrhizal inoculation.

The remaining plants (langka, upland rice and cacao) were classified as not dependent on mycorrhiza. Responses of these plants varied from intermediate, to no response at all to mycorrhizal inoculation. Correlation analysis showed that mycorrhizal infection was not related to the total biomass, height, stem diameter and nitrogen and phosphorus content and uptake of these plants.

C. Host Specificity and Host Preferences

Tables 6, 7 and 8 also show the evaluation of the presence of host specificity for all the three VA mycorrhizal species. Host specificity was defined by Harley and Smith (1983) as the condition wherein a given species of fungus forms mycorrhizal relationship only with a specific host plant. If the fungus forms mycorrhizal associations with many plants it is considered as "not specific". All the three VA mycorrhizal fungi were evaluated to be non-host specific. They all formed mycorrhizal infection as verified by the Gridline Intersect Method (Giovanetti and Mosse 1982) used in evaluating mycorrhizal infection. Even the non-mycorrhizal dependent crops such as upland rice, langka and cacao were observed to have mycorrhizal root infections. Mycorrhizal infection observed were usually in the form of vesicles and arbuscules.

The different responses of the hosts to the three VA mycorrhizal species may be due to the host preferences by the mycorrhizal fungi. This was also suggested by many researchers when one fungus improved growth of one plant better than another (Mosse 1975; Fox 1971-72). Mosse (1975) cited that the preferential association between certain plants and fungal species can be evaluated with respect to combinations which produced the greatest plant growth stimulation, the greatest colonization and maximum sporulation.

D. Fertilizer effect and large cotyledons

The possible reason why upland rice was not influenced by mycorrhizal inoculation was the very good response of the plant to fertilizer application. Rice plants responded greatly to the added fertilizer and with their fibrous root system, they were able to have access to the nutrients applied. Growth of the plant was no longer limited by the nutrient deficiency of the soil, thus mycorrhizal inoculation did not have any effect on the growth of the plant. This was similar to the report of Rhodes (1981) who stated that "if the supply of organic nutrients is not limiting the growth of non-mycorrhizal plants, then mycorrhizal inoculation will add nothing."

A common characteristic of plants not dependent on mycorrhiza and those only intermediately dependent on mycorrhiza, particularly cacao, mahogany and langka, is the very large cotyledons of their seeds. In the initial stages of seedling growth, the plants might have depended much on the stored food. Host photosynthates were channeled to the formation of the above-

ground biomass and not on root formation, such that the large food reserves may have delayed mycorrhizal association between the fungi and the plant. Furthermore, there might not have been enough infection sites for the fungus to enter and form mycorrhizal associations due to the relatively few and sparse roots.

E. Mycorrhizal Dependency Based on Percentage Relative Increase in Total Biomass

Figure 1 presents the mycorrhizal dependency of the 18 crops tested based on percentage relative increase in total biomass over the fertilizer alone treatment. Percentage relative increase in total biomass was computed by dividing the total biomass of the plant receiving fertilizer treatment alone over the average biomass of the inoculated plants then multiplied by 100. The higher the percentage relative increase over the fertilizer treated plants alone, the more the plant is not dependent on mycorrhiza. Plants not dependent on mycorrhiza had relative increases in total biomass of 90-107%, plants intermediately dependent on mycorrhiza had relative increases in total biomass of 76-87% and the plants dependent on mycorrhiza had 7-53% relative increase in total biomass over plants treated with fertilizer alone.

CONCLUSIONS AND RECOMMENDATIONS

1. It is concluded that growth of plants in very infertile areas can be improved with application of small amounts of fertilizer and inoculation with VA mycorrhiza.
2. The three VA mycorrhizal fungi were equally effective in improving growth of the plants and were not host specific. Thus, any of the three species can be used to inoculate plants and can still give good growth performance.
3. Further studies on factors which determine host preference should be done.
4. Further studies on the response of plants to mycorrhiza in other problem soils such as saline affected soils, mining areas and the like under Philippine conditions should be done to fully evaluate the potentials of mycorrhizal associations.

Table 1. Chemical and initial mycorrhizal population of Annam soil, collected from Carranglan, Nueva Ecija

SOIL PROPERTIES	ANALYSIS
pH	5.35
Organic Matter (%)	1.08
Total Nitrogen (%)	0.05
Available Phosphorus (ppm)	5.34
Exchangeable K (me/100 g)	1.71
Exchangeable Ca (me/100 g)	7.95
Exchangeable Mg (me/100 g)	3.06
CEC (me/100 g)	17.02
Native Mycorrhizal Population (organisms per gram air dry soil)	44.0

Table 2. Growth parameters measured, method used and time of measurement

Parameter	Method	Time of Measurement
Plant Height	From the base of the stem to the tip of the apical bud with a metric ruler	Monthly
Flowering date	Observation on the earliness of flowering between treatments	Duration of experiment
Stem diameter	Measured at the base of the root collar with a vernier caliper	2nd month
Pod/Cob Yield	Weighed using a Mettler balance after oven drying at 60^{0}C for 48 hours	Harvest
Root/Shoot/Nodule and total biomass	Weighed using a Mettler balance after oven drying at 60^{0}C for 48 hours	Harvest
Mycorrhizal infection	Taking fine root segments, preserving and fixing in Formalin - acetic-acid-solution (FAA) then clearing and staining using the procedure of Philipps and Hayman 1970. Infection count was done using the Gridline Intersect Technique by Giovanetti and Mosse 1980.	Harvest
Nitrogen Content	Analyzed using the Kjeldahl Method	Harvest
Nitrogen Uptake	Nitrogen content multiplied with the total plant biomass	Harvest
Phosphorus Content	Analyzed using the Molybdo-Vanadate Method	Harvest
Phosphorus Uptake	Phosphorus content multiplied with the total plant biomass	Harvest

Table 3. Responses of six agricultural crops to inoculation with three different VA mycorrhizal species

Agricultural Crops	Control	60-60-60	*Gl. etunicatum* 60-60-60	*Gl. macrocarpum* 60-60-60	*G. margarita* 60-60-60	Correlation with Myc. Infection
1. Upland rice						
Total biomass (g) *	1.96 b	10.82 a	10.33 a	11.48 a	11.62 a	0.18 ns
Tiller count (no/plt) *	3.00 b	7.00 a	7.00 a	8.00 a	7.00 a	0.18 ns
Height (cm) *	47.20 b	60.50 a	62.60 a	65.00 a	64.60 a	0.06 ns
2. Corn						
Total biomass (g) *	2.05 c	7.24 b	13.49 a	15.16 a	12.41 a	0.59 *
Cob yield (g) *	0.20 b	193.50 b	955.30 a	825.90 a	744.20 a	0.63 *
Height (cm) *	55.60 c	88.40 b	107.40 ab	112.90 a	107.80 a	0.53 *
3. Mungbean						
Total biomass (g) *	0.24 b	0.36 b	4.74 a	5.03 a	5.57 a	0.85 *
Pod yield (mg) *	0.10 b	163.90 b	2512.00 a	26.01 a	3157.00 a	0.84 *
Height (cm)	11.10 b	12.80 b	25.60 a	26.60 a	25.40 a	0.83 *
4. Peanut						
Total biomass (g) *	2.16 c	2.73 c	8.02 b	8.32 ab	9.91 a	0.93 *
Pod yield (g) *	0.82 b	1.18 b	3.79 a	3.42 a	3.53 a	0.81 *
Height (cm) ns	17.80	15.40	18.20	20.60	19.50	0.32 *
5. Soybean						
Total biomass (g) *	1.30 c	2.91 b	6.01 a	4.80 a	5.70 a	0.69 *
Pod yield (g) *	0.25 b	0.91 b	2.52 a	1.91 a	2.27 a	0.66 *
Height (cm) *	52.30 b	87.80 a	110.00 a	98.60 a	110.90 a	0.43 *
6. Eggplant						
Total biomass (g) *	0.03 b	1.63 b	6.33 a	6.51 a	5.62 a	0.92 *
Height (cm) *	1.80 b	6.30 b	15.00 a	14.40 a	14.20 a	0.89 *

Table 4. Responses of six fruit trees to inoculation with three different VA mycorrhizal species

FRUIT TREES	Control	60-60-60	*Gl. etunicatum* 60-60-60	*Gl. macrocarpum* 60-60-60	*G. margarita* 60-60-60	Correlation with Myc. Infection
1. Guava						
Total biomass (g) *	0.05 b	0.11 b	0.43 a	0.40 ab	0.43 a	0.60 *
Stem diameter (mm) *	1.20 b	1.30 b	2.00 ab	1.90 ab	2.20 a	0.61 *
Height (cm) *	3.30 c	6.80 bc	11.80 ab	12.30 ab	14.20 a	0.68 *
2. Cacao						
Total biomass (g) ns	1.08	1.92	1.74	2.10	1.55	0.23 ns
Stem diameter (mm) ns	4.90	6.10	6.10	6.20	5.70	0.19 ns
Heigth (cm) ns	16.10	18.30	17.50	18.80	17.10	0.16 ns
3. Langka						
Total biomass (g) ns	6.34	6.74	7.78	7.70	7.11	0.04 ns
Stem diameter (mm) ns	7.30	7.20	7.20	7.30	6.70	0.31 ns
Height (cm) ns	49.20	49.40	53.50	53.60	53.70	0.15 ns
4. Citrus						
Total biomass (g) *	0.23 b	0.24 b	0.31 b	0.72 a	0.72 a	0.57 *
Stem diameter (mm) *	1.20 b	1.70 ab	1.40 ab	1.60 a	2.10 a	0.40 *
Height (cm) *	5.70 b	5.70 b	8.10 b	11.00 ab	11.60 a	0.65 *
5. Papaya						
Total biomass (g) *	0.06 b	1.62 b	6.39 a	6.45 a	6.72 a	0.82 *
Stem diameter (mm) *	1.60 b	3.00 b	10.10 a	11.90 a	11.90 a	0.87 *
Height (cm) *	7.00 b	11.20 b	34.00 a	32.30 a	34.60 a	0.91 *
6. Guyabano						
Total biomass (g) ns	1.05	1.11	1.44	1.24	1.69	0.41 *
Stem diameter (mm) ns	4.20	3.70	4.10	3.80	4.40	0.12 ns
Height (cm) *	22.70 b	23.40 ab	27.00 ab	24.70 ab	28.30 a	0.47 *

Table 5. Responses of six forest trees to inoculation with three different VA mycorrhizal species

	FOREST TREES	Control	60-60-60	*Gl. etunicatum* 60-60-60	*Gl. macrocarpum* 60-60-60	*G. margarita* 60-60-60	Correlation with Myc. Infection
1	*A. manqium*						
	Total biomass (g) *	0.15 b	0.52 b	3.77 a	3.12 a	4.37 a	0.57 *
	Stem diameter (mm) *	1.10 b	1.00 b	2.80 a	2.70 a	3.20 a	0.66 *
	Height (cm) *	6.20 b	7.00 b	23.30 a	21.60 a	27.10 a	0.73 *
2.	*P. falcataria*						
	Total biomass (g) *	0.21 b	0.27 b	3.91 a	3.74 a	4.67 a	0.89 *
	Stem diameter (mm) *	1.40 c	1.20 c	3.60 b	3.60 b	4.20 a	0.93 *
	Height (cm) *	2.30 b	3.30 b	17.30 a	16.90 a	18.50 a	0.89 *
3	Raintree						
	Total biomass (g) *	1.11 b	1.02 b	2.97 a	3.39 a	3.40 a	0.70 *
	Stem diameter (mm) *	2.80 b	3.10 ab	3.80 a	3.80 a	3.60 ab	0.50 *
	Height (cm) *	20.30 c	22.10 bc	31.70 ab	34.70 a	34.30 a	0.64 *
4.	*A. Auriculiformis*						
	Total biomass (g) *	0.20 c	0.47 bc	0.98 abc	1.34 a	1.13 ab	0.65 *
	Stem diameter (mm) *	1.20 b	1.40 b	2.00 ab	2.40 a	2.40 a	0.63 *
	Height (cm) *	10.50 c	12.10 bc	20.20 ab	22.90 a	21.20 a	0.63 *
5	Mahogany						
	Total biomass (g) *	2.18 b	2.89 b	3.23 ab	3.08 ab	3.63 a	0.37 *
	Stem diameter (mm) ns	3.50	3.50	4.20	3.90	3.80	0.16 ns
	Height (cm) ns	24.70	22.60	24.00	22.00	25.30	-0.16 ns
6.	Kariskis						
	Total biomass (g) *	0.14 b	0.12 b	2.10 a	1.70 a	1.86 a	0.73 *
	Stem diameter (mm) ns	1.30 b	1.40 b	2.80 a	3.00 a	2.30 a	0.73 *
	Height (cm) *	5.10 b	5.60 bb	46.50 a	45.20 a	41.10 a	0.79 *

Table 6. Summary of the responses of six agricultural crops to inoculation with three different VA mycorrhizal species, their dependency on mycorrhiza and evaluation of the presence of host specificity

AGRICULTURAL CROPS	*Gl. etunicatum*		*Gl. macrocarpum*		*G. margarita*		MYCORRHIZAL DEPENDENCY
	% Inc. vs 60-60-60	Graded response	% Inc. vs 60-60-60	Graded response	% Inc. vs 60-60-60	Graded response	
1. Upland rice							
Total biomass *	0	none	6	none	7	none	not
Tiller count *	0	none	14	interm.	0	none	dependent
Height *	4	none	7	none	7	none	
2. Corn							
Total biomass *	86	high	109	high	70	high	highly
Cob yield *	394	high	327	high	1447	high	dependent
Height *	21	interm.	28	interm.	22	interm.	
3. Mungbean							
Total biomass *	1217	high	1297	high	1447	high	highly
Pod yield *	1434	high	1488	high	1827	high	dependent
Height *	100	high	108	high	122	high	
4. Peanut							
Total biomass *	194	high	205	high	263	high	highly
Pod yield *	221	high	190	high	199	high	dependent
Height ns	18	interm.	34	interm.	27	interm.	
5. Soybean							
Total biomass *	106	high	65	high	96	high	highly
Pod yield *	177	high	110	high	149	high	dependent
Height *	25	interm.	12	interm.	26	interm.	
6. Eggplant							
Total biomass *	288	high	299	high	245	high	highly
Height *	138	high	129	high	125	high	dependent
Presence of host specificity	not specific		not specific		not specific		

Legend : 0-10% increase = not dependent
11-40% increase = intermediately dependent, >40% increase = highly dependent

Table 7. Summary of the responses of six fruit trees to inoculation with three different VA mycorrhizal species, their dependency on mycorrhiza and evaluation of the presence of host specificity

FRUIT TREES	*Gl. etunicatum* % Inc. vs 60-60-60	*Gl. etunicatum* Graded response	*Gl. macrocarpum* % Inc. vs 60-60-60	*Gl. macrocarpum* Graded response	*G. margarita* % Inc. vs 60-60-60	*G. margarita* Graded response	MYCORRHIZAL DEPENDENCY
1. Guava							
Total biomass *	291	high	264	high	291	high	highly
Height *	54	high	46	high	69	high	dependent
2. Cacao							
Total biomass ns	0	none	9	none	0	none	not
Height ns	0	none	3	none	0	none	dependent
3. Langka							
Total biomass ns	14	interm.	14	interm.	6	none	not
Height ns	8	none	9	none	9	none	dependent
4. Citrus							
Total biomass *	29	interm.	200	high	200	high	highly
Height *	42	high	93	high	191	high	dependent
5. Papaya							
Total biomass *	294	high	298	high	315	high	highly
Height *	203	high	188	high	209	high	dependent
6. Guyabano							
Total biomass ns	30	interm.	12	interm.	52	high	interme-
Height *	15	interm.	6	none	21	interm.	diately dependent
Presence of host specificity	not specific		not specific		not specific		

Legend : 0-10% increase = not dependent
11-40% increase = intermediately dependent, >40% increase = highly dependent

Table 8. Summary of the responses of six forest trees to inoculation with three different VA mycorrhizal species, their dependency on mycorrhiza and evaluation of the presence of host specificity

FOREST TREES	*Gl. etunicatum*		*Gl. macrocarpum*		*G. margarita*		MYCORRHIZAL DEPENDENCY
	% Inc. vs 60-60-60	Graded response	% Inc. vs 60-60-60	Graded response	% Inc. vs 60-60-60	Graded response	
1. ***A. Manqium***							
Total biomass *	625	high	500	high	740	high	highly
Height *	230	high	251	high	287	high	dependent
Stem diameter *	180	high	170	high	220	high	
2. ***P. falcataria***							
Total biomass *	1348	high	1285	high	1630	high	highly
Height *	424	high	412	high	461	high	dependent
Stem diameter *	200	high	200	high	250	high	
3. **Raintree**							
Total biomass *	191	high	232	high	233	high	highly
Height *	43	high	57	high	55	high	dependent
Stem diameter *	23	interm.	23	interm.	16	interm.	
4. ***A. auriculiformis***							
Total biomass *	109	high	185	high	140	high	highly
Height *	67	high	89	high	75	high	dependent
Stem diameter *	73	high	71	high	71	high	
5. **Mahogany**							
Total biomass *	12	interm.	7	none	26	interm.	interme-
Height ns	6	none	0	none	11	interm.	diately
Stem diameter *	20	interm.	11	interm.	9	none	dependent
6. **Kariskis**							
Total biomass *	1650	high	1317	high	1450	high	highly
Height *	812	high	786	high	706	high	dependent
Stem diameter *	87	high	100	high	53	high	
Presence of host specificity	not specific		not specific		not specific		

Legend : 0-10% increase = not dependent
11-40% increase = intermediately dependent, >40% increase = highly dependent

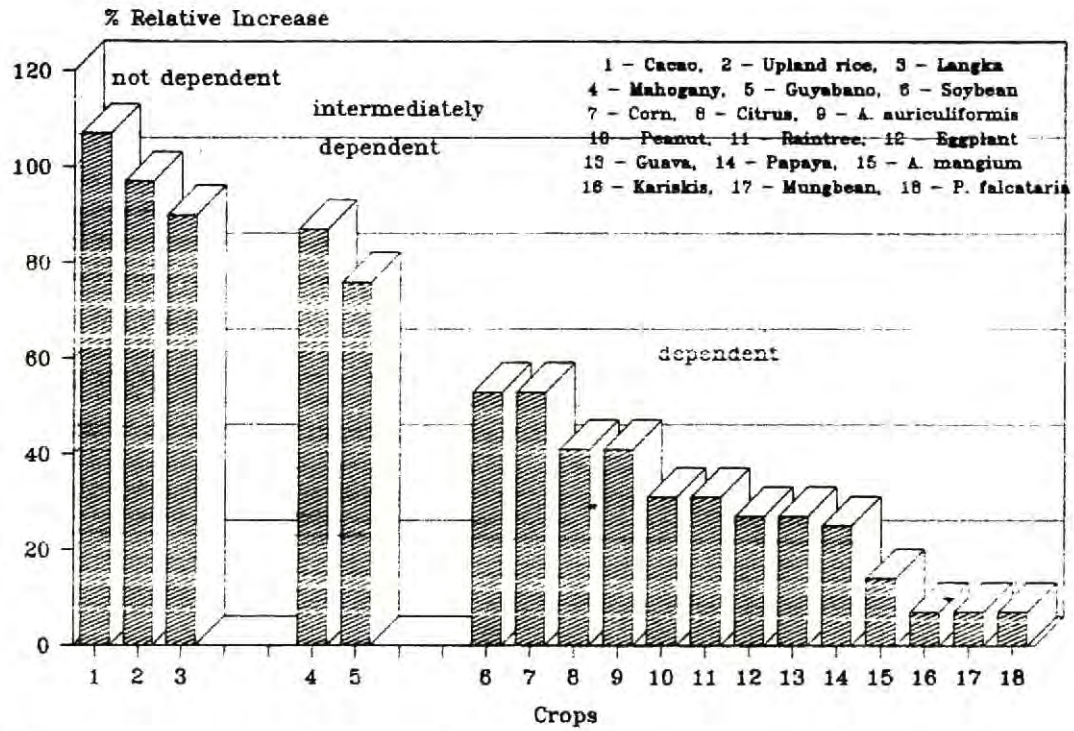

Figure 1. Mycorrhizal dependency of the 18 selected crops based on percentage relative increase of total biomass over the fertilizer alone treatment

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Urea-treated Straw With Limited Supplementation for Sustained Ruminant Production in Developing Countries

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ABSTRACT

The state-of-the-art of urea-treated straw feeding technology is presented, focusing on strategic supplementation to optimize animal productivity in sustainable production systems for developing countries.

The treatment procedure and the effects of urea incubation on the feeding value of rice straw are described, particularly on the improved digestibility and nitrogen content of straw. Comparative animal responses to urea treatment vis-a-vis urea-molasses supplementation are shown. An account of the efforts to extend the technology to farmers in selected Asian countries is given with special reference to the Philippines.

Practical supplements and appropriate supplementation strategies are discussed. Recent findings on milk and meat production of urea-treated straw fed livestock with limited supplementation are reviewed. Finally, sustainable animal production systems involving rice straw utilization are described.

INTRODUCTION

Abount 80% of the ruminants in developing countries are kept in mixed animal/cropping systems on small farms. The small-hold livestock producers are basically crop farmers keeping some goats/buffaloes/cattle as a major source of food for the family, as draft power as well as source of manure for the crops. Because of limited land holding expected to be able to support the cash requirement of the family, whatever the small piece of land the farmer tills is prioritized primarily for food/cash crop production. Improved pastures, therefore, do not exist and will never have a place among the small-hold farming systems in this part of the world. His animals have to be contented with crop residues supported with forages from marginal lands. From rice and corn alone, it can be computed from production data (**28**) that approximately 12 million metric tons of rice straw and corn stover are generated yearly. Trung (1987) pointed out that the current cattle and buffalo population in the country could be doubled through full utilization of fibrous agricultural residues which are currently being left rotten or burnt in the field.

This paper discusses the issues on sustainable livestock production systems in developing countries involving the utilization of fibrous agricultural residues with emphasis on urea treatment and limited supplementation. Research data generated by the Dairy Training and Research Institute (DTRI- UPLB) and other institutions in developing Asian countries wil constitute the scientific basis for this paper.

UREA TREATMENT: WHY? WHAT CAN IT DO?

1. Nutritional constraints of rice straw

Rice straw and other fibrous residues consist mainly of the structure components of plants. Lignin in fibrous residues is closely associated with cell wall polysaccharides and acts as a physical barrier to microbial breakdown, hence its low digestibility/energy values. Rice straw, likewise, contains very low crude protein (3-5%) which is below the critical level of 7% dietary protein required for acceptable voluntary feed intake. The ash content, although high, is made up largely of silica. The levels of Ca, P and Mg available from rice straw are usually lower than the range of 0.2-0.8% required for the normal growth and

fertility of ruminants. The same is true with trace elements like Co, Cu, etc. (**9**).

Because of the poor nutritive value, rice straw alone could at most support liveweight maintenance. Pretreatments and/or supplementation of rice straw are, therefore, essential to bring about production. There are a number of chemicals-largely alkalis - that have been found to be capable of breaking down the lignopolysaccharide bonds (**39**).

The following discussion centers on urea, a ready source of alkali to farmers in developing countries. Urea has been used to treat straw in Bangladesh (**30**), India (**13**), Indonesia (**8**), Sri Lanka (**27, 33**), the Philippines (**19,40,41,42,43**) and Thailand (**46**). With adequate moisture and suitable temperature conditions, microbes which produce urease are capable of degrading urea to ammonia, which eventually forms into ammonium compounds (ammonium carbonate, bicarbonate or hydroxide) which then permeate the straw.

2. Exogenous urease: a past concern

Through plastic bag experiments on urea treatment, it was thought that the breakdown of urea into ammonia and, subsequently ammonium compounds, would take at least 21 days to realize the treatment effects. In this connection, research in the early '80s endeavored to identify natural sources of urease to cut down treatment time form 21 to 5 days (**11, 15**). Along this line, we found that dried poultry manure (DPM) was a better source of urease compared to *Gliricidia sepium* leaves (**19**). With the inclusion of 4 - 12% of DPM in 4% urea-treated straw, within three days, straw digestibility was increased by 12 % while its crude protein content doubled (**19**).

Urea treatment of straw in large heaps/silos done in Sri Lanka indicated that because of higher temperatures maintained by the heaps compared to those in small plastic bags, full treatment effects can be achieved in 7 days (**11**) without additional source of urease. Although the issue of exogenous urease has been put to rest, the urease sources (e.g. *Gliridicida*, DPM) can always be regarded as valuable supplements to improve straw's feeding value.

With the present state of knowledge, the use of 4% urea, a straw to water ratio of 1:1 and airtight storage for at least 7 days seems to be suitable for the tropics (**11**).

3. Intake and digestibility

A good effect of ammoniation is nitrogen enrichment, i.e. N content of rice straw is roughly doubled after treatment (**15,19**). This, therefore, has a positive effect on intake (**20,39**). Increases in digestibility brought about by urea treatment have not been consistent; as low as 2-6 percentage units (e.g. **15,20**) or as high as 10 percentage units (**19**) have been recorded. The variation in digestibility may be attributed to differences in temperature, moisture content, urea concentration, treatment conditions and duration. These interrelated factors influence the growth of microorganisms responsible for degrading urea and hence the concentration of ammonium compounds.

4. Urea treatment vs supplementation

Several experiments were reviewed (**9**) in which urea treatment and supplementation were compared based on animal responses (Table 1). Supplementation has generally been found to increase the feeding value of straw compared to untreated, unsupplemented controls and, in some instances, as effective as pretreatment. The effect of urea treatment could not be felt, however, if both groups received relatively generous supplementation (e.g. 1% LW concentrate, **4**).

Urea treatment would generally increase the quality of straw to maintenance level and supplements would be required to bring about production. It is clear that as long as straw remains a substantial part of the diet, then urea-treated straw has substantial advantages over untreated material (**17,27,43**). On the other hand, the effects of treatment may be lost if straw constitutes only a small portion of the diet.

In terms of feed cost, it has been estimated that urea treatment costs 60% less than concentrate supplementation of untreated straw in order to get a similar energy intake (**20**).

FEED RESOURCES OF SMALL-HOLD DAIRY FARMERS AND ADOPTION OF UREA- TREATED STRAW TECHNOLOGY

The Philippines is perhaps one of the countries in Asia where feed resource potentials have not been fully exploited.

