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ABSTRACTS of PAPERS
Presented during the
25th NAST Annual Scientific Meeting

*Addressing the Demographic Crisis
in the Philippines*

9-10 July 2003

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25th ANNUAL SCIENTIFIC MEETING

Addressing the Demographic Crisis in the Philippines

25th ASM: 9-10 July 2003; Manila Hotel

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PLENARY PAPERS

BASIC HEALTH SERVICES AND POPULATION GROWTH

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The continued high rate of population growth in the Philippines has serious consequences for basic health service delivery in terms of cost and quality. The estimated 2,000,000 Filipino babies added to the population each year will require added resources for immunization, disease control, and hospital services at all levels. Given the fact that the highest fertility rates are among the poorest 40% of the population, it is expected that service demands will be greatest in government facilities that are even now struggling to maintain service quality while dealing with more clients.

Conversely, improvements in basic health services can potentially reduce population growth rate by its influence on fertility rates. Improved MCH is known to be associated with lower fertility as child survival improvements. More directly, an aggressive family planning program that makes available all modalities for fertility regulation and prevention of unplanned or unwanted pregnancies will help couples to attain desired reduced family sizes.

A population policy that advocates a two-child family to increase contraceptive prevalence, promotes appropriate family planning methods to achieve an ideal contraceptive method mix, and encourages private sector collaboration can reduce total fertility rate to the replacement rate of 2.1. If this rate is achieved within four or five years, the Philippines can realistically hope to reduce population growth rate to manageable levels and even target zero population growth by the year 2025 or soon after.

Keywords: population growth, basic health services, fertility, MCH, family planning methods

**THE ECONOMIC IMPACT OF THE DEMOGRAPHIC
CRISIS: ITS IMPLICATIONS ON PUBLIC POLICY**

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The Philippines is “over-populated” not in relation to its natural carrying capacity but in relation to the performance of its economy and government. Clearly, it would be better to improve the performance of the government and the economy than to just get government involved in fertility choices of households. However, given the history of the performance of both government and the economy, population policy can clearly help improve the nation’s welfare. Government must provide public goods and services and its capability to deliver them is affected by population growth. Moreover, the impact on government of high fertility may be even more serious than suggested by the average level of total fertility rate since children’s education is closely correlated with their parent’s education and poorer and less educated parents tend to have more children.

Government’s capability to meet the needs of the country’s growing population has been impaired by a weak economy and high levels of public debt. Due to high expenditures on interest payments and weak tax collections, the government’s deficit is high and its level of indebtedness may become unsustainable even at present inadequate levels of spending on basic social services and infrastructure.

Government’s ability to meet the needs of the population will clearly be improved if fertility can be brought down. Fertility can be reduced significantly without resorting to coercive policies. Poor and less educated parents have higher fertility than average, but their desired fertility is much lower than their actual fertility. Population policy can go a long way simply by helping people attain their desired family sizes.

Keywords: demographic crisis, public policy, basic social services, fertility, population policy

TECHNICAL SESSIONS

AGRICULTURAL SCIENCES

EMERGING SWINE PRODUCTION TECHNOLOGIES TO KEEP PACE WITH INCREASING POPULATION

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This paper presents some analyses and recommendations on how the swine subsector, as it continues to dominate the livestock sector and hence make up a significant proportion of the agricultural landscape, can become a logical and potent springboard in addressing the demographic crisis in the country. It also provides a framework showing the vital link between population, poverty and food security, with the contention that unless poverty is significantly reduced, the goal to attain food security remains a distant reality. This paper further presents a comprehensive discussion and vital recommendations on the role of emerging swine production technologies in meeting the protein requirements of the present and future generations of Filipinos, as well as in providing livelihood opportunities to empower the poor and the disadvantaged sector of the society.

During the last 10 years, R&D programs and initiatives were able to generate scientific and technological breakthroughs, which have significantly contributed to the improvement of swine production in the country. These include, among others, genetic and reproduction improvement through artificial insemination (AI), nutrition and feeding management, and animal health care. However, much still needs to be done to maximize the potential of these technologies, particularly for the backyard raisers.

With a projected population of 111 million by year 2025, the swine industry in the next 22 years must triple its pork production (2.8 million MT by 2025) to meet the projected demand (2.3 million MT by 2025). The ultimate task ahead is for all industry players to be able to encourage and empower hog farmers and farmer organizations to attain increased productivity and production efficiency, improved product quality, and reduced production cost toward an efficient, viable, and sustainable swine industry. The interventions required

from the industry players include: policy interventions; R&D/S&T interventions; technology/information delivery services; and market and input support services.

Vital to this goal is the *political will* of each and every player – government and private sectors alike – to participate in a concerted effort to uplift the plight of backyard raisers in the rural areas who account for 77 percent of the current swine inventory.

Keywords: swine, production technologies, strategies, genetic improvement, artificial insemination, nutrition, feeding management, animal health care.

THE RICE PROBLEM IN THE PHILIPPINES: TRENDS, CONSTRAINTS, AND POLICY IMPERATIVES

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The comparatively poor performance of the rice sector in recent years is microcosm of the state of Philippine agriculture. Both domestic policies and institutions have constrained efficiency and raised the “cost of doing business” in agriculture, thereby blunting productivity growth and eroding the country’s competitiveness in the global marketplace. Rice has become more expensive in the Philippines than in other developing East Asian countries, owing principally to the government’s ill-advised self-sufficiency objective. Liberalizing rice trade enhances the welfare of the poor, especially landless workers and urban consumers, although the short-term cost to the rice sector in terms of reduced incomes and labor displacement may be quite substantial. However, when this is combined with public investment in productivity-enhancing support services (particularly R&D and irrigation), rice trade liberalization is a win-win proposition.

Keywords: Philippine agriculture, productivity growth, self-sufficiency, rice, trade liberalization

**CORN IN THE PHILIPPINES: FEEDING THE
POPULATION BEYOND THE PRESENT**

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For the past several decades, white corn consumption has been limited to Southern Philippines, mostly in Cebu area. And yet white corn has a big potential to supplement the supply of food grain in the country. Nutritionally it is better than rice. Due to its "slow release" property, it is the staple of athletes and is recommended for diabetics. Corn, a C4 plant, is also more efficient photosynthetically than rice, a C3. Being an upland crop, it is basically rainfed and therefore does not need expensive irrigation facilities which is a must in rice. Corn could also be grown even in marginal environments but certainly could yield very high under optimum growing condition. The grain, however, has the stigma of being the food of the poor. With proper information campaign, however, and hopefully, subsequent acceptance, white corn has a big potential to supply the staple grain need of the Filipino populace expected to be 200 million in year 2050. With our farmed area not expected to increase and the foreseen water crisis, corn has a big role to play in our national economy and life.

Keywords: corn, consumption, slow release, rainfed, information campaign

**SUSTAINABLE MARINE FISHERIES PRODUCTION
IN THE PHILIPPINES**

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The fisheries sector plays an important role to the economy of the country. In the coastal and marine waters as well as aquaculture, it provides substantial benefits to the Filipino nation, not only on food and essential nutrients but also substantive employment and sustenance, and valuable foreign exchange for the country's developing economy. The total fisheries production is contributed by the three (3) sectors, namely; the aquaculture sector, municipal sector and commercial sector. In 2001, the marine landings were about 66% or 1,946,074 M.T. of the total fisheries production. Fishermen (small-scale and commercial) use various types of gears, with heavy concentration in inshore/municipal waters where production is highest. Despite the stable figures of catch and exports of the fisheries, this sector faces serious challenges in the management of fisheries. Overexploitation of coastal resources and other factors have been reported in various documents and forums, and the problem still continues. As a contribution to sustainable marine fisheries production, this paper discusses an overview of the fisheries resources, contribution of fisheries by sector, the major problems of the industry, sustainable fishing technologies, and fisheries management approaches and key recommendations. Sustaining the country's fisheries and coastal resources requires urgent and concerted action by responsible authorities and the wider participation of stakeholders in all levels. In this context, successful interventions must be required for the effective implementation of a wide range of measures as well as shifts in management perspectives.

Keywords: fisheries, sustainable, fishing technologies, management, production

**TOWARDS SUSTAINABLE AQUACULTURE
IN THE PHILIPPINES**

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The Philippines has one of the highest per capita fish consumption in the world. However, in recent years the national total fish production could not meet this per capita requirement. There are three fishery resources, namely commercial and municipal fisheries and aquaculture, but only aquaculture offers the potential to fill the gap between increasing demand and supply. Further growth and development of aquaculture is faced with problems which can jeopardize its sustainability. Sustainable aquaculture requires that these key constraints are properly addressed. It is only then that the potential for growth and sustainability of Philippine aquaculture can be realized.

Keywords: aquaculture, Philippines, food security, environment

BIOLOGICAL SCIENCES

MECHANICAL, CHEMICAL AND SURGICAL METHODS OF CONTRACEPTION

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There are several methods of contraception. They may be classified as natural or artificial, temporary or permanent. The natural and temporary methods are the Cervical Mucus, Calendar Rhythm, Basal Body Temperature (BBT), Sympto-Thermal and the Lactational Amenorrhea (LAM). All these are considered periodic abstinence except the Lactational Amenorrhea Method (LAM). The artificial and temporary methods are the Barriers – both mechanical (condom and diaphragm) and chemical (spermicides), Hormonal (pills, injectables and implants) and the Intrauterine Contraceptive Devices (IUCD). The permanent methods are surgical sterilization procedures such as bilateral tubal ligation for women and vasectomy for men. This paper focuses its discussion on the Mechanical, Chemical and Surgical methods, and the Intrauterine Contraceptive Devices.

Keywords: methods of contraception, mechanical, surgical, chemical

**HORMONAL CONTRACEPTION: AN APPROACH TO THE
DEMOGRAPHIC CRISIS IN THE PHILIPPINES**

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This paper aimed (1) to analyze the acceptance of hormonal contraceptives and the performance of women-users in the Philippines; (2) to discuss the development of the hormonal contraceptives; (3) to discuss recent evidence about health benefits and risks and issues in oral contraceptive use; (4) to discuss new benefits and new drugs; (5) to discuss hormonal contraceptive for the male. The hormonal contraceptive methods in the form of oral contraceptive pill and injectables enjoy the first choice of Filipino women who use contraceptive methods. It was found that discontinuation rates are due to side-effects and health concerns. The method failure for the hormonal contraceptive methods is 5.4 % on the first year. The prospect of immediate future use is higher than the other methods.

Keywords: hormonal contraceptives, injectables, reproductive control methods

**SEX PRESELECTION IN ANIMALS: CURRENT
METHODS AND APPLICATIONS**

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Advancement in sexing technologies when used with other animal reproductive technologies presents opportunities to boost food production from animal sources and thus increases the availability of animal protein in the Filipino diet. The ability to predetermine the sex of offspring before and after

fertilization of the ovum would allow farmers to raise animals of the desired sex based on their breeding needs and market demands. This paper reviews current sperm sexing technologies such as flow cytometry or cell sorting, H-Y antigen detection, and detection of sex-specific proteins on the sperm surface as well as embryo sexing technologies such as chromosome analysis, polymerase chain reaction and other methods. Moreover, it discusses how sexing technologies can further enhance other reproductive technologies namely artificial insemination, embryo transfer, in vitro fertilization, embryo splitting and cryopreservation, and the potential applications of these technologies in animal production.

Keywords: DNA, embryo, flow-cytometry, sex chromosomes, sperm

CHEMICAL, MATHEMATICAL AND PHYSICAL SCIENCES

DEMOGRAPHICS AND EDUCATION

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From data obtained during the work on Division Elementary Development Plans (DEDP) for the 20 poorest provinces in the Third Elementary Education Project (TEEP) and from more recent data of the Department of Education, we would like to explore the challenges posed by demographics on providing elementary education to all Filipino children. The data show us:

1. The pressure on number of teachers, classrooms and budget
2. High dropout rates in the poorest provinces (about 20% in the first two grades)
3. Health problems especially lack of water and toilets}
4. Problems of distribution
5. Diversity of the system

For example, too many students and not enough classrooms in urban areas

and, on the other hand, classrooms without students in some rural areas because there are not enough students to meet minimum requirements.

Some conclusions are that the size, diversity and complexity of the problems do not allow for centralized solutions. It is important to seek solutions on the division or district level.

Keywords: demographics, elementary education, diversity, DEDP, TEEP

WATER SUPPLY IN THE PHILIPPINES: CEBU AS OBJECT OF A CASE STUDY

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This paper intends to show that the understanding of nature's water supply system is essential for a sound management of a water distribution system. The focus of the paper is Cebu City with its direct surrounding, because it is a contained supply and consumption system that has some historical data.

From 1911 until World War II Cebu relied on surface water (Buhisan dam) and groundwater (Jagobiao spring) for its distribution system. When the cleanup of the war damage reached Cebu, deep wells were added to the system. The inspiration of the Buhisan dam produced two feasibility studies with plans for two high dams. The growing demand has been followed by a further exploitation of ground water resources by government and private entities. The progressive sea water intrusion proves that the narrow coastal aquifer is under stress.

Over-extraction of ground water from the coastal aquifer does not really lower the water table, because the sea resupplies without limits. The problem is that 1% seawater mixed with 99% fresh water establishes 250 ppm Cl⁻, which is the upper limit acceptable according to WHO guidelines. Two percent seawater produces 500 ppm Cl⁻, a concentration which the local population does not accept. The sad irony is that Cebu talks about sufficient surface water in its own backyard while it acts to permanently destroy the ground water source that can supply one third of its needs.

The paper consists of 3 parts. First a description is given of the period 1910 to 1974. During this period a correct and sufficient first step was not followed by the necessary in-depth study. The second part covers the period from 1974 until the present during which substantial foreign input analyzed the local problem and pointed out the solution. During this period much of the science of water (hydrology) was absorbed through local hard labor to the point that "water engineers" can be trained locally. The third part points out what has to be done today to ascertain that Cebu, City and island, will have potable water for its people.

Keywords: water management, Cebu, ground water

ENGINEERING SCIENCES AND TECHNOLOGY

COMPETITIVENESS IN R&D

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In today's globalizing economies, both the developed and developing countries acknowledge the importance in investing on education and manpower resources to propel economic growth. The country's state of industrialization and economic growth entails a corollary demand for highly skilled manpower including scientists and researchers to bring the desired progress.

In line with the need to develop human resource in science and technology, the Department of Science and Technology (DOST), particularly through the Philippine Council for Industry and energy Research and Development (PCIERD), has been stimulating and supporting research activities in identified priority areas. Linkages among the academe, industry and government agencies have been strengthened to effectively carry out programs and projects.

Keywords: competitiveness, industrialization, S&T programs

COMPETITIVENESS IN EDUCATION

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The information technologies (IT), globalization, and the movement towards knowledge-based economies are the major forces now pushing and pulling at the Philippine educational system. Amidst such an environment, Philippine engineering and technology schools must directly compete for students, faculty, research funding and outsourced services against other schools in the world and even against foreign-owned schools on Philippine soil. At the same time the schools must support the efforts to make the Philippine economy globally competitive not only by supplying properly-educated human resources but also by supplying new knowledge and applying such knowledge successfully. The competitiveness of Philippine technological education ultimately lies in the volume and the quality of its intellectual capital as reflected in its curricula, faculty qualifications, scholarly works, R & D outputs and its technical extension services to the community and industry. While it may be argued that the academic degree programs in leading schools are at par with those of other countries, it would be difficult, if not impossible, to make a case for research capabilities. Philippine schools have a lot of catching up to do. The generally weak economy constrains capability-building. Students and their families cannot afford to pay the level of tuition, nor can government afford to allocate the resources, necessary for the schools to attain world-class quality in instructions, not to mention research. The "economics" of paucity of resources is a bit involved because education can be a savior as much as it is a victim of the general economic condition. How may this vicious cycle be disrupted? Philippine technological schools can be a savior if they are able to smartly handle the opportunity presented by the country's comparative advantages, in IT-related fields, for example. But any attempts at improving competitiveness must reckon with the predominantly privately-owned nature of Philippine education. The government, for its part, has recently relaxed the regulatory environment. It is entirely up to the schools to squander or make good use of the newfound freedom.

Keywords: globalization, knowledge-based economics, competitiveness, Philippine education, technological education

**GLOBAL COMPETITIVENESS IN ENGINEERING
AND TECHNOLOGY PRACTICE**

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The paper first points out the many aspects of global competitiveness, then identifies the limit of discussion to global competitiveness in Engineering and Technology Practice. The various stakeholders are identified. Indicators of global competitiveness are identified and discussed, distinguishing between those applicable to individual Filipinos and to Filipino entities (companies/firms). Conclusions on the current competitiveness of Filipinos and Filipino entities are presented. The various issues affecting competitiveness are presented and analyzed. Finally, recommendations to improve or achieve global competitiveness are presented, including specific detailed course of actions and identifying the implementing agencies or organizations.

Keywords: global competitiveness, engineering, and technology practice

HEALTH SCIENCES

**THE NATIONAL HEALTH INSURANCE PROGRAM
IN THE FACE OF THE DEMOGRAPHIC CRISIS**

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The population of the Philippines is growing at an annual rate of 2.36 percent or an additional 1.5 million Filipinos everyday. If the growth rate remains at such level, the population would double in 29 years. The population structure is triangular suggesting a high young age dependency. Due to population

momentum, the country is expected to have a young population in the next three decades.

Actual fertility is one and half births more than replacement fertility and one birth more than desired fertility, suggesting unmet need for family planning. Unwanted fertility remains high due to inadequate access to FP supplies and services and as a result of the devolution of responsibility for services to the local government units.

The country is one of the developing countries that is expected to make the demographic transition between 2015 and 2025. The population will be characterized by a peak ration of workers to dependent population. Past mortality and fertility gains coupled with rising life expectancy and improvement in the health situation will cause an irreversible and inevitable graying revolution, the increase in the elderly population. The Philippines has to be ready for this looming crisis. The National Health Insurance Program is a potential system that can help meet this challenge with its feature of universality, with an increasing membership base, improved benefits provision, and expanding administrative infrastructure. It will soon have an effective and efficient information and communication system that will complement the reengineered business process, and most important of all, the ability to leverage its robust financial position for better delivery of quality health care by both public and private health care providers.

Universal health insurance coverage can help bring back the glorious days of an integrated health care system that the Philippine populace truly deserves. When the country is confronted with the demographic dividend, PhilHealth can help the country get rich before it gets old.

Keywords: insurance, demographic crisis

SOCIAL SCIENCES

**USING THE CAPABILITIES APPROACH TO
ANALYZE ACCESS TO INFORMATION AND
COMMUNICATION TECHNOLOGIES (ICTS) BY THE POOR¹**

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This paper applies Amartya Sen's "capabilities approach" to the access and use of ICTs. An important issue raised by the Capabilities Approach is that while access to a basic good, in this case information and communication technologies (ICTs), is a prerequisite to its usage, individual differences, capabilities and choice also play a role on the use, value and application of these goods. As such, the paper investigates the extent to which people have access to ICTs, the characteristics of people who make use of it, and how and for what ends they are utilized. Based on household surveys conducted in urban and rural barangays in Puerto Princesa City, it attempts to analyze access beyond the traditional method of considering teledensities and number of Internet service providers (ISPs), but instead focuses on key demographic traits in a community and how these influence their capabilities, functioning and freedoms with respect to ICT use.

Keywords: ICT, information, communication technologies, teledensities, capabilities approach

**PROBING THE DECISIONS BEHIND INDUCED
ABORTION IN THE PHILIPPINES**

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Often a subject of emotional debates that unleash strong and opposing views, abortion, in particular, induced abortions, remain a health concern deserving public health policy action. While there have been several studies on the causes and consequences of abortion, data on a scale that would generate reliable estimates of the prevalence of abortion for the whole country remains scarce. This paper uses a mix of available data on abortion in the Philippines and compliments the profiles of women who have had abortions with life stories to give the abortion statistics the needed human face. The compelling circumstances surrounding the hard decisions to terminate unwanted pregnancies show that Family Planning Program interventions on preventing unwanted pregnancies have a potential of reducing induced abortions. Given the combination of the secrecy of abortion decisions and procedures and the limited capacity of our health system to provide post-abortion care and treatment due to limited resources to meet competing health needs, it is crucial that imperfections in the use of the more effective methods of family planning are addressed to prevent unwanted pregnancies, an event in women's lives that push them into preventable complications and ill health effects of induced abortions and at worst, maternal deaths.

Keywords: induced abortion, health policy, unwanted pregnancies, post-abortion care

**DEMAND FOR HOUSING IN METROPOLITAN CITIES
OF THE PHILIPPINES**

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This paper adds to the existing literature on the housing demand behavior of households in the Philippines. Unlike previous studies on housing demand, the paper compares major metropolitan cities – Metro Manila, Metro Cebu and Metro Davao and uses a panel set of households instead of single year household data in the analysis. The results show that housing demand for owners or amortizing owners is income elastic for both poor and non-poor households in the key metropolises of the country. Even chronically poor households are willing to spend more of income on improvements in tenure and dwelling conditions. The rate of improvements, however, is also affected by location. Tenure change and improvements in dwelling in Metro Cebu and Davao City are modest compared to Metro Manila. In the case of renter households, demand for housing is income inelastic. Renter-households have less incentive to spend a higher proportion of additional income on housing. These findings suggest that shelter design projects of government should adopt a more realistic and variable basis of households housing expenditure. It also suggests the need to develop the low cost rental housing market where the bulk of subsidies should be channeled instead of programs on homeownership. This will not only provide efficient targeting but lessen housing in illegal settlements.

Keywords: housing demand, housing consumption, urban housing

WOMEN, MIGRATION AND REINTEGRATION

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The paper is about the action research on the Social Cost of Migration and Possibilities for Reintegration done by Atikha-Balikabayani. The research was conducted among migrant women in Hong Kong and Italy and their families in San Pablo City, Laguna and Mabini, Batangas.

The objectives of the action research were: 1) assess the impact of migration on migrants, their families and communities and 2) mobilize the various stakeholders to work together and craft a comprehensive OFW Reintegration Program.

The research showed that despite years of hard work, majority of the migrant women do not have substantial savings and have no immediate plans of returning home for good. It was also noted that husbands of migrant women are unable to take on the "feminine responsibility" of managing the household. Migrant returnees had difficulty in adjusting to the estranged relations with their children and husbands and the lack of economic opportunities in the Philippines.

To enable the OFW to rejoin their families and maximize the gains from migration, the various stakeholders must provide community based assistance. The psychosocial and economic preparation of the OFWs for their eventual return must be addressed.

keywords: migration, reintegration, social cost, feminine responsibility, migrant women

POSTER SESSION

AGRICULTURAL SCIENCES

ASD No. 1

GROWTH PERFORMANCE AND YIELD OF SELECTED STRAIN (GIFT) NILE TILAPIA (*OREOCHROMIS NILOTICUS* L.) IN LOWLAND IRRIGATED RICEFIELDS INTEGRATED WITH AZOLLA AND MALLARD DUCK

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Nile tilapia (*Oreochromis niloticus* L.) is commonly cultured in the Philippines and has high consumer acceptability. Different Nile tilapia strains exist in the country and the genetically improved farmed tilapia (GIFT) selected strain is widely cultured at present. The pond performance of this strain is well documented but there is a lack of information on its performance in a well-managed lowland irrigated integrated rice-fish culture. Hence, our study evaluated the growth performance and yield of the selected GIFT strain in integrated rice-fish culture with (+) and without (-) herbicide and molluscicide (HM), azolla and duck.

Five treatments: conventional rice-fish culture (RFHM), rice-fish (RF), rice-fish-azolla (RFA), rice-fish-duck (RFD) and rice-fish-azolla-duck (RFAD) were conducted in fifteen 300-m² plots with fish refuge in three cropping seasons. All treatments except RFHM were not applied with HM. GIFT strain Nile tilapia (density and weight: 10,000 fingerlings ha⁻¹ and 13-17 g, respectively) was cultured for 83 days. Mallard ducks (400 ha⁻¹) were housed over the refuge while azolla served as in situ food for Nile tilapia. Treatment effects: HM, azolla and duck and the interaction of azolla and duck on growth and yield were analyzed.

After 83 days, the specific growth rate (SGR) of Nile tilapia in the treatment -HM was 33% higher than in the treatments +HM ($P < 0.0001$) due to the strong effect of azolla and ducks based on three trials. Mean SGR from

treatments +azolla was 21% higher than -azolla whereas treatments +ducks was 83% higher than -ducks ($P < 0.0001$). Nile tilapia yield in the conventional rice-fish culture was 195 kg ha⁻¹ and increased by 33% +azolla; 1.9 times +ducks; and 2.2 times +azolla and duck ($P < 0.0001$). These findings demonstrated that growth and yield of GIFT strain Nile tilapia in the conventional rice-fish system can be significantly increased by integration with azolla and duck and without the use of herbicide and molluscicide.

Keywords: Nile tilapia, rice-cum-fish culture, azolla, mallard duck, natural resources management

ASD No. 2

**GEOGRAPHICAL DISTRIBUTION AND FREQUENCY
OF ALBUMIN, TRANSFERRIN, AND α -2 MICROGLOBULIN
ALLELES AMONG ANGLO NUBIAN, NATIVE
GOATS AND THEIR F₁ CROSSES**

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This paper reports the geographical distribution and the frequency of albumin (*Alb*), transferrin (*Tf*), and alpha 2 - microglobulin (*S2 α*) alleles among the Anglo Nubian, Native goats and their F₁ crosses in Luzon Island, Philippines. The blood serum protein polymorphisms were obtained from heparinized blood samples of 718 goats from 32 farms in 18 provinces using the using vertical polyacrylamide gel electrophoresis (PAGE).

The frequency of *Alb*-A and *Tf*-A alleles was similar in the Anglo Nubian, Native goats and F₁ crosses, ranging from 62 to 66%. The *S2 α* -A alleles however, were highest in the Anglo Nubian (72%) than the Native (67%) and F₁ crosses (62%). Native goats particularly adapted to the local subsistence level of management and environmental conditions and which have the largest number and highest density of goat populations in the country, represent a unique reservoir of genetic resources for their continuous genetic improvement. A high degree of similarity is found among Native goats in farms/

provinces along routes accessible to large and popular public auction markets such as in Padre Garcia, Batangas and Urdaneta City, Pangasinan. Our data revealed a pattern of introgression of imported Anglo Nubian alleles in local programs to upgrade the Native goats in the countryside, probably originating from Department of Agriculture Regional Field Units (DA-RFUs) and/or institutional herds of major state colleges and universities. Analysis of the geographical distribution of blood protein alleles provided a clear picture and importance of Anglo Nubian introgression in strategically located goat breeding/dispersal centers to rapidly create and expand hybrid zones in an outward direction. Marketing routes, phenotypic preferences by goat farmers, adaptation to specific habitats and to production and management conditions are the main factors explaining the current distribution of various blood protein alleles of goats in the island of Luzon.

Keywords: Albumin, alpha 2-microglobulin, Anglo Nubian, transferrin, F₁ cross, Native goats

ASD No. 3

**SUBSEQUENT EFFECTS OF INTRARUMINAL
SOLUBLE GLASS BOLUS ON PLASMA CALCIUM,
PHOSPHORUS AND MAGNESIUM CONTENT OF GRAZING
DOES UNDER BACKYARD CONDITIONS IN SELECTED
AREAS IN NUEVA ECIJA, PHILIPPINES**

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The effects of intra-ruminal administration of soluble glass bolus (SGB) containing selenium (Se), copper (Cu) and cobalt (Co) on the blood mineral content was determined among 60 grazing upgraded goats raised under backyard condition. The animals were fed only with available feed resources within the paddy field and mango orchard. The subsequent effects of SGB supplementation on plasma Ca, P, and Mg were determined for 12 months. Plasma mineral concentrations were determined using Inductively Coupled Plasma

Spectrophotometer (ICPS) after wet ashing with nitric acid.

SGB administration did not affect the Ca, P, and Mg contents in the blood. Except for the marked increase in the plasma Mg level of animals in the control group during the early stage of the trial, there was no clear indication of monthly variations in plasma mineral concentrations among animals with or without the bolus supplementation. Results also revealed that plasma Ca concentrations of the animals appeared to be more stable than P and Mg. On the other hand, plasma Ca concentration showed seasonal variation. The plasma P concentrations for both groups were significantly lower during the rainy season than during the dry season.

The normal plasma Ca, P, and Mg concentration suggests that the available feed resources under a typical rice-based and mango orchard farming conditions could provide adequate amount of these essential elements to support gestation and lactation. Hence, Ca, P or Mg imbalance is unlikely to happen even without SGB supplementation.

Keywords: soluble glass bolus, plasma minerals, goals

ASD No. 4

WATER QUALITY ANALYSIS AND UTILIZATION OF SMALL FARM RESERVOIRS (SFRs) FOR AQUACULTURE IN REGION III

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This research project was conducted to improve aquaculture production in Region III through water quality analysis and utilization of small farm reservoirs (SFRs) for sustainable community development.

A total of 23 farmer-cooperators (FCs) from various municipalities of the region were involved in the study after seminar-orientation. Two different culture systems, extensive (GMT) and semi-intensive (GMT, GIFT and FAC Selected Lines) randomly assigned were tested using the genetically improved strains of *Oreochromis niloticus* (Nile tilapia). Growth monitoring is done every month and

water analysis is bi-monthly or weekly if necessary while pesticide residue and metal detection were performed prior to each culture system.

In phase I, results showed that most SFRs have water pH within the ideal range for fish culture while DO and BOD were at tolerable levels. Phosphate values are less than 200 ppm while two sites gave higher than the allowable values but corrective measures were done at once. Abucay, Bataan and Talugtug, Nueva Ecija showed positive results for Organophosphorous using Rapid Field Kit (RFK) however, Gas Chromatographic analysis confirmed the presence of the same for Talugtug, Nueva Ecija. Step-wise regression model identified percentage recovery related to phosphate and sodium as the main contributors, but phosphate as the lone predictor of average body weight (ABW) and average growth rate (AGR).