This could be grossly attributed to the dwindling cattle and buffalo population which is a consequence of low priority of the livestock commodity among the government's development programs. Nevertheless, with the introduction of dairy animals in some milkshed areas, the value of rice straw is gaining appreciation among dairy farmers. In a recent survey involving 32 small-hold dairy farmers, 22 (or 69%) actually used straw as feed for their milking cows (**25**).

In a urea-treated straw feeding project, feed resources of 22 adoptors were surveyed and shown in Table 2. Majority of the farmers depended heavily on straw feeding, followed by grazing under coconuts and marginal lands, while only 2.5% of the total feed DM was derived from improved pastures. Another important observation was that feed sourcing came largely from off-farm areas rather that within the farm proper (**44**).

As soon as the benificial effects of urea treatment of straws were discovered, animal scientists and extension workers lost no time in trying to bring the technology to the village level. Constrained by limited land to support a progressive livestock industry, Bangladesh was the first country (1980) to introduce urea treatment to small farmers with financial assistance from local and international sources. The adoption by farmers was evaluated (**32**). Assisted by the Dutch government, Sri Lanka launched a straw utilization project focusing on urea treatment in 1982 (**16**). The same effort was noted in India (**22**); Thailand (**29**), and the Philippines (**26,41**). Farmers in those countries readily accepted the technology at the beginning. Continued adoption, however, was not noted. For example, among 145 Bangladesh farmers in the Pabna milkshed area, only 13% fed urea- treated straw continuously in 1981 (32).

Through the financial assistance of PCARRD, researchers at the DTRI-UPLB attempted to introduce urea treatment to small-hold dairy farmers in Laguna on a limited scale in 1987. Among the 22 adoptors of urea-treated straw feeding technology, 7 carried on with the practice (32%) while the remaining 15 farmers (68%) tried only once or twice. The latter nevertheless stated that they would again do the treatment because apparently they were convinced about the feeding value of treated straw. Ninety-one percent (**20**) felt that they derived the following benefits from the technology: time saving (45%); good substitute for grass even during rainy months (25%); increased appetite of animals (15%); improved milk yield and quality (10%);

and fully utilized straw (5%). The same number of adoptors (20 or 91%) stated they would recommend that other farmers try the technology (**26**).

PRACTICAL SUPPLEMENTS

Because of poor nutritive value earlier pointed out, rice straw, when fed alone, could not support productive functions. Urea treatment improves straw quality to the level of fair quality grass, which may bring about low productivity. The use of supplements in such a feeding situation will further enhance animal performance. The subsequent discussion identifies supplements that are readily available at the village level while supplementation strategy will be dealt with thereafter.

1. Concentrate supplements

Unlike situations in developed countries where grains and protein meals are available at low costs for feeding livestock liberally, grains in the developing countries are widely used as human food and for monogastric animal feeding. Farmers raising ruminants, therefore, have to be contented with industrial by-products for which poultry and swine raisers also compete. It is, therefore, conceivable that small amounts of concentrate by-products may be used judiciously in feeding systems to promote production through maximized utilization of fibrous basal feeds.

A number of by-products are shown in Table 3. Although they are classified as energy and protein supplements because the latter group has crude protein values of more than 20%, both groups do provide not only energy and protein but also vitamins and minerals. The nutritive values of these ingredients vary considerably depending on the sources, methods of processing and the degree of adulteration.

Animal proteins have a higher by-pass value than protein supplements from plant sources. Among energy supplements, pulps, corn, sorghum and brans are more slowly fermented in the rumen compared to tuber meals and molasses, some of which also tend to escape rumen fermentation (**7**). Rice bran may contain from small amounts to as much as 50% of rice hull, hence its protein value may be as high as 14% or as low as 4%.

Likewise, protein meals left after the commercial extraction of oil from nuts/seeds have a lipid content of from 1 - 10%. Solvent extraction produces a meal of only 1-4% fat compared to 6-10% for mechanical extraction. While a protein meal with high fat content generally has lower than expected CP value, the loss in protein may be well compensated for. Fat has a gross energy content of about 2.5 times that of protein and carbohydrates of the same weight and a higher efficiency of utilization within the body. Intake of a given weight of fat provides approximately seven times more usable energy for growth than the same weight of mature roughage (**21**). O'Kelly (1985) compared two isonitrogenous and isocaloric diets having different fat contents (2.5 vs 9.2%) for 120-day steer fattening. He reported 20 kg heavier weight traceable from 10% higher net energy intake from the steers eating high fat diets. On the other hand, the disadvantages are: high fat concentrates being associated with developed rancidity and the high cost of fats versus other energy sources.

2. Green forage supplements

The principal constraint of using concentrates as a supplement at the village level is the cost involved in purchasing the feeds. Perhaps, this may be overcome in many instances if concentrates are replaced by green forages which are available on or near the farm at almost no cost, except for the time involved in cutting and hauling. Systems in which green forages can be generated for small farmers without the need for establishing permanent pastures have been discussed (**10,35,37,45**). It is inevitable that when green forages are considered in this context, attention is focused on the legumes. However, protein-rich crop residues, such as cassava tops, banana leaves, sweet potato vines, etc. should not be overlooked.

Tropical legumes generally contain high levels of protein and all minerals except sodium. Although the digestibility of legumes is not higher than grasses, voluntary intake is generally higher due to shorter rumen retention times (**36**).

While most legumes are high in protein, one important difference among them is the considerable variation in their solubility. The resistance to protein degradation in the rumen has been attributed to high tannin content (**10**).

Another variable feature among the legumes is sulphur content. This element, in adequate concentrations, is required to achieve optimum fiber breakdown in the rumen (**1**) and important not only for bacterial growth but also for development of fungi. By providing S and other rich substrates to the rumen, legumes could play an important role in promoting the activity of both bacteria and fungi which subsequently digest fiber of low-quality materials.

APPROPRIATE SUPPLEMENTATION

The two supplementation strategies, viz. liberal and limited, were presented in this forum a few years ago (**38**). While very good growth and lactation responses can be expected from liberal supplementation, their application is confined to situations wherein supplements are readily available at low costs, coupled with high cost of hauling crop residues, e.g. commercial dairy/beef operations. For production at the village level where animal holdings of small farmers constitute 70-90% of the livestock population in the Asian countries, consideration needs to be given to restricting the use of supplementary concentrates, as they are usually in short supply, to levels which maximize the use of fibrous feeds. In this regard, the use of "balanced rations" to meet nutrient requirements has been questioned (**12,38**). For small-holders, it is much more realistic to adopt the feed-budget approach whereby the use of supplements would be rationed to improve utilization of fibrous feed resources for optimizing livestock productivity.

1. Substitution effect of supplementation

The decrease in roughage DM intake per unit of supplement DM given is known as the substitution rate (SR), calculated as:

$$SR = \frac{\text{Decline in roughage intake (kg)}}{\text{Increase in supplement given (kg)}}$$

This SR may be negative when the supplement stimulates roughage intake, indicating a true supplementation effect. When supplements high in RFC are given, the substitution rate usually ranges from 0, where the concentrate has no effect on

roughage intake, to 1.0 or more, where the roughage intake decreases by an amount equal to or more than the concentrate given.

2. Limited concentrate supplementation

In an attempt to compile available literature on straw supplementation, it was noted that, except at low levels of supplementation (10-20%), the amount of straw consumed decreased as the level of supplementation increased (**9**). Table 4 summarizes animal responses to urea-treated straw feeding with limited supplementation obtained from recent experiments conducted in Asian countries. These findings confirm an earlier statement made that urea-treated straw needs supplementation to support production with efficient feed utilization (**5,34**). Superiority of fish meal over oil cake or its combination with rice bran in promoting growth of young animals was demonstrated (**31**). Growth of older cattle (**42**) and milk production of dairy cows in late lactation (**11**), on the other hand, were not affected by protein sources (copra meal vs fish meal) or level of supplementation (0.3 vs 0.6% LW for the heifers and 1:2.5 vs 1:3.5 concentrate to milk ratio for the dairy cows).

3. Supplementation with green forages

Ideally, a legume supplement should maintain or increase voluntary intake rather than substitute for the basal ration. Experimental evidence, however, has shown that with untreated crop residues, forage supplements substituted for the basal feeds even when they were only 10-15% of the diet (Table 5). The situation was less clear with treated straw and more research in this area is needed. Perhaps the most pertinent point from the information presented is that, in some experiments, legume supplements had little effect on digestibility even when they comprised a significant proportion of the diet (Table 5). This indicates that the quality of forage supplements might not always be high, and that they may be best included as small amounts of the diet to provide specific nutrients such as RFC, nitrogen, minerals and vitamins. Considering the substitution effect, time availability and supply constraints, in so far as small-hold resources are concerned,green forages, particularly legumes, may be best included at levels of not more than 25% of the dry matter ration.

SUSTAINABLE RUMINANT PRODUCTION SYSTEMS INVOLVING CROP RESIDUES

With the population explosion taking place in most developing countries, resources, especially land, are being spread more and more thinly. This situation, aggravated by the debt burden, makes planning for development in this part of the world an extremely difficult task. Sustainable livestock production, along this line, must involve the full exploitation of cheap, lasting and locally-available resources with the maximum use of solar, not fossil fuel, energy. This necessitates the integration of ruminant production with crops. These two components complement and supplement each other in an integrated farming system. Large ruminants provide draught power and manure as fertilizer for crop production while crops provide residues and by-products for ruminant feeding.

1. Sustainability versus high input and productivity

Jackson (1981) proposed a realistic model for animal agriculture in Bangladesh (Fig. 1). The important feature of this system is the efficient utilization of the energy captured by vegetation -- nothing is wasted. It is self-reliant, not dependent on additional energy inputs, which is an asset for a country that must import much of its petroleum. By way of contrast, the Western system uses large inputs of fossil fuel energy in the form of fertilizers, hence its much higher yields. High crop yields, coupled with fewer people to feed, mean a surplus of cereals which are used for livestock feeding together with imported oilcake. This, coupled with a pleasantly conducive environment, brings about very high livestock productivity. Our attention in the past half century has exclusively focused on the positive aspects of Western agriculture - its high productivity per unit area of land and head of livestock. Needless to say, we have not succeeded in reaching this goal. Indeed, we can not succeed because among other reasons, petroleum costs too much (**12**). Although it may be true that the Bangladesh model is an exaggeration of the Philippine situation in terms of pressure on use of land and other resources, the message is that if we are not conscious about conserving resources and go on with the western model, bankruptcy is just around the corner.

2. Feed resource from rice production: An intervention

To support more livestock units in cropped lands, research in farming systems has successfully identified several strategies to augment feed supply. Intercropping, sequential cropping, ley farming and alley cropping involving food-forage crops were reviewed (**35,37**). Table 6 provides rough estimates of feedstuffs (crop residue and forages) generated from rice-based farming systems. Carrying capacity (animal unit equivalent to 450 kg liveweight) greatly increases with the introduction of forage crops after rice harvest; this is especially true with one rice crop a year. While grasses would yield twice as much biomass compared to legumes, the latter further provides food grain and enriches the soil. It should be pointed out that the introduction of forages after rice harvest does not reduce rice yield in the subsequent crop.

Feed resources for small-hold livestock production could further be augmented through the introduction of fodder trees, established as living fences. Calub (1988) estimated that 200 trees in a hedgerow or a living fence of Gliricidia planted 1m apart could provide 25% daily feed requirement of a 300 kg cattle on a year-round basis. This is premised on 0.47 kg DM/cut/tree for a 60-day cutting interval. The availability and utilization of tree fodders in the Philippines have been reviewed (**45**).

3. The three strata forage system (TSFS)

The TSFS is a technology of producing fodders from forage crops, shrubs and trees in a cash crop based area. One unit of TSFS (Fig. 2) is 2500 m^2 wide, consisting of: (1) 1,600 m^2 core area for cash crops (e.g. corn, soybean, cassava); (2) 900 m^2 peripheral area (first stratum) planted to grasses and ground legumes for wet season feeding; and (3) 200 m circumference area planted to alternating shrubs (2nd stratum) and trees (3rd stratum) for mid-dry and late-dry season feeding, respectively (**23**). Corn stovers and cassava tops are fed straight after harvest while soybean straw and cassava stems are stored for feeding during the late dry season.

In summary, one TSFS unit (good for a 300 kg cow) consists of 0.16 ha cash crop for human (with residues for livestock), 0.09 ha pasture, 2000 shrub legumes and 422 fodder trees. Stall-fed cattle is integrated in the second year after the

establishment of the first two strata. The third stratum takes three years to establish.

Since more and better quality of forages are available, the cattle growth is 12% faster and feed conversion, 29% better compared to traditional practice by farmers. The system has been in operation since 1984 and has established 180 units in dryland areas of Bali, 10 units in East Java and 1 unit in Southeast Indonesia (**23**).

CONCLUSION

While it is true that fibrous agricultural residues are poor quality feedstuffs, their feeding values can be greatly improved through urea treatment and/or supplementation for meat and milk production. Appropriate supplementation involves the use of small quantities of legumes and/or concentrate to maximize voluntary intake of fibrous feeds for optimal production of the animal.

In the context of sustainable agriculture in developing countries of Asia, taking into consideration the high cost of energy and shrinking land resource, the most sensible approach is to integrate livestock with the current crop production systems involving smallholders. Feed resources in these systems can greatly be improved through technology intervention without compromising the yield of the primary crop.

Table 1. Responses of growing and lactating animals fed with urea treated vs untreated rice straw

PARTICULARS	UNTREATED STRAW	UREA-TREATED STRAW
Sahiwal heifers (166 kg) with 6 kg grass silage + 0.54 concentrate/day **(27)**		
Dry matter intake		
Straw, kg/d	2.1	2.8
Total, kg/day	3.8	4.6
Liveweight gain, g/d	73	346*
Feed/gain, g/d	53	13
Zebu (121 kg) with 1 kg grass + 0.42 kg concentrate/day **(17)**		
Straw, kg/d	2.9	3.7
Total, kg/d	3.5	4.2
Liveweight gain, g/d	125	310*
Feed/gain	28	14
Holstein grades (175 kg) with 1.6 kg 13% CP concentrate/day[1] **(4)**		
Dry matter intake		
Straw, kg/d	3.0	2.9
Total, kg/d	4.6	4.5
Liveweight gain, g/d	670	650
Feed/gain	6.8	6.9
Brahman grades (190 kg) with 1 kg fresh grass and 0.85 kg 16.6% CP concentrates/day[1] **(43)**		
Dry matter intake		
Straw, kg/d	4.4	4.8
Total, kg/d	5.3	5.7
Liveweight gain, g/d	190	290*
Feed/gain	28.5*	20.2
Surti buffaloes with 1 kg concentrate/day **(27)**		
Dry matter intkae, %LW	2.8	3.7
Liveweight change, g/d	-93	+59
Milk yield, kg/d	2.2	3.0*
Milk fat, %	6.7	7.5
Calf LW gain, g/d	165	295*
Calf milk intake, kg/d	0.95	1.03

[1] Straw in the untreated group was sprayed with urea-molasses.

* : $P<0.05$

Table 2. Feed resources of dairy farmers practicing urea-treated straw feeding (N = 22)

Sourcing	Estimated available feed dry matter Tons/yr	Percent
Rice straw		
On farm	81.7	16.3
Off farm	195.2	38.9
Under coconuts		
On farm	45.1	9.0
Off farm	89.5	17.8
Marginal land	69.6	13.9
Improved pasture	12.5	2.5
Others	8.5	1.5
TOTAL	502.1	100.00

Table 3. Some commonly available agro-industrial by-products

Ingredients	DM (%)	CP (% DM)
Energy		
Molasses	75	4
Rice bran	88	11
Cassava chips	89	1.4
Corn bran	87	10
Sweet potato chips	90	12
Pineapple pulps	88	5.5
Soybean residue	46	6.8
Protein		
Copra meal	91	22
Brewer's spent grains	89	22
Soybean pulps	88	22
Soya oil meal	88	44
Meat and bone meal	94	54
Fish meal	89	60
Cassava leaf meal	91	25

Table 4. Dry matter intake (DM), average daily gain (ADG), daily milk yield (DMY) and feed conversion efficiency (FCE) of animals fed urea-treated straw (UTS) with limited supplementation[1]

Particulars	DMI, % LW			
	Straw	Total	ADG, g	FCE
Merino crossbred lambs, 28 kg LW-fish meal (FM) and/or Lucerne hay (LH) to UTS **(34)**				
UTS	1.6	1.6	-13	-
UTS + 75g LH	1.7	1.9	03	175
UTS + 75g FM	1.7	1.9	09	59
UTS + 75g LH + 75g FM	1.7	21	27	22
Cattle, 100 kg LW-Basal diet includes urea-lime treated straw (UTS) + 2 kg grass + minerals **(31)**				
Basal	-	-	150	21
Basal + 300 g oil cake (OC)	-	-	190	20
Basal + 300g kOC + 300g rice bran (RB)	-	-	250	15
Basal + 150g fish meal (FM)	-	-	360	10
Basal + 150g FM + 300g RB	-	-	350	11
Cattle, 175 kg LW-Basal diet includes UTS restricted to 2.2% LW + 1 kg napier grass **(5)**				
Basal	-	-	190	47
Basal + 200g OC	-	-	370	15
Basal + 600g OC	-	-	510	10
Brahman bulls, 200 kg LW-14% CP concentrate to UTS **(46)**				
UTS + 1 kg conc.	3.0	3.5	468	14.3
UTS + 2 kg conc.	2.8	3.6	840	9.5
Dairy replacement heifers, 270 kg LW-Basic supplements to UTS include 1 kg grass + minerals **(42)**[2]				
FM + RB, 0.7 kg/d	1.8	2.3	460	12.4
Copra meal (CM) + RB, 0.7 k/d	1.8	2.3	420	13.3
Late lactating cows - Basic supplements to UTS include 2 kg grass + mineral **(18)**[3]				
			DMY, kg	
FM + RB, 3.5 kg/kg milk	2.2	2.8	6.0	1.3
CM + RB, 3.5 kg/kg milk	2.1	2.8	5.3	1.5

[1] Urea-treated rice straw (UTS) was fed *ad lib* in all experiments unless otherwise indicated.

[2] No significant changes in response when the concentrate was doubled

[3] No significant changes in response when the concentrate intake was increased to 2.5 kg/kg milk

Table 5. **Dry matter intake (DMI), digestibility (Digest.), average daily gain (ADG) and feed conversion efficiency (EFC) of animals fed straw with forage supplementation (DM basis)**

Particulars	DMI, %LW Straw	DMI, %LW Total	Digest. %	ADG g	FCE
Rams, 20 kg LW - Rice straw (RS) vs urea-treated straw (UTS) with fresh Leucaena (L) **(4)**					
Para grass	3.4	3.4	51.1	86	9.0
UTS	3.3	3.3	45.7	37	18.6
RS - UM	3.7	3.7	50.4	40	19.4
UTS + 285g L	2.1	3.3	59.9	63	11.6
RS - UM + 285g L	2.5	3.7	49.2	60	13.4
Sheep 25 kg LW - RS with Cassava leaves (C) or Leucaena (L) **(6)**					
RS	2.5	2.5	44	-	-
RS + 0.25 g C	2.2	3.2	51	-	-
RS + 0.13 g L	2.1	2.6	47	-	-
RS + 0.40 g L	1.5	3.0	53	-	-
Bulls, 100 kg LW - RS vs UTS with Gliricidia (G) **(9)**					
RS	2.7	2.7	47	-113	-
RS + 0.6 kg G	2.5	3.1	49	-94	-
RS + 1.1 kg G	2.2	3.3	55	10	-
UTS	3.2	3.2	41	-28	
UTS + 0.3 kg G	3.1	3.4	45	63	-
UTS + 0.5 kg G	3.4	3.9	50	134	-
Steers, 150 kg LW - RS with Verano stylo (V) **(9)**					
RS	2.1	2.1	-	-165	-
RS + 0.3 kg V	2.2	2.5	-	11	332
RS + 0.6 kg V	2.2	2.9	-	60	78
RS + 0.9 kg V	2.21	3.0	-	104	45

Table 6. Feed resources from 1 hectare of paddy field (ton DM/ha/yr) and estimated carrying capacity (animal unit) with and without technology intervention

CULTIVATION METHOD	Rice Straw	Animal Unit	Green Forages[1] Bunds	Legumes	Grasses	TOTAL
Traditional						
One crop	2.5	0.7	0.2	-	-	0.2
Two crops	5.0	1.4	0.2	-	-	0.2
Three crops	7.5	2.1	0.2	-	-	0.2
Forage crops after rice						
One crop	2.5	1.7-2.4	1.0	2.5	5.0	3.5-6
Two crops	5.0	1.9-2.2	1.0	1.0	2.0	2-3
Three crops	7.5	2.4	1.0	-	-	1.0

Computed from (35) and (37).

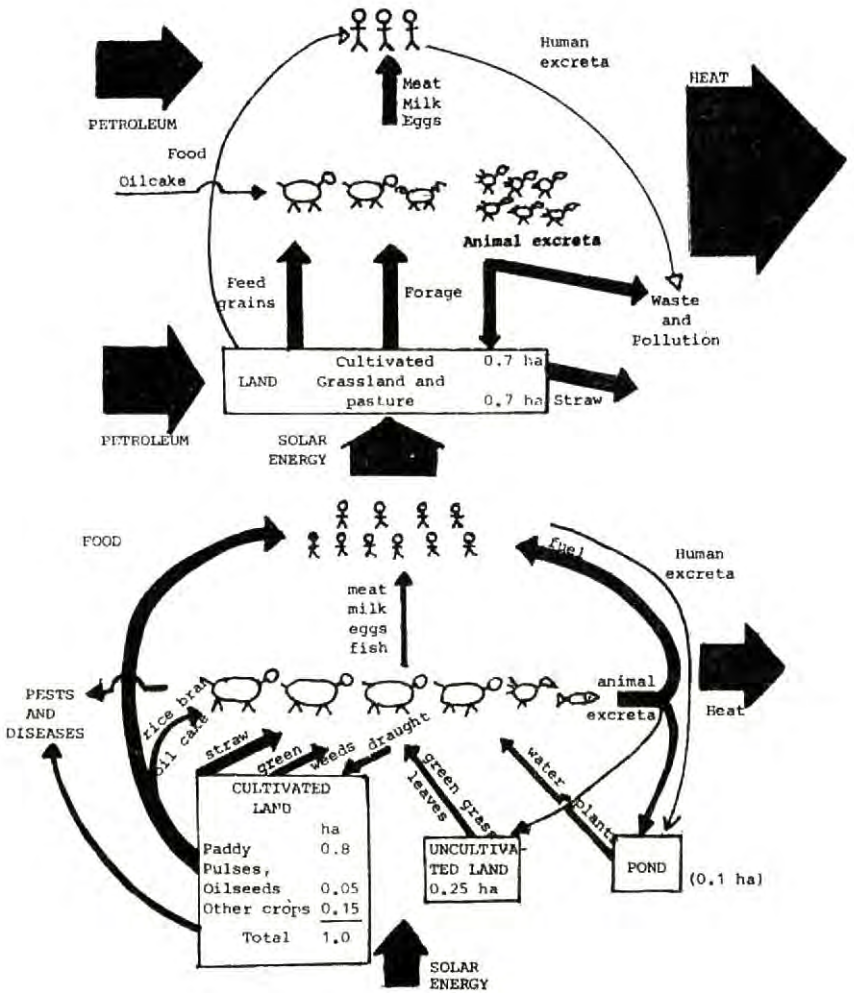

Figure 1. Comparative livestock production models: Western agriculture's (upper) vs Bangladesh's (lower). Adapted from Jackson (1981)

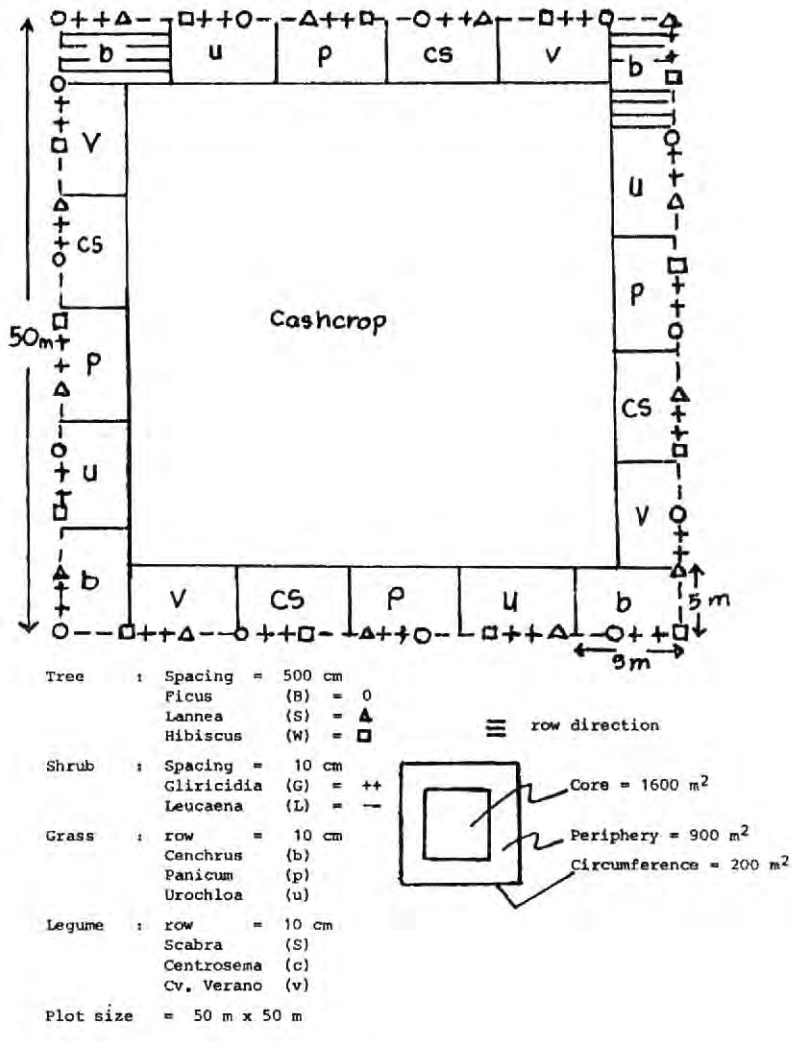

Figure 2. Planting arrangement of grasses, legumes, shrubs and trees in the TSFS

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Response of Crop Plants to Enhanced UV-B Radiation and Possible Implications on the Rice Crop

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ABSTRACT

Increases in solar UV,particularly UV-B (280-320 nm) waveband, have been observed due to the erosion of the stratospheric ozone layer by gases like chlorofluorocarbons. Ozone depletions of as much as 10% on a global scale and 4% in tropical rice-growing areas have been detected. The consequent increase in UV-B radiation may affect agricultural production in general and rice production in particular. UV-B has been shown to affect growth, photosynthesis, plant pigments, morphology and anatomy of plants, while interspecific and intraspecific differences have been reported.