Phase II revealed that total ammonia level remained below the safe level while heavy metals, Cu and Hg were minimal and within the tolerable limit set by USFDA. No detectable residue was detected for both analyses for pesticide residue. Copper, predator, fish strain, mercury, BOD, hardness, DO and potassium ion were predictors of percentage recovery using the same regression model. Further, predator and strain as predictor of ABW while strain was the lone predictor variable of AGR.

Both type of culture systems exhibited high percentage recovery though lower fish density than the carrying capacity resulted to better growth rate and fish average body weight (ABW). Combination of rain, deep well and natural spring as sources of water is beneficial to tilapia and water exchange of at least 2-3 times every culture period yielded better harvest. Generally, SFRs water in the region is classified hard and relatively safe, an indication of its suitability for fish culture.

Keywords: water quality, SFRs, reservoirs, small farm reservoirs, aquaculture

ASD No. 5

**CONTROLLED-RELEASE FERTILIZER (CRF) FOR
LAHAR AFFECTED AND COARSE-TEXTURED
AGRICULTURAL SOILS**

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Pyroclastic deposits emitted by Mt. Pinatubo in 1991 are currently being utilized as a medium for crop production, however, since lahar deposits are considered marginal due to poor physico-chemical properties, low yield, high input requirement and high nutrient losses became a consequence. Production and testing therefore of a new fertilizer material suitable to overcome nutrient losses resulting from the coarse textured characteristics of the deposits were undertaken. Specifically, it aimed to determine the physical and chemical properties of coarse textured soil and lahar deposits, determine the release pattern and percent release of fertilizer nutrients from controlled release fertilizers (CRF), measure crops response to controlled release fertilizer and evaluate/identify advantages and constraints to CRF usage in both coarse textured soil and lahar deposit.

The depth of lahar deposition ranged from 60 cm to more than 150 cm. Soil texture is mostly sandy clay loam with low water holding capacities and low available water (3.53 – 22.7%). Soil temperature can also go as high as 65 to 70°C during summer months. The deposits are strongly (pH 4.35) to slightly acidic (pH 6.7) with very low total N, adequate P and highly variable amount of exchangeable K ranging from deficient to adequate. Sulfur content is high which poses H₂S toxicity particularly to more sensitive crops such as rice and mungbean. Since lahar soil texture is mostly sandy clay loam, rapid percolation of water and leaching of nutrient particularly nitrogen and potassium are highly possible.

Using soil medium, the formulation released all its N content under submerged condition at 45 DAI. Not all the N in complete fertilizer was released

in lahar deposit under submerged condition even beyond 85 DAI.

Phosphorus and potassium remaining in coated complete fertilizer though did not reach zero level, was much lower in submerged compared to upland condition at 65 DAI. A slower rate of K₂O release was observed in mineral media compared to pure water.

In the case of muriate of potash, a linear behavior of K released exist both under upland and submerged condition.

Using CRF in onion, NPK application was reduced to only half of the rate using conventional fertilizers (COF). Full substitution by CRF outyield plants applied with either full COF, partially substituted COF or those applied in combination with organic fertilizer.

Addition of organic fertilizer at the rate of 4.5 tons per hectare together with full COF did not show any positive effect on the growth and yield of onion as compared to those applied with full CRF during the first trial.

Generally, onion applied with 4.5 tons/ha + CRF had higher N and K uptake than the COF treated plants. In the same manner tomato had higher N uptake when fertilized with CRF. The rest of the fertilized plants had comparable NPK uptake.

Keywords: lahar, controlled-release fertilizer, organic fertilizer, organic fertilizer

ASD No. 6

FLY ASH FOR AMELIORATING ACID SOILS AND INCREASING CROP YIELD

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Fly ash is a residue of coal burning in thermal power stations. This waste product has been found to be useful in agriculture in modification of soil bulk density, improvement of water holding capacity of soil, optimization of soil pH, improvement in crop yield, as source of micronutrient supplement to soil and creation of conducive condition for better plant growth (Fly Ash Mission,

1998).

The potential of utilizing fly ash to ameliorate acid soils was evaluated in San Ildefonso, Bulacan. Fly ash was compared with other soil ameliorants such as rice hull ash, compost and spent absorbent. Pot and field experiments were conducted to compare the ameliorating property of the different amendments.

In pot experiment, result showed that fly ash is as effective as the other amendments as shown by increased plant survival and yield particularly at an application rate of 60 t/ha. Soil pH was increased by 2 to 2.5 pH units from 4.8 to 6 and 6.5.

In field experiments, application of fly ash at 10t/ha increased the yield of pak-choi by 245% or from 5.25 to 18.3 t/ha. The increase in yield was attributed to greater availability of nutrients brought about by an increase in pH from 4.8 to 5.8.

Lower yields of pak-choi were produced from application of rice hull ash (15.91 t/ha) and compost (11.98 t/ha).

Keywords: fly ash, soil amelioration, pak-choi

ASD No. 7a

**EFFECTS OF MULCHING MATERIALS AS A COMPONENT
OF AN INTEGRATED PEST MANAGEMENT (IPM)
STRATEGY FOR THE CONTROL OF TWO MAJOR
INSECT PESTS OF OKRA (*Abelmoschus esculentus* Linn.)**

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In growing high value vegetable crops, farmers control insect pests by using a very potent systemic insecticide sprayed in large dosages. This threatens both the farmer and his environment. Thus, this study aims to develop an economical and environmentally sound IPM strategy to control two major insect pests of okra, namely: the cotton leafhoppers (*Amrasca biguttula* Ishida) and the melon aphids (*Aphis gossypii* Glover).

Silver plastic mulch gave the lowest mean insect population, lowest mean damage ratings, tallest plants, and high yield consequently obtaining the highest gross income. However, rice straw mulch gave the highest return on investment due to its low cost. If silver plastic mulch were to be made durable enough that it may be re-used then its high investment cost would inevitably be outweighed by the benefits it offers. Another possibility of compensating its high cost would be to use silver plastic mulch where ratooning is involved. Based on the results of the study however, rice straw mulch would be the most recommended mulch.

Since a minimum dose (20 ml) of the insecticide showed no significant differences with the higher dosages used, the combination of rice straw mulch and Fipronil at 1000 a.i. per hectare is recommended.

Keywords: mulching, integrated pest management, IPM, okra

ASD No. 7b

**MECHANIZING THE PROCESSES IN DEVELOPING
BIOBASED FARM INPUTS**

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Rice and rice-based farming system in the Philippines are becoming cost-intensive due to increasing cost off-farm and non-renewable inputs. Indigenous materials in the farm and household that are sustainable should be recovered from a biobased system of developing ecological farm inputs.

PhilRice has developed a system in converting (physical, thermal & biological) indigenous materials from farm biomass and biodegradable household wastes. Physically, a drum-type manually operated chopper/mixer-cum composter was developed for a cluster of households. Also a convertible thresher-shredder/chopper was improved by sharpening both sides of the threshing teeth and disconnecting the blower. Result showed the acceptable chopping length of 9.6, 7.0, 6.2 cm of rice straw, kangkong and ipil-ipil branches, respectively.

Thermally, rice hull is carbonized by simple open-type carbonizer using a perforated oil drum with chimney, producing a black colored substance with uniform particle size. Ten bags of rice hull make 6-7 bags carbonized rice hull (CRH) in four hours.

Biologically, a biogas digester was designed to produce methane or alcogas. The system uses a 150-L plastic container and commercial gas control mechanism. The process anaerobically ferments a mixture of farm biomass and kitchen garbage to produce gas for cooking.

Keywords: indigenous materials, physical, biological and thermal conversions

ASD No. 8

**EFFECT OF WEEDING LEVELS ON THE INCIDENCE
OF *Amrasca biguttula* (ISHIDA) ON EGGPLANT**

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This study was conducted to determine the effect of three weeding levels on the incidence of the eggplant leafhopper, *Amrasca biguttula* (Ishida) on resistant and susceptible eggplant varieties. Insect and natural enemy populations were counted and weeds were sampled randomly per plot from 30-90 days after transplanting (DAT).

The numbers of insects and natural enemies (leafhoppers, ants, aphids, spiders, flea beetles, whiteflies) were counted and the number of individuals per weed species were also counted, ranked and classified as broad or slender.

Results showed that fewer insect pests and more natural enemies occurred in the regulated and unweeded treatments. Although it was in the unweeded treatments where fewest insects occurred, weed species diversity was higher in the regulated weeding treatments.

The available data support:

- a) that regulated weeding is compatible with the use of resistant varieties for eggplant pest management.
- b) that the presence of weeds affords diversity in the eggplant farm and renders conditions favorable for natural enemies but not for pests like leafhoppers; and
- c) that regulated weeding maybe more economical as a practice than total weeding, realizing the costs farmers incur for weeding in clean culture.

Keywords: eggplant, *Solanum melongena* L., leafhopper, *Amrasca biguttula* (Ishida), resource concentration, natural enemy hypothesis

ASD No. 9

**EVALUATION OF THE PROCESSING QUALITIES OF
SQUASH (*Cucurbita moschata* Duch. ex Lamk)**

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Recent researches on breeding works should not only consider improved agronomic, horticultural qualities and resistance to insect pests and diseases but also its ultimate utilization, otherwise, increasing yield without corresponding increase in utilization will just result to wastage. At present, squash products are prepared from just any available varieties from the market resulting to inconsistent sensory characteristics and end products. Development of squash products will thus require a comprehensive study of the most suitable varieties to ensure that uniform end products will result from its use.

Local collections from major squash-growing areas in the country and commercial varieties were subjected to sensory evaluation, physico-chemical and nutritional analyses and tested for their suitability in the development of squash products, namely, frozen squash slices, puree, and flour at the IFST Sensory Evaluation Laboratory. For squash slices, color, texture and cohesiveness of mass are important parameters for consideration. Among the samples evaluated, San Marcelino-1, Ormoc-3, Tinuning-1, and Asingan-1 were the promising materials that can be used in the study of frozen slices. Consistency is very important in preparation of puree. San Marcelino-1 was the most consistent and the process of pureeing was relatively easy possessing homogenous appearance. Botolan-1, San Marcelino-1 and Ormoc-1,2 had the highest starch content of 21.17, 20.66 and 20.16%, respectively. San Marcelino-1 had the highest amylose content while highest total sugar content was obtained from Tinuning-1 and Botolan-1. The moisture content of samples ranged from 75.19-96.31% wet basis while the total soluble solids ranged from 3.90-14.05°Brix. Flours were prepared from samples and San Marcelino-1 gave the highest drying yield. The samples differed in particle sizes. Those particles that passed thru sieve mesh no. 80 were considered acceptable as composite flour for noodle making. Preliminary evaluation of Tinuning-1 flour (>80) in combination with wheat flour showed promising results for noodle making.

Keywords: *Cucurbita moschata* (Duch. ex Lamk), sensory evaluation, physicochemical analyses, processing

ASD No. 10

***Bacillus Cereus*: A NEW BIOLOGICAL N₂-FIXING
ORGANISM AND ITS UTILITY AS BIOFERTILIZER
IN COTTON PRODUCTION¹**

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The bacterial isolate, *Bacillus cereus*, from the rhizosphere of the grass weed *Cenchrus echinatus*, is a non-symbiotic N₂ fixer. This is the first time that this species is reported of its capability to fix atmospheric nitrogen. Used as cottonseed inoculant, it significantly affected the germination percentage, seedling vigor index and fresh seedling weights of the cotton variety, UPLC-2.

Both screenhouse and field conditions showed that ½ RR combined with N-BOF by *B. cereus* gave comparable agronomic performance and seedcotton yield of UPLC-2 with the recommended rate (RR) treatment. Cotton plants of the said treatments grew luxuriantly, and distinctly developed the minor fruit-bearing branches, i.e. the Ax Sy and Se Ms Sy, in addition to the mainstem sympodia.

The quantity of inorganic fertilizer recommended for cotton that can be substituted by the nitrogen bio-enriched organic fertilizer (N-BOF) by *B. cereus* was within the range of 37.5 to 65 kg N/ha.

Keywords: *Bacillus cereus*, Biological Nitrogen Fixers (BNF), *Cenchrus echinatus*, biofertilizer, UPLC-2, sympodial branches, seedcotton, plant maps

ASD No. 11

**BIOLOGICAL AND CULTURAL MANAGEMENT OF SOME
MAJOR INSECT PESTS OF EGGPLANT**

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The effectiveness of several component control tactics such as the use of biological control agents (*Trichogramma chilonis*, *Orius tantillus* and microbial isolates: *Metarhizium anisopliae* and *Beauveria bassiana*), cultural control (mechanical removal of infested shoots and fruits and planting of culinary herbs) were evaluated against major insect pests of eggplant at the Central Experiment Station, UPLB. Integration of component control approaches including the application of selective insecticide (thiamethoxan) was also evaluated and compared with farmers' practice in Asingan, Pangasinan and in Balete, Batangas.

An isolate of *Beauveria bassiana* (Bb-1) gave 87% leafhopper mortality in 6 days while *Metarhizium anisopliae* (Fmll) gave 45% mortality in 9 days.

Okra was found to be a preferred host of leafhopper than eggplant. Therefore, okra was used as trap crop where application of thiamethoxan was directed (drenched at the rate of 100 ml per hill) at one week after sowing instead of spraying eggplant to control leafhoppers. This control method was found equally effective as the farmer's practice in controlling leafhoppers. Likewise, the three weekly field releases of *Orius* at the rate of one nymph (immature predator about to become adult) starting at three weeks after transplanting (WAT) was comparable with farmers practice (weekly calendar spraying of recommended insecticide, deltamethrin) against *Thrips palmi*.

Basil intercropped with eggplant (approximately 20% of the total plant populations) appeared to have potential in reducing the infestation of aphids and leafhoppers shown by the lower density of the pests and higher yield.

The results of the integration of selective insecticide, thiamethoxan (drenched 1 WAT at 100 ml per hill), weekly releases of *Trichogramma chilonis* (40,000 parasitoids/ha/release starting 45 DAT) and sanitation (mechanical removal of damaged shoots and fruits) suggest that the approach can be an alternative management tactic against leafhoppers and fruit and shoot borers.

Keywords: *Trichogramma*, biological control agents, leafhopper, shoot of corn borer, culinary

ASD No. 12

**COST-EFFECTIVE MECHANIZATION FOR
CROP DIVERSIFICATION**

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Crop diversification improves productivity and profitability. Mechanizing this will help increase land and labor productivity. Since machines are cost-intensive investment, they should be used effectively and efficiently, combining several functions to reduce their fixed and variable costs. In this regard, mechanization inputs that can handle several crops simultaneously will help farmers in uplifting their living conditions.