Much of the research on UV-B in the past decade centered on the physiological and biochemical responses and environmental conditions. Few studies were undertaken under field conditions. Information is still insufficient to assess the risks of UV-B to rice. Results show varietal differences in rice response varying from: stunting; increased silica wax and flavonoid content of leaves; degradation of stomates, etc.. The resulting effect of these changes in the rice plant and the possible venues for the mitigation of and adaptation to the effects of UV-B are discussed.

The stratospheric ozone layer is decreasing at an alarming rate. This is mainly the result of the use of chlorofluorocarbons (CFC), the most common chemical for refrigerants and aerosols. Current estimates of ozone depletion during the next century range between 5 and 9% (**87**). The stratospheric ozone is the primary attenuator of solar ultraviolet radiation. A reduction of the ozone layer results in a very specific increase in shortwave radiation (UV-B, 280-320 nm). Absorption by ozone of shorter wavelengths (UV-C, 200-280 nm) is so great that a small fraction of the present ozone layer is sufficient to block radiation of UV-C. The absorption by ozone of longer wavelengths (UV-A, 320- 400 nm) is so weak that changes in ozone are of no consequence. The main concern, therefore, is the UV-B radiation.

In the rice growing regions of Asia, ozone column thickness has declined to 1-4% over the past 20 years (**140**). The highest UV-B levels at the earth's surface are found in the tropics, where the ozone layer is naturally thinnest and prevailing solar angles are highest. With ozone depletion, UV-B levels in the tropics are expected to exceed those experienced in recent history (**37**).

The most obvious effect of UV-B on human beings is skin cancer. UV-B is also known to cause cataracts and wrinkles on the skin. In plants, a wide variety of responses have been recorded. The biological effects of UV-B on plants have been reviewed by several scientists (**35, 61**). For field crops in particular, the effect of UV-B has been reviewed by Teramura in 1983 (**112**). This review is an update of Teramura's review with emphasis on the implications of enhanced UV-B irradiance on the rice crop, one of the most widely planted crops in the world under diverse environmental situations. Rice is planted in plains and high- altitude areas, under dry and flooded conditions (even in more than two meters of water), using a large and diverse group of cultivars.

This would mean that a wide range of cultivar differences from UV-B irradiance may be expected.

Studies involving the whole range of UV radiation were made in the early thirties and even in the late seventies (**61**). More recent studies indicate very definite responses between UV-B and UV-C so that earlier studies where UV-B and UV-C were not differentiated in the treatments would be difficult to interpret. This review deals mainly with UV-B. To date, very

little work has been conducted under field conditions for crop plants,even less on their yield and only a handful on rice. While most of the described UV-B effects were destructive or inhibitory to the plant system, plants also showed nondamaging responses to UV-B. These responses may be a protection from UV damage.

The responses recorded by different workers using different plant species or cultivars are summarized in Table 1 (after Teramura **112**). UV-B radiation affects the photosynthetic processes, through both photosystems I and II, the carboxylating enzymes, stomatal resistance, chlorophyll concentration, soluble leaf proteins,lipids and carbohydrate pools. UV-B radiation has also been shown to affect the anatomy and morphology of the plant, such as plant stunting, reduction in leaf area and increase in leaf thickness. Cell and tissue damage such as chlorosis, bronzing and necrosis also occur in sensitive plants. The best documented effect of UV-B radiation is the stimulation of flavonoid biosynthesis which is thought to be a protective response to the deleterious effects of UV-B. Flavonoids are UV-B-absorbing compounds found in the epidermal layer of plants.

Results of many experiments using a variety of plants definitely show alteration in plant growth and physiological processes with increased levels of UV-B. Generalizations were made in this review. However, one must bear in mind that the UV-B influence rate and dose used varied and that reactions may differ with cultivar, stage of growth and condition of the plants.

A. Physiological and biochemical effects

Photosynthesis. Among species and among cultivars, large differences in response to UV-B irradiation occurred. Nevertheless, the general result is the reduction in net photosynthesis by UV-B radiation through the primary photochemical events and electron transport reactions, the dark reactions fixing carbon into reduced compounds, the dark respiration, stomatal resistance and the attendant CO_2 exchange (Table I). All these can affect the carbon balance of the crops although species and cultivar differences have been reported.

In comparing photosynthetic rates of leaves with similar chronological ages, some caution must be observed. In soybean, UV-B irradiation resulted in earlier attainment of leaf ma-

turity and maximum photosynthetic capacity (**114**). Thus, low photosynthetic rates at later stages of growth may be recorded for plants exposed to UV-B since the peak level has been reached and the plants are senescing when compared to the control plants. The reduction in photosynthetic activity paralleled the decrease in Hill activity, indicating the sensitivity of Photosystem II (PSII) to UV-B radiation (**12, 136**). Although cyclic photophosphorylation (PSI) was also somewhat sensitive to UV-B radiation, the primary effect involved PSII. The net reduction in photosynthesis also coincided with structural damage to the chloroplast. The reduction in photosynthesis with enhanced UV-B was manifested through reduction of photosynthetic pigments, protein and RUPB carboxylase activity (**138**). These led to less growth in UV-B-sensitive crop plants. PEP carboxylase activities in *Zea mays* L. (cv Golden Cross Bantam) have been reported to be suppressed with large dosages of UV-B; they were, however, only enhanced by low dosages (**137**).

Most early studies on the effect of UV on photosynthesis used lights with broader spectrum (**112, 142**). Different wavebands of the UV spectrum have shown different effects so that earlier findings were difficult to interpret and were excluded from this review. However, the studies were cited in Table I.

A reduction in photosynthesis was accompanied by a significant increase in respiration rate. In *Rumex patientia* L., dark respiration rates were significantly higher in UV-B-treated plants (**102**).

Electron micrographs of *Pisum sativum* L. exposed to UV-B radiation indicated structural damage to chloroplasts as well as other organelles (**33**). This was not so in *Rumex patientia* L. where chlorophyll concentration remained unaltered even after 22 days of UV-B treatment (**102**).

The reaction of the rice crop to high UV-B irradiation in terms of net photosynthesis is complicated since growth stages, canopy reactions, levels of visible radiation before and during treatment, cultivar differences and other factors have to be considered. For example, many plant species are more sensitive to UV-B irradiation when grown under low levels of visible radiation (**76, 122**) and this would be the case with the rice crop grown during the wet season (as compared with that grown during the dry season).

In soybean, stomatal conductance was reported to have been increased and, at another stage, to have been decreased by UV-B (**81**). Varietal differences in reaction were noted.

In rice, we found that stomatal resistance increased with enhanced UV-B (**39**). This is partly the result of stomatal closure and the collapse of the stomata especially in UV-B-sensitive cultivars. Stomates of rice plants exposed to UV-B opened later than those in the control plants when returned to normal conditions (**39**). This response may indicate less efficient CO_2 exchange and lower transpiration rates in UV-B-sensitive rice plants. With closed stomates, the attendant higher leaf temperatures and lower CO_2 decrease the photosynthetic rates and eventual biomass production in rice.

Soluble proteins/DNA. UV-B is readily absorbed by nucleic acid and protein chromophores so that these substances are easily damaged by UV-B. Their participation in the plant response to UV-B has been documented (**35, 85, 52**). Our studies showed that total amounts of nucleic acid and soluble proteins were reduced significantly in UV-B-sensitive rice cultivars (Table 2). Studies have also shown that plants have the ability to repair UV-induced DNA damage (**74**).

Carbohydrates. UV-radiation significantly reduced total nonstructural carbohydrates in tomato, cabbage and collard but had no effect on peanut and corn (**50**). Nothing had been reported on rice.

Nonphotosynthetic pigments. Flavonoids in the leaf epidermis selectively filter sunlight so that much of the damaging UV-B radiation is removed while most of the photosynthetically active radiation is transmitted. The increase in the production of flavonoids in the leaf tissue due to UV-B irradiance can reduce the UV-B flux received at potentially sensitive sites such as chloroplasts and nuclei. This may represent an adaptive response to UV-B radiation. Much of the UV-B attenuation on the leaf epidermis was removed upon methanolic extraction of the epidermis, suggesting that phenolic compounds such as flavonoids are important in the absorption of UV-B radiation. Flavonoids are ideal UV screens since they are nearly transparent in the visible region and thus allow PAR while possessing high absorption coefficient in the UV region. Resistant cultivars generally produce more flavonoids with UV-B radiation (**78, 112, 128**) However, in radish seedlings, the increase in flavonoids could not protect them from high UV-B radiation levels (**78**).

Epidermal transmission of UV-B was lowest in plants growing in regions with high, naturally occurring UV-B flux; it then increased as UV-B radiation diminished along a latitudinal gradient (**112**). SInce rice plants are cultivated in such places as Uruguay, South America (35^0 S latitude), Northeastern China (50^0 N latitude) and from altitudes below sea level in Kerala, India to above 2000 m in Kashmir (**148**), great variability in response may be expected from this wide ecologial distribution. This aspect requires more extensive investigation.

The results for UV-B-induced anthocyanin formation in corn (**16**) and sorghum (**43**) show that the response of various cultivars may vary considerably within a single species. Large quantities of anthocyanins were found not only in the shoots but also in the roots of corn seedlings (**49**).

Some reports indicate that UV-B irradiation induced flavonoid synthesis and that a blue light photoreceptor may also be involved (**16, 42, 44, 144**). Flavonoid increase in rice leaves has been reported (**39**) and the increase is greater in the tolerant cultivars.

Plant hormones. UV-B treatment of spinach leaves showed lower endogenous gibberellin activities at the period of active growth of the plants (**70**). UV-B had no significant effect on the amount of abscisic acid in *Rumex patientia* L. (**69, 133**).

Ion transport. The amount of ^{65}Zn translocated from the cotyledons of cotton seedlings to the newly developing shoot was twice as great in control seedlings as that in UV-B-treated plants (**3**). In UV-B-treated rice plants, the reduction in electrical conductivity of leaves measured after boiling may be an indirect evidence of ion transport inhibition (**39**). Such an inhibition would affect the nutrition of the rice plant and the subsequent biomass production.

Cellular/chromosome. The rate at which cell division occurs is determined generally by the sequence of DNA replication. Since DNA, RNA and proteins are UV-B chromosomes (**52**), these are likely targets of UV radiation. In *Rumex patientia* L., UV-B primarily affects cell division rather than cell expansion. Less number of cells results in a smaller leaf. The cell size of the palisade and the epidermis are similar in the control and treated plants (**41**).

UV-B increased water permeability in *Allium cepa* (**67**) and the UV- B dose necessary was much higher than those affecting the cytomorphological parameters of the cell. In *Allium cepa*, low

dosage of UV-B resulted in plasmolysis, acceleration of protoplasmic streaming and rounding of mitochondria. Higher dosages resulted in decreased protoplasmic streaming, swelling of the endoplasmic reticulum and increased water permeability.

B. Morphological/anatomical effects

Stunting. One of the most commonly observed effects of UV-B radiation on seedling growth is stunting or dwarfing (Table 1). This is primarily due to the decrease in internode length. In cereals, stunting may also be due to shorter leaf blade and leaf sheath since the measure of plant height depends on the length of leaf sheath and blade. The decrease in leaf area is accompanied by a decrease in leaf length.

UV-B-induced growth reductions are associated with changes in cell division and/or cell elongation. An interaction with growth regulator indole-3-acetic-acid (IAA) was demonstrated in hypocotyls of sunflower seedlings. IAA absorbs UV-B and can be converted to various photo-oxidation products (**126**). One of these products, 3-methyl-oxindole, inhibits hypocotyl growth when applied exogenously.

According to Biggs and Kossuth (**19**), rice height was unaffected by UV-B but Basiouny (**11**) reported shorter plant height for variety 'Caloro'. Our preliminary observations showed a wide range of responses by different rice cultivars. Nine out of 30 cultivars had stunting with Amarelao and IR45 showing the greatest reduction while Fujisaka 5, Cafuringa 1 and ROK5 showed the greatest increase in height (**6**).

If UV-B reduces internode elongation, this would be critical for deepwater rice cultivars since internode elongation is necessary for their survival. Deepwater rice is planted in around 20 million hectares, mostly in India, Bangladesh, Thailand and Vietnam. SInce it is the only crop planted during the monsoon season, survival of the crop means survival of the farmer.

Leaf area. Leaves are sensitive to environmental stresses such as low and high temperatures, drought, mineral deficiency and toxicity and excess salt. It is not surprising that UV-B radiation generally reduces leaf area (Table 1). In a growth chamber study using over 70 unrelated crop species and cultivars, it was found that leaf area was significantly reduced in over 60% of the plants (**19**). However, UV-B had little effect on rice and other cereals. In the most sensitive plants, 60-70% reduction in leaf expansion was recorded. Such large reductions were found only in

studies utilizing very low PAR (**19**). Under field conditions, leaf expansion was substantially increased by moderate UV-B radiation in rice (**20**). In some species, apparently different responses were obtained among growth chamber-vs-field grown crops. Our preliminary findings showed both decreases and increases in leaf area in 30 rice cultivars (**6**).

Specific leaf weight (SLW). Plants adapt to UV-B radiation by increasing the SLW; the upper leaf tissue layers thus act as anatomical screens or filters. Thicker leaves have a greater proportion of their chloroplasts at greater depths in the leaf tissue and they are better shielded from UV-B radiation. However, increase in SLW did not always correspond with UV-B radiation resistance (**19**). This is to be expected in as much as different species have different or many mechanisms for tolerating UV-B radiation. Therefore, increase in SLW alone cannot be assumed to be an indicator of UV radiation stress. In soybean, visible irradiation during leaf development is important in altering UV-B sensitivity of the photosynthetic system. The thicker leaves produced at high visible irradiation or outside the greenhouses and growth chambers had less damage from subsequent UV-B radiation (**13,32,36,47,102,139**).

Leaf structural characteristics and protective pigment levels, rather than chloroplast characteristics, appear to be responsible for tolerance to UV-B among species and among cultivars. If additional flavonoids can be induced by increased solar UV-B radiation, the sensitivity to UV-B of field-grown plants could be reduced further.

Epidermal transmission/wax content. Attenuation of damaging UV-B radiation (280-320 nm) in the upper epidermis reduces the penetration of UV-B radiation to the mesophyll where damage to physiologically sensitive targets can occur. The epidermis can attenuate up to 95% of the incident UV-B radiation and yet transmit between 70 and 89% of the visible radiation of 400-700 nm (**96**). In *Oenothera stricta*, a high-elevation tropical plant, no significant reduction in epidermal transmittance of visible radiation was observed as a result of UV-B exposure (**96**). The plasticity in epidermal UV-B transmittance results from production of flavonoid and related phenolic compounds in the tissue. After UV-B exposure, the absorbance of UV-B radiation in flavonoid extract solutions from epidermal and mesophyll tissues significantly increased by as much as 100

and 35%, respectively, without reduction in epidermal transmittance of visible radiation.

Most leaf waxes do not absorb within the UV and the visible wavebands and are excellent leaf protectants against microorganisms and water loss. Waxes also enhance light reflectance and scattering. UV-B irradiation increased the total wax on the leaf surface by about 25% in barley, bean and cucumber seedlings (**108**). The aldehydes, detected as minor constituents of the wax, increased twofold, mostly on the adaxial surface on the leaf. The wax layer might be thicker merely as a consequence of smaller leaf area and of constant endogenous wax production.

In the wax biosynthesis of cucumber leaves, the distribution pattern of alkanes was greatly influenced by UV-B irradiation. Short-chain alkanes were increased while long-chain alkanes were depressed (**123**) by UV-B irradiation.

Preliminary studies on rice leaf epidermis using scanning electron microscopes showed that enhanced UV-B treatment tended to increase wax content of the leaves (**40**). Since the UV-B treatment also decreased the leaf area, the increase may be a consequence of the smaller leaf area. The increase in leaf surface wax may be important if increase in temperature accompanies increase in UV-B irradiance.

Leaf bronzing, glazing and chlorosis. According to Teramura's review (**112**), UV-B radiation produces bronzing, scorching, glazing or chlorosis in leaves of susceptible plants such as soybean, pea, cotton and cucumber (Table 1). However, these symptoms are nearly always associated with plants grown under either high UV-B (**128**) or moderate UV-B but low PAR. Scanning electron microscopy of leaf surface showed deformed epidermal structures in plants showing scorching (**128**). Basiouny (**11**) reported leaf necrosis in the rice cultivar 'Caloro'. In field studies with high UV-B supplied by unfiltered lamps, these symptoms have not been reported. Our studies showed no chlorosis in rice cultivars for the levels of treatment used (**39**).

Seedling emergence and growth. UV-B radiation had no effect on the percentage of seed germination in the species tested (**11,63**). This may be due to the inability of UV-B to penetrate the growing media or the seed coat. However, once the seed has germinated, seedling emergence was delayed 2-3 days by UV-B radiation in almost all the species tested (**11**). The cotyledons in some plants emerged late from the seed. Extended UV-B expo-

sure can result in abnormal seedling growth in many species and, consequently, cause short, stubby roots, increased pigmentation and abnormal curvatures of the shoots (**63**). Although germination is not particularly sensitive to UV-B, the developing seedling is extremely sensitive.

Total chlorophyll and chorophyll a/b ratios were reduced in UV-B- irradiated plants during the vegetative stage. In contrast, total chlorophyll was increased by UV-B during the reproductive stage (**121**). Under natural conditions, plants will probably be more sensitive to UV-B during the early stages of growth than at later stages.

No significant changes in chlorophyll content were reported in different cultivars of rice at the seedling stage by the Spad method (**39**) while chlorophyll content by the acetone method increased (Table 2). The effect of UV-B irradiation on the chlorophyll content of rice at the later growth stages is not known.

Biomass production/partitioning. Total biomass represents a long- term integration of all growth processes and subtle effects of UV-B radiation may accumulate and result in significant effects. Unfortunately, most studies on UV-B radiation do not include the root biomass so that one of the effects of UV-B -- which may be in the distribution of the biomass -- is not reflected in the results. Nevertheless, studies showed that the total plant dry weight was often substantially reduced by UV-B radiation (**17,102,112, 133, 135**).

Rice variety 'Caloro' produced less fresh and dry weight per plant and also less ash weight per plant as a result of UV-B irradiation (**11,133**). Our studies in rice showed that in general, total dry weight (which included the root biomass) was reduced by UV-B radiation in 30 cultivars. Marked decreases in shoot weight were observed mostly in IR cultivars. UV-B also induced a shift in biomass partitioning with enhanced allocation to the leaves during the vegetative stage. Reduction in total biomass was not always correlated with reduction in plant height or leaf area, especially in grasses where leaves were oriented vertically rather than horizontally (**9**).

Crop Yield. Ultimately, we are interested in the effect of UV-B irradiation on crop yield or the economic yield of a crop. Very few studies have been conducted under field conditions. Most experiments using crop plants were conducted in growth

chambers or in greenhouses and, because of space limitation, the plants were usually not grown to maturity. The crop yield response is the key factor in assessing the impact of partial stratospheric ozone depletion.

Of the 10 crop species tested, yield was unaffected in nine crops despite massive UV-B doses and only a significant reduction in fruit number of pepper was observed in one of two experiments (**54**). In another study using eight crops, only broccoli was affected (**4**); still in another experiment using six crops, no significant effect was reported (**46**). No effect in bean and tomato was reported (**10**) although yield of corn was significantly greater. However, other workers said that corn yield was not affected (**4**, **54**). Until these discrepancies are resolved, the field experiments, to date, are of limited use (**112**).

Studies of Biggs and Kossuth (**20**) showed no effect of low levels of UV-B irradiation on yield of rice and other crops. However, yield was consistently reduced at the highest UV-B enhancement level for all test crops. The possible effect of UV-B irradiation on rice crop yield is difficult to predict since cultivar differences and environment parameters are most diverse in this crop compared with other major crops.

Plant type. Using different models, leaf area indices and leaf angles, it was predicted that penetration of UV-B radiation would be much greater in erect-leaf than in horizontal-leaf canopies (**1**). This simulation has definite implications to the rice plant type -- erect leaves for the modern, high-yielding varieties in contrast to the long and droopy leaves of the traditional varieties. The modern cultivars have a canopy that would be more receptive not only to solar radiation but also to the attendant UV-B radiation. In this case, the deleterious effects of UV-B might be greater.

In developing a rice cultivar tolerant of high UV-B radiation, the different plant traits responsible for tolerance should be incorporated and each trait should be examined independently. This is similar to the development of flood-resistant rice cultivars. Many morphological and physiological factors are involved and as many positive traits as possible should be incorporated.

Most of the rice traits measured in terms of response to enhanced UV-B radiation showed a wide range of cultivar differences, thus selection can be made and breeding for a plant type tolerant of UV-B is possible.

Reproduction. Flowering responses of plants to UV-B irradiance have been reported in several crops. It was found that more

flowers were produced when UV-B was excluded by mylar plastic films or glass in *Melilotus* (**60**), *Trifolium dasyphyllum* (**34**) and *Tagetes* (**62**). A clear UV-B fluent rate and fluence-dependent inhibition of photoperiodic flower induction were observed in the long-day *Hyoscyamus niger* (**92**). In contrast, no significant effect was found on the flowering of *Petunia*, on the tassling of maize or on heading of *Sorghum bicolor* (**54**).

In soybean, the transition period between vegetative and reproductive growth was the stage most sensitive to enhanced UV-B radiation (**116**). The time of flowering was delayed by 1-3 days by UV-B radiation in all species tested by Basiouny (**11**). The flowers of UV-B-irradiated plants were smaller, fewer and less vital than flowers of untreated plants.

Specific leaf weight increased during vegetative growth but was unaffected by UV-B during reproductive growth (**116**).

Since UV-B may cause inhibition of elongation, panicle exsertion in the rice plant may also be delayed by UV-B irradiation or panicles may not be fully exserted as a consequence. This would make threshing difficult.

C. Differences in response

Interspecific differences. There are large differences in response to UV-B radiation between genera and species (**19**). Since a variety of responses has been recorded, a combination of morphological, anatomical and physiological processes can easily provide different sensitivities in different species. It has been reported, however, that monocotyledons as a whole seem to be less affected by UV-B than dicotyledons (**111, 128, 132**). This difference might be partially due to the vertical leaf orientation and the basal leaf sheaths which provide protection to the meristematic region in the monocots (**132**). Some broad-leafed species with C_3 type of carbon assimilation were more susceptible to UV-B than the narrow-leafed species with C_4 type of photosynthesis (**12**).

Some plant families like the Cruciferae have many species which are extremely sensitive to UV-B while the Poaceae (a grass family) has relatively resistant species (**19**). Also, alpine species are more resistant than lowland species (**110**). Epidermal transmission of UV-B was lowest in plants growing in regions of high, naturally occurring UV-B flux; it increased as UV-B radiation diminished along a latitudinal gradient.

Variations in plant responses may be the results of changes in microclimate, differences in repair or protection mechanisms, diversity in conditions prevailing during the growing season and stage of plant development.

Intraspecific differences. The more important aspect in the mitigation of UV-B is the large varietal differences in response to UV-B within species (**4, 17, 29, 45, 112, 135**). In cucumber, the intraspecific differences in UV-B sensitivity are related to inherent differences in the accumulation of UV-absorbing compound (**82**). This suggests that there is a potential for genetically modifying future cultivars to minimize the deleterious effects of UV-B or to optimize possible beneficial effects. Biggs and Kossuth (**19**) reported cultivar differences in rice. The reasons for the cultivar variability are not completely understood.

Our studies of four rice cultivars showed cultivar differences in the response of rice to enhance UV-B (**39**). Differences in plant height, leaf area and length, dry weight, as well as in chlorophyll, soluble protein, nucleic acid, flavonoid, silica, root-oxidizing activity and ion concentration in the leaves were observed (Table 2).

D. Environmental interactions

Visible radiation. The greatest differences in total dry matter production resulting from UV-B radiation were found in moderately shaded conditions for soybean but in full sunlight for wheat (**111**). The study emphasized the importance of the interaction between UV-B and PAR and the need to measure PAR to critically evaluate the effects of UV-B radiation on plant growth under natural conditions.

Leaves that developed under reletively low visible radiation (as in cloudy weather) are generally thinner and are, therefore, more susceptible to inhibition of photosynthesis by UV-B radiation (**76, 133, 139**). The implication is if the visible radiation is low during UV-B irradiation, this will result in greater photosynthetic depression than when visible irradiation is high. However, radiation during pretreatment can play a larger role in altering UV-B sensitivity of photosynthesis than the visible flux applied during the UV-B irradiation, as found in soybean (**139**). High visible flux given concomitantly with UV-B after low visible radiation treatment resulted in greater photosynthetic depression than when low visible flux was presented with the UV-B

irradiation. High visible flux alone resulted in no detectable photosynthesis inhibition.

During the monsoon season, the rice plant may be subjected to a week of low visible irradiation followed by high visible irradiation concomitant with high UV-B irradiation. The damage from UV-B to the new leaves formed, which are thinner, is expected to be higher, based on the findings in soybean.

Water stress. UV-B had no effect on the internal water relations of soybean (**118**). However, in *Allium cepa,* UV-B increased water permeability in the cell (**67**) through membrane lipid and plasmalemma breakdown.

In soybean, the intraspecific response differences in UV-B treatments between two seasons of planting were thought to be related to the frequency of drought and overcast skies (**81**). In another study, increased levels of UV-B in soybean had no effect on leaf area, total plant dry weight and net photosynthesis in plants subjected to water stress (**80**). The insensitivity may be related to anatomical and biochemical changes induced by water stress, such as increase in the concentration of UV-B-absorbing compounds in the leaves and leaf thickening, both of which can lessen UV-B penetration to the photosynthetic system.

Water deficit in rice plants is common in upland and rainfed lowland rice. There is, therefore, a need for intensive research on the effect of enhanced UV-B irradiance on the water relations of the rice plant.