A multi-purpose power unit and attachments that will perform subsequent farm operations such as precision seeding, weeding, and harvesting were developed and evaluated. First, the power unit consists of a handle-frame, transmission, and cage wheel-skid assemblies. The transmission system of the machine was a direct-coupled shaft mechanism from the prime mover (1:2.5 ratio of chain & sprocket, and 50:1 worm reduction gears) to reduce the engine speed of 1600 rpm to 15~34 rpm required for its respective attachments. Second, the 6-row precision seeder attachment has a 19-kg seeding rate fabricated from local and recycled materials that substantially reduced its initial cost. Third, the brush cutter-type rice harvester attachment acceptably laid the rice stalks in windrows. It had an effective swath (cutting width) of 1.8 meters (7 hills/row) with a field capacity of 400 m²/hr, and cutting height of 3.5 cm. Labor productivity was doubled. Fourth, the power weeder attachment consists of rotor blades adapted from a rototiller design. Test revealed its potential weeding performance. Further improvement and adaptation trials are being done.

Keywords: cost-effective mechanization, crop diversification, multi-purpose power unit, precision seeding, weeding, harvesting.

ASD No. 13

**THE EFFECT OF INTERCROPPING SWEET POTATO
AND POLE SITAO ON INSECT PEST AND
NATURAL ENEMY POPULATION**

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Pole sitao is an important legume in the Philippines. However, pole sitao is attacked by a number of insect pests causing damage from seedling to maturity resulting to tremendous reduction of yield. Weeds also cause yield loss by directly competing with crops or intensify the problems of diseases, insect and other pest by serving as host. On the otherhand, crop fields with dense weed cover and high diversity usually have more beneficial insects than do weed free field.

This study was conducted at the Central Experiment Station of the UP Los Baños from December 2001 to April 2002 to determine the effect of sweet potato intercrop on the pole sitao insect pest complex and the natural enemies.

There were four treatments namely: 1) control plot; 2) 2 sitao and 1 camote; 3) 3 sitao and 1 camote and 4) 4 sitao and 1 camote. A randomized complete block design was used. Each plot measures 5 x 6 square meters and these were replicated four times per treatment.

Insect pest and natural enemies were monitored on a weekly basis through the use of sweep net and actual count. Leafminers, leafhoppers, flea beetles, snout beetles and cutworms were the pest observed using sweep net. The order of decreasing density of pest are: control (30.6) > 2 sitao + 1 sweet potato (23.6) > 3 sitao + 1 camote (13.7) > 4 sitao + 1 camote (10.3).

The natural enemies observed were: ladybird beetles, Ichneuomonid wasp, spiders, praying manthis and myrid bug. The order of decreasing density of natural enemies were: 2 pole sitao + 1 camote (1.3) = 4 sitao + 1 camote (1.3) > Control (.90) > 3 sitao + 1 camote (.70).

The actual count of pest was done on aphids, leafhoppers, flea beetles and katydids. The order of decreasing density of pest were: control (465) > 3 sitao + 1 camote (367.5) > 4 sitao + 1 camote (269.8) > 2 sitao + 1 camote (254.3). The ladybird beetle and spiders count were: 3 sitao + 1 camote (3.0) > 4 sitao + 1 camote (2.75) > 2 sitao + 1 camote (1.0) > control (.25).

The results showed that pole sitao alone had high pest population count and low natural population count while those that have camote intercrop showed the reverse. The findings suggest that camote provides diversity and refugia for natural enemies which in turn reduce the pest population through predation and parasitism.

Keywords: pole sitao, sweet potato, intercrop, natural enemy, pest population, parasitism, predation

ASD No. 14

**OCCURRENCE OF *Spodoptera litura* (FABRICIUS) AND
Helicoverpa armigera (HUBNER) ON EGGPLANT AND
COMPARATIVE STUDY OF DAMAGE WITH THE
SHOOTFRUIT BORER, *Leucinodes orbonalis* GUENEE**

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The insect pests of eggplant (*Solanum melongena* Linn.) in the Philippines were first compiled from previous publications by Gabriel in 1969 and later updated it in 1997. He treated the pests of eggplant together with those of sweet pepper (*Capsicum frutescens* L.), pungent pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon lycopersicum* (L.) Karsten) under the lumped heading "Nightshade Family (Solanaceae). However, this compilation as far as eggplant and other solanaceous crops are concerned is incomplete.

In Gabriel's compendium, the common cutworm, *Spodoptera litura* (Fabricius) and the corn earworm or tomato fruitworm, *Helicoverpa armigera* (Hubner), are two of the lepidopterous pests listed under solanaceous crops. *S. litura* was listed as among those chewing on young branches and leaves while

H. armigera was listed as among those chewing insect on fruits, i.e. as the tomato fruitworm. On eggplant, the only insect listed on fruits is the shootfruit borer, *Leucinodes orbonalis* Guenee.

However, during our screening trials to evaluate the resistance of eggplant to *L. orbonalis* and leafhoppers for our DA-BAR and IPM-CRSP PhilRice projects, the common cutworm and the corn earworm were observed for the first time feeding on whorls (curled, immature leaves that form loose heads) and flower buds of eggplant. They were heavily feeding on these parts of the test plants. The field trials were conducted in Aliaga, Nueva Ecija and Asingan, Pangasinan. As far as known, there have been no reports on the occurrence of these two pests at several stages of eggplant growth and development. The occurrence of the two pests, although not surprising as they are polyphagous, are, therefore, new records for eggplant.

The nature of damage of *S. litura* and *H. armigera* was also compared with the most serious and the number one insect pest of eggplant, *L. orbonalis*. The damage caused by the former unfortunately was even more serious compared to that caused by the shootfruit borer.

Keywords: eggplant, *Solanum melongena* L., *Solanaceae*, common cutworm, *Spodoptera litura* (Fabricius), corn earworm, tomato fruitworm, *Helicoverpa armigera* (Hubner), shootfruit borer, *Leucinodes orbonalis* Guenee, pest resistance

ASD No. 15

EFFICIENT INDUCTION OF SOMATIC EMBRYOGENESIS AND MULTIPLE SHOOTS IN AVOCADO (*Persea americana* MILL.) AND EFFECTS OF GAMMA RAYS ON AVOCADO CULTURES

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Avocado is a popular and promising fruit crop but lacks formal breeding in the Philippines. The objective of the study is to improve plant regeneration systems (somatic embryogenesis and shoot organogenesis) in avocado to allow efficient generation of somaclonal variants and induction of mutants by gamma

ray irradiation. Immature zygotic embryos of five locally grown avocado genotypes were aseptically cultured in 'M14' medium (MS basal medium + 5.0 mg/L 2,4-D and 0.5 mg/L BA) and 'B₃P' medium (B5 major salts, MS minor salts and 0.1 mg/L picloram). Percentage callus formation was higher with the use of 'B₃P' medium (48.3 to 61.9 %) as compared with M14 medium (10.0 to 40.5 %). After the first subculture (SC1), embryogenic cultures were selected in genotypes 'Semil' and 'Mainit', and transferred every 3 to 4 weeks on three media namely, 1) 'B₃P'; 2) 'RA₃' (MS + 0.1 TDZ and 0.5 GA₃); 3) 'BA₂' (MS + 2.0 BA + 1.0 IBA) for induction of somatic embryogenesis. The radiosensitivities of embryogenic cultures of 'Mainit' and 'Semil' were determined by exposing embryogenic masses to increasing doses of gamma rays (0, 10, 20 and 30 Gy). Proliferation rates were enhanced at a dose of 10 Gy but exposures to 20 and 30 Gy resulted in approximately 50 and 30 % reduction in proliferation rates of 'Mainit' and 68 and 15 % reduction in proliferation rates of 'Semil', respectively, as compared with the control. Somatic embryos at cotyledonary stage from 8-month-old cultures (irradiated and non-irradiated control) are now placed on the maturation medium as part of continuing experimentation on the requirements for germination and plant regeneration. On the other hand, germinating seedlings from aseptically cultured zygotic embryos from mature fruits of 'San Felix' were exposed to gamma rays (0 to 50 Gy). The resulting shoot cultures are now being propagated in vitro by culturing nodal cuttings on B5 basal medium with 1.0 mg/L BA until M₁V₄ generation.

Keywords: irradiation, mutation, plant regeneration, somaclonal variation, tissue culture

ASD No. 16

**IN VITRO STUDY ON CALLUS INDUCTION AND
PLANT REGENERATION OF GRAMMATOPHYL
SCRIPTUM (ORCHIDACEAE)**

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Rizal Technological University's Conservation of Philippine Native Orchids Project involves the optimization of the most suitable medium for

mass propagation of Philippine orchid species, somatic embryogenesis and plant regeneration with the use of plant growth regulators. Somatic embryogenesis and further plant regeneration were observed using young leaves and node of *in vitro* cultured plantlets of *Grammatophyllum scriptum*, a native Philippine orchid. The explants were cultured in modified Vacin & Went (MVW) medium supplemented with different levels of auxin. The range of 2,4 - dichlorophenoxyacetic acid concentration is from 0 to 10 μ M. Callus formation was observed to occur in all auxin concentrations except in MVW with no auxin supplementation. Instead, root formation with the presence of root hairs was observed to occur. Calli formed in the different set-ups were all subcultured after three months in MVW without plant growth regulators. The most favorable results were observed. The callus most suitable for plant regeneration was obtained from MVW supplementation with 4 μ M 2,4-dichlorophenoxyacetic acid. In this set-up, most profuse and highest percentage of callus formation (67%) was observed. In addition, calli obtained were also suitable for plant regeneration.

Keywords: *Grammatophyllum scriptum*, *in vitro* culture, callus, somatic embryogenesis

ASD No. 17

**INDUCED CALLUS FORMATION OF HYBRID
Dendrobium cv. 'Clomen White' ANDTHERS BY
COLD PRETREATMENT AND 2,4-D SUPPLEMENTATION**

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The application of anther culture (AC) techniques in orchids has a great potential for revolutionizing orchid breeding technology and propagation. Anther culture of *Dendrobium* 'Clomen White' was investigated to identify its potentials in orchid breeding programs.

Anthers derived from three different bud size ranges namely: (01) 0.90 x 0.40 cm to 1.40 x 0.70 cm; (2) 1.50 x 0.75 cm to 1.85 x 1.00 cm; and (3) 2.0 x 1.10 to 2.40 x 1.30 cm, were subjected to cold shock pretreatment at 4°C in 5,

10, and 15 days duration. Calli were obtained in all treatments but highest (24.17%), over-all performance was observed at bud size ranges of 2.0 x 1.1 to 2.4 x 1.3 cm when cultured in B5 (Gamborg et al, 1968) with 1 mg L⁻¹ 2,4-dichlorophenoxol acetic acid (2,4-D) for 30 days in the dark. Further optimization of the culture condition was conducted using anthers from bud size ranges of 2.0 x 1.1 to 2.4 x 1.3 cm. Anthers were subjected to cold shock pretreatment at 4°C in different durations (5, 10 and 15 days) and cultured in B5 medium with different levels of 2,4-D (1,2 and 3 mg L⁻¹ medium). Callus induction was observed after 5 days of inoculation and was significantly higher on cultures with the following treatments: (a) on B5 + 2-3 mg L⁻¹ 2,4-D without flower bud cold pretreatment (21% & 17.5%); B5 without 2,4-D and buds subjected to 15 days cold shock pretreatment (26.7%). The results of this study showed, cultural requirements of *Dendrobium* for anther culture, and opened up an opportunity for further researches in breeding orchids.

Keywords: *Dendrobium*, anther culture, cold shock pretreatment, 2-4, D, breeding

ASD No. 18

**GENETIC TRANSFORMATION OF BANANA FOR
BUNCHY TOP VIRUS RESISTANCE**

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Banana bunchy top disease is the most devastating viral disease of bananas in the Philippines. Development of BBTV resistant banana by conventional breeding is difficult since most commercially important cultivars are sterile and triploid. Thus, genetic transformation of banana with viral genes, to develop resistant plants, was applied to banana-BBTV system. The coat protein gene (CP) was amplified from local BBTV isolates using specific primers. PCR amplification of total nucleic acid extracts generated a 589 bp product. The development of gene constructs was carried out by cloning the coat protein gene of BBTV into a plant transformation vector pBI 121. Compact and embryogenic calli and somatic embryos were initiated from immature male inflorescence explants of Lakatan. Small male inflorescence explants (~1-2mm)

initiated earlier and higher percentage of embryogenic calli formation than the 3-5mm inflorescence. Plantlet regeneration was observed on both compact and friable calli. The conditions for transient transformation (using particle inflow gun) of calli, somatic embryos and shoot tips were optimized. The effect of distance and helium gas pressure on transient transformation was studied. Compact embryogenic calli bombarded at the 15 cm and pressures of 900- 1000 kpa gave the highest percentage of transient transformation with more than 50% of cultures showing Gus expression. Shoot tips bombarded using the same conditions showed no Gus expression. Since banana cultures are not so sensitive to antibiotic kanamycin, the sensitivity of the banana cultures to antibiotic geneticin, another antibiotic in place of kanamycin, was evaluated. Embryogenic calli and plantlets were sensitive to geneticin in the range of 50-100mg/L. All explants died at 150mg/L geneticin after 20 days of culture. Transformation of embryogenic calli with the BBTV CP gene and using the optimized bombardment parameters are currently being undertaken. Bombarded calli are now in selection and regeneration medium.

Keywords: Banana bunchy top virus, transformation, somatic embryo, coat protein, particle bombardment

ASD No. 19

CONTAINED FIELD TESTING OF TRANSGENIC RICE WITH XA21 GENE FOR RESISTANCE TO BACTERIAL BLIGHT

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Preliminary field testing of transgenic IR72 lines with Xa21 gene for bacterial blight resistance was conducted during the 2002 WS to determine gene expression for resistance to bacterial blight under field conditions, compare the agronomic performance of transgenic and non-transgenic rice, and assess further the horizontal gene flow to non-targeted rice plants. Seeds of transgenic rice TT103 (IRRI transgenic line), IR72-82-3-13-2-2 and IR72-82-3-42-4-6 (PhilRice transgenic lines), untransformed IR72; IRBB21, a conventionally bred line with Xa21 gene; and IR24, a susceptible control were evaluated. Bacterial blight susceptible purple rice was planted in between varieties (3 rows) and around

the periphery of the field (10 rows) that served as buffer and indicators for horizontal transfer of Xa21 gene. Species of Sesbania trees were planted outside the 1.2 m fence surrounding the field as pollen barrier. Inoculation was done at maximum tillering stage following the clipping method using PXO79 race 3 (Maligaya isolate) and rated for percentage diseased leaf area (%DLA) 14 and 21 days after. The agronomic traits: days to flowering and maturity, plant height at maturity, percentage productive tillers, panicle length, weight of 1000 grains and the harvest index were obtained to determine possible phenotypic change between transgenic and non-transgenic IR72. IR24 showed the highest mean %DLA as expected. The untransformed IR72, which contain some genes for resistance to Xoo, showed an intermediate response, IRBB21 showed a moderately resistant response, and the transgenic lines were resistant to moderately resistant. Yield component data in the wet season of 2002 revealed that the transgenic lines were comparable to the untransformed IR72 in days to flowering and maturity, percent productive tillers, plant height, 1000 grain weight and harvest index. At present, the second season of field testing is being conducted this 2003 DS.

Keywords: transgenic rice, field testing, bacterial blight, IR72, genetic engineering

ASD No. 20

**AGROBACTERIUM-MEDIATED TRANSFORMATION
OF DAVAO 'SOLO' PAPAYA FOR PRSV (PAPAYA
RINGSPOT VIRUS) RESISTANCE**

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Transformation of plants with viral genes has been utilized to produce resistance in agriculturally important crops. Development of transgenic papaya resistant to PRSV in Hawaii has been successful, however, resistance did not hold for Asian isolates of PRSV. *Agrobacterium*-mediated transformation of papaya is currently being undertaken to produce papaya resistant to the local strain of PRSV. Studies have shown that resistance is highly specific and depends on the relatedness of the transgene and the challenging virus. Hence, primer pairs based on PRSV Philippine isolate were made to amplify the coat protein (CP) and replicase (N1b) genes of PRSV by RT-PCR. The amplified CP and N1b genes were cloned into pMON plant expression vector and then transformed

into *Agrobacterium tumefaciens* strain ABI by electroporation. Somatic embryos obtained from Davao 'Solo' papaya were co-cultivated with the transformed and activated *Agrobacterium* for three days. After 3 months on selection medium containing kanamycin, embryos were selected and cultured on regeneration and rooting media. One hundred and fifty putative transgenic plants (transformed with CP) were then acclimatized and potted-out in the biological containment level 2 (BL2) greenhouse. Ninety putative transgenic plants were mechanically inoculated with PRSV. Three lines remained healthy and showed no symptom while others showed various levels of disease severity. The surviving transgenic lines and uninoculated lines are now planted in a contained screenhouse to generate R1 seeds for resistance evaluation and molecular analysis.

Keywords: papaya ringspot virus, transformation, somatic embryo, coat protein, replicase gene, *Agrobacterium tumefaciens*

ASD No. 21

**GLYPHOSATE TOLERANCE OF TRANSGENIC
CORN (ROUNDUP READY CORN HYBRID NK603)
UNDER SCREENHOUSE CONDITIONS**

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The glyphosate-tolerance of Roundup Ready (RR) hybrid NK 603, a transgenic corn, was evaluated for herbicide susceptibility/tolerance in comparison with isohybrid C818 corn under contained screenhouse conditions.

The hybrids were sprayed with glyphosate at application rates of 0.72 or 1.44 kg acid equivalent/ha and at various crop stages, i.e. 15, 30, and 15 and 30 days after planting (DAP) with effects evaluated in comparison with respective untreated checks.

Both hybrids exhibited comparable seedling emergence and vigor. Regardless of dosage rates, the RR hybrid NK 603 corn was not affected in terms of plant height, number of leaves per plant, extended leaf length and degree of chlorosis, while isohybrid C818 corn was adversely affected and died at 7 days after spraying. However, morphological abnormalities such as twisted, curled or constricted outer apical leaf sheath resulting in deterred shoot

development or shortened leaf blades were noted on RR hybrid NK 603 corn sprayed at any rate at 30 DAP. This indicates a weakened glyphosate-tolerance of the transgenic corn to herbicide application when applied at later stage.

The performance of Roundup Ready corn NK 603 should be evaluated under field conditions, along with economic analysis to warrant commercial value.

Keywords: transgenic corn, isohybrid, glyphosate-tolerance, agronomic characters

ASD No. 22

**INSECT PEST MANAGEMENT STUDIES FOR PAK-CHOI
Brassica chinensis L. PRODUCTION IN THE PHILIPPINES**

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Insect pest management studies were conducted against major insect pests of pak-choi such as diamondback moth, *Plutella xylostella* and cabbage webworm, *Hellula undalis*. The studies were: 1) identification of local natural enemies of major insect pests on pak-choi 2) effective crop management studies for pak-choi and 3) use of net barriers to reduce diamondback moth and cabbage webworm damage on pak-choi.

The insect pest found on pak-choi were *Plutella xylostella*, *Hellula undalis*, *Spodoptera litura*, *Spodoptera exigua* and grasshoppers. The major predators of adult Lepidopterous insect pests were dragonflies and damselflies, but larvae predators were pentatomid bug and spiders. Wasps from the family Braconidae and flies from Tachinidae were present. Parasitism of insect pest larvae was nil for *Hellula undalis* and *Plutella xylostella* in all plots however in *Spodoptera litura*, there was one larva infected by a fungus of unknown etiology and unknown pathogenicity and one larva parasitized by a Dipteran. Natural enemies were few and failed to build up in the non-sprayed plot.

Foliage damage by diamond back moth (DBM) and cabbage webworm (CWW) larvae in researcher managed plots was significantly less than on farmer

practice plots and nil pesticide plots. There were no differences in foliage damage between farmer managed and untreated plots. The farmer practice plots received nine pesticide applications; six insecticides and three fungicides. In contrast, the researcher managed plots received four applications; three insecticides and one fungicide. Farmer sometimes mixed two pesticides in the spray tank to economize time, however no additive effect from the mixture was noted. The quantity (li/ha) of pesticide product used in farmer practice plots was 70.6 percent higher than in researcher managed plots.

The number of DBM and CWW larvae inside the net barriers was less than the number in the open field. Infestation of DBM was observed 14 days after seeding (DAS) in the open field while in the net barriers DBM was noted 28 DAS. Percentage damaged plant increase in time in the open field. Pak-choi inside the nylon net barrier house were prevented from insect infestation and plant damages compared to the open field. However, adult flea beetles were able to pass through the holes of 16 mesh, therefore this mesh is not suitable for flea beetle control.

Diseases and insect pests must be correctly identified to improve crop management. Net barrier, using 32 mesh significantly reduced thrips, DBM and CWW populations.

Keywords: banana bunchy top virus, transformation, somatic embryo, coat protein, particle bombardment

ASD No. 23

**WOCAT AS A TOOL FOR EFFECTIVE PLANNING,
MONITORING, AND EVALUATION OF
SOIL AND WATER CONSERVATION PRACTICES**

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The World Overview of Soil and Water Conservation Approaches and Technologies (WOCAT) programme, which was launched in 1992 by the World Association of Soil and Water Conservation (WASWC), has developed a standardized framework for the assessment and evaluation, as well as promotion of exchange of knowledge, of soil and water conservation (SWC) technologies and approaches world-wide. WOCAT results and outputs are accessible via the Internet (www.wocat.net), in the form of books and maps, or on CD-ROM.

WOCAT examines advantages and disadvantages of SWC systems and why technologies were accepted or rejected by local users. Data are collected through three comprehensive questionnaires: on SWC Technologies, on SWC Approaches, and on SWC maps. The resulting information system and analytical tools, through the WOCAT database, overviews and maps, provides a useful framework and a tool for decision-makers and project planners responsible for SWC project design, implementation, monitoring and evaluation. Through the global network involving international and national institutions and programs, the valuable knowledge on SWC is being exchanged and made available.

In the Philippines, the Philippine Overview of Conservation Approaches and Technologies (PHILCAT) was organized through a Special Order by the Secretary of the Department of Agriculture in September 1999. It is an interagency committee of eleven member agencies/institutions and two professional societies/organizations for WOCAT and Asia Soil Conservation Network (ASOCON) in the Philippines.

In Thailand, the Thailand Overview of Conservation Approaches and Technologies (THAICAT) is based at the Land Development Department of the Ministry of Agriculture and Cooperative in partnership with other government and non-government agencies. A number of approaches has been collected since the a workshop in September 1996. With present updating, it is expected that up to 12 technologies and 12 approaches would be completed within 2001.

The presentation and the paper will demonstrate the program, its tools and how they can be applied, and some of the results obtained from different countries in the world.

Keywords: soil and water conservation, WOCAT, SWC technologies, SWC approaches, SWC maps

ASD No. 24

CONSERVATION TILLAGE SYSTEMS IN CORN PRODUCTION

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The potential of conservation tillage systems to improve corn yields while reducing production costs and conserving soil and water resources was tested through on-farm trials in three sites, namely: San Jose, Mindoro Occidental, Calabanga, Camarines Sur and Mahaplag Leyte. A total of 11 farmer-partners cooperated in the conduct of the trial. Treatments include conventional tillage, minimum tillage, zero tillage and farmer's tillage practices, which are tested either as single factor or in factorial with variety or fertilizer. Results in Mindoro and Leyte reveal that variation in grain yield is mainly due to tillage practices. Moreover, conservation tillage practices i.e., zero and minimum tillage, resulted to the higher grain yield of about 5.29 t ha⁻¹ and 3.95 t ha⁻¹ for both

Mindoro and Leyte, respectively. In the case of Camarines Sur, yield was not significantly affected by any of the treatments combinations applied (tillage x variety). Zero tillage obtained higher grain yield (2.88 t ha⁻¹) followed closely by minimum tillage with 2.79 t ha⁻¹. From these trials it is evident that conservation tillage management generally resulted to higher grain yield compared with the farmer's tillage practice. Conservation tillage practice is thus a viable option for corn production. Benefits obtained in adopting conservation tillage will be further realized in terms of economic returns, where highest net benefit is obtained due to lower production costs. In addition, when the soil surface is left undisturbed, soil moisture is conserved at a time when dry periods are a problem.

Keywords: conservation tillage, conventional tillage, minimum tillage, zero tillage, on-farm trials, corn

ASD No. 25

CLONING OF THE PETUNIA *Restorer of Fertility (Rf)* GENE: IMPLICATIONS IN BASIC AND APPLIED PLANT RESEARCH

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Cytoplasmic male sterility (CMS) and fertility restoration are active areas of plant research because they offer a unique opportunity to elucidate the molecular interaction between the nuclear and mitochondrial genomes, and are crucial in enhancing crop productivity by exploiting heterosis in hybrid crops. While a number of mitochondrial CMS-causing genes have been cloned and characterized, corresponding nuclear *Restorer of fertility (Rf)* genes have remained elusive. Using map-based and candidate gene approaches, the *Petunia Rf* gene was cloned and designated as *Rf-PPR592*, the first cloned restorer gene that directly suppresses the expression of a mitochondrial CMS gene.

Rf-PPR592 encodes a 592-amino acid (AA) protein, which includes a stretch of 29-AA mitochondrial-targeting sequence. Almost the entire protein

is arranged into 14 copies of the pentatricopeptide repeat (PPR) motif, a recently discovered motif found in a large gene family in *Arahidopsis*. An adjacent homologous gene, *Rf-PPR591*, did not restore fertility. The non-restoring recessive allele, *Rf-PPR592*, has a 530-nucleotide deletion in its promoter region. However, open reading frame swapping indicates that its inability to restore is due to changes in the coding region.

The cloning of the petunia *Rf* gene would facilitate studies on the origin and other possible functions of *Rf* genes in plant development. In addition to map position, our results provide other important clues for cloning *Rf* genes in other species, including the presence of mitochondrial targeting signal and PPR motif, and the reduction of proteins encoded by the CMS genes. Cloning of *Rf* genes in major crops would greatly facilitate the production and identification of suitable and improved restorer lines by marker-aided selection and/or DNA transformation, as well as modification of the current 3-line method of hybrid seed production into a simpler 2-line method by putting the *Rf* genes under the control of inducible promoters.

Keywords: mitochondrial cytoplasmic male sterility, *Oryza sativa*, petunia restorer gene

ASD No. 26

**ANALYSIS OF TUNGRO VIRUS-RICE INTERACTION BY
DIFFERENTIAL DISPLAY RT-PCR AND ISOLATION OF cDNA
FRAGMENTS ASSOCIATED WITH TUNGRO RESISTANCE**

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Rice tungro disease (RTD), characterized by stunting and discoloration of the leaves, is known to be the most widespread and destructive disease of rice in the Philippines and Southeast Asia. The RTD is a composite disease caused by two kinds of viruses - rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV), which are commonly transmitted by the greenleafhopper (GLH) vector insect (*Nephotettix virescens*). The use of rice

tungro virus (RTV) resistant rice varieties is an important approach to control the RID.

Current knowledge and information on rice-tungro virus interaction at the molecular level is still very limited. The specific host genes that are expressed during the viral infection process and the role of these genes in pathogen recognition and elicitation of the resistance reaction in the host plant have not been specifically identified. Therefore, research on gene expression in *planta* during rice tungro virus infection will provide substantial answers to the questions regarding the nature and mechanism of RTV resistance in rice. Based on the knowledge of the molecular basis of this interaction, it is possible to design an effective approach for breeding against the RTV resistance.

Recently, we have embarked on the isolation of genes involved in RTV resistance mechanism through a gene expression-based approach. RTV-resistant and RTV-susceptible near isogenic lines were analyzed by differential display RT-PCR (Liang and Pardee, 1990). RNA was extracted from the RTV-infected and uninfected resistant and susceptible isolines. At 72 hours after inoculation by virus-carrying GLH, at least 20 RT-PCR bands were identified as RTV-rice interaction specific from amplifications involving 40 primer combinations, i.e. cDNA fragments that were present only in RTV-infected resistant line. The differential PCR products were isolated and purified for further characterization and sequence analysis to establish their identity and possible roles in RTV resistance.

Keywords: rice tungro virus resistance, differential display RT PCR

ASD No. 27

**BIOACCUMULATION AND BIOCONCENTRATION OF
PB IN THE TISSUES OF *Zea mays* L. IPB VAR. 911**

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Plants selectively take up and accumulate heavy metals at varying mobilities. The bioaccumulation and bioconcentration of heavy metals are greatly affected by the intrinsic capabilities of plants to uptake and store heavy metals in their tissues, and their interaction with the physico-chemical environment. The bioaccumulation (BA) and bioconcentration factor (BCF) of Pb in the root and shoot tissues of *Zea mays* L. IPB var. 911 seedlings were investigated in potted field conditions and were analyzed using Atomic Absorption Spectrophotometry (AAS). Results indicated significant difference on the BA of Pb in the root and shoot tissues of corn in various Pb(NO₃)₂ treatments. Significant (P<0.05) increase of Pb accumulation (2930 µg g⁻¹) in the root tissue was observed at 5000 mg kg⁻¹ treatment. Moreover, significant (P<0.05) BA values of 46.67, 43.54 and 50.93 µg g⁻¹ of Pb in the shoot tissues were recorded at 100, 2000 and 5000 mg kg⁻¹ treatments, respectively. Bioconcentration factors (BCF) of 0.760, 0.450, 0.697 and 0.935 at control, 100, 2000 and 5000 mg kg⁻¹ treatments were determined in the root tissues, respectively. Likewise, the highest root BCF of 1.25 was registered at 500 mg kg⁻¹ treatment. Compared to root, much lower BCF values of 0.205, 0.746, 0.038, 0.035, 0.016 in the shoots were recorded at the control, 100, 500, 2000 and 5000 treatments, respectively. Greater bioaccumulation values and enhance BCF suggest that *Zea mays* L. IPB var. 911 might be used for the clean-up of Pb-contaminated soils.