Ecology/competition. Predicted penetration of UV-B radiation was much greater in erect-leaf than in horizontal-leaf canopies. This finding would indicate that the modern, high-yielding rice cultivars with erect leaves would have UV-B radiation penetrating down to the lower leaves. Whether the lower leaves are more sensitive or not would also determine the effect of UV-B radiation. The lower, more mature rice leaves have lower photosynthetic rates (**149**) and the effect of UV-B may not be as critical.

In pine, the effects of enhanced UV-B were less for those species indigenous to higher elevations, implying the presence of natural adaptations to UV-B (**110**).

Competitive interaction studies show that smaller species usually benefited under UV-B stress, presumably as a result of relatively reduced UV-B flux in the shade of the taller species (**53**). In many communities, complex species interrelationships

invalidate the concept of discrete, competing species pairs. However, a ricefield with serious infestation of broad-leaf weeds may show greater damage to the rice crop than to the broad-leaf weeds. The possible stunting of the rice crop, a general effect on all cultivars so far (**39**), and the reduction of UV-B flux to the weeds by the relatively taller rice plants will probably result in greater domination of the weed species especially those less sensitive to UV-B.

UV-B has shown deleterious effects on the morphology, anatomy and physiology of the rice plants. However, cultivar differences were noted and this provides strong indications of mitigating the effect of elevated UV-B in the future.

Table1. Summary of the effects of UV-B radiation on crop growth (Modified from Teramura 1983)

	References
Physiological/biochemical effects	
Photosynthesis	2, 7, 12, 17, 18, 21, 33, 37, 47, 71, 76, 78, 80, 81, 90, 96, 100, 101, 102, 103, 104, 112, 113, 114, 117, 121, 122, 125, 133, 136, 139
Hill reaction	2, 12, 33, 50, 65, 73, 90, 136
Electron transport	33, 58, 65, 90, 93, 94
RuBP carboxylase	2, 25, 50, 76, 130, 137, 138
PEP carboxylase	2, 137
Dark respiration	21, 33, 102, 117, 121
Transpiration/stomatal resistance	17, 18, 21, 33, 47, 76, 80, 81, 88, 89, 101, 102, 112, 113, 117, 118, 120, 121, 122
Photosynthetic pigments	2, 12, 24, 33, 50, 57, 70, 76, 80, 81, 90, 101, 102, 114, 121, 122, 125, 128, 136, 138, 139
Soluble proteins/DNA	2, 12, 14, 15, 35, 47, 49, 59, 74, 84, 86, 91, 98, 105, 127, 128, 131, 134, 137, 138, 142
Lipids	46, 72, 107, 112, 128, 129
Carbohydrates	3, 4, 46, 50, 112
Nonphotosynthetic pigments	5, 7, 14, 15, 16, 24, 36, 37, 42, 43, 44, 47, 49, 55, 56, 57, 68, 71, 74, 75, 76, 77, 78, 80, 82, 84, 90, 96, 97, 100, 113, 114, 122, 127, 128, 136, 138, 139, 142, 143, 144, 145, 147
Plant hormones	23, 25, 37, 69, 70
Ion transport	3, 93
Cellular/chromosome	2, 38, 41, 61, 67, 80, 134

Table 1. Cont . . .

Morphological/anatomical effects	
Stunting	2, 8, 11, 12, 29, 48, 79, 80, 82, 110, 112, 115, 116, 122, 124, 135, 136, 138, 142
Leaf area	2, 8, 21, 28, 29, 41, 48, 66, 68, 70, 76, 80, 81, 82, 84, 100, 102, 103, 111, 112, 114, 115, 116, 121, 122, 128, 135
Specific leaf weight	7, 29, 47, 66, 76, 80, 82, 84, 112, 114, 116, 121, 139
Epidermal transmision/wax content	1, 22, 47, 51, 74, 96, 97, 104, 108, 109, 123
Bronzing/glazing/chlorosis	2, 17, 28, 29, 55, 63, 95, 117, 128
Growth stages	11, 55, 61, 63, 81, 106
Biomass production/partitioning	8, 9, 11, 12, 17, 19, 20, 21, 28, 29, 33, 48, 53, 66, 70, 72, 79, 80, 82, 84, 100, 102, 106, 110, 111, 112, 113, 115, 116, 121, 122, 124, 133, 135, 136
Crop yield	20, 30, 70, 112, 115, 119, 122
Plant type	1, 141
Reproduction	11, 20, 26, 27, 37, 116, 122
Response differences	
Interspecific (species differences)	2, 8, 11, 12, 19, 20, 28, 50, 63, 79, 110, 112, 113, 128, 133, 135, 137
Intraspecific (cultivar differences)	16, 28, 29, 64, 71, 82, 84, 112, 113, 115
Environmental interactions	
Visible radiation	7, 10, 29, 48, 76, 102, 111, 113, 117, 122, 133, 139, 146
Water stress/microclimate	67, 76, 80, 81, 113, 116, 118, 120, 121, 122, 127
Ecology/competition	1, 7, 8, 9, 18, 37, 48, 53, 96, 99, 110, 122, 133
Mineral stress	31, 83, 113, 122

Table 2. **Physiological changes of rice in response to enhanced UV-B treatment for 4 weeks. Each value is average of 24 plants (39).**

Parameter	Cultivar			
	IR30	IR45	IR64	IR74
Plant height (cm)	-*	-*	-*	-*
Maximum root length (cm/plant)	-	+	-	-
Root volume (cm^3/plant)	+	-	-	-**
Tillers (no./plant)	+	-	+	-**
Leaf area (cm^2/plant)	-	-*	-	-**
Leaf dry weight (g/plant)	-	-*	-	-**
Sheath dry weight (g/plant)	-*	-*	-	-**
Root dry weight (g/plant)	-	-*	-*	-**
Shoot dry weight (g/plant)	-*	-*	-	-**
Dry weight (g/plant)	-	-*	-	-**
Specific leaf weight (g/m^2)	+	-	+*	+
Relative growth rate (mg/g/d)	-	-*	-*	-**
Net assimilation rate (g/m^2/d)	-*	-**	+*	-*
Shoot/root ratio	+	-	+	+**
Chlorophyll content (mg/g fresh weight)	+*	+	+*	+
Root activity (mg α-naphthylamine/h/g)	-*	-*	-*	-*
Soluble protein (mg/g fresh weight)	-**	-*	-*	-**
Nucleic acid (g/g fresh weight)	-	-*	-	-*

*, ** Significant at the 0.05, and 0.01 levels, respectively, according to Student's T-test.

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HEALTH SCIENCES

Antimicrobial Activity, Antimutagenicity, Cytotoxicity and Neuroactivity of Some Philippine Marine Sponges and Tunicates

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ABSTRACT

Sponge and tunicate samples collected from Bolinao, Pangasinan are in various stages of extraction by solvent partitioning and purification by chromatography. Biological screening for antimicrobial activity, antimutagenicity, cytotoxicity and neuroactivity is performed on the crude extracts and semi-pure fractions. Diagnostic thin-layer chromatography and H-NMR spectroscopy are used to determine the profile and nature of compounds present in these samples. Preliminary results indicate that 15 sponges and 4 tunicates are active against certain gram-positive bacteria, gram-negative bacteria and fungi. Crude and Kupchan (hexane, choloroform, methanol and aqueous) extracts showed zones of inhibition ranging from 7 - 15 mm against ***B. subtilis*** *(G+),* ***M. luteus*** *(G+),* ***S. aureus*** *(G+),* ***S. pyrogenes*** *(G+),* ***S. viridans*** *(G+),* ***P. vulgaris*** *(G-),* ***P. aeroginosa*** *(G-),* ***E. aerogenes*** *(G-),* ***E. coli*** *(G-),* ***K. pneumoniae*** *(G-),* ***C. albicans*** *(f),* ***C. utilis*** *(f) and* ***S. cerevisiae*** *(f). A dose-dependent response was demostrated in several cases. Antimutagenicity testing using the Rec assay system, with repair-proficient (Rec+) and repair-deficient (Rec-) strains of* ***B. subtilis*** *and with*

quinoline as positive control, showed 10 crude sponge and tunicate extracts with potential antimutagenicity activity. The cytotoxicity assay performed using the fertilized eggs of the starfish ***Protoreaster nodosus*** *indicates that some extracts have cytolytic activity, while others may contain DNA or protein synthesis inhibitors. The intracranial injection of extracts into mice elicited specific neuroactivities ranging from stimulation to depression. Preliminary H-NMR of some bioactive extracts showed interesting and novel chemical features. More material is currently being purified for complete structural elucidation of the bioactive compounds in these extracts.*

INTRODUCTION

The search for drugs from the sea in the last 10 years has yielded an enormous number of novel bioactive, secondary metabolites. Compounds from marine invertebrates such as bryozoans, sponges, echinoderms, coelenterates and tunicates, accounted for over 58% of all compounds studied up to 1985 (**7**). Compounds isolated from these animals can be classified into: non-nitrogenous metabolites such as terpenes, sterols and steroids, polyketides and macrolides, carotenoids: and nitrogenous metabolites such as small aromatic amines, amino acids -- aromatic and nonaromatic; peptides -- linear and cyclic, conservative and derivatized; nucleosides, isonitriles and alkaloids.

Sponges, the most primitive marine invertebrates, are prolific producers of terpenoid and nitrogenous metabolites. Various biological activities have been observed in many species: a large percentage is antibacterial; while others have antifungal, antiviral, cytotoxic, antitumor, icthyotoxic and insecticidal properties. It is argued that sponges have developed these novel secondary metabolites for defense and survival. Tunicates, on the other hand, belong to the phyliogenetically higher division of chordates. Among marine invertebrates, they have the highest percentage of isolated nitrogenous compounds and one of the lowest numbers of compounds studied so far. Being in the same phylum as higher animals such as fish, amphibians, reptiles, birds and mammals, they may contain compounds with greater specificity and affinity for receptors in these animals.

Tremendous interest has been generated in the field of marine natural products since the isolation of the novel arabinonucleosides, spongouridine and spongothymidine, from the sponge *Tethya crypta* (**1**). These compounds served as models for the development of the potent antiviral and antitumor drugs, Ara A and Ara C. Other compounds from the sea have since shown great potential as new marketable therapeutic and diagnostic drugs. Among these are the discodermins, antimicrobial peptides from the sponge *Discodermia kiiensis* (**11**); the didemnins, antiviral and anticancer cyclic peptides from the Carribean tunicate *Tridemnum sp.* (**15**); and manoalide, an anti-inflammatory, analgesic sesterterpene from the sponge *Luffariella variabilis* (**16**). Okadaic acid, a polyether macrolide isolated from the sponge *Halichondria okadai* (**6**), showed *in vitro* anti-cancer activity in its early evaluation. More recent studies, however, show a tumor-promoting activity based on its phosphatase- inhibitory property, which is now being used to unmask protein kinase C activity in cancer-related tests (**3**).

Taxonomic identification and antimicrobial screening of some marine sponges from three areas in the Philippines have been reported (**14**); but further purification and structure elucidation of bioactive compounds, as well as other types of biological screening, have not been undertaken. Studies on Philippine tunicates, moreover, are completely lacking. In this study, we report preliminary findings of the biological screening of several Philippine marine sponges and tunicates. A bioassay- directed strategy may lead to novel compounds with biomedical potential. Purification and structure elucidation of some compounds are currently underway; and further evaluation of activities using more specific and powerful assays has been started in collaboration with Dr. Chris M. Ireland, Department of Medicinal Chemistry, University of Utah.

METHODS

Sponges and tunicates were collected by scuba diving from Bolinao, Pangasinan from 1988-90 at depths ranging from 5-60 feet. These were kept in ice and frozen. Sponges were identified to the genus level by Jane Fromont at Sir George Fisher Centre, James Cook University, North Queensland, Australia (Table 1, Fig. 1); while tunicate samples are still awaiting identification.

Work-up and methodology used for sponges and tunicates were identical (Fig. 2). Leads towards the elucidation of the structure and function of bioactive compounds were pursued synchronously in the course of purification of extracts. Frozen samples were iyophilized, ground, extracted in methanol twice overnight and filtered. The crude methanol extract was subjected to a Kupchan partitioning scheme using solvents of increasing polarity (**10**). This resulted in four Kupchan extracts, namely: hexane, chloroform, methanol and water. The Kupchan extracts were rotaevaporated to dryness; residues were weighed and reconstituted with appropriate solvent to the desired concentration.

Diagnostic thin layer chromatography was performed on crude and Kupchan extracts. All extracts were routinely spotted on silica gel-60 sheets. Crude and chloroform extracts were developed in chloroform/methanol (9:1); while hexane extracts were developed in hexane/ethyl acetate (1:1). The more polar methanol and aqueous extracts were also spotted on C-18 (reverse phase) plates and developed with various proportions of methanol/water or acetonitrile/water (9:1). Detection was done under UV 366 nm and 254 nm and with three color reagents, namely: chlorine-toluidine, ninhydrin and vanillin (**12**).

Kupchan extracts (K) with interesting tlc profiles and results from bioassays were subjected to one or a combination of various chromatographic flow methods: vacuum liquid chromatography (VLC), column chromatography (CC), flash column chromatography (FC) (**18**) and high performance liquid chromatography (HP). The choice of solid support material such as silica gel-60, Sephadex LH-20, or C-18 (reverse phase) silica gel, as well as the type of solvent system and gradient used depended on the nature of the sample. Fractions from these steps were concentrated and again subjected to tlc bioassays. Samples were coded based on their purification history. For example, S1KhCC30 is column chrom fraction 30 of the Kupchan hexane extract of the crude extract of sponge S1.

The proton-NMR spectra of selected extracts and fractions were provided by Dr. Chris M. Ireland, Department of Medicinal Chemistry, Univesity of Utah, Salt Lake City, USA.

Samples were screened for biological activity using the following assays: the disk diffusion method for antimicrobial activity, the differential rec assay system with *Bacillus subtilis* for antimutagenicity, the starfish fertilized egg assay for cytotoxicity and the intracranial mouse bioassay for neuroactivity.

Disk Diffusion Method for Antimicrobial Activity. The antimicrobial assay was performed on five gram-positive bacteria, five gram-negative bacteria and three fungi. Crude and Kupchan extracts, as well as VLC fractions, were tested using the standard disk diffusion-streak method (**5,13**). The most positive results from this test were confirmed using the disk diffusion- top agar method. Three different concentrations of the sample were used to demonstrate a dose effect.

Rec Assay System with *Bacillus subtilis* for Antimutagenicity. Extracts were tested for their mutagenic and antimutagenic effects using wild, H17(Rec +) and mutant, M45(Rec -), strains of *B. subtilis* (**9**). The method was adapted for antimutagenicity testing by comparing the effects of a standard mutagen, quinoline, with that of a mixture of quinoline and extract. Straight streaks of the microorganism were compared with the standard wiggle streak. The assay was further developed into a seed or lawn method whereby all samples could be tested on equal or comparable concentrations of the microorganism and each sample compared for equal concentrations of the (Rec +) and (Rec -) strains.

Starfish Embryo Assay for Cytotoxicity. Starfish eggs and sperm were obtained from dissection of the starfish *Protoreaster nodosus* from Bolinao. Eggs were released from their follicles upon addition of 1-methyl-adenine and fertilized (**4**). Upon formation of the fertilization membrane, these were added to the samples in minicell wells. Cell division for the first eight hours was monitored using an inverted microscope. The method was then modified by using microwells, each containing only 5-12 fertilized eggs and a smaller amount of the sample. Percentage scoring of effects observed was made on a fewer number of cells.

Intracranial Mouse Bioassay for Neuroactivity. Extracts were dried and residues reconstituted in appropriate solvents: NSS for the polar fractions and DMSO or cyclodextrin (Molecusolv) for the nonpolar fractions. Samples were injected intracranially or intracerebrally to 2-4 week-old Swiss Webster mice (**2**). The Hippocratic Method of multidimensional observation and scoring of effects on the CNS and of subjective and general observations was adopted (**17**). A modified table of neuroactivity testing was devised.

RESULTS AND DISCUSSION

Antimicrobial activity. Preliminary results showed at least 15 sponges and 4 tunicates with antimicrobial activity (Tables 2 and 3). Crude and Kupchan extracts were active against *B. subtilis (+), M. luteus (+), S. aureus (+), S. pyrogenes (+), S. viridans (+), P. vulgaris (-), P. aeroginosa (-), E. aerogenes (-), E. coli (-), K. pneumoniae (-), C. albicans (f), C. utilis (f) and S. cerevisiae (f).* The zones of inhibition are not indicated in Table 2 because the crude extracts were of unknown concentration and thus relative values are not significant. Since this is a preliminary screening, all extracts which showed a thin growth or clear line around the disk were also reported.

The antimicrobial activities in the crude extracts were shown to be distributed among the different Kupchan extracts (Table 3). Zones of inhibition ranged form 7-15 mm, generally smaller than those for the standard antibiotic disks. The activities of the most active Kupchan extracts were confirmed and demonstrated in dose-response experiments using the top agar method (Fig. 3). Further screening has been performed on semipure column chromatography fractions S1KcVLC, S1KcVLC-CC, S1KmCC, SGVLC-CC, T1KcCC, but the results are not included here.

The search for antimicrobial agents has taken a definite direction in developed countries. While anti-viral drugs are high on priority, gram-negative infections are still among the most common, serious and pathogenic. Their quickly changing patterns of resistance and susceptibility need to be adressed with the constant search for novel drugs. While gram-positive infections have practically been eradicated in affluent societies, they cannot be disregarded in areas of poor health and environmental conditions like the Philippines. They are many and difficult to treat, and resistant strains have emerged recently, like the heteroresistance of Staphylococci. Yeast, on the other hand, are the most common fungal infections, being aggravated by the extensive use of broad-spectrum antibiotics, immunosuppressive corticosteroids and antitumor agents.

Antimutagenicity. The Rec assay system has been successful in detecting and evaluating a number of environmental mutagens, when used with other microbial test systems. The use of bacteria as the repair test for mutagens provides a sim-

ple, inexpensive, preliminary assay for mutagenicity. *Bacillus subtilis (G+)* is useful because it has a cell membrane that is more permeable to chemicals than some gram-negative bacteria.

Extracts were tested for their mutagenic as well as antimutagenic effects (Fig. 4)). This was done differentially on mutant, recombinationless, repair-deficient M45(Rec-) versus wild, recombination repair-proficient H17(Rec+) strains. The *rec* character or genes are activated upon exposure of the cell to DNA-damaging agents. These, in turn, induce the expression of the SOS genes responsible for a network of cellular repair responses. Mutant, recombinationless bacteria then are usually more sensitive to inhibition than the wild type. Extracts showing increased lethality (larger zone of inhibition) on (Rec-) in comparison with (Rec+) cells may have caused cellular DNA damage (**9,19**).

Because of difficulty encountered with the standard wiggle streak, a straight streak was used in the second batch of samples to make the density of microorganisms uniform and to facilitate the measurement of the zone of inhibition. No zone of inhibition was observed in the presence of any extracts alone; thus none were mutagenic. A large zone of inhibition was observed for the mutagen quinoline alone (Fig. 4). A reduction in the zone of inhibition was observed for several extracts when applied with quinoline. This indicates a weakening or reversal in the inhibition or mutagenic effect of quinoline on the microorganisms. Thus, the extracts are considered antimutagenic. It is noted that the (Rec+) strain showed a smaller zone of inhibition than the (Rec-) strain for each case, consistent with the fact that the (Rec+) strain is capable of cellular recombination repair.

The rec assay method was further modified into a seed or lawn method. Concentrations of the two strains of microorganisms in the top agar were made approximately uniform for all samples. This was achieved by making a serial dilution of the inoculated top agar to the appropriate concentration that would produce a thin lawn. Concentrations were compared by reading the absorbance at 540 nm. This method was used to screen samples from a later batch which showed interesting H-NMR spectra. Among these, sample S28 (unidentified) by itself was shown to cause inhibition; mutagenic activity was later traced to S28KcFC fractions which caused inhibition alone and increased the inhibition of quinoline.

Cytotoxicity. The starfish embryo assay measures the cytotoxic effects of samples on starfish fertilized eggs undergoing mitosis or embryogenesis. Results showed some crude and Kupchan extracts with dramatic effects on early mitotic cell division, such as: retardation or absence of cell division, lysis of fertilization or egg cell membranes and formation of polynucleated cells and abnormal cells (Tables 4 and 5, Fig. 5). Cytolytic agents are clearly present in some samples; while others may contain DNA synthesis, RNA synthesis and protein synthesis inhibitors or cytokinetic inhibitors.

Table 5B is the result of a modification on the method of Fusetani. Microcell wells and microvolumes of fertilized egg suspension and samples were used to monitor effects more quantitatively. The whole microwell containing only 5-12 fertilized eggs was within full view of the microscope field at a time; thus cells were more accurately observed. Samples of S28KcFC, S1KhFC, T1KcCC26HP and T17KcFC have recently been tested using this micromethod.

The starfish egg assay is meant to be paired with another assay, the starfish oocyte maturation assay, which involves a meiotic process. The value of the paired assays lies in the ability to distinguish among different mechanisms of cytotoxicity. DNA synthesis inhibitors block the mitotic division of embryos at the one-cell stage but do not affect meiotic maturational divisions of oocytes. Protein synthesis inhibitors, on the other hand, arrest both oocyte maturation and embryo cleavage; while RNA synthesis inhibitors allow embryo development up to the 64-128 cell or morula stage. Microtubule and microfilament assembly inhibitors, as well as cytolytic agents, can likewise be distinguished.

However, the oocyte maturation assay has not been performed due to the absence of or difficulty in identifying immature eggs at the germinal vesicle stage. At this point, the differential effects of an extract on meiosis and mitosis have not been demonstrated; thus it cannot be confirmed which samples specifically contain DNA, RNA or protein synthesis inhibitors.

Neuroactivity. The intracranial mouse bioassay was used to screen for neuroactive compounds. Almost all sponge and tunicate samples showed interesting neuroactivities ranging from CNS depression to stimulation. Several samples caused death with varying time. Table 6 presents the range of activities observed for some chloroform extracts and their column

chromatography fractions, the former being the most neuroactive among the Kupchan extracts. The bioassay was used for primary screening to provide a profile of the toxicity and pharmacological activity of extracts. An intracranial or intracerebral (i.c.) injection introduces the sample directly into the central nervous system, in contrast to the intraperitoneal (i.p.) route, which affects peripheral neuromuscular systems. The i.c. route detects a wider range of effects because its target is central. It is sensitive to those compounds which have difficulty crossing the blood-brain barrier and are weak or ineffective when applied intraperitoneally or intravenously. Symptoms observed show that peripheral neuromuscular systems in other organs and tissues are also affected.

Nature and profile of some bioactive components. Because of the great number of bioactive fractions and components found, priorities were established based on quantity of sample, strength of bioactivity and the nature of compounds. Tlc profiles provided information on polarity from Rf values and the presence of functional groups from color reagent reactions.

Earlier, the nonpolar hexane fractions, some of which were also bioactive, were given low priority. Interest was confined to more polar, nitrogen-containing compounds, which are less well-studied and more likely to be novel. The chlorine-toluidine color reagent specific for amines, imines, amides, imides and ammonium salts was used to detect nitrogenous compounds; while aromatic and conjugated groups were detected by UV at 254 nm and 366 nm. Polar components from the Kc and Km extracts had an Rf range of 0.3-0.4 on silica gel-60 tlc developed in chloroform/methanol (9:1).

Initially, three neuroactive compounds, two from sponge S1 (*Stylopus sp.*) and one from tunicate T1 (unidentified), were purified. However, the H-NMR and mass spectra showed that they were not novel but known, low molecular weight, aromatic amines. Biogenic amines are known to be present in marine animals; some of them are not only polar but also charged like homarine, the compound isolated from T1, which is believed to function in marine animals as an anti-salt stress factor. Among the biogenic amines are the catecholamines such as the neurotransmitter epinephrine. It is, thus, not surprising that the small aromatic amines from sponges and tunicates, which may have structures similar to epinephrine, are neuroactive.

More recently, interest has been focused specifically on novel peptides. These peptides are of intermediate polarity and show an Rf of about 0.6-0.8 on silica gel-60 tlc developed in chloroform/methanol (9:1). To confirm the presence of peptidal and aromatic functions, crude extracts with interesting tlc profiles are now further screened using H-NMR spectroscopy. These groups show up in highly characteristic patterns and regions in the H-NMR spectrum.

The tunicate T17, tentatively identified as *Lissoclinum bistratum*, has yielded at least two new cyclic peptides that are different but closely related to the family of known *Lissoclinum* peptides, the bistramides (**8**). Tentative structures have been assigned but are awaiting confirmation pending completion of other NMR experiments. The H-NMR spectrum of one semi-pure fraction shows the peptidal patterns in the 7.5-9.0 ppm region (Fig. 6). Bioassays are currently being performed on T17 fractions and recently T17 fractions have been shown to be active against human colon cancer cells *in vitro*.

Another sample, sponge S28 (unidentified), showed a promising tlc profile in the crude extract; this was confirmed to contain interesting peptidal groups by H-NMR (Fig. 7). In addition, S28KcFC10-14 is neuroactive and mutagenic. Purification and structure elucidation of this sample are currently underway.

CONCLUSION

The search for novel bioactive compounds from sponges and tunicates, in its initial phase, consists of two main methodologies: purification and bioassays. This project was initiated with the objective of searching for various biological activities in these marine animals that could be studied for biomedical and pharmacological application. However, a bioassay- guided search generally leads one to known compounds. And so recently, more emphasis has been given to the chemistry by screening extracts with tlc and H-NMR. The project should eventually lead to the structural elucidation of novel, bioactive compounds, whose molecular targets can then be pursued.

Table 1. Taxonomy of sponge samples
A piece of fresh sample showing surface, internal and basal tissue, as well as unusual features, was preserved in 70% ethanol. Field data such as depth, substrate type and locality and a picture were provided.