Keywords: bioaccumulation, bioconcentration, corn, heavy metals

ASD No. 28

**MECHANIZING THE PROCESSES IN DEVELOPING
BIOBASED FARM INPUTS**

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Rice and rice-based farming system in the Philippines are becoming cost-intensive due to increasing cost off-farm and non-renewable inputs. Indigenous materials in the farm and household that are sustainable should be recovered from a biobased system of developing ecological farm inputs.

PhilRice has developed a system in converting (physical, thermal & biological) indigenous materials from farm biomass and biodegradable household wastes. Physically, a drum-type manually operated chopper/mixer-cum composter was developed for a cluster of households. Also a convertible thresher-shredder/chopper was improved by sharpening both sides of the threshing teeth and disconnecting the blower. Result showed the acceptable chopping length of 9.6, 7.0, 6.2 cm of rice straw, kangkong and ipil-ipil branches, respectively.

Thermally, rice hull is carbonized by simple open-type carbonizer using a perforated oil drum with chimney, producing a black colored substance with uniform particle size. Ten bags of rice hull make 6~7 bags carbonized rice hull (CRH) in four hours.

Biologically, a biogas digester was designed to produce methane or alcogas. The system uses a 150-L plastic container and commercial gas control mechanism. The process anaerobically ferments a mixture of farm biomass and kitchen garbage to produce gas for cooking.

Keywords: indigenous materials, physical, biological and thermal conversions

BIOLOGICAL SCIENCES

BSD No. 1

ESTABLISHING A NEW CODON PREFERENCE TABLE FOR THE COCONUT (*Cocos Nucifera* L.)

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The triplet codes of 18 sequences derived from ten genes isolated from coconut (*Cocos nucifera* L.) were tallied. Relative frequency percentages of the individual codons encoding each amino acid were calculated. The highest percentage of the codon for each amino acid was chosen as that amino acid's most preferred codon. Comparative analysis to determine whether a change of codon preference and degeneracy exists was done between the published codon preference table (Nakamura et al, 2000) and from a new one generated from the sequence data of the coconut genes. A similar analysis was done for the sequence data of the genes derived from the normal and the makapuno phenotypes.

Analysis between the published codon preference table and the table derived from this study showed that 30% of the amino acids retained the existing published preference codon, 35% of the amino acids prefer a non-degenerate codon while 35% had a change of codon preference especially at the third nucleotide position of the codon. Moreover, arginine prefers a degenerate codon with an additional change at the first nucleotide position over the reported codon preference.

Analysis between the genes derived from the normal and makapuno phenotypes shows that 70% of the amino acids utilize the same codon across the two phenotypes; 5% has a distinction with the normal coconut phenotype preferring a degenerate codon while its makapuno counterpart utilizes a non-degenerate codon; and, 25% of the amino acids had a change in codon preference

between the two phenotypes.

This is a pioneering study in establishing the coconut codon preference table, a molecular biology tool much-needed by researchers in gene discovery and other applications in instances where molecular biology data is limiting.

Keywords: codon preference, coconut, *Cocos nucifera* L.

BSD No. 2

**DETECTING ISOFORMS OF GENES INVOLVED IN
FATTY ACID SYNTHESIS IN COCONUT (*Cocos nucifera* L.)**

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The RACE (Randomly Amplified cDNA Ends) method is a very useful and accurate technique in detecting isoforms of various genes. In coconut, several genes are involved in fatty acid synthesis and among them are: phosphatidic acid phosphatase (PAP), acyl carrier protein (ACP) and beta-keto acyl (ACP) synthase 3 (KAS 3). To check for the presence of isoforms of each gene at the 4, 5 and 6 month old coconut endosperms, the RACE method was used.

Forward primers of both ACP and KAS 3 were designed from the highly conserved amino acid region based on previous publications. The forward primer of PAP was designed based on multiple sequence alignment of known PAP sequences. Reverse primer used for all three genes was provided by the RACE kit.

Initially, cold-start PCR was used and a 300 bp band was obtained for ACP in the 4 mo old coconut endosperm. No distinguishable band was obtained for PAP and KAS 3. Both Pap and KAS 3 were further subjected to an improvised hot-start and touchdown PCR, which yielded three bands with sizes 700 bp, 500 bp and 400 bp, respectively for PAP at the 6 mo. old coconut endosperm while two bands with sizes 725 bp and 425 bp, respectively were obtained for KAS 3 in both the 5 and 6 month old coconut endosperm.

The results obtained indicate the presence of each of the gene and their isoforms at varying ages of the coconut endosperm. Furthermore, the present results are consistent with the results obtained using the coconut cDNA library.

Keywords: Randomly Amplified cDNA Ends (RACE), phosphatidic acid phosphatase (PAP), acyl carrier protein (ACP), Δ -ketoacyl (ACP) synthase 3 (KAS 3), isoforms, PCR

BSD No. 3

**COCONUT (*Cocos Nucifera* L.) EXPRESSES
GUS IN SELECTED PLANT PARTS**

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This study stems from our research to develop a transient expression analysis system using embryogenic callus derived from zygotic embryos of coconut. The transient expression analysis is necessary to establish some physical parameters such as distance (between the gun and the target tissue) and pressure of the helium gas in the optimization of a particle gun bombardment system for transformation in coconut. pBI121 is a binary vector which contains the *GUS* gene and is driven by a 35S CaMV promoter. This 13 kb plasmid was propagated in *E. coli*, and the isolated plasmids were purified using a commercial purification kit. The purified plasmids were coated onto tungsten and bombarded directly to embryogenic callus derived from zygotic embryos of coconut using a particle inflow device. After particle bombardment, expression of the *GUS* gene was assayed using standard histochemical staining protocols. *GUS* expression through the formation of blue spots scattered randomly was observed in embryogenic calli bombarded with pBI121-coated tungsten and with tungsten only. Contamination was ruled out by including some controls in the experiments. Different portions of the coconut (endosperm, embryo, young leaves, young stem, haustorium) were histochemically assayed for endogenous *GUS* or *GUS*-like activities and the immature endosperm, young stem and haustorium showed positive reaction for *GUS* activity. These results show that coconut contain endogenous *GUS* activity and therefore *GUS* may not a suitable reporter gene assay for transient expression analysis in coconut.

Keywords: coconut, *Cocos nucifera* L., *GUS*, transformation

BSD No. 4

**BULKED SEGREGANT ANALYSIS (BSA): A RAPID PROCEDURE
FOR IDENTIFYING AFLP MARKERS IN THE SPECIFIC REGIONS
OF THE RICE TUNGRO SPHERICAL-VIRUS DISEASE
RESISTANCE GENE LOCUS IN RICE (*Oryza sativa* L.)**

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The most important viral disease that causes yellowing and stunting in rice plants, inflicting heavy losses on rice is the Tungro virus disease. The disease is caused by a composite of two viruses, the rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV). The green leafhopper (*Nephotettix virescens* Distans) is the major transmitter of the rice tungro virus disease. In this study, genetic mapping of the tungro spherical virus (RTSV) resistance gene was undertaken using the bulked segregant analysis (BSA). The BSA involves comparing two pooled DNA samples from a segregating population derived from a single cross, delineating the R gene to a narrow region through the identification of candidate Amplified Fragment Length Polymorphism (AFLP) markers.

In the genetic analysis, the F₂ and F₃ populations from the cross between TI-11-8, a TN1 line with introgressed R gene from ARC11554, and a susceptible line R4-40/RCN13-19-94 were grown for DNA isolation and tungro phenotypic screening. Two hundred eighty-six F₃ families were evaluated for tungro reaction through ELISA after RTSV inoculation. A bulked segregant analysis for amplified fragment length polymorphism (AFLP) was performed on the 16 highly resistant and 16 highly susceptible F₂ plants that were identified based on the ELISA scores of their corresponding F₃ families. The AFLP profile of the R and S pools and the four parentals were compared in each of the forty pairs of PstI and MseI primers as well as forty pairs of EcoRI and MseI primers used. Seven polymorphic bands were identified for PstI/MseI primer combinations while five were identified for the EcoRI and MseI primer pairs. Hence, based on the mapping results these AFLP markers may lie within 0.3 cM from the R gene.

Keywords: Tungro, AFLP, bulked segregant analysis, green leafhoppers, markers

BSD No. 5

**MOLECULAR ANALYSIS OF MITOCHONDRIAL GENOMIC
VARIATION BETWEEN CYTOPLASMIC MALE STERILE (CMS)
AND FERTILE LINES OF MESTIZO HYBRID RICE**

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This study analyzed the genetic polymorphism between the cytoplasmic male sterile (CMS) and maintainer lines of Mestizo hybrid rice. Total genomic DNA was isolated from IR58025A and IR58025B, the CMS and maintainer lines, respectively. The mini-prep CTAB DNA isolation method was employed to extract and purify high quality DNA from the two rice varieties. Five PCR primers based on the atp6- ORF region of Bo- type mitochondrial genome was used to amplify mitochondrial DNA using total genomic DNA as template. PCR products were size- separated in 1% agarose gel electrophoresis. Variation in the PCR profiles (DNA fingerprints) between the A and B line was visualized and documented after ethidium bromide staining. Polymorphism was only detected in the PCR products with mit1 as primer. No polymorphism was detected in PCR products with mit2- mit5 as primers. It can be said that mitochondrial DNA can be used to detect variation in the A and B lines of Mestizo hybrid rice. It is suggested that more primers must be used to detect more polymorphism.

Keywords: genetic polymorphism, CMS, maintainer line, PCR, primers, mitochondrial genome, electrophoresis

BSD No. 6

HIGH THROUGHPUT SCREENING OF THE BACTERIAL ARTIFICIAL CHROMOSOME (BAC) LIBRARY USING THE THERMAL ASSYMETRIC INTERLACED PCR (TAIL-PCR) FOR THE PHYSICAL MAPPING OF TUNGRO SPHERICAL VIRUS RESISTANCE GENE IN RICE (*Oryza sativa* L.)

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Tungro is a viral disease that inflicts heavy losses on rice and is caused by rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV). It is transmitted by the green leafhopper (*Nephotettix virescens* Distans). In this study, physical mapping was conducted to identify clones that will guide the map-based cloning of the resistance (R) gene identified in ARC 11554 to RTSV, the primary causal organism.

Two rice BAC libraries were screened using the thermal asymmetric interlaced PCR (TAIL-PCR). The TAIL-PCR has been developed for the isolation and amplification of insert end sequences of BAC clones. The TAIL-PCR strategy used three nested specific primers in successive reactions together with a shorter arbitrary degenerate (AD) primers so that the relative amplification efficiencies of specific and non-specific products can be thermally controlled. Probes used for library screening were derived from the previously mapped RFLP markers around the tungro R gene. Marker CDO456 bound to BAC4705, marker C708 hybridized to BAC17N19, and marker CDO783 cleaved to BAC19P8. Recurrent BAC end isolation by TAIL-PCR and library screening identified 11 more clones at the CDO456 locus, 13 more BAC clones at the C708 locus, and 4 more clones at the CDO783 locus. The selected BACs at C708 have an average insert size of 75 kb ranging from 35 to 105 kb as determined by pulse-field gel electrophoresis of the *Not*I digest. Preliminary assembly of the clones suggests a contig size of 190 to 335 kb. The advance toward the R gene from the C708 locus will be assessed based on the genetic mapping of the contig ends. Once cloned, the gene is envisioned to be utilized in the rapid development of tungro resistant varieties through genetic engineering.

Keywords: BAC, cloning, contigs, library, physical mapping, resistance, tungro

BSD No. 7

**GENE DISCOVERY FOR PEST RESISTANCE IN CORN:
BACTERIAL AND INSECT-GUT SPECIFIC CHITINASES**

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Transgenic plant technology can be a useful tool in the development of resistant crops by introducing novel resistance genes into plant species. To date, two main strategies for the generation of insect-resistant plants have been employed. One approach is to use the entomocidal bacterium *Bacillus thuringiensis* (Bt) as a source of resistance genes and the other is to deploy other insect-resistance genes present in other organisms.

Chitinases are hydrolytic enzymes that can degrade the peritrophic membranes of larval midguts. Genes encoding chitinases could be used for stacking with the Bt gene. At least seven isoforms of the chitinase gene were isolated and cloned from a local strain of *Serratia marcescens*, an enteric insect pathogen. Partial cDNAs corresponding to midgut-specific chitinase were also isolated from larval tissues of corn borer isolated in Los Baños by RT-PCR. One of the seven isoforms of the bacterial chitinase gene is already a full length gene and being characterized prior to stable transformation in corn. Based on sequence analysis, it is homologous to ChiA (a secreted extracellular chitinase).

Keywords: chitinase, corn, *Serratia marcescens*

BSD No. 8

**CHITINASE PRODUCTION BY *Serratia marcescens*
AND ITS POTENTIAL CONTROL OF THE SHEATH
BLIGHT PATHOGEN OF CORN**

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Plant pathogenic fungi like *Rhizoctonia solani*, a causal organism of sheath blight of corn creates a major problem in boosting agricultural production. The use of microbial products like the enzyme chitinase can be a promising alternative to lessen the incidence of fungal infestation and at the same time lessen environmental hazards brought about by chemical use. This study aimed to optimize the cultural conditions of the wild type strain of *Serratia marcescens* for increased chitinase production and to test its effectiveness against *R. solani*.

A chitinolytic wild type *S. marcescens* LPM42 BIOTECH 1749 was grown by batch fermentation in varied cultural conditions to produce chitinase. The amount of crude chitinase excreted in the culture medium was assessed turbidimetrically through its ability to release N-acetylglucosamine (GlcNAc) after reaction with colloidal chitin obtained from crab shells. Incubating the culture broth containing 5% (v/v) inoculum, 0.6% (v/v) colloidal chitin without Tween 80 at an initial pH of 7.0, for 48 hr at room temperature with continuous shaking (158 rpm) were the optimum conditions for chitinase production of *S. marcescens* (0.004 units/ml).

Laboratory experiments to control the growth of *R. solani* Kuhn showed that soaking the sorghum seeds coated with fungal mycelia for 3 to 5 hours in undiluted crude chitinase extract showed significant reduction in mycelial proliferation after 12 hr of incubation. Complete inhibition of mycelial development was observed after 12 hr soaking in undiluted crude chitinase extract. No growth was observed even when the incubation was extended for 48 hr. These results demonstrated the potential of using even the crude form of the chitinase enzyme for the biocontrol of *R. solani*.