Class Demospongiae (unless otherwise stipulated)

SAMPLE	ORDER	FAMILY	GENUS
S1	Halichondrida	Hymeniacidonidae	Stylopus
S2	Axinellida	Axinellidae	
S3	Hadromerida	Suberitidae	Suberites
S4	Axinellida	Axinellidae	Axinella
S5	Axinellida	Axinellidae	
S6	Nepheliospongida	Petrosiidae	Petrosia
S7 (same S9)	Poecilosclerida	Myxillidae	Lissodendoryx
S8	Astrophorida	Pachastrellidae	Pachastrella
S9 (same S7)	Poecilosclerida	Myxillidae	Lissodendoryx
S10	Dictyoceratida	Thoractidae	
S11	Dictyoceratida	Dysideidae, mainly a matrix full of cyanobacteria	
S12	Nepheliospongida	Petrosidae	Xestospongia
S13	Axinellida	Respaillidae	Echinodictyum
S14	Haplosclerida	Niphatidae	
S15	Hadromerida	Spirastrellidae	Spirastrella
S16	Class Calcarea		
S17	Class Demospongiae, mainly siphonous green algae		
S18	Axinellida	Agelasidae	Agelas
S19	Dictyoceratida, thin mainly sandgrains		
S20	Dictyoceratida	Spongiidae	
S21	Petrosida	Petrosiidae	Xestospongia
S22	Axinellida	Axinellidae	
S23	Petrosida	Petrosiidae	Xestospongia
S24	Petrosida	Petrosiidae	Strongylophora
S25	Petrosida	Petrosiidae	Xestospongia
S26	Class Calcarea		
S27	Haplosclerida	Niphatidae	
SA (same S1)	Halichondrida	Hymeniacidonidae	Stylopus
SB	Axinellida	Axinellidae	
SC (same S5)	Axinellida	Axinellidae	
SD	Petrosida	Petrosidae	Xestospongia
SE	Poecilosclerida	Myxillidae	Lissodendoryx
SF	Haplosclerida	Niphatidae	
SG	Hadromerida	Suberitiidae	Suberites

Table 2. Antimicrobial screening of crude extracts

METHOD, MEDIA, & CONDITIONS Disk diffusion method - streak method, tryptic soy agar (TSA) slants and stock plates. Mueller-Hinton Agar (MHA) test plates standard inoculation and incubation (37 overnight) conditions.CONTROLS - standard antibiotic disks. Penicillin G, 10 *ug* Amikacin, 10 *ug*; Polymixin B, 300 *ug* Erythromycin, 30 *ug* Chloramphenicol, 30 *ug*; Tetracycline, 10 *ug*; extract solvent-methanol. SAMPLES - 10 *ul* of unknown concentration of crude methanol extract per disk. 6 mm disks, two trials per sample. MICROORGANISMS. from the National Science Research Institute (NSRI) and Institute of Public Health (IPH), U.P.

GRAM POSTIVE BACTERIA:

BS - *Bacillus subtilis*
ML - *Micrococcus luteus*
SA - *Staphylococcus aureus*
SP - *Streptococcus pyrogenes*
SV - *Streptococcus viridans*

GRAM NEGATIVE BACTERIA:

PV - *Proteus vulgaris*
PA - *Pseudomonas aeroginosa*
EA - *Enterobacter aerogenes*
EC - *Escherichia coli*
KP - *Klebsiella pneumoniae*

FUNGI;

CA - *Candida albicans*
CU - *Candida utilis*
SC - *Saccharomyces cerevisiae*

EXTRACT	SUSCEPTIBLE MOs	EXTRACT	SUSCEPTIBLE MOs
S1	G + : BS, ML, SP, SV G - : PA, EA, EC, KP F : CA, CU, SC	S12	G + :BS, ML, SA, SP, SV G - : PV, EA F : CA ,CU, SC
S2	G + :BS, ML, SP, SV	S13	G + : ML, SA, SP, SV F : CA, CU, SC
S3	G + :ML F : CU, SC	S14	G + : SP, SV F : CU
S4	G + : BS, ML, SA, SP, SV G - : EA,KP F : CA, CU, SC	S15	G + :SV G - : PV F : CA
S5	G + : BS, ML, SA G- : EC F : CU	S17	G + : ML
		S19	G + : BS
S6	G + : ML	T1	G + : BS, ML, SA
S7	G + : ML, SA F : CA, CU	T3	G + : ML
S8	F : CU	T4	G + : BS, ML
S10	G + : BS, ML, SA G- : SV	T5	G + : BS

Table 3. Antimicrobial screening of Kupchan extracts

METHOD, MEDIA, CONDITIONS, MICROORGANISMS (LEGEND), ANTIBIOTIC STANDARDS (POSITIVE CONTROLS). DISK SIZE AS IN TABLE 2. NEGATIVE CONTROLS: solvents-hexane, chloroform, methanol, water. SAMPLES: 80 μg residue or 40 μl of Kupchan extract per disk; two replicates per sample. LEGEND:

(+) - clear line around disk

+ + - zone of thin growth around disk exceeds 1 mm

+ - thin growth around disk

= - no zone of inhibition

	ZONE OF INHIBITION, mm							
Extract	**BS (G+)**	**ML(G+)**	**SA (G+)**	**EA (G-)**	**EC (G-)**	**KP (G-)**	**CA(r)**	**CU(r)**
S1-Kh		(+)	+		+	+		
S1-Kc			+			+		
S1-Km		8.5	11.25	7.75		7.5	+	+
S1-Ka		8.75	+					+
S2-Kh		+	+		+	+		+
S2-Kc			+					(+)
S2-Km		+	(+)	8.0		7.5	(+)	+
S2-Ka		(+)	(+)					
S3-Kh		+	(+)		+	+		
S3-Kc			+		7.75			(+)
S3-Km		+		8.0		7.5	+	(+)
S3-Ka			+					
S4-Kh		(+)	+		+	+		
S4-Kc			+		7.5			(+)
S4-Km		+	9.5	8.25		7.5	(+)	(+)
S4-Ka		+	(+)					(+)
S6-Kh		(+)	+		+	+		+
S6-Kc			+		+			
S6-Km		8.5	8.5	8.0		7.5	+	(+)
S6-Ka			(+)					
S7-Kh		+	+		+		+	+
S7-Kc	+							
S7-Km		(+)	+		+	+		
S7-Ka			+					
S12-Kh		(+)	+		+		+	
S12-Kc	9.0	10.5	+					+
S12-Km			+		+	+		
S12-Ka			+					
S13-Kh			+		+			
S13-Kc								
S13-Km	+	(+)	+		+	+		
S13-Ka			+					
S14-Kh			+		+			
S14-Kc								
S14-Km			+		+	+		
S14-Ka			+					
S15-Kh			+		+			
S15-Kc								
S15-Km			+		+	+		
S15-Ka								
hexane		(+)	+		+	(+)	(+)	(+)
chloroform								
methanol			+		(+)			
water			(+)					

Table 4. Cytotoxicity screening of crude extracts. Effects on mitotic division of starfish fertilized eggs

METHODS as described in Fusetani, N. 1987. Egg follicles from slit female starfish ***Protoreaster nodosus*** were washed with natural sea water (NSW) 4X and eggs were released upon addition of 20-100 μl .001M 1-methyl-adenine. Active sperms from slit male starfish were added to mature eggs. Upon formation of the fertilization membrane,500 μl of the fertilized egg suspension was immediately added to each sample in a minicell well. SAMPLE used was about 0.1 μg residue of crude methanol extract (variable volumes) made up to 500 μl with NSW. Final total test volume was 1.0 μl. CONTROLS: Aphidicotin, cycloheximide, vinblastine, flourouracil, hydroxyurea, NSW, methanol. Observation was made using a Reichert microstar inverted microscope (magnification 60 X 10) for the first, second, fourth and eighth hours.

CRUDE EXTRACT	CYTOTOXIC EFFECTS
SPONGES	
S1, S17, S20	cell division retarded
S2, S6, S8	no cell division, polynucleated cells
S3	cell division retarded and irregular
S5, S7, S16, S18	cell division irregular
S9, S10	cell division irregular, egg and fertilization membrane lysed
S12, S13	no cell division, polynucleated cells, irregular cell shapes, fertilization membrane lysed
S14	egg and fertilization membrane lysed
S19	no cell division, egg membrane lysed
TUNICATES	
T1, T3, T4	cell division irregular
T2, T7	cell division irregular, polynucleated cells
T5	no cell division, egg membrane lysed
T6	no cell division, polynucleated cells

Table 5. Cytotoxic effects of Kupchan extracts

METHODS and POSITIVE CONTROLS as in Table 4. A. NEGATIVE CONTROLS. NSW hexane methanol, water. SAMPLE. 3 mg residue from Kupchan extract in final test volume of 1.0 ml. B. The method was scaled down to the micro level. 25 *ul* of fertilized egg suspension (containing 5-12 cells) was added to sample in a microcell well. Extracts were dried of Kupchan solvent KA and Km residues were redissolved in normal saline (NSS). Kc residues were redissolved in cyclodextrin (Molecusolv). SAMPLE: 25 *ug* residue in 25 *ul* NSS or Molecusolv. Final test volume was 50 ml. NEGATIVE CONTROLS NSS, Molecusolv, NSW. The number of fertilized eggs at zero hour and the number or % of cells showing specific cytotoxic symptoms at each observation time were recorded.

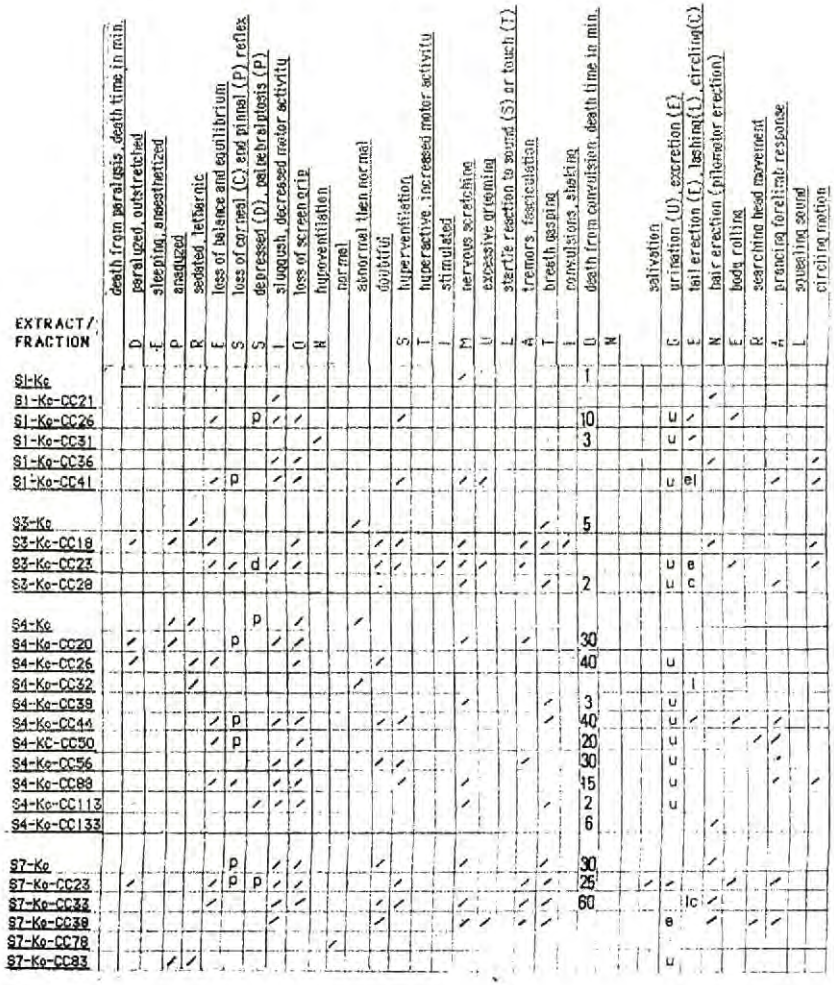
LEGEND:

CD - cell division normal
CDN - no cell division (cd)
CDR - cd retarded
CDI - cd irregular & unequal
CSI - cell shapes irregular
CDA - cleavage furrows present but cd aborted
FML - fertilization membrane lysed
EML - egg membrane lysed
PNC - polynucleated cells

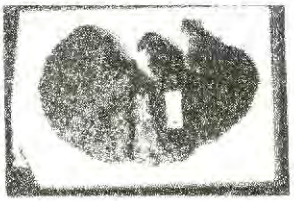
EXTRACT	EFFECT	EXTRACT	EFFECT	EXTRACT	EFFECT
A. SPONGES					
S1Kh	CSI,PNC	S1Km	CDN,PNC	S1Ka	CD
S2Kh	CDN, PNC	S2Km	EML	S2Ka	CDN,PNC
S3Kh	CDR, CDI	S3Km	CD	S3Ka	CD
S4Kh	CDR, CDI	S4Km	CDA,EML	S4Ka	EML
S6Kh	CDI	S6Km	CDA,EML	S6Ka	CDI
hexane	CDI	methanol	CD	water	CD
NSW	CD				
B. TUNICATES					
T1Kc	CDN	T1Km	CDN	T1Ka	CDR,CDI
T2Kc	CDR	T2Km	CDR,CDI	T2Ka	CDI
T3Kc	CDN	T3Km	CD	T3Ka	CDI
T4Kc	CDR,CDA,CSI	T4Km	CDN,CSI	T4Ka	CDN,CSI
T5Kc	CDR,CSI	T5Km	CDR,CDI	T5Ka	CDR,CDI
T6Kc	CDN	T6Km	CDR	T6Ka	CDR,CDI
T7Kc	CDR	T7Km	CDR,CDI	T7Ka	CDR
Molecusolv	CDR	NSS	CD	NSS	CD
NSW	CD				

Table 6. Neuroactivity screening of Kupchan chloroform extracts and fractions from column chromatography

METHOD: Intracranial injection into 3-4 week old Swiss Webster mice. SAMPLES: Kc extracts and KcCC fractions. (silica-gel 60 column), 1 $\mu\ell$ of unknown concentration of sample per gram body weight mouse extracts & fractions were dried of solvent and resuspended in DMSO. CONTROLS: caffeine-stimulant, phenobarbital-depressant, DMSO-stimulated for first 30 min., normal after. Observation and scoring done for first two hours at 30 minute intervals.



Stylopus sp. (S1)

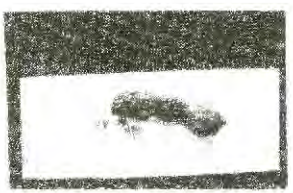


Lissodendoryx sp. (S7)

Suberites sp. (SG)

Axinellidae sp. (S4)

Xestospongia sp. (S21)



Unidentified (S28)

Unidentified (T1)

Unidentified (T2)

Lissoclinum sp. (T17)

Figure 1. Some sponges and tunicates collected

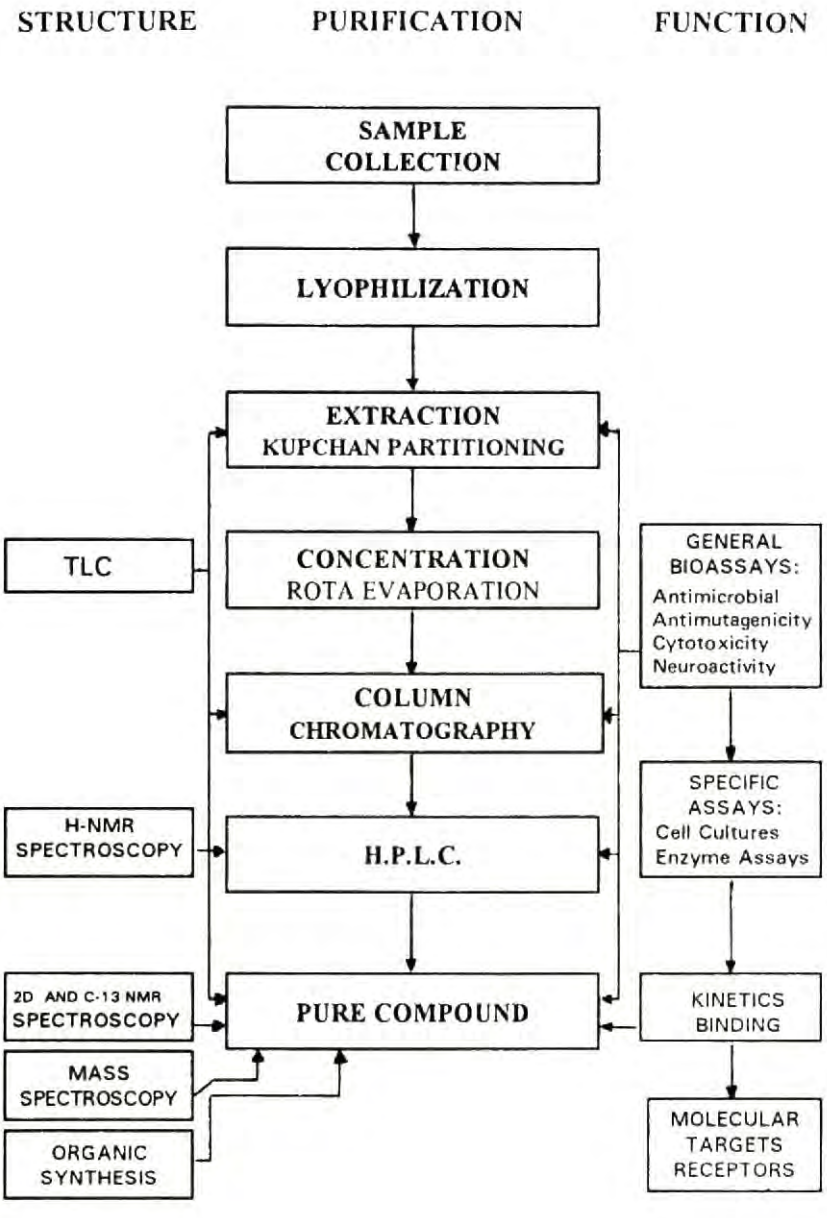


Figure 2. **General workup and methodology for bioassay-guided natural products research**

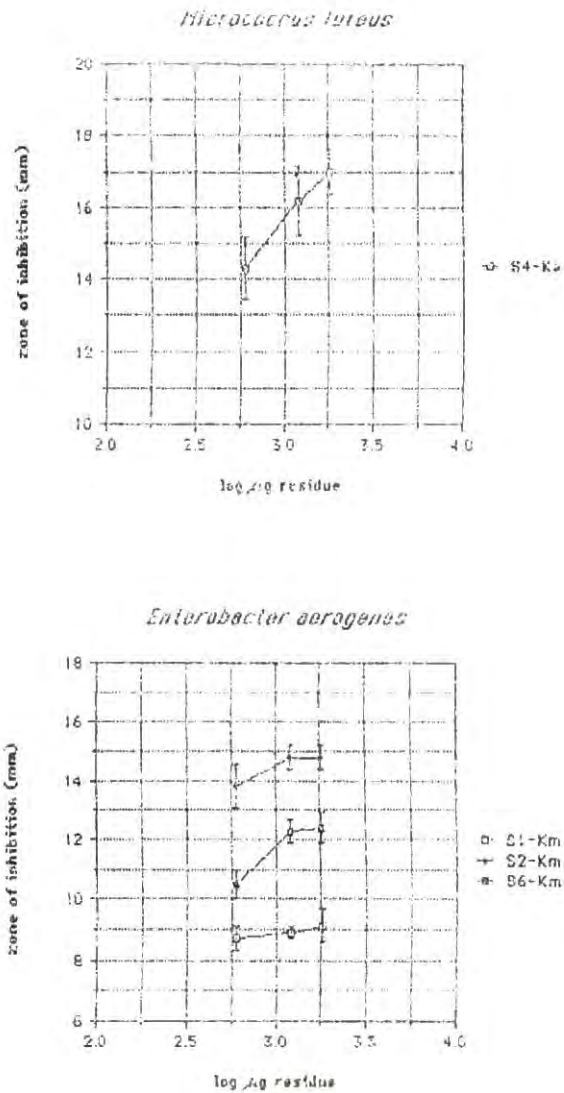
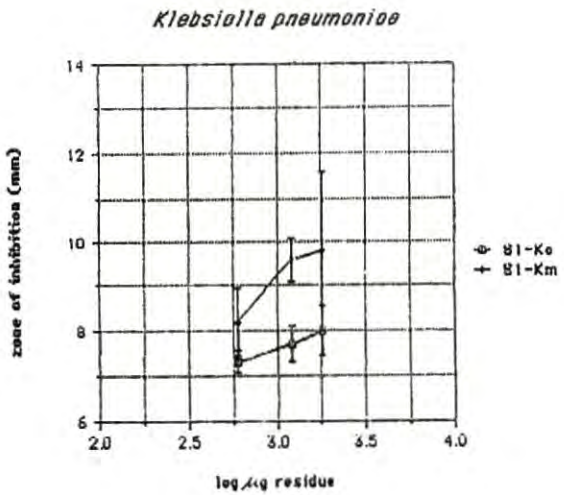
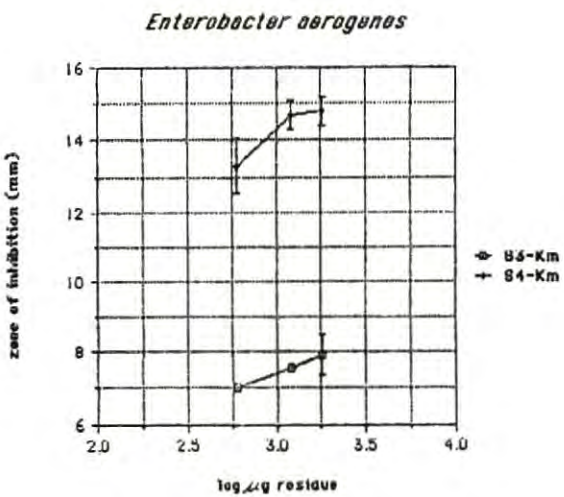


Figure 3. Antimicrobial dose effects of selected Kupchan extracts

METHOD: Disk diffusion-top agar method; MEDIA: TSA-base agar, Bactoagar-NaCl (6 g each per liter)-top agar; MICROORGANISM: A loopful is mixed with top agar, 5 μl of which is poured onto base agar. SAMPLES: 10 μl, 30 μl of 60 $ng/\mu l$ Kupchan extract or 0.6, 1.2, 1.8 ng doses; 5 replicates per dose. CONTROLS as in Tables 2 & 3.

Figure 3. Continued





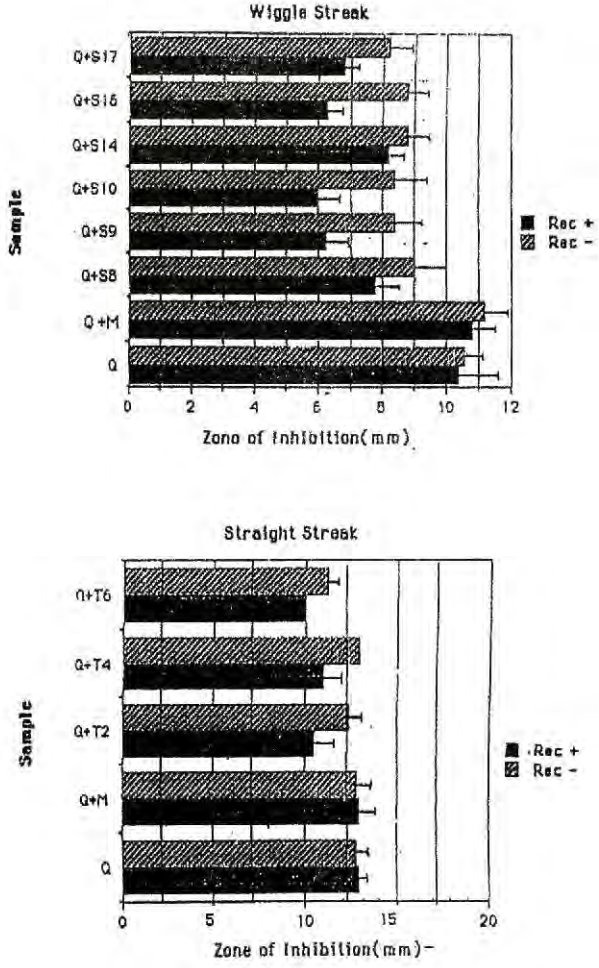
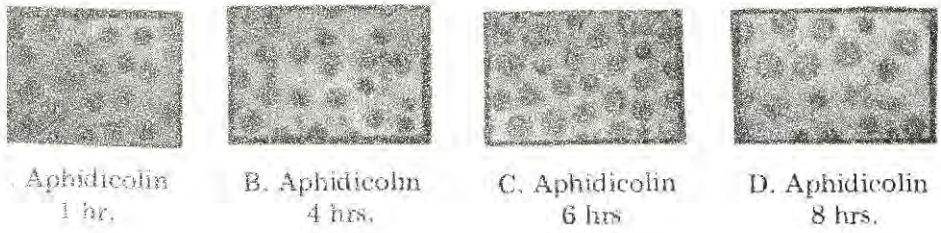


Figure 4. Antimutagenicity screening of crude extracts METHODS as described in Kada, T. et al. 1980. Differential Rec assay system with *Bacillus subtilis*, rapid streak method without metabolic activation. MICROORGANISMS; H17 Rec + and M45 Rec- strains of *B. subtilis* from Dr. C.L. Sylianco, Institute of Chemistry, U.P. Inoculum applied in a wiggle or straight streak. SAMPLES and CONTROLS: 50 μl of unknown concentration of crude extract: 50 μl of mutagen quinoline: 50 μl of crude extract plus 50 μl of quinoline; 50 μl of solvent methanol plus 50 ml of quinoline, 5 replicates per sample. LEGEND Q-quinoline, M-methanol, Rec + - wild, repair-proficient strain, Rec= - mutant, repair deficient strain.

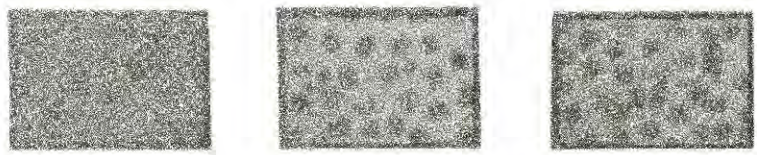


A. Aphidicolin 1 hr.

B. Aphidicolin 4 hrs.

C. Aphidicolin 6 hrs

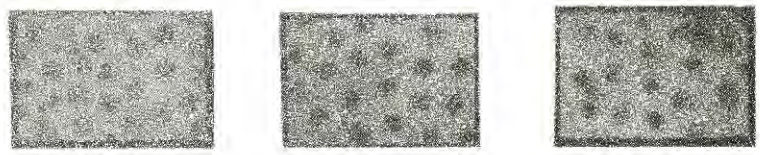
D. Aphidicolin 8 hrs.



E. S3

F. S7

G. S9



H. S12

I. S2Kh

J. S2Km

Figure 5. Cytotoxic effects (photographs) of standard and some crude and Kupchan extracts

A-D. Aphidicolin, 3 μg, DNA synthesis (DNA polymerase) inhibitor, blocks mitotic cell division at the one-cell stage; E. S3 - CDN, CDR; F. S7 - CDI; g> S9 - CDI, EML, FML; H. S12 - CDN, PNC, CSI, FML; I. S2Kh - CDN, CDA, PNC; J. S2Km - EML (LEGEND as in Table 5)

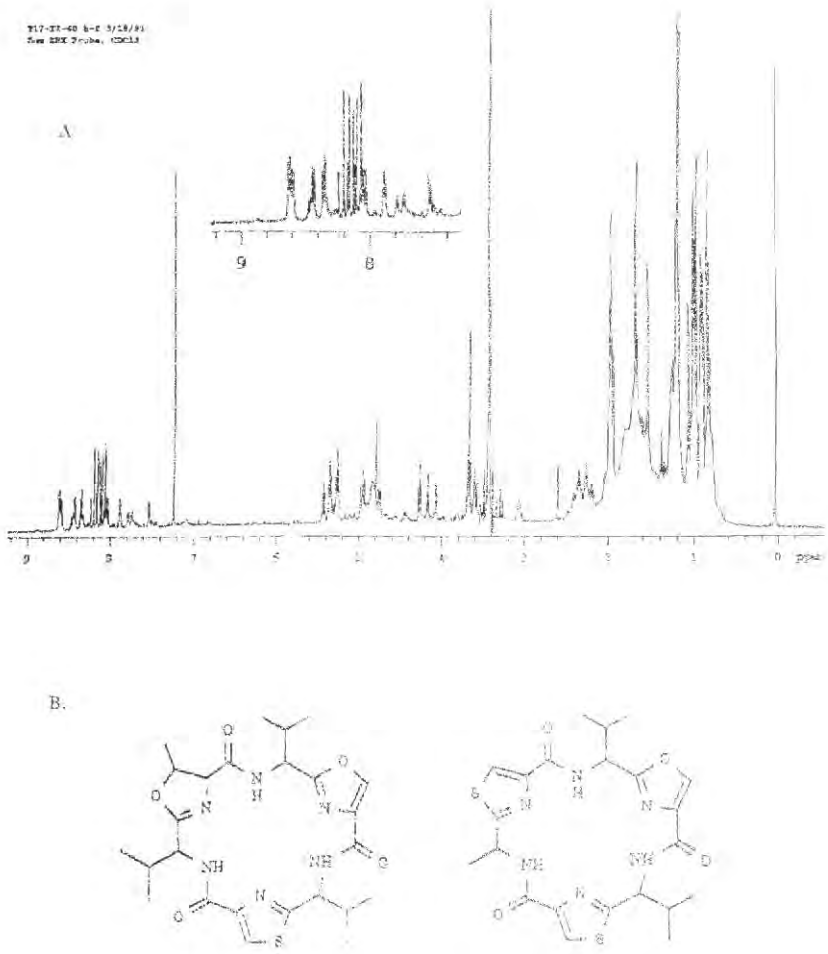


Figure 6. H-NMR of semipure fraction of *Lissoclinum sp.* (T17) and tentative structures of new cyclic peptides

A. SAMPE: fraction T17KcFC61-100; peptide region:7.5-9 ppm; B.tentative structures of peptides (deduced from DEPT, COSY, HMOC, HMBC by Mark P. Foster, Department of Medicinal Chemistry, University of Utah).

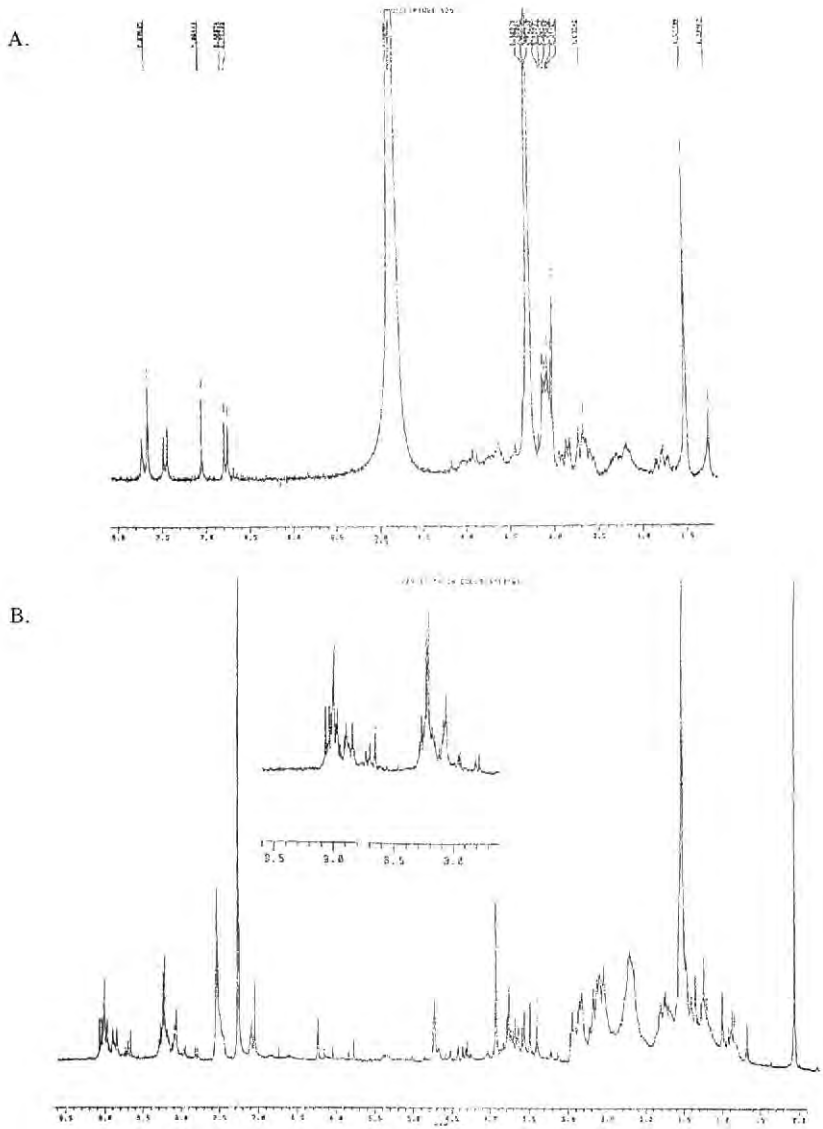


Figure 7. H-NMR of crude extract and semipure fraction of S28 (unidentified)

A. Crude extract S2B; B. semipure fraction S28KcFC11-14

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Alkaloid Studies on Selected Philippine Plants

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ABSTRACT

A research program for the chemical and biological studies of Philippine plants was conceived. The program calls first for the conduct of a field survey for alkaloids. The study includes the isolation of pure alkaloids for structure elucidation and for possible biological characterization. The program also calls for safekeeping of herbarium specimens of all plants surveyed. These are properly identified and authenticated by a botanist.

The following studies are the results of the alkaloid field surveys conducted in selected areas of Luzon from 1982 to 1983 and central, southern and northern areas of Palawan.

The plants studied for their alkaloid contents are the following: ***Ipomoea muricata*** *(L.) Jacq. (Convolvulaceae);* ***Melicope triphylla*** *(Lam.) Merr. (Rutaceae);* ***Microcos philippinensis*** *(Perk.) Burrett;* ***Antidesma fructiferum Elm. (Euphorbiaceae); Talauma gitingensis Elm. (Magnoliaceae); Lilium philippinense*** *Baker* ***(Liliaceae); and Pandanus amaryllifolius Roxb. (Pandanaceae).***

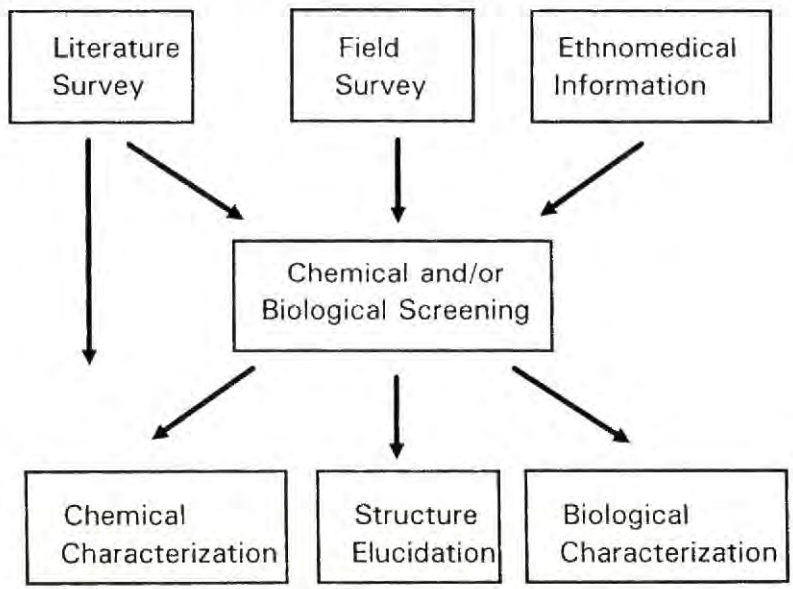
We have a treasure-house of plants in the virgin forests of the Philippines as well as in our immediate surroundings. Many of these plants have not been explored for their chemical constituents and their biological properties; if ever they are used for their ethnopharmacologic effects, many of these reputed effects have not been substantiated by scientific evidence.

Identifying the drug potentials of plants and establishing the scientific validity in the use of plants for medicinal purposes give more than enough materials for our natural products researchers - the botanists, the biologists and the chemists - to investigate.

In addition, what better reasons can we give for the need to preserve and propagate our many plants, wild or otherwise, than to show in strong and valid scientific terms, the usefulness and the value of these plants as drug materials or as sources of basic chemicals for synthesis.

Our goal is to search for alkaloids, antimicrobials, analgesic and anti-inflammatory principles in plants and to conduct the studies in a mutidisciplinary approach. The end view of our studies is to establish some scientific basis on the use of the plant materials for primary health care.

Our program of studies may be illustrated as follows:



One phase of our work involves a field survey of plants for the presence of alkaloids. A systematic search for alkaloids from plants has led us to scour selected areas and virgin forests of Palawan and Mindoro. More than 1000 plant samples have been collected and field tested for alkaloids using the Culvenor-Fitzgerald method (**3**).

A total of more than 70 samples, belonging to different genera, gave positive results for alkaloids (**1,8**). A list of plants found to be highly positive for alkaloids is shown in Table 1.

The plants found positive for alkaloids were collected in bulk. Herbarium specimens were prepared for all the plants assayed. These were properly identified and deposited at the UST Herbarium.

Confirmatory laboratory assays were conducted using the Farnsworth-Euler thin-layer chromatographic method (**5**). This method detects polar and non-polar alkaloids and also eliminates false positive results.

Selection of plants for priority studies was based on the positive results of the survey, the absence of literature reports and the availability of the plant material.

Some plants prioritized for the study of their alkaloid content include:

Ipomoea muricata (L.) Jacq. (Convolvulaceae)
Melicope triphylla (Lam.) Merr (Rutaceae)
Microcos philippinensis (Perk) Burrett (Tiliaceae)
Antidesma fructiferum Elm. (Euphorbiaceae)
Talauma gitingensis Elm. (Magnoliaceae)
Lilium philippinense Baker (Liliaceae)
Pandanus amaryllifolius Roxb. (Pandanaceae)

Air-dried plant samples were ground in a Wiley mill while fresh samples were chopped in a blender. These were exhaustively extracted by cold percolation with 95% ethanol or 80% methanol until the marc was negative for alkaloids. The combined alcohol extracts were concentrated, under reduced pressure at temperatures not higher than 50°C, until they became thick and syrupy.

The concentrated extract was defatted by successive extraction with diethyl ether and dilute acid. The combined acid layers were made alkaline with aqueous ammonia to pH 10 and exhaustively extracted with chloroform or with ethyl acetate.

The organic layer was washed, dried and the solvent evaporated. The residue, containing the crude tertiary alkaloids, was subjected to a series of chromatographic separations until pure alkaloids were obtained.

The aqueous base layer, if positive for alkaloids, was concentrated under vacuo. The residue, containing the quaternary alkaloids, was subjected to different chromatographic methods to isolate and purify the alkaloids.

The structure elucidation of the pure alkaloids was conducted using chemical and physical methods. Chemical methods included functional group analysis through spray reagents that gave distinct colored spots in thin-layer chromatograms. Physical methods included melting point determination, optical activity measurement and spectroscopic methods such as UV, IR, ^{1}H n.m.r., ^{13}C n.m.r. spectroscopy and mass spectrometry.

The alkaloids isolated and identified are listed in Table 2. Prior to these studies, no reports of the isolation of these alkaloids were reported for the plants mentioned.

Pandanus amaryllifolius, commonly known as 'pandan mabango' was a real challenge. A novel alkaloid appears to have been isolated with the molecular structure $C_{18}H_{25}N_3O_2$. Structural features were obtained from spectroscopic data. Crystallographic analysis appeared to have confirmed our findings. Results of this study are given in a separate paper.

What makes phytochemical work meaningful is the biological property associated with the plant, obtained either through ethnomedical information or by way of the results of the biological assay conducted on the plant extract.

Listed below are some of the results obtained in the biological assay conducted on the alcohol extract of the plant material:

I. muricata	antibacterial and analgesic
A. cydoniafolia	antifungal and anti-inflammatory
M. philippinensis	antimicrobial
A. fructiferum	antibacterial
L. philippinense	antibacterial and anti-inflammatory

While these phytochemical studies appear basic in nature, a view from a higher level will show that the entire exercise has been useful in the training of our organic chemists in disciplined and appropriate research and in appreciating our rich natural plant resources.

We may have acquired the knowledge and the skills and the values for natural products research but if there is no strong desire, no political will to see that these studies lead to plant drug development, then our studies may be an exercise in futility. We are faced with more challenging problems, to name a few:

- the wanton destruction of our trees and forests and their rich diversity of plant and animal life;
- the need for sensitive and meaningful bioassays, sensitive enough to assay small amounts of the pure natural product; and
- the need to spearhead and support the development of the plants studied into useful products.

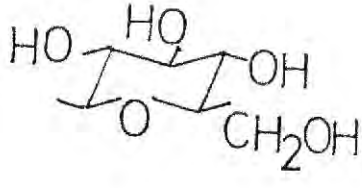
Table 1. Plants found highly positive for alkaloids

Plant Family	Botanical name
Acanthaceae	*Odontenema nitidum* (Jacq) O. Kuntz
Alangiaceae	*Alangium chinense* (Lourd) Hrms. & Rebd.
Anonaceae	*Phaeanthus ebracteolatus* (Presl.)
Apocynaceae	*Alstonia scholaris* (L.) R. Br.
	Catharanthus roseus (L.) G. Don
	Erbatamia divaricata (L.) Bark
	Ervatamia pandacaqui (Poir)
	Kibatalia gitingensis (Elm) Words
	Kopsia arborea (Blume)
	Tabernamontana subglobosa (Merr.)
Campanulaceae	*Isotoma longiflora* (Mill.) Presl.
Caricaceae	*Carica papaya* Linn.
Convolvulaceae	*Ipomoea muricata* (L.) Jacq.
Flacourtiaceae	*Caeseria trivalvis* (Blco.) Merr.
Lauraceae	*Litsea glutinosa* (Lour.) C.B. Rob.
Leguminosae	*Cassia spectabilis* L.
Menispermaceae	*Cissampelos pareira* L.
Pandanaceae	*Pandanus amaryllifolius* Roxb.
Rutaceae	*Lunasia amara* Blco.
Solanaceae	*Nicotiana tabacum* L.
Tiliaceae	*Microcos philippinensis* (Perk) Burrett

Table 2. Alkaloids isolated from selected plants and their structure

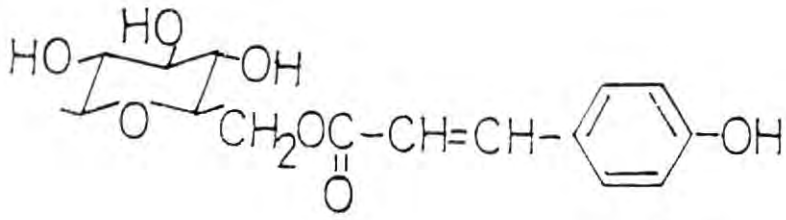
Name of Plant	Plant Part	Where collected	Stucture	Reference
Ipomoea muricata	seeds	UST Botanical Garden	[1], [2], [3]	(7)
Melicope triphylla	leaves	Los Baños, Laguna	[4]	(8)
Adhatoda cydoniaefolia	leaves	Diadi, Nueva Vizcaya	[5}	(8)
Microcos philippinensis	leaves	Diadi, Nueva Vizcaya	[6]	(2)
Antidema fructiferum	leaves	Los Baños, Laguna	[7]	(6)
Talauma gitingensis	leaves	Trideint Mines, Palawan	[8]	
Lilium philippinense	leaves	Sagada, Mountain Prov.	[9]	(4)

[1] R = H Ipalbidine

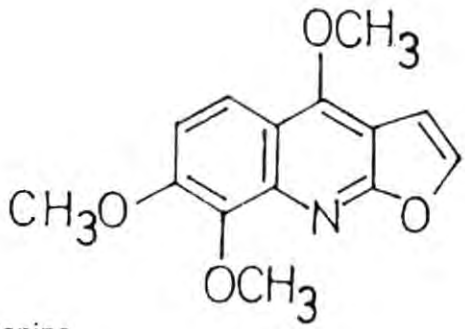
[2] R = 

Ipalbine

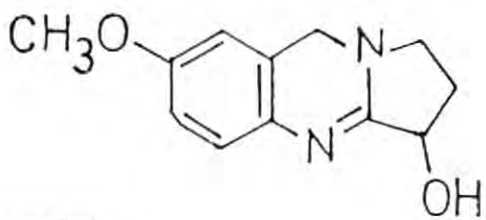
[3] R =



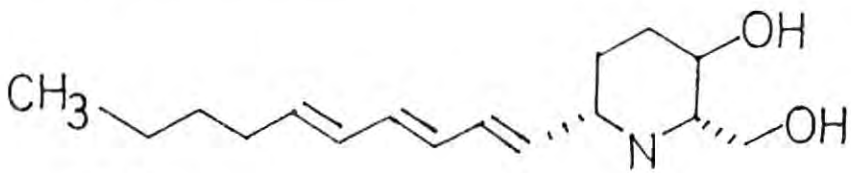
Ipomine



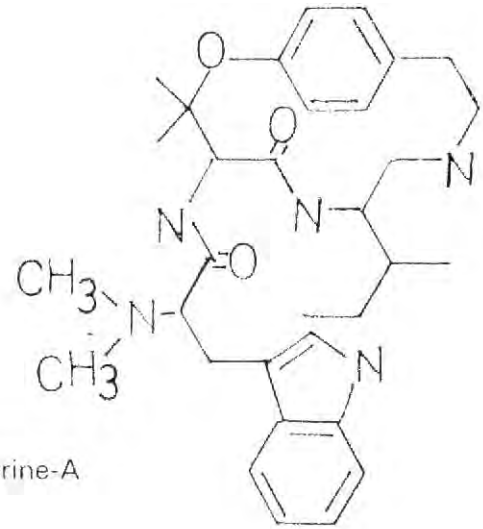
[4] Skimmianine



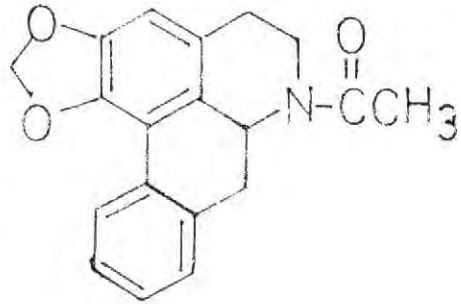
[5] 7-Methoxyvascine



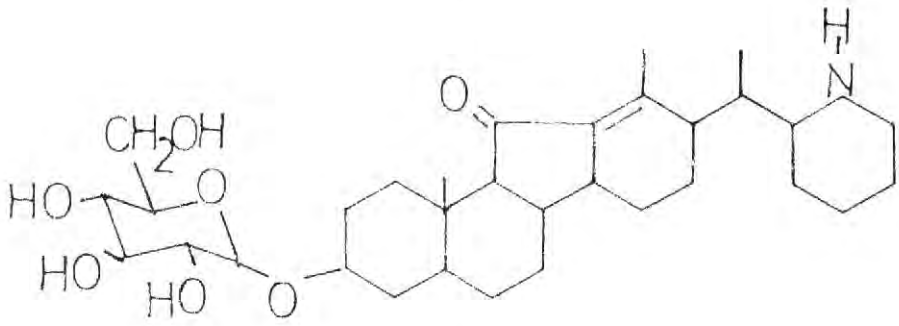
[6] Micropine



[7] Discarine-A



[8] Anonaine



[9] a steroidal glycosidic alkaloid

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Antigenotoxic Effects on Bone Marrow Cells of Refined Coconut Oil Administered Simultaneously With Azaserine, Dimethylhydrazine, Dimethylnitrosamine, Benzo(a)pyrene, Methylmethanesulfonate and Tetracycline*

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ABSTRACT

Methylmethanesulfonate, dimethylnitrosamine, dimethylhydrazine, benzo(a)pyrene, azaserine and tetracycline induced the formation of micronucleated polychromatic erythrocytes in bone marrow cells of experimental mice, an indication that these chemicals exhibited chromosome-breaking effects and are therefore, genotoxic.

Simultaneous administration of refined coconut oil reduced micronuclei formation indicating that coconut oil has antigenotoxic activity against methylmethanesulfonate, dimethylnitrosamine, dimethylhydrazine, benzo(a)pyrene, azaserine and tetracycline. The antigenotoxic activity of refined coconut oil was far superior to that of soybean oil (low and high peroxide value)

* Supported by the Philippine Coconut Research and Development Foundation

INTRODUCTION

It has been shown that (1), methylmethanesulfonate, (2), di- methylnitrosamine, (3), dimethylhydrazine, (4), azaserine and (5), benzo(a)pyrene are chemical carcinogens that alter the structure of DNA, the genetic substance of the living cell. Tetracycline is a well known teratogen (6), Azaserine can induce pancreatic carci- noma while 1,2-dimethylhydrazine is a colon carcinogen. Benzo- (a)pyrene can induce skin and liver cancer. Methylmethanesulfonate and dimethylnitrosamine are carcinogens which alkylate DNA (7).

Studies published several years ago suggested the inhibitory effects of dietary coconut oil on the development of colon tumors (8), liver tumors (9), pancreatic carcinoma (4), mammary tumors (10) and skin cancer (5). However, these findings were ignored by those who have tried to malign coconut oil. These were long-term studies that were done for long periods of time.

This report deals with short-term approaches in studying the effect of coconut oil on the genotoxicity of methylmethanesulfonate, dimethylnitrosamine, dimethylhydrazine, benzo(a) pyrene, azaserine and tetracycline.

MATERIALS AND METHODS

Methylmethanesulfonate, dimethylnitrosamine, dimethylhydrazine, azaserine, benzo(a)pyrene and tetracycline were purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A.

Fetal calf serum was purchased from Grand Island Biological Supply, Grand Island, New York, U.S.A.

Swiss Webster mice were supplied by the College of Veterinary Medicine, University of the Philippines.

The micronucleus test (11) was used in studying the genotoxicity of methylmethanesulfonate, dimethylnitrosamine, dimethylhydrazine, benzo(a)pyrene, azaserine and tetracycline. The same method was used in determining the antigenotoxicity effects of refined coconut oil.

A completely randomized design was used in studying genotoxicity and antigenotoxicity. Ten mice were used for each

experimental group. Two administrations for each test system were done using an oral gavage, 24 hours apart. Six hours after the second administration, the experimental mouse was killed by cervical dislocation. The femur was removed and bone marrow cells were flushed out using fetal calf serum. The cell suspension was centrifuged and the supernatant discarded.

The cells were mounted on slides, stained and micronucleated polychromatic erythrocytes were counted under the microscope.

For antigenotoxic studies, refined coconut oil (0.5 ml/20 gram body weight) was administered simultaneously with the test mutacarcinogen. Refined coconut oil as well as soybean oil (low and high peroxide) were used in the antigenotoxicity trials.

Peroxide values of the test oils were analyzed using the method described by Sully (**12**). Soybean oil with high peroxide value was prepared using the method of Ault (**13**).

RESULTS AND DISCUSSION

Significance of data indicated in Tables 1, 2 and 3 has been statistically validated using ANOVA and Duncan's multiple range test.

Table 1 shows that the clastogenic or chromosome-breaking potential of soybean oil, both low and high peroxide, is greater than that of coconut oil. The clastogenic potential of coconut oil is lower than the blank. These results suggest that coconut oil does not possess chromosome-breaking effects. This is indicated by the very small number of micronucleated polychromatic erythrocytes produced when coconut oil was given by oral gavage. The formation of micronucleated polychromatic erythrocytes is lower than that produced spontaneously.

Soybean oil, with low peroxide, has chromosome-breaking effects. Its clastogenic potential is enhanced when the peroxide content is increased. This is a consequence of the high content of linoleic acid in soybean oil. Being polyunsaturated, it easily forms peroxides and free radicals when incubated in the presence of oxygen. Peroxides and free radicals are very reactive with DNA (deoxyribonucleic acid) (**7**).

Table 2 indicates that the carcinogens (e.g. azaserine, benzo- (a)pyrene, dimethylhydrazine, dimethylnitrosamine,

methylmethanesulfonate and the teratogen, tetracycline), induced the formation of micronucleated polychromatic erythrocytes, suggesting that these test systems fragmented the chromatin material of the bone marrow cells. These test substances are therefore genotoxic to bone marrow cells.

The genotoxicity to bone marrow cells of azaserine, benzo(a)pyrene, dimethylhydrazine, dimethlynitrosamine, methlymethanesulfonate and tetracycline was inhibited by coconut oil as shown in Table 3. The inhibitory effects of coconut oil is far superior to that of soybean oil. When the peroxide value of soybean oil is increased, its inhibitory effects is reduced. These results clearly suggest that the antigenotoxic effect of coconut oil exceeds that of soybean oil. This activity of coconut oil is indicated by the highly significant reduction in the formation of micronucleated polychromatic erythrocytes in bone marrow cells.