Keywords: *Serratia marcescens*, *Rhizoctonia solani*, chitinase, chitin

BSD No. 9

MOLECULAR SYSTEMATIC STUDIES OF THE PHOTOSYNTHETIC RHIZOBIA ISOLATED FROM AESCHYNOMENE SPP. USING *nifH* AND *nodA* GENE SEQUENCES

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The rhizobia isolated from *Aeschynomene* species have the ability to produce photosynthetic pigment, aptly termed as the photosynthetic rhizobia. These photosynthetic species belong to the α -2 Proteobacteria and are phylogenetically related to the non-phototrophic *Bradyrhizobium* species and the phototrophic *Rhodospseudomonas palustris* based on 16S rRNA sequence analysis. Their very unique biological characters, possessing the nodule forming and the photosynthetic abilities of *B. japonicum* and *Rps. palustris*, respectively, have led us to think that the photosynthetic rhizobia are the “missing link” between the two species. As such, these bacteria are good models for studying the evolution of nodulation, nitrogen fixation and photosynthesis in the α -Proteobacteria.

In this study, we performed a phylogenetic analysis of the genes involved in nitrogen fixation (*nifH*) and nodulation (*nodA*) in comparison with that of the 16S rRNA and the intergenic spacer region (ITS) to investigate the possible origin and evolution of their unusual characteristics. The 16S rRNA and ITS phylogenies showed that the photosynthetic rhizobia are mainly monophyletic and closely related to *B. japonicum* and *Rps. palustris*. The *nifH* phylogeny placed the photosynthetic rhizobia in a monophyletic group with the strains of *B. japonicum*, but far from the *Rps. palustris* strains. The *nodA* from the photosynthetic rhizobia, on the other hand, were highly conserved and phylogenetically distant from those of other rhizobial species. These results suggest that the nitrogen fixing photosynthetic rhizobia may have evolved with the nitrogen fixing *B. japonicum* from a common ancestor, while their *nodA* gene might have been acquired from a different species in the latter part of their evolution. The ultimate conservation of their *nodA* gene from species grown in separate geographical regions also suggests that this gene might have co-evolved with its host plant.

Keywords: photosynthetic rhizobia, *Aeschynomene*, *nifH*, *nodA*, 16S rRNA, phylogeny

BSD No. 10

**MONOMORPHICITY OF *RALSTONIA SOLANACEARUM*
BANANA STRAINS IN THE PHILIPPINES**

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Ralstonia solanacearum causes two distinct diseases in bananas, the moko and bugtok. There are considerable differences in the symptomatology, distribution and epidemiology of bugtok and moko diseases in the Philippines but this has been established to be due to difference in the varieties infected. Analysis of genetic variation using molecular biology methods have shown that banana strains in the Philippines are homogenous except for some strains wherein the difference is very minimal (Ilagan, 1996). However, isolates used were limited only to Mindanao, majority of which came from Davao and Bukidnon. There is, therefore, a need to verify whether new banana isolates from the Philippines will give the same DNA types.

Seventy-seven new banana isolates of *R. solanacearum* obtained from Davao, Sultan Kudarat and Iligan City were all pathogenic on tomato (Yellow Plum). Using primers 759/760 and M114 in polymerase chain reaction (PCR), the expected 281 bp and 2.28 Kb products of 759/760 and M114, respectively, were obtained confirming their being *R. solanacearum* and banana strains. PCR analysis using REP primers produced 10 to 15 bands ranging from 298 to 5090 bp. Of the 81 isolates, 73 were found to have genetic profiles similar to that of the reference strains Bu24W and MoD6. Seven new strains possessed an extra band at about 3563 bp. This band is completely distinct from the extra bands present in the RA-02 haplotype thus creating a new haplotype. Therefore, there are now three haplotypes in the population of banana strains. This difference, however, is still minimal confirming the monomorphicity of the banana strains. An abaca isolate tested produced a genetic profile that was completely distinct from the banana isolates of *Ralstonia solanacearum* using REP primers.

Keywords: bacterial wilt, moko, bugtok, *Ralstonia solanacearum*

BSD No. 11

**TAXONOMIC CHARACTERIZATION OF A NOVEL
RHIZOBIAL STRAIN ISOLATED FROM ROOT
NODULES OF ENTADA PHASEOLOIDES**

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Rhizobia are symbiotic bacteria capable of eliciting root and stem nodules on leguminous plants, where they reduce atmospheric nitrogen to ammonia to the benefit of the plants. Initial studies on rhizobial diversity in Okinawa, Japan revealed that MAFF 210191 isolated from root nodules of woody legume *Entada phaseoloides* exhibited phylogenetic characteristic distinct from other rhizobia. However, phenotypic characteristics of this isolate have not been described. Thus, this study was done to characterize and examine the taxonomic position of MAFF 210191 using polyphasic approach. Results showed that MAFF 210191 belong to the slow-growing group of rhizobia, displaying biochemical, physiological and chemotaxonomic characteristics quite different from the other rhizobia. Phylogenetic analysis based on 16S rDNA sequences showed that this isolate formed a separate node far from other root-nodulating bacteria. Analysis of its *nodA* gene (encodes an acyltransferase involved in nodulation) sequences showed a high homology with that of *Rhizobium tropici* CFN 299. These results suggest that MAFF 210191 occupies a unique phylogenetic position distinct from other rhizobia, and probably a new genus of nodule-forming bacteria. In light of these, characterization of symbionts of yet unexplored legumes such as *E. phaseoloides* reveals additional rhizobial species. Such undertakings may significantly contribute to the understanding of the origin and evolution of the rhizobium-legume symbiosis, and open new perspectives for environmental and agricultural applications.

Keywords: polyphasic taxonomy, rhizobia, root nodules, 16S rRNA, *Entada phaseoloides*

BSD No. 12

**GENOME IDENTIFICATION OF SELECTED TABLE AND
COOKING-TYPE BANANA (*Musa* Sp.) CULTIVARS IN
THE PHILIPPINES THROUGH ISOZYME ANALYSIS**

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Isozyme profiles of twenty one table-type and six cooking-type banana cultivars were studied. The different isozymes include malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), phosphoglucoisomerase (PGI), and Phosphoglucomutase (PGM). Different banding patterns were observed. Isozyme patterns in the different cultivars to determine its genome (19) were compared with eight (8) newly collected cultivars to determine its genome identity. The genomes of the five table-type cultivars were identified. Based on MDH and PGD, the genome of Latundan Puti is AAB, the genome of Manibun and Magipod is AAA. The four isozymes were not useful in identifying the genomes of the three cooking-type cultivars namely, Balatay, Bataan and Dumanese. Unweighted Pair Group Method using Averages Cluster Analysis confirms the correct genome of the five table-type cultivars.

Keywords: table-type bananas, cooking-type bananas, isozymes, malate dehydrogenase, phosphogluconate dehydrogenase, phosphoglucoisomerase, phosphoglucomutase

BSD No. 13a

**SCREENING OF DNA POLYMERASE FROM
LOCAL HYPERTHERMOPHILES**

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DNA polymerase is the enzyme involved in amplifying segments of DNA in an in-vitro technique termed as Polymerase Chain Reaction or PCR. Because of its speed and specificity, the PCR technique has found numerous applications in molecular biology research, epidemiological and forensic studies, disease diagnosis, and pathogen detection. This study aimed to screen the DNA polymerase in local hyperthermophiles.

A total of 150 isolates from hot springs and mud springs were obtained by streaking onto three different media, namely: modified artificial sea water (ASW) medium, nutrient broth and DSM medium. Twenty isolates have been purified but only 10 remained in stable conditions using the modified *Thermus* medium. Most of these isolates were found to grow at 85 °C.

Detection of a homologous DNA polymerase gene from the genomic DNA templates were done using *Thermus aquaticus* (*Taq*) polymerase gene (TP) primers. The positive control which was the *Taq* pol recombinant clone, *pTaq*, produced the expected 2.5 kb fragment. Isolates 62d, 62c, 60b2 and 62h2 each produced an amplified product 1.2 kb in size. Isolate 571aa and 10e had approximately 1.5 kb product whereas the Mud spring isolates had < 1 kb as its major amplified product.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein lysates showed that only three out of the eleven protein precipitate of the thermophilic bacterial isolates exhibited the protein band of approximately 90 kd. These initial results gave us the information on which isolate to choose for the purification of the protein and cloning of the DNA polymerase gene in *E. coli*.

Keywords: DNA polymerase, thermophilic bacteria, PCR-based screening

BSD No. 13b

**NEUTRAL PROTEASES OF THERMOPHILIC
Bacillus sp. ISOLATED FROM THE MUDSPRINGS,
MOUNT MAKILING, PHILIPPINES**

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Six thermophilic *Bacillus* sp. (3SM-23, 4MM-2, 4MM-22, 5-SM-6, 7MM-8 and 7MM-16) from the Mudsprings, Mount Makiling, Los Baños, Laguna, were selected and screened for neutral protease production using modified soybean cake extract broth. Crude enzymes produced by the *Bacillus* sp. were assayed using 0.6% casein and results obtained showed that three isolates, 3SM-23, 7MM-8 and 7MM-16, have high neutral protease activity (NPU) of 183.9, 128.7 and 154.1, respectively. Crude neutral proteases (CNP) from these isolates have maximum proteolytic activity at pH 7 and temperature of 55°C for 3SM-23 and 7MM-16, and at 40°C for 7MM-8. CNP were stable over a pH range of pH 4 to 7 for 3SM-23, 4 to 8 for 7MM-8 and 5 to 7 for 7MM-16. On one hand, the enzymes were stable at temperatures 20 - 60°C for 3SM-23, 20 - 50°C for 7MM-8 and 20 - 55°C for 7MM-16.

Keywords: Neutral protease, *Bacillus* sp., thermophilic bacteria

BSD No. 14

**PREDICTION OF POTENTIAL CYTOTOXIC LYMPHOCYTE (CTL)
EPTIOPES IN MALARIA ANTIGENS USING COMPUTATIONAL TOOLS**

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Induction of cytotoxic T cell (CTL) response against *Plasmodium falciparum* through the use of antigenic determinants as vaccines can greatly

reduce malaria infections worldwide. CTL responses have been theorized to be important in controlling liver stage malaria infection. Identification of T cell epitopes through experimentation alone is laborious and expensive, creating the need for computational tools that can decrease the number of candidate epitopes to make T cell epitope identification faster. Four proteins expressed by the parasite during this stage, namely liver stage antigen-1 (LSA-1), liver stage antigen-3 (LSA-3), merozoite surface protein-1 (MSP-1), and thrombospondin-related anonymous protein (TRAP), were searched for candidate T cell epitopes through the use of prediction servers available online. The following software were used in the study: ProPred1, RANKPEP, SYFPEITHI, PREDEP, and an unnamed prediction tool. For the HLA class I alleles known to be prevalent in the Philippines, 40-180 epitopes from each of the four proteins were predicted by the methods used. Some of these epitopes have already been experimentally tested by others. Analysis of promiscuity was also done to identify epitopes that can potentially be presented by multiple alleles of MHC class I molecules from different loci. Seven epitopes from TRAP appear to be promiscuous, while none were found for LSA-1. Selected T cell epitopes among the hundreds predicted can then be tested in *in vitro* binding experiments to confirm their specificity.

Keywords: malaria, T cell epitope, HLA class I, prediction, LSA-1, LSA-3, MSP-1 and TRAP

BSD No. 15

**CONSTRUCTION OF SHUTTLE PLASMIDS WHICH CAN BE
EFFICIENTLY MOBILIZED FROM *Escherichia coli* INTO THE
CHROMATICALLY ADAPTING CYANOBACTERIUM,
*Fremyella diplosiphon***

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In some strains of cyanobacteria the composition of the light-harvesting antennae is determined by the color of available light. The mechanisms of this chromatic adaptation involves the regulation of gene expression by red and green

light and has been most studied in *Fremyella diplosiphon* (*Calothrix* sp. FCC7601) a filamentous cyanobacterium for which there has been no reported means of genetic manipulation. We have constructed shuttle plasmids which can be efficiently mobilized by RP4 from *Escherichia coli* into *Fremyella diplosiphon* and which can be recovered from transconjugant *F. diplosiphon* and turned to *E. coli* by transformation. The ability of these plasmids to replicate in *F. diplosiphon* is conferred by an 8.0-kb DNA fragment isolated from pFDA, a plasmid native to *F. diplosiphon*. To create these shuttle plasmids from *oriV* and *bom* from pBR322, *cat* from pACYC184 and *aphA* from pACYC177. pJCF22 lacks sites for the restriction enzymes *Fdi*I and II. Transconjugants *F. diplosiphon* containing shuttle plasmid pJCF62 are resistant to chloramphenicol and highly resistant to the aminoglycosides, G418 and neomycin. When *aadA* from the omega interposoa was incorporated into a shuttle plasmid transconjugant *F. diplosiphon* could also be selected with streptomycin or spectinomycin. In *F. diplosiphon* shuttle plasmid pJCF62 replicates with a minimum copy number of seven. The *oriV* for replication in *F. diplosiphon* was localized to a 2.8-kb region within the cyanobacterial part of pJCF62. The presence on a shuttle plasmid of a single recognition site for *Fdi*I reduced the efficiency of mobilization into *F. diplosiphon* by 5-to 10-fold. Restriction at this site was prevented when the *E. coli* donor strain in the mating contained the enzyme *Eco*4711 methylase.

Keywords: cyanobacteria, chromatic adaptation, *Fremyella diplosiphon*, shuttle plasmids, *Escherichia coli*

BSD No. 16

GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES SPECIFIC TO THE CD33 CELL SURFACE ANTIGEN OF LEUKEMIC MYELOID HL-60 CELLS

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The CD33 antigen is a 67 kD cell surface sialoadhesin molecule that is primarily expressed on normal progenitor monocytic and mature myeloid

hematopoietic cells, but not in non-myelomonocytic nor in non-hematopoietic cells. Because CD33 serves as a marker for myeloid progenitor cells and most leukemic myeloid cells, monoclonal antibodies (mAbs) that are reactive with this CD33 glycoprotein cell surface antigen serve as effective agents in the immunotherapy of acute myeloid leukemia (AML). In this study, mAbs specific to CD33 expressed by HL-60 cells were generated and characterized. M195 hybridoma cells, producing murine anti-CD33 IgG_{2a} mAbs, were grown in vivo and injected into primed BALB/c mice for propagation in ascitic fluid. The anti-CD33 mAbs were then purified from the extracted ascitic fluid by fast performance liquid chromatography (FPLC) on Protein G columns. Enzyme-linked Immunosorbent Assay (ELISA) was performed to characterize the binding affinity of the purified anti-CD33 mAbs to CD33-positive human leukemic myeloid HL-60 cells. The purity and the molecular weight of the anti-CD33 mAbs were subsequently determined by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE). The generated, characterized, and purified anti-CD33 mAbs are useful in the production of cytotoxic drug-antibody bioconjugates for drug therapy and ex-vivo purging of bone marrow prior to autologous transplantation. Ultimately, the engineering of these anti-CD33 bioconjugates by pepsin digestion and mild reduction into F(ab') fragments optimizes the specificity to leukemic myeloid cells displaying AML, and decreases immunogenicity to normal myeloid cells.