Azaserine, benzo(a)pyrene and dimethylnitrosamine are well-known alkylating agents of DNA, the genetic substance of the living cell (**17**). This alkylating activity can induce the fragmentation of DNA of the chromosomes inducing the formation of micronucleated polychromatic erythrocytes. It is possible that coconut oil reduced the alkylating activity of these carcinogens, by a mechanism that has yet to be elucidated.

It is also possible that coconut oil enhanced the repair of DNA that was altered structurally by the test mutacarcinogens.

CONCLUSION

Coconut oil, a saturated oil, exhibited antigenotoxic activity against five carcinogens and a teratogen. Its antigenotoxic activity is far superior to that of soybean oil, a polyunsaturated oil.

ACKNOWLEDGMENT

Financial support from the Philippine Coconut Research and Development Foundation is gratefully acknowledged.

Table 1. A comparison of clastogenic potential of refined coconut oil and soybean oil

	No. of micronucleated polychromatic erythrocytes per thousand ± S.D.
Coconut Oil, refined *	1.53 ± 0.90
Soybean Oil, low peroxide **	3.77 ± 1.71
Soybean Oil, high peroxide ***	4.52 ± 0.53
Control, no oil	1.93 ± 0.72

* Coconut oil = 0 peroxide value

** Soybean oil, low peroxide, 13.97 ± 3.25 meq/kg

*** Soybean oil, high peroxide, 30.60 ± 1.31 meq/kg

Table 2. Clastogenic or chromosome-breaking effects of azaserine, benzo(a)pyrene, dimethylhydrazine, dimethylnitrosamine, methlymethanesulfonate and tetracycline

	No. of micronucleated polychromatic erythrocytes per thousand ± S.D.
Azaserine (9.4 mg/kg)	7.63 ± 1.79
Benzo(a) pyrene (47 mg/kg)	7.30 ± 1.08
Dimethylhydrazine (18 mg'kg)	8.22 ± 1.96
Dimethlynitrosamine (10 mg/kg)	8.52 ± 1.56
Methlymethanesulfonate (10 mg/kg)	6.00 ± 1.30
Tetracycline (55 mg/kg)	8.93 ± 3.21
Control (distilled water)	1.93 ± 0.72

Table 3. **Effect of refined coconut oil and soybean oil on genotoxicity of azaserine, benzo(a)pyrene, dimethylhydrazine, dimethylnitrosamine, methlymethanesulfonate and tetracycline**

	No. of micronucleated polychromatic erythrocytes per thousand ± S.D.
Azaserine (AZ) alone	7.63 ± 1.79
AZ plus coconut oil	2.03 ± 0.85
AZ plus soybean oil (LP) *	3.43 ± 0.83
AZ plus soybean oil (HP) **	5.59 ± 1.30
Benzo(a)pyrene (BP) alone	7.30 ± 1.08
BP plus coconut oil	3.00 ± 1.47
BP plus soybean oil (LP)	4.57 ± 1.87
BP plus soybean oil (HP)	5.59 ± 1.30
Dimethylhydrazine (DH) alone	8.22 ± 1.56
DH plus coconut oil	2.80 ± 1.07
DH plus soybean oil (LP)	4.63 ± 1.10
DH plus soybean oil (HP)	6.63 ± 1.46
Dimethylnnitrosamine (DMN) alone	8.52 ± 1.56
DMN plus coconut oil	1.83 ± 1.02
DMN plus soybean oil (LP)	3.87 ± 1.93
DMN plus soybean oil (HP)	7.24 ± 3.23
Methylmethanesulfonate (MMS) alone	6.00 ± 1.30
MMS plus coconut oil	2.00 ± 1.04
MMS plus soybean oil (LP)	3.57 ± 0.93
MMS plus soybean oil (HP)	5.74 ± 1.37
Tetracycline (Tet) alone	8.93 ± 3.21
Tet plus coconut oil	1.70 ± 0.76
Tet plus soybean oil (LP)	4.70 ± 1.36
Tet plus soybean oil (HP)	6.48 ± 1.79

* LP = low peroxide value
** HP = high peroxide value

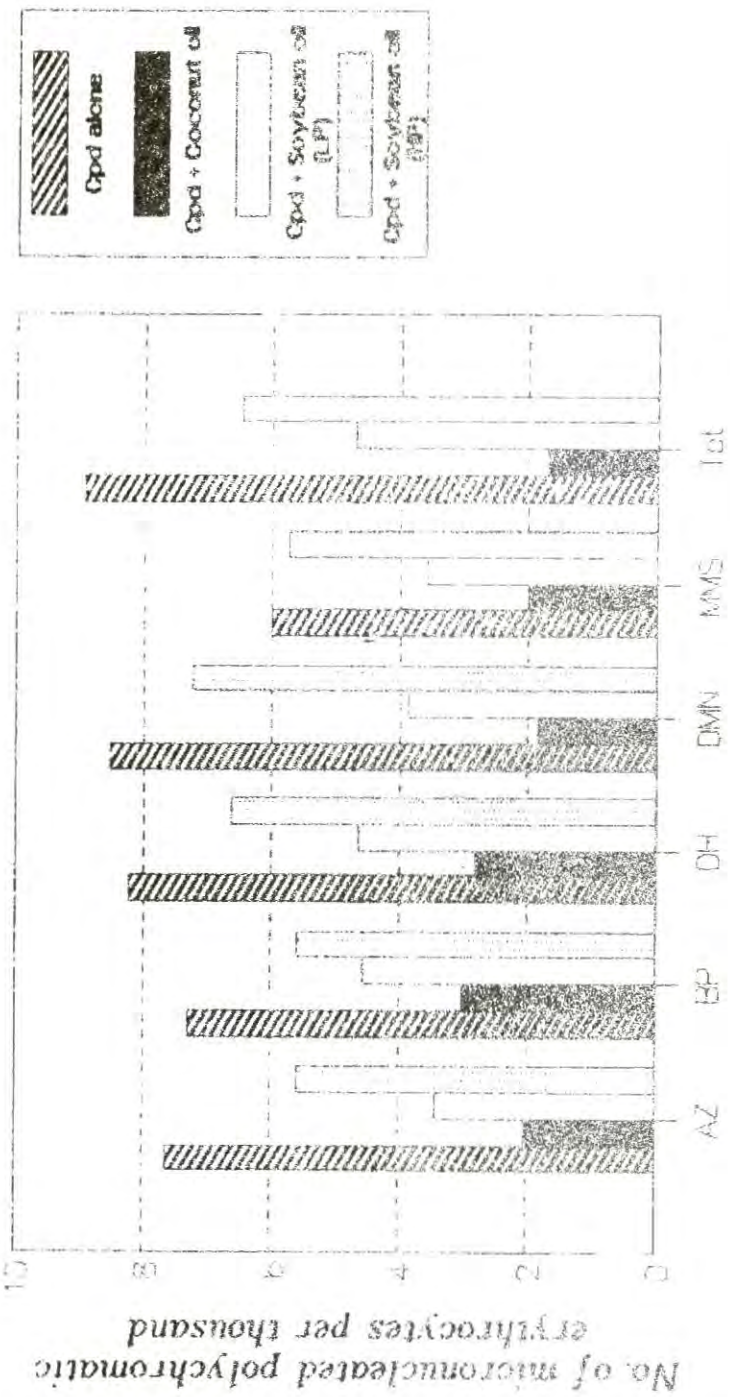

Figure 1. Effect of coconut oil and soybean oil on the genotoxicity of azaserine (AZ), benzo(a)pyrene (BP), dimethylhydrazine (DH), dimethylnitrosamine (DMN), methylmethanesulfonate (MMS) and tetracycline (Tet)

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Biochemical and Nutritional Qualities of Several Philippine Indigenous Food Legumes

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ABSTRACT

*This review paper provides the highlights of a six-year comprehensive study on the biochemical and nutritional qualities of several underutilized legumes indigenous to the Philippines. These legumes include: pigeon pea (**Cajanus cajan**), jackbean (**Canavalia ensiformis**), sword bean (**Canavalia gladiata**), sam-samping (**Clitoria tenatea**), batao (**Dolichos lablab**), sabawel (**Mucuna pruriens**), lima bean (**Phaseolus lunatus**) and rice bean (**Vigna umbellata**).*

Among the analyses conducted were for proximate chemical composition, amino acid composition, levels of antinutritional factors such as polyphenols, trypsin inhibitors, lectins or phytohemagglutinins, flatulence factors and phytates. Toxic or potentially toxic substances were also investigated.

A survey was made on the uses of these legumes and the extent of their uses. Problems in the utilization of and other potential areas of research on these legumes were identified.

INTRODUCTION

Food legumes have become a major source of dietary proteins in developing countries. Although the protein content of legumes ranges from 20-50%, the presence of antinutritional factors and the limiting levels of sulfur-amino acids lower their nutritional quality.

In 1984, our laboratory started a comprehensive study of the biochemical and nutritional qualities of several undertutilized legumes indigenous to the Philippines, namely, pigeon pea *(Cajanus cajan)*, jackbean *(Canavalia ensiformis)*, sword bean *(Canavalia gladiata)*, tam-sampong *(Clitoria ternatea)*, batao or hyacinth bean *(Dolichos lablab)*, sabawel *(Mucuna pruriens or M. Conchinchinensis)*, lima bean *(Phaseolus lunatus)* and rice bean *(Vigna umbellata)*.

To accomplish our goals, we determined the proximate chemical composition, amino acid composition, levels of antinutritional factors such as polyphenols, trypsin inhibitors, lectins or phytohemagglutinins, flatulence factors and phytates. Toxic or potentially toxic substances, like cyanogenic glycosides, alkaloid and saponins, were likewise investigated.

As part of the breeding team of the Institute of Plant Breeding, we undertook this research to provide baseline information on these legumes, and, thus expand the genetic resources for improvement of pest and disease-resistance, nutritional quality, yield and tolerance to stresses of our traditional legumes. These legumes are a major potential source of food and livelihood for our people. Thus, these studies are expected to provide a sound basis for the wider utilization of these relatively unknown and underutilized legumes.

NUTRITIONAL QUALITY

The nutritional quality of a protein depends primarily on its capacity to satisfy the requirements for nitrogen and essential amino acids both for maintenance of health and for net protein synthesis of a growing body. Several reviews and books on nutritional evaluation of proteins are available (Pellet and Young 1980; Cereal Chem. 1977). Analysis of nutritional quality usually consists of determination of protein and amino acid composition, as well as animal (rat) assays to determine protein efficiency ration (PER), biological value (BV), nitrogen protein utilization and true digestibility. However, because of the expense, large amounts of samples and long periods of time needed for such animal assays, other less expensive and more rapid methods have been developed. Two of these are used in this study, namely, relative nutritive value (RNV) and *in vitro* protein digestibility (IVPD).

Proximate Chemical Composition

Protein (18 - 30%) and carbohydrates (50 - 60 %) are the major constituents of the mature seeds of 33 samples comprising seven legume species (Table 1) (Mendoza et al. 1990). *Vigna umbellata* had the lowest protein content of 17.42 - 17.56%, while *Canavalia ensiformis, Canavalia gladiata, Mucuna pruriens* or *M. conchinchinensis* and *Clitoria ternatea* had similar protein contents of 28 - 30%. Fat content ranged from 1.2 - 3.7%. *Dolichos lablab, Phaseolus lunatus* and *Canavalia gladiata* had the largest (5-6%) difference in protein and carbohydrate contents.

The proximate compositions of the immature and mature leaves and pods were also obtained: moisture (70-90%), carbohydrates (15 - 18%), proteins (3-10%), fibers (2%), fat (2-4%), ash (0.5- 5%).

Amino Acid Composition

As expected, most of the legumes had met as first limiting amino acid (Table 2) with leu, lys, thr or tyr as the second limiting amino acids (Laurena et al. 1991). Almost all the accessions of all indigenous legumes have chemical scores of the aromatic amino acids phe and tyr greater than 100 except for the three accessions of swordbean. The chemical score or amino acid score is defined as the ratio of mg of essential amino acid (EAA) in one gram of test sample to the level of EAA in one gram reference protein (milk or egg) as set by FAO/WHO (1973). The amino acid that shows the lowest score is termed "limiting amino acid" and the ratio obtained is the score.

Results show the variability in amino acid content of various accessions of each of the legumes studied. Some of these observations on the essential amino acids are as follows: for *D. lablab,* met 1.5-5.8; trp, 0.05-0.21; thr, 3.1-5.2; phe, 3.6-7.2%; lys, 3.1-11.4%; val, 4.1-7.8; leu 3.9-4.0; ile, 4.2-6.6; tyr, 4.2-6.6. for *C. ensiformis:* met, 1.2-1.6; trp, 0.09-0.12; thr, 3.4-4.5; phe, 3.6-5.4; lys, 1.8-5.3; val, 4.3-5.8; leu, 3.1-4.0; ile, 3.6-4.9; tyr, 2.1-4.1; for *C. gladiata:* trp, 0.06-0.09; thr, 3.0-5.3; phe; 1.5-3.7; lys, 7.0-8.5; val, 3.7-6.6; leu, 2.6-5.2; ile, 2.9-6.2; and try, 1.5-2.3; for *P. lunatus:* met, 0.6-1.3; trp, 0.10-0.18, thr 2.6-5.8; phe, 3.4-7.3; lys, 6.6-9.8; val , 3.7-6.6; leu, 2.7-5.0; ile, 3.5-6.6; tyr, 1.8-4.1. for *M. pruriens:*

met, 1.0-1.4; trp, 0.05- 0.07; thr, 2.4-3.9; phe, 2.7-5.0; lys, 2.4-5.2; val, 3.4-5.6; leu, 2.6-3.7; ile, 3.4-5.8; and tyr, 3.8-5.3.

Complete amino acid composition data are available in the reference of Laurena et al. 1991a.

In Vitro Protein Digestibility

Because of the limited amounts of samples available, a micro- biological assay using *Tetrahymena pyriformis* instead of the rat assay was utilized to further evaluate the nutritional quality of the indigenous legumes. A correlation of 0.947 has been obtained between relative nutritive value (RNV) and protein efficiency ration (PER) for eight foods (Landers 1975). In contrast to chemical score, biological tests, such as RNV, are able to detect the presence of toxic factors in a test food.

The IVPD of the raw mature seeds of seven different legumes studied ranged from 70 - 78% (Table 3) (Laurena et al, 1991a). All three accessions of lima bean had low IVPD (70-71%) while jack bean IVPD had a wider range of 72-76%. These results for raw seeds are similar to those reported for other legumes such as mung bean (Barroga et al. 1985), cowpea (Laurena et al. 1984), horse gram and moth bean (Satwadhar et al. 1981). Cooking, such as boiling, was shown to increase the IVPD of these legumes, suggesting the presence of heat-stable factors, such as trypsin inhibitors, which lower IVPD.

Relative Nutritive Value (RNV)

Plant proteins are lower in true protein digestibility (81-96%) than animal proteins such as egg or milk proteins (PAG 1975). Rat assays are usually utilized to determine true digestibility. However, proteolytic enzymes have been used to assay *in vitro* protein digestibility (IVPD)(Hsu et al. 1977; Saterlee, et al. 1979).

The RNV of the raw mature seeds of the indigenous legumes ranged from 11 - 66% (Table 4) (Laurena et al, 1991a). Boiling and roasting to cooked condition resulted in large increases in RNV, 68-94% and 51-89% m respectively. Raw mature seeds of *D. lablab* had relatively higher levels of RNV (33 - 66%) compared to others. *C. gladiata* A-12 had the lowest

value of 11.3%. These low levels suggest the presence of toxic constituents in the raw seed which are inactivated by heat treatment. Boiling (wet treatment) was more effective than roasting (dry treatment). The heat labile toxic constituents could be proteins such as lectins and trypsin inhibitors.

Levels of Anti-nutritional Factors and Potential Toxins

Table 5 shows a summary of the screening for antinutritional factors in the indigenous legumes tested. None of the legumes were found to be high in condensed tannins and cyanogenic glycosides (Laurena et al. 1991b). Condensed tannins are known to lower nutritional quality by binding with proteins, thus decreasing their digestibility. Cyanogenic glycosides, when hydrolyzed in the presence of specific enzymes, will release cyanide which inhibits the electron transport system.

Phytates were found to be higher than normal in hyacinth bean, jack bean and lima bean (6 - 11.6 mg phytate/g). Amounts of phytate lower than 5 are considered to be harmless. Phytic acid or myoinositol hexaphosphate can form insoluble phytate-metal complexes, thus prevents absorption of minerals by the body. Phytate-protein complexes also result in reduced solubility of the proteins which can affect their functional properties.

Trypsin inhibitors were found to be highest in hyacinth bean and sabawel (14 - 27 units/mg) but these are still low compared with soybean. Trypsin inhibitors are proteins which inhibit proteolytic enzyme activity, specifically the enzyme trypsin.

Oligosaccharides, which are known to be the flatulence factors in legumes, were highest in mature seeds of sam-samping, hyacinth bean and sabawel (Revilleza et al. 1990). Saponins were highest in sam-samping.

On the other hand, alkaloids were high in mature and immature leaves of pigeon pea, jackbean and sword bean. Lectins or hemagglutinins were highest in jack bean, sword bean and hyacinth bean.

Saponins have antiphysiological activity: they lower food intake due to poor palatability or irritation of the gastrointestinal tract and/or inhibition of enzymes and reduced availability of nutrients due to the formation of a saponin-mineral complex. The

toxic effect of lectins may be due to their ability to bind in specific receptor sites on the intestinal epithelial cells which causes non-specific interference with the adsorption of nutrients across the intestinal wall.

SURVEY OF USES

Uses of Legumes

The food uses of the seven legumes are quite extensive (Table 6). A common way of preparing the mature seeds of the legumes seems to be "ginisa". Immature pods and immature leaves are used in different vegetable dishes as component in meat dishes such as "sinigang" and "nilaga" and noodle dishes. Boiled mature seeds, specifically of tapilan, may also be used as dessert, e.g. a component of "halo-halo". Mature seeds of sabawel are also roasted and used as substitute for coffee as similarly noted by Basuel and Valdez (1989).

Among the legumes studied, hyacinth bean and lima bean are used in the most number of dishes. Their varied uses also reflect regional dish preferences. For example, "pinakbet", "bulanglang" or "dinengdeng" are favorite dishes in Northern Luzon and make use of edible parts of both hyacinth bean and lima bean, as well as other legumes in the study. On the other hand, "ginataan" is the more popular way of using these legumes in the Bicol region. Jack bean, known as "Lambajong" in the Visayas and "Pataning Dagat" in the Tagalog Region was the subject of studies by science clubs in Southern Leyte (Lugatiman and Ruiz 1981). The plant, reported to be a favorite feed for rabbits, was shown to be a source of various products such as substitute meat, flour, vegetable, beverage, jelly, etc. From its stems, fibers were obtained for home decors, slippers, mats, rugs, bags and others. In this survey, only jack bean and sword bean were noted to cause dizziness when eaten raw or not well-cooked. This could be due to high levels of the lectin concanavalin A present in jackbean and an immunologically similar constituent in sword bean (Altamirano and Mendoza 1991).

A few respondents noted that some of the legumes in the study, e.g. hyacinth bean, rice bean, sabawel and jackbean, are used as animal feed. Basuel and Valdez (1989) observed that sabawel leaf meal incorporated in broiler diets at 5 - 10% resulted in body weight, gain in weight feed conversion and return

above feed cost which were comparable to the control. Higher levels of 15% and 20% produced lighter birds. The potential use of these legumes as animal feed deserves careful investigation.

Availability

While hyacinth bean, lima bean and rice bean were noted to be available in the market and usually grown in the backyard for home consumption, the rest were not available in the market. Several commented that their being less available was a major factor for these legumes coming second to mung bean.

Extent of Use

Among the seven legumes, hyacinth bean and lima bean had the highest recognition/usage rates of 78% and 59% (Table 7). These are followed by rice bean (27%), sam-samping (11%) and sword bean (4%). Respondents from Region 1 reported use of all seven legumes included in the survey. Notably, only respondents from Region I reported usage of sam-samping. So far, jack bean's usage had been reported only by respondents from Regions I, II and III. However, jack bean, also known in the Visayas as "Lambajong" and in the Tagalog Region as "Pataning Dagat" was the subject of studies by science clubs in Southern Leyte (Infoscience 1981) and is perhaps also utilized by people in the area. An accurate picture of the extent of utilization of these legumes is still to be expected once this study is completed.

CONCLUDING REMARKS

This investigation has provided a wealth of information on the biochemical and nutritional qualitites of several of our indigenous legumes. Hopefully, the results of this work will be helpful to plant breeders, food scientists, nutritionists, agriculturists, other researchers, students and teachers as a take-off point for further studies that could lead to wider utilization of these legumes.

Notably, one major problem in utilizing many of these legumes is their indeterminate habit, thus, their cultural management is labor-intensive and expensive especially if done at larger than backyard scale. They are all generally acceptable but a

few need pre-cooking treatment to remove toxic principle and/or bitter taste. It is notable also that the people of Region II utilize all the indigenous legumes studied.

Aside from popularizing these legumes as foodstuff, one strategy for their wider utilization is to obtain high value chemicals or components from them for the food and other industries. For example, there should be follow-up studies of the lectins, saponins, alkaloids as well as galactomannans of these legumes.

Certainly, this study has only barely touched the rich Philippine flora from which our country can derive much needed resources.

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Table 1. Proximate chemical composition of different plant parts of several Philippine indigenous food legumes

Moisture (%)	Fat (%)	Protein (% N x 6.25)	Ash (%)	Fibers (%)	Nitrogen Free Extract (%)
Batao (*Dolithos lablab* L.)					
Immature leaves (5)	80.8-82-1	2.2-3.7	4.0-4.4	1.1-1.9	1.2-1.6	7.5-9.8
Mature leaves (5)	57.8-65.4	3.9-4.6	8.1.9.9	3.2-4,4	3.2-3.6	14.2-20.1
Immature pods (5)	86.0-89-2	0.8-1.1	2.4-3.4	0.7-0.9	1.1-1.5	5.6-7.2
Mature pods (5)	80.1-84.2	1.1-1.5	2.1-2.7	1.0-1.2	1.6-2.2	9-12.6
Mature seeds (5)	6.8-7.4	1.7-2.1	18.5-23.2	4.1-4.2	4.8-5.5	60.2-62-8
Pigeon Pea (*Cajanus Cajan* (L.) Mill sp.)					
Immature leaves (1)	69.8	4.2	6.5	1.6	2.4	15.5
Mature leaves (1)	67.4	4.9	6.8	2.7	2.7	14.6
Lima bean (*Phaseolus lunatus* (L.) Macf.)					
Immature leaves (8)	82.2-84.4	2.1-3.0	3.3-4.2	1.3-1.5	1.0-1.2	6.0-9.1
Mature leaves (8)	79.9-81-4	2.8-3.9	3.2-4.0	1.2-1.9	1.1-1.9	8.1-11.1
Immature pods (8)	79.3-84.4	1.1-1.5	2.2-2.9	1.1-1.4	1.4-1.9	5.6-10.0
Mature pods (8)	75.6-81.8	1.1-1.5	1.9-3.0	1.2-2.0	1.7-2.2	11.6-16.2
Mature seeds (9)	9.3-10-2	1.2-1.6	20.2-26.1	4.4-4.8	3.2-3.7	55.1-60.7
Jackbean (*Canavalia ensiformis* (L.) DC)					
Inmature leaves (3)	76.8-79-8	2.4-2.9	5.3-5.7	2.3-2.6	1.4-1.6	8.8-10.4
Mature leaves (3)	75.4-77.8	2.8-3.1	5.7-6.2	2.3-2.8	1.6-2.0	9.8-10.5
Inmature pods (3)	74.5-79-1	1.6-1.9	3.1-3.4	1.2-1.3	1.6-1.7	12.7-18.0
Mature pods (3)	20.7-74-1	1.6-2.0	3.0-3.4	1.8-1.9	2.9-3.1	15.9-19.6
Mature seeds (3)	7.9- 8.2	1.2-2.6	27.8-29.4	4.1-4.6	4.3-5.2	49.5-51.9
Sabawel (*Mucuna pruriens* L. (DC)					
Inmature leaves (3)	78.3-80.1	2.5-3.6	5.3-5.7	1.3-1.6	1.4-1.8	8.4- 9.6
Mature leaves (3)	62.1-67.4	3.9-4.8	8.1-8.9	3.3-3.6	2.3-3.7	14.0-17.8
Inmature pods (3)	76.5-78.9	17-2.0	3.7-3.9	1.1-1.3	1.5-1.6	12.7-15.2
Mature pods (3)	70-8-72-4	1.9-2.1	4.1-5.2	1.7-1.9	3.1-3.5	15.2-15.5
Mature seeds (3)	7.2- 7.8	2.5-2.7	28.2-29.4	4.0-4.1	3.2-4.4	51.8-53.8

Table 1. Continued

	Moisture (%)	Fat (%)	Protein (% N x 6.25)	Ash (%)	Fibers %)	Nitrogen Free Extract (%)
Sam-samping (*Clitoria ternatea* L.)						
Inmature leaves (1)	80.1	1.9	6.1	1.5	1.1	9.2
Mature leaves (1)	72.7	2.5	7.8	1.9	1.6	13.6
Sword bean (*Canavalia gladiata* (Jacq.) DC)						
Immature leaves (4)	78.6-80.	82.4-2.7	4.7-5.6	1.92.6	1.2-1.7	8.8-10.1
Mature leaves (4)	76.6-77.9	2.6-2.9	5.3-5.8	2.4-3.0	1.5-1.8	8.3-10.6
Immature pads (4)	83.9-84.8	1.1-1.3	1.7-1.8	0.9-1.0	0.8-10	10.4-11.3
Matture pads (4)	69.1-71.8	1.-2.6	3.1-3.7	1.6-1.8	2.9-3.6	17.8-19.4
Matture pseeds (4)	8.1-8.8	2.2-2.4	22.6-28.1	4.2-4.7	5.4-6.9	49.6-56.3
Rice bean (*Vigna umbellata* (Thumb.) Ohwi & Ohashi)						
Immature leaves (2)	74.6-74.9	2.6-2.8	4.5-4.7	1.4-1.8	1.4-1.5	14.8-14.9
Mature leaves (2)	74.1-75.7	2.5-2.8	4.4-4.7	2.4-2.6	1.6-1.8	13.0-14.4
Mature seeds (3)	8.9- 9.3	3.4-3.7	17.4-17.9	4.3-4.8	5.9-6.9	58.7-59.3

aBased on data from Mendoza et al., 1990
Numbers in parentheses refer to number accessions studied.

Table 2. Chemical score of several Philippine indigenous legumes

Sample	Essential amino acids (% AA or g AA/100 g. sample							Limiting Amino Acid	
	Met	Thr	Phe & Tyr	Ile	Lys	Leu	Val	First	Second
Canavalia ensiformis									
Jackbean A-1	>54	112	115	120	96	57	116	met	leu
A-3	>28	92	137	97	98	44	86	met	leu
A-5	>31.4	107	117	115	49	48	104	met	leu
A-6	>26	85	97	90	45	37	82	met	leu
A-8	>26	87	148	122	40	44	106	met	lys
8-8	>14	107	125	110	33	47	98	met	lys
Canavalia gladiata									
Swordbean A-4	>20	132	52	155	154	74	32	met	phe & tyr
A-9	>20	82	95	85	134	50	74	met	leu
A-12	>11	77	87	72	127	37	98	met	leu
Dolichos lablab									
Hyacinth A-45	>48.6	85	115	75	122	46	78	leu	met
bean A-51	>62.8	130	188	165	84	70	156	met	leu
A-52	>17.4	100	168	112	93	56	98	met	leu
A-57	>11.43	92	135	132	56	57	120	met	lys

Table 3. *In vitro* protein digestibility (IVPD) of raw mature seeds of several Philippine indigenous legumes

Sample		IVPD (%)
Canavalia ensiformis		
Jack bean	A - 1	75.44
	A - 3	75.60
	A - 5	74.61
	A - 6	75.36
	A - 8	76.61
	8 - 8	72.23
Canavalia gladiata		
Sword bean	A - 4	74.02
	A - 12	72.62
Clitoria ternatea		
Sam-samping	7 - 2	76.19
Dolichos lablab		
Hyacinth	A - 45	73.71
bean	A - 51	70.18
	A - 52	71.19
	A - 57	78.57
Mucuna pruriens		
Sabawel	A - 2	72.19
Phaseolus lunatus		
Lima bean	A - 535	71.00
	A - 537	70.31
	A - 544	71.45
Vigna umbellata		73.48
Rice bean		74.30

From Laurena et al. (1991 a).

Table 4. Relative nutritive values (RNV) of raw, boiled and roasted mature seeds of several Philippine indigenous legumes

Sample		Relative nutritive value (%)		
		Raw	Boiled	Roasted
Canavalia ensiformis				
Jackbean	A-1	47.39 ± 6.40	85.31 ± 5.08	60.46 ± 5.17
	A-3	25.31 ± 4.05	75.74 ± 5.64	76.22 ± 6.30
	A-5	17.26 ± 0.00	83.32 ± 2.39	61.36 ± 6.48
	A-6	40.23 ± 4.85	82.16 ± 4.38	74.10 ± 5.97
	A-8	28.00 ± 3.02	78.40 ± 3.74	71.07 ± 5.48
	8-8	18.54 ± 2.22	59.19 ± 3.05	50.56 ± 0
Canavalia gladiata				
Sword bean	A-4	23.19 ± 4.66	83.99 ± 4.85	69.91 ± 1.73
	A-9	16.09 ± 2.02	86.38 ± 5.22	58.17 ± 3.13
	A-12	11.29 ± 2.19	86.69 ± 3.44	83.18 ± 0
Clitoria ternatea				
Sam-samping	7-2	54.09	90.86 ± 3.53	88.49 ± 4.60
Dolichos lablab				
Hyacinth bean	A-45	65.97 ± 3.69	94.20 ± 3.40	82.13 ± 4.30
	A-51	32.99 ± 3.52	92.3 ± 3.79	76.46 ± 1.63
	A-52	42.39 ± 3.34	84.39 ± 3.70	84.93 ± 1.51
	A-57	48.39 ± 3.11	87.76 ± 2.98	79.19 ± 4.34
Mucuna pruriens				
Sabawel	A-2	38.01 ± 1.58	81.87 ± 5.50	81.58 ± 9.30
	A-5	34.76 ± 5.03	79.97 ± 5.35	62.55 ± 2.30
	A-542	51.23 ± 5.40	83.01 ± 4.25	77.17 ± 6.13
Vigna umbellata				
Lima bean	28	22.62 ± 2.64	79.42 ± 1.97	60.40 ± 6.72
	46	42.42 ± 6.11	67.68 ± 5.80	55.57 ± 5.35

From Laurena et al.(1991a)

Table 5. Summary of screening Philippine indigenous legumes for antinutritional factors

Factor	High levels in legume (part)
Condensed tannins	none* (all edible parts)
Phytic acid	hyacinth bean, jack bean lima bean (6 11.6 mg phytate P/g) (mature seeds)
Cyanogenic glycosides	none (all edible parts)
Trypsin inhibitor activity sabawel (mature seeds)	hyacinth bean
Oligosaccharides	sam-samping, hyacinth bean, sabawel (mature seeds)
Saponins	sam-samping (mature seeds)
Alkaloids	pigeon pea, jack bean and sword bean (mature and immature leaves)
Lectins	jackbean, sword bean and hyacinth bean (mature seeds)

* None among the legumes tested was high in this factor.

Based on data from Laurena et al. (1991 b) and Mendoza (1989).

Table 6. Food uses of seven Philippine indigenous legumes

Legume	Parts	Food Uses
Hyacinth bean *Dolichos lablab* **(Batao, bulay, parda, harabilla, Kadyos)**	mature seeds	ginisa
	immature pods	vegetable dish; vegetable component of sinigang nilaga, lumpia, pansit pinakbet
	immature leaves	vegetable component of sinigang, nilaga, lumpia and other vegetable dishes
Lima bean *Phaseolus lunatus* **(Patani, palaminko, betsuelas, utung, perkules, pataning dagat)**	immature seeds	ginisa; cooked with coconut (ginataan)
	mature	boiled, ginisa; vegetable component of pinakbet; halo-halo
	immature pods	vegetable dish; vegetable component of sinigang gang, nilaga, ginataan, pinakbet may be added to all types of dishes as vegetable
Rice bean *Vigna umbellata* **(Tapilan. munggo)**	mature seeds	boiled beans; ginisa; component of halo-halo
	immature leaves	vegetable component of sinigang, nilaga or tinola
	immature pods	vegetable component of sinigang, salad, pansit
Sabawel *Mucuna pruriens* **or cochinchinesis (sabawel, cocoa)**	mature seeds	ginisa, roasted as coffee
Jack bean *Canavalia ensiformis* **(Pataning espada)**	mature seeds	ginisa
	immature leaves	vegetable component of dinengdeng, pinakbet, ginataan

Table 6. Continued

Legume	Parts	Food Uses
Sam-samping *Clitoria ternatea* (Sam-samping; Kum-kumpitis; competes)	mature seeds	ginisa; mixed with pinakbet
	immature leaves	blanched; added to vegetable dishes
	immature	added to pinakbet, sinigang, nilaga, salad other vegetable dishes
Sword bean *Canavalia gladiata* (Pataning espada)	mature seeds	
	immature leaves	added to sinigang, nilaga, salad
	immature pods	added to ginisa, sinigang

Local names in parenthesis

Table 7. Survey of usage of seven Philippine indigenous legumes

Region	Number of respondents	Number of respondents reporting use of						
		Jack bean	Sword bean	Sam-samping	Hyacinth bean	Sabawel	Lima bean	Rice bean
I	8	1	1	5	7	3	5	2
II	6	2			4	1	4	
III	5	1	1		4		3	1
IV	12	1			10	1	6	4
V	5				4	1	5	
VI	2						1	2
VII								
VIII	2				2			1
IX	3				3		1	
X								
XI	3				3		1	
XII								
	46	5	2	5	36	7	27	10
% of Total		11	4	11	78	15	59	22

POSTER PAPERS

Development of Palay Purity Tester

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ABSTRACT

The Palay Purity Tester was fabricated, modified and developed to come up with a reliable equipment capable of measuring the purity of palay sample under the NFA's procurement standard classification. The fabricated model was tested and evaluated using palay samples with predetermined purity of A (97.0% and 95.0%), B (94.0%, 92.0% and 90.0%) and C (89.0% and 87.0%). All samples carried a moisture level of 14%. Test and evaluation were conducted to determine the tester's efficiency at three different blower openings (1/2, 2/3 and 3/4 open), its reliability and adaptability at NFA palay procurement operations.

Results showed that there was a numerical discrepancy between the actual purity of the sample and the meter reading. However, taking into consideration the purity level (A, B and C used in palay procurement operations) of palay samples used, the tester's reading complied with the required purity level of the sample being tested indicating applicability to NFA procurement operation.

Statistical analysis showed that the 2/3 blower opening gave the best reading with the least standard error of 1.779. This result therefore shall be used as the working data for the calibration of the tester's autoweigher to project the true reading.

A Proposed Model for the Waste Utilization Value

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ABSTRACT

The Waste Utilization Value (WUV), a concept introduced by one of the authors (Jose), is the value of a waste in monetary units per unit measure. The value is positive if the waste is profitably utilized or negative if the waste is hazardous or a pollutant. A model for wastes having positive WUVs is being proposed. Sixteen of the 30 wastes surveyed for the buying price in Metro Manila were considered. "Discriminant Analysis" was used. The following factors that affected the WUV were chosen -- availability, separation, handling, product, demand, cost, technology and profit factors. The model correctly classified 15 of the 16 types of wastes into predicted group membership.

On the Dynamics of Resource-Consumer-Toxicant-Systems: Models of Reproductive Effort and Resulting Offspring of an Individual Organism

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ABSTRACT

Dynamic models for an individual organism's reproductive effort and resulting offspring were developed and analyzed. These models were based on life history theory and bioenergetics of an organism. Two indices, namely, the organism's ***energy utilization index*** *and* ***reproductive index,*** *were defined and discussed; the former was used as indicator of stress resulting from energy investment in reproduction, the latter as indicator of reproductive success or failure. The organism's threshold reproductive effort (level of reproductive effort that optimizes the energy utilization index) was likewise defined. The dependence of reproduction on available food was also determined. To illustrate the ideas in this study specific forms of the general model were developed for* ***Daphnia*** *and the metals arsenic and cadmium.*

[1] This research was supported by the Mindanao State University Research Center under URC Project Number 295-89.

Morpho-Histochemical Studies of Some Medicinal Ferns

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ABSTRACT

*Five species of medicinal ferns found in Bukidnon, viz., **Blechnum orientale** Linn., **Pteridium aquilinum** (L.) Kuhn., **Sphenomeris chinensis (L)** Maxon, **S. chinensis** (L.) **Maxon var. rubens** Amoroso et Medecilo com. nov., **Oleandra maquilinguensis Copel.** are described morpho-anatomically and their medicinal values are discussed. Likewise, the active constituents and their distribution within the plant tissues and organs were determined through histochemical tests. Active principles such as alkaloids, amygdalin, tannin, saponin, formic acid, tartaric acid and oxalic acid were observed to be present from detectable to very abundant.*

Localization of Zinc in the Gonadal Tissues of *Tilapia Nilotica* Linn.

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ABSTRACT

Subcellular localization of zinc in gonadal tissues was done in the microsomal fraction of adult fish exposed to 17 ppm zinc for 14 days. The elution pattern of the cytosolic fraction of treated ovary and the testis indicates zinc bioaccumulation. Elution peak two corresponds to cytochrome C, characteristics of metallothionein binding with zinc.

Gonads of zinc exposed fish show altered morphological structures. The oocytes were vacuolated and devoid of yolk and the thecal cells were disintegrated. There was dramatic reduction in oocyte number. The testis of juvenile fish remained immature and showed only spermatogonia and proliferation of the connective tissues.

Competition of Water Hyacinth [*Eichlornia Crassipes* (Mart.) Solms] with *Hydrilla Verticillata Royle* and *Pistia Stratiotes* Linn.

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ABSTRACT

Hydrilla verticillata *can control the growth of* ***Eichlornia crassipes*** *to some extent when nutrients are limited. Although a floating weed,* ***E. crassipes*** *needs to anchor its roots to absorb nutrients from the bottom. Failure to do so affects its growth rate.*

Pistia stratiotes *can grow better than* ***E. crassipes*** *if both plants are placed in a container with limited amount of nutrient and space. It does not, however, completely suppress the growth of* ***E. crassipes.***

Strain Improvement of Selected Species of Edible Fungi

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ABSTRACT

An efficient method for mutagenesis using UV irradiation and nitrous acid treatment was developed for ***Volvariella volvacea Agaricus bisporus*** and ***Lentinus edodes****. The Holliday technique for mutant identification and the characterization of auxotrophic mutants was used. Vegetative mycelia of high temperature tolerant strain of* ***A. bisporus*** *and* ***L. edodes*** *were isolated and used in breeding trials.*

Methods for the isolation of protoplast from ***V. volvacea*** *using commercial enzyme preparation were described. The regeneration and reversion frequency of protoplasts for this fungus was evaluated. A system for genetic analysis was also done using available mutant strains.*

The production of fruit bodies using different substrates for the three species was investigated. Environmental factors affecting fruit body production were determined and analyzed.

Chromium From Leather Tanning Effluent

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ABSTRACT

The possibility of using lime liquor in a waste complementation process for recovery of chromium from leather tanning effluent was explored. High recovery rates of 98.2%, 96.5% and 96.6% for the bluish green compound, green pigment and basic chromium sulfate, respectively, were obtained in this study. The process involved is efficient, relatively simple and cheap.

Organogenesis from Leaf Callus of Mungbean (*Vigna Radiata* L Wilczek) and Mothbean (*Vigna Aconitifolia* Jacq Marechal)

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ABSTRACT

*Callus cultures of mungbean (**Vigna radiata** L. Wilczek) cvs. Pag-asa 2 and Pag-asa 3 were established from leaf explants cultured on modified Murashige and Skoog's (MS) medium (1962) supplemented with 2.0 mg/l 2,4 dichlorophenoxy acetic acid (2,4-D) and 0.5 mg/l benzylaminopurine (BAP). Regeneration of roots was observed upon transfer of the compact green calli to MS medium with different hormonal combinations.*

*In mothbean (**Vigna aconitifolia** Jacq Marechal) acc. 1892 calli were established from leaf explants cultured on B5 basal medium (Gamborg et al. 1968) supplemented with 1.0 mg/l 2,4-D and 0.4 mg/l kinetin. Regeneration of shoots was observed upon transfer of calli to L6 medium (Kumar et al. 1988) with 1.0 mg/l zeatin or a combination of 0.5 mg/l each of zeatin and BAP.*

Endosperm Culture of Calamansi (*X Citro Fortunella Mitis*), a Progress Report

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That seedless calamansi could be derived directly by regeneration of plants from the triploid endosperm was first theorized by RC Barba and LF Pateña in 1976. Plantlets were then regenerated from callus culture of calamansi seed tissues (Pateña, Barba and Estrella 1978). The seed tissues used did not contain the endosperm, hence, subsequent studies traced the development of the endosperm as the seed matured. Results of initial work showed that the endosperm developed about two weeks after pollination, first nuclear and at later stages, cellular (Pateña 1980). Between 1-2 months after pollination, greater development of the endosperm occurred. This period was identified to be a good stage of collecting seeds for the ***in vitro*** *culture of endosperm to obtain triploid plants for seedlessness (Pateña 1980). In 1987, the endosperm was excised and cytologically identified (Avenido and Barba 1987). From 1983 to 1989, calli were established from the nucelli-endosperm (NE) tissues and plantlets were regenerated (Avenido, Zamora, Barba and Pateña 1991). Present efforts are concentrated on efficiently excising the endosperm, regenerating plantlets from the endosperm - derived callus and cytologically triploid plantlets.*

Branch Cutting Propagation of Five Bamboo Species Using IBA

Manuel Castillo

ABSTRACT

Increasing demand for bamboo necessitates the most efficient and effective nursery propagation and plantation cultural operation of different bamboo species. Culms are wasteful; branch cuttings are technologically economical and easier to handle. Combined Indole-Butyric Acid hormonal effects on rooting of five bamboo species performance were evaluated.

Species, type of branch and position of cutting were found to be highly significant in hormonal propagation of bamboo. More live cuttings with well developed shoots were found on the basal section of the primary branch treated with 100 ppm IBA. Likewise, the highest number of live cuttings were in the primary branch position.

Micropropagation of Banana and Rattan for Mass Distribution

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Two biotechnological techniques developed at the Institute of Plant Breeding (IPB) -- one by Damasco and Barba (1984) and the other by Pateña, Mercado and Barba (1984) -- were adopted by the IPB National Seed Foundation ***In Vitro*** *Propagation Laboratory for mass propagation of banana and rattan, respectively. The techniques involve: 1) the* ***in vitro*** *culture of shoot tips in the case of banana, and seeds in the case of rattan; 2) allowing the shoots to grow, proliferate and form roots; and 3) transplanting the plantlets to potting mix. With these techniques, a production capacity of 12,000 and 5,000 plantlets of banana and rattan, respectively, is targetted annually. Tissue-cultured banana plants that are free of banana bunchy top virus (BBTV) disease are now on sale at P15 (plants with 3-4 leaves) and P20 (plants with 5-8 leaves) each. Rattan plants will also be on sale toward the end of the year at P45 each.*

Tissue Culture of Garlic and Shallot

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ABSTRACT

Shoot tip explants (4-6 mm) of 11 strains of garlic (8 from the Philippines and 3 from Indonesia) and 19 strains of shallot (12 from the Philippines, 4 from Indonesia, 1 from Laos and 2 from Thailand) were initiated ***in vitro.*** *Initial results showed that addition of 4 mg/l 6-benzylaminopurine (BAP) to the culture establishment media [Murashige and Skoog's (MS) medium of formulation (Pateña et al.)] favored more vigorous growth. Multiple shoots were obtained using MS medium with 1-2 mg/l 2- isopentenyladenine (2-ip) and 0.5 mg/l napthaleneacetic acid (NAA) in case of garlic and 0.5 mg/l 6-benzylaminopurine (BAP) and 0.5 mg/l NAA in case of shallot* benzylaminopurine *(BAP) and 0.5 mg/l NAA in case of shallot.*

Mannitol added to the medium at 1-2% effectively controlled the rapid growth of leaves and thus minimized subculturing. Subculture of shoots was limited to three passages, after which the cultures died or formed bulbs ***in vitro.***

Somaclonal Variation and Induced Mutation in Ramie

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*Plant formation using shoot tip and single node explants of ramie (**Boehmeria nivea**), Acc 2 and 20, was obtained in ramie medium (RM), which is composed of modified (1/2 strength micronutrients) Murashige and Skoog's (MS) formulation supplemented with myo-inositol, sucrose and coconut water. RM is a modification of the medium used in embryo culture of bamboo (Zamora and Gruezo 1990). With the use of internode explants of Acc 2, 13, 18 and 20, compact, yellow to green nodular calli composed mostly of tracheids were obtained in modified hormone-free MS medium. Plantlet regeneration from calli was not observed.*

*Chemical mutagens, sodium azide (NaN_3) and ethylmethylsulfonate (EMS) were used to induce variability **in vitro**. NaN_3 at concentration 1.0 mg/l was found to be lethal to shoot tip and single node explants of Acc 2 and 20. EMS at the highest concentration (0.10%) tested was not. EMS-treated plantlets, when transplanted to potting mix, survived.*

The study is in progress and efforts are concentrated on regenerating plantlets from calli via somatic embryogenesis or organogenesis, obtaining chemically-mutated plants and evaluating these plants for high yield and good quality (low denier) fiber.

Development of Tissue Culture Techniques for Woody Species, Durian (*Durio zibethinus*), Mussaenda (*Mussaenda sp cv Dona Luz*) and Derris (*Derris elliptica*)

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ABSTRACT

*This study aims to determine the tissue culture requirements of three woody species: durian (**Durio zibethinus**), mussaenda (**Mussaenda sp** cv Dona Luz) and derris (**Derris elliptica**).*

Callus was induced from durian stem explants 38 days after inoculation onto Murashige and Skoog's (MS) medium (1962) supplemented with 0.5 mg/l each of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP). Loose, friable and vitrescent calli were initiated on the abaxial surface of leaf squares, midvein sections, petiole segments, stem sections and shoot tips of mussaenda cv Dona Luz. Calli grew a week after inoculation onto the same medium used for durian. In derris, callus was formed from internodes, nodes and axillary buds three weeks after inoculation onto two kinds of medium, hormone-free R medium (Pateña et al. 1978) and Shenck and Hildebrandt (SH) medium (1972) with either 1.0-2.5 mg/l BAP or 0.5-2.5 mg/l naphthaleneacetic acid (NAA).

Organogenesis was limited to root formation in derris and mussaenda on NAA-enriched SH medium. In derris, roots formed after 3-5 weeks in culture while in mussaenda, roots formed after a month in culture.

This study is in progress to complete the requirements for ***in vitro*** *culture of these three woody species.*

Reaction of Recovered Tungro-infected Taichum Native I Rice Plants to Elisa

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ABSTRACT

Tungro is the most destructive and widely distributed virus disease of rice in the Philippines. It is present and equally destructive in all the rice-producing countries of South and Southeast Asia. The viral nature of the disease has been established and confirmed through transmission, electron microscopy and serology, There have been observations and reports that symptoms of the disease disappear after a certain growth period. As virus-infected plants rarely, if ever, recover, the disappearance of tungro symptoms is ascribed to masking. However, no evidence has been presented to support this claim. Others held the opposite interpretation that the disappearance of tungro symptoms in the field and in the greenhouse is a manifestation of recovery.

Even Taichung Native I rice, a variety most susceptible to tungro, recovers from the disease as expressed by its normal vegetative growth with its tillering capacity restored and stunting reversed. Plants which have apparently recovered look as normal as their healthy counterparts. Leaf sections from original TNI plants infected with tungro showed positive reactions indicating the presence of bacilliform, spherical viruses and

their combination when indexed by ELISA (ELISA assays were conducted at the International Rice Research Institute). In first ratoon TNI plants, positive reaction only to baciliform virus was detected through ELISA. No trace of either virus was present in second ratoon plants when indexed by ELISA. Results of this and previous studies strongly suggest that the disappearance of tungro symptoms is a true and complete recovery and not a masking phenomenon.

Recovery from the disease seems to be affected by the variety, severity of symptoms and vegetative stage of infected plants. The principal requirement for recovery to occur is the exclusion of insect vectors from visiting and colonizing the diseased plants. The abnormal behavior of tungro-infected plants by recovering at any time of the year, naturally, en masse and permanently after freeing them from their insect vector leads the senior author to speculate on the possible involvement of insect toxin in tungro syndrome.

Chemical and Biological Studies on *Mikania cordata* (Burm. f.) B. L. Robinson

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ABSTRACT

*Chemical and biological studies were done on the plant **Mikania cordata** (Burm. f.) B. L. Robinson in an attempt to establish the scientific basis for its medicinal uses. Upon isolation and structure elucidation, the plant has yielded sesquiterpene lactones of the germacranolide type, flavonoids, sterol glucosides and glucosyl ceramides.*

While the crude chloroform extract exhibited antimicrobial and antimycobacterial properties, the purified chloroform extract indicated ***in vivo*** *anti-inflammatory effects. Enhanced antimicrobial activities, as well as anti-inflammatory effects, were detected in the fraction containing the mixture of sesquiterpene lactones.*

Scandenolide, the major sesquiterpene lactone isolated, was found to inhibit the production of the inflammatory mediators leukotriene B_4 and platelet activating factor (PAF) by isolated rat peritoneal leukocytes. However, the toxicity shown by the crude extract would limit the use of this medicinal plant.

A Multi Media Instructional Kit on Philippine Medicinal Plants

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The research aimed to develop an instructional kit for use in the teaching of Philippine medicinal plants. It employed the experimental and descriptive methods, dealing with developmental investigation and administration of a survey questionnaire.

The following conclusions were arrived at.

1. Information on Philippine medicinal plants can be transmitted through the print medium, through visuals and through the medium of sound.
2. The following audio-visual materials are practical components for an instructional kit on Philippine Medicinal Plants: botanical specimens, including herbaria, crude drugs and leaf collections; bottled herbal preparations; flat pictures; colored slides and transparencies; tapes; reading matters; and selected laboratory apparatus.

A kit guide in manual form can serve as a means of listing and explaining the contents of the instructional kit.

The kit guide is instructional in itself, providing additional activities to reinforce the teaching/learning of the various aspects of herbal technology.

From the above conclusions, it can further be generalized that educational technology can improve the quality of education through: (1) greater individualization of instruction; (2) a greatly enriched library of teaching materials; and (3) possible cost reductions.

RECOMMENDATIONS

1. There is a need to produce prototypes of this Instructional Kit on Philippine Medicinal Plants for distribution to involved agencies/institutions. These audio-visual materials can be utilized in the formal school system, as well as in informal set- ups.

2. This system may increase levels of awareness, interest and performance of students enrolled in science courses, particularly general science and botany.

3. Due to the lack of trained health workers in the remote rural areas, community workers may be taught, on a voluntary basis, to help improve health conditions through a more comprehensive primary health care program.

4. It is suggested that community programs involving health instruction include herbal technology specifically through non- formal classes.

5. Within the scope of the formal schooling system, it is recommended that each school allocate a portion of its campus for a Herbal Garden where each species of the common Philippine medicinal plants could be grown. The garden could be used for instruction and research purposes, as well as a source of crude drugs. Each representative plant should have a nameplate where the basic data on its identity and use are printed.

6. It is highly recommended that schools/faculty/students produce short films covering various aspects of herbal medicine. The following are suggested:

a. Agricultural aspect;

b. Phytochemical screening of Philippine medicinal plants;

c. Microbiological screening of Philippine medicinal plants;

d. Pharmacological screening of Philippine medicinal plants; and

e. Pharmaceutical aspect.

7. There is a need to establish a computerized Data Bank of Philippine medicinal plants for systematic and expeditious retrieval of data.

8. A follow-up study on the effectiveness of the instructional kit on Philippine medicinal plants in the teaching-learning situation is hereby proposed.