Keywords: CD33, HL-60, M195, monoclonal antibody, hybridoma, acute myeloid leukemia

BSD No. 17

TAXONOMIC REASSESSMENT OF LOCAL ANTIBIOTIC- AND ENZYME-PRODUCING *Bacillus* ISOLATES BASED ON PHENOTYPIC CHARACTERISTICS

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The taxonomy of fifty-four locally isolated antibiotic- and enzyme producing *Bacillus* isolates deposited at the Philippine National Collection of Micro-

organisms (PNCM)-BIOTECH were reassessed in this study since they were only partially identified prior to deposition in the culture collection. Results from thirty-nine phenotypic tests were analyzed using simple matching coefficient to construct a dendrogram. The dendrogram showed twelve clusters discerned at 80% similarity level - cluster I consisted of *B. cereus* and related species, clusters II to V included the *B. subtilis* and related species, and clusters VI to XII appeared to be single-member clusters. Molecular analysis using 16S rRNA-specific PCR primers further differentiated the isolates belonging to cluster I (*B. cereus* group) from those of clusters II to V (*B. subtilis* group). Based on these analyses, forty-three isolates of *Bacillus* maintained their original identities, while five isolates were named at the species level. Nine isolates were misclassified prior to deposition, and were re-identified and renamed.

Keywords: *Bacillus*, taxonomy, phenotype, cluster analysis

BSD No. 18

**DENITRIFYING BACTERIA IN THE SEDIMENT OF
CAGED AND UNCAGED SITES IN LAKE TAAL**

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Nitrate loss through microbiological denitrification is one way of bioremediation of nitrate-rich environments. The total counts of denitrifying bacteria in the sediment of caged and uncaged sites in Lake Taal were determined using the most probable number (MPN) technique with 10x diluted nutrient broth + KNO₃ (NBN) as medium. Phenotypic and biochemical tests using established procedures and the API 20 NE system were done on pure cultures isolated from highly diluted MPN cultures showing positive nitrate reduction. Results show that the caged site had MPN of 1.8×10^4 per g dry wt sediment while the uncaged site had 2.4×10^1 . The % of denitrifying bacteria of total aerobic heterotrophic bacteria in the caged site was 0.08%, in the uncaged site 0.0005%. Except for one, the isolates were gram-negative rods, motile, and oxidative. The predominant denitrifiers in the caged sites were identified as *Pseudomonas aeruginosa* and

Aeromonas hydrophila, in the uncaged site, *Chryseomonas luteola*. These bacteria must play an important role in the sites by reducing the nitrate level in the environment.

Keywords: Denitrifying bacteria, Lake Taal, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Chryseomonas luteola*

BSD No. 19

**TAXONOMY, DISTRIBUTION AND TEMPORAL
CHANGES IN THE ABUNDANCE OF PHYTOPLANKTON
IN TAAL LAKE, BATANGAS**

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Taal Lake, a volcanic lake and one of the known tourist spots in the Philippines has a significant contribution to the country's economy being also one of the sources of large amount of commercial fishes distributed to nearby provinces. The introduction of commercial fish cages and the river inputs have contributed a great deal of disturbance in the lake's ecosystem. To investigate the lake's water quality and phytoplankton composition, a study was conducted from October 1999 to July 2000. Parameters such Ammonium-Nitrogen ($\text{NH}_4\text{-N}$), Nitrate-Nitrogen ($\text{NO}_3\text{-N}$), soluble Phosphate (P_{s}) and Total Phosphorous (P_{T}), temperature, light penetration, pH, total dissolved solids (TDS) and conductivity were measured from the three sampling sites. Qualitative and quantitative composition of the phytoplankton from the identified sampling stations were also studied including chlorophyll *a* (chl *a*) concentrations.

Significant increase in $\text{NH}_4\text{-N}$ was observed from June and July, 2000. Significant increase in P_{T} , P_{s} and $\text{NO}_3\text{-N}$ were also observed during the dry season from the monthly collections conducted. Though there was a significant increase in the nutrient hold of the lake, there was no significant correlation between the nutrients and the chl *a* content of the three collection sites in the

lake. On the other hand, TDS ($p < 0.05$), pH, and temperature ($p < 0.001$) showed positive correlation with chl *a* concentration. There was no significant difference between depth observed among the nutrients and other physico-chemical factors. No significant increase in the monthly chl *a* collected but significantly high amount of chl *a* was observed from 2.5 m and 5 m depth. *Ceratium*, centric diatoms, *Aulacoseira* and *Merismopedia* showed significantly high individual cell count among the rest of the collection months considered for counting which also holds true to chl *a* concentration. The four significantly dominant genera mentioned above also showed positive correlation with TDS, pH, temperature, $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^- \text{-N}$ generated from the Canonical Correspondence Analysis (CCA).

Keywords : Taal Lake, phytoplankton, nutrients, water quality

BSD No. 20a

PHILIPPINE WILD MACROFUNGI WITH COMMERCIAL POTENTIAL: CONTINUING SEARCH AND CHALLENGE

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The Philippines as a tropical country is endowed with very rich and diverse flora and fauna which are still under utilized and undiscovered from their economic potential. Wild edible macrofungi for instance are naturally found growing on forest litters, fallen logs and leaf debris. These macrofungi are usually being ignored due to lack of technical information about their edibility by the local folks and due to the unavailability of production technology. In our attempt to domesticate these wild edible species, our group has initiated the collection, identification and rescue of their mycelia. We have been successful in the development of production technology for *Collybia reinakeana*, a virtually unknown edible species of wild mushrooms that usually inhabits the forest floor of Puncan, Carranglan, Nueva Ecija. It can be grown on composed rice straw - based substrates having a pH of 6.0 with >70% moisture content at

an optimum temperature of 30°C and 85% relative humidity. Four edible species of macrofungi which are known by the Aetas of Mt. Nagpale, Abucay, Bataan were also collected and rescued. These are *Schizophyllum commune*, *Ganoderma lucidum*, *Auricularia* sp. and *Mycena* sp. These mushrooms grow best at a pH range of 5.5-6.0 in a coconut water medium.

Keywords: *Collybia reinakeana*, *Ganoderma lucidum*, *Schizophyllum commune*, wild edible mushroom

BSD No. 20b

APPLICATION OF PURE AND MIXED POPULATIONS OF EFFLUENT- DERIVED MERCURY-RESISTANT BACTERIA IN BIOREMEDIATION

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Toxic heavy metal contamination of the environment is one of the most destructive forms of chemical pollution. Reports of fish kills and illnesses in certain fishing villages in Iligan City suspected of being caused by mercury poisoning serve as an impetus for identifying and ascertaining point sources of the toxic pollutant(s) and for developing strategies for remediation.

Our study focuses on the use of biosorptive properties of microorganisms for bioremediation applications. Four five hundred milliliter of effluent water and four 500-gram sediment samples were obtained from an effluent outlet conveying wastewater from two chemical plants in Iligan City. Serial dilutions were prepared and 0.1 ml portions were pour-plated using MS agar medium containing from 10 to 40 ppm of HgCl₂ for screening. The plates were then incubated at 37° for 48 hours. Biosorptive efficiency for mercury was performed in MS broth with 20 ppm HgCl₂. Mercury concentrations of the broth and cell pellet were measured using atomic absorption spectrophotometry (AAS) after 0, 24, 72 and 120 hours.

Isolate 4D showed a relatively high biosorptive efficiency of 78.56% after 72 hours when used as a single population. Isolates 2D and 1S exhibited increased biosorptive efficiency when used as a mixed population with a 1:1 ratio exceeding 90% after 72 hours.

We have therefore isolated bacterial strains that could be excellent candidates for bioremediation in heavy metal-contaminated environments. Further isolate characterization and design of cell immobilization systems for use in industrial effluent outlets are on-going endeavors in our laboratory.

Keywords. bioremediation, biosorptive efficiency, atomic absorption spectrophotometry, cell immobilization

BSD No. 21

**DETECTION OF MICROCYSTIN FROM CRUDE EXTRACTS OF
Microcystis Sp. COLLECTED FROM LAGUNA DE BAY AND
ITS EFFECT ON TILAPIA (*Oreochromis niloticus* L.) FINGERLINGS**

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Laguna de Bay is extensively being used for aquaculture and is considered an important source of livelihood for families living along its coastline. At present, the lake faces problems such as runoff, and siltation. Moreover, dumping of domestic and industrial wastes contributes to the formation of blooms of cyanobacteria which may be capable of producing toxins in the lake.

The most common cyanobacterial toxin encountered in fresh water is the cyclic heptapeptide microcystin which inhibits protein phosphatase type 1 (PP1) and type 2A (PP2A), which can both be found only in eukaryotic organisms. Microcystin is also a hepatotoxin which causes severe hepatic haemorrhage and possibly liver cancer.

In this study, crude extracts of a *Microcystis* sp. were assayed for the presence of microcystin. Moreover, the pathologic effect of the cyanobacteria on tilapia fingerlings (*Oreochromis niloticus* L.) was also determined.

Masses of the cyanobacterium were collected and extracted using absolute methanol. Microcystin was then detected by intraperitoneal injection on ICR strain laboratory mice. The mice showed classical symptoms of microcystin in-

toxication which indicated the presence of the toxin. The presence of microcystin was confirmed by thin layer chromatography (TLC) with the elution of toxic spots having retardation factor (RF) values of 0.660, 0.701, and 0.7045 which are close to literature values.

Meanwhile, three to four month-old tilapia fingerlings were fed with fresh cells of the *Microcystis* sp. for seven days. Deaths were observed after the 5th and 3rd day of exposure to concentrations of 1g and 2g cyanobacterial cells per liter, respectively. Hotelling's Trace statistical analysis showed that the treatments had a significant effect on the accumulated deaths of the fingerlings. Histopathological examinations revealed pronounced effects on the liver with the hepatocytes becoming enlarged and vacuolated, with the nuclei displaced at the periphery, and with lesions on all hepatic lobules.

Keywords: microcystin, *Microcystis* sp., tilapia, *Oreochromis niloticus* L., cyanobacteria

BSD No. 22

GLUTATHIONE-S-TRANSFERASE PROFILE OF CAGED-CULTURED TILAPIA IN LAKE TAAL

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Tilapia farming has emerged as a major global industry for food production in the 20th century and cage culture has been one of the rearing methods extensively practiced at present. This study presents the glutathione S-transferase (GST) profile of tilapia cultured in Leviste at Lake Taal, Batangas and relates it with the water quality conditions. GST is a detoxification enzyme found in the liver and levels of it are increased during stress.

The GST activity of tilapia liver samples from Leviste was determined for the months of October, November 2001, and January 2002. Fish were acclimatized in the laboratory for 24 hours. Liver samples were dissected, homogenized in cold phosphate buffer. Homogenate was ultracentrifuged at approximately 100,000g at 4° C. Supernatant containing the cytosolic GST was stored under ultra-low

refrigeration for subsequent analysis. Phosphate buffer and 2 mM glutathione were added to diluted samples. The spectrophotometer was set at 340nm and the enzyme activity was measured as the absorbance change/minute. GST levels steadily increase from October to January, that is, 0.04, 0.09, and 0.12 dA/min, respectively. The increase in GST values is correlated with the steady decline of water temperature in the succeeding months October to January (29.6, 28.4, 26.6 °C). As to dissolved oxygen and pH, lowest values of 2.97 mg/l and 7.9, respectively coincided with the GST peak of 0.12. This suggests that fish in cultured cages sampled in January seemed to be the most stressed ones.

Keywords: glutathione -S-transferase, liver, tilapia, culture cages, Lake Taal, Batangas

BSD No. 23

**ERYTHROCYTE VALUES OF CAGED NILE TILAPIA IN
LAKE TAAL INFECTED WITH GILL FLUKES**

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Oreochromis niloticus or Nile Tilapia is the predominant fish cultured in Lake Taal. Erythrocyte values and metazoan parasites of caged Nile Tilapia from the lake were studied from August 2001 to May 2002. Seven to 20 specimens were collected monthly for eight months from two sites in the lake. Monthly total erythrocyte counts and % hematocrit of Site 1 (Quiling) fish ranged from 1.41 to 1.97 x 10⁶ mm⁻³ and 20.8 to 37.0 %, respectively. Site 2 (Leviste) values ranged from 1.54 to 2.14 x 10⁶ mm⁻³ and 23.5 to 38.2%, respectively. *Cichlidogyrus* spp. were recovered from the gills of most fish from both sites. Monthly prevalences and mean intensities of infection in Site 1 fish ranged from 77.8 to 100% and 7.7 to 46.8 parasites, respectively. In Site 2, values ranged from 86.7 to 100% and 10.2 to 134.7 parasites, respectively. Red cell values of fish from the two sites were similar and fall within the range of normal values for teleosts. High prevalences of infection in samples were observed from both sites, the higher mean intensity values in Site 2 may reflect poorer cultural conditions in the site such as overcrowded cages.

Keywords: Nile Tilapia, Lake Taal, *Cichlidogyrus*, erythrocytes, hematocrit, gill parasites

BSD No. 24

**IMPORTANT INSECT PESTS OF SELECTED LIVE
ORNAMENTALS IN NURSERIES AND NATURAL STAND**

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Live ornamental plants like *Mussaenda*, *Aglaonema* and palms are now becoming important crops with export potential. These crops can be grown as potted plants that command higher price. The plants are used as indoor and outdoor decors and landscape. Little is known as to the occurrence of insect pests on these ornamentals.

Based on the survey conducted in ornamental nurseries and natural stand of the crops from most part of Luzon including Palawan, the Visayas (Tagbilaran City and Ubay Bohol; Cebu City and Mandaue City) and in Mindanao (Davao City), showed that mealybugs, *Pseudococcus* sp. and aphids, *Aphis gossypii* Glover, are the most serious insect pests of *Mussaenda*. Occasionally, tussock moth larvae, *Orgyia australis postica* Walker occur at a damaging level particularly during rainy months. The red spider mites, *Tetranychus* sp. is also serious in greenhouses during the summer months. On *Aglaonema*, mealybugs and scale insects are the more dominant insect pests collected while the bagworms occasionally occurred in high number causing considerable damage on Manila palms and Champaign palms.

Survey on the crop protection practices of crop nurseries showed that nursery owners are more concerned of plant diseases than insect pests. Whenever insect pests are observed, owners spray available insecticide blanket to all ornamentals grown in the nursery.

Keywords: insect pest, live ornamental, *Mussaenda*, *Aglaonema*, palms, mealybugs, aphids, tussock moth larva, red spider mites, bagworms.

BSD No. 25

**GENETIC VARIATION IN NATURAL POPULATION OF HONEYBEES
Apis cerana F. IN MT. MAKILING AND ALONG LAGUNA
DE BAY AREAS USING ISOZYME ANALYSIS**

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Starch gel electrophoresis of 45 nursed bees per feral colony of *A. cerana* F. from 3 localities in an upland area, Mt. Makiling and lowland areas along Laguna de Bay showed polymorphism for acid phosphatase-2 (Acph-2), alkaline phosphatase (Alph), esterases (Est) and malic enzyme (ME) and monomorphism for acid phosphatase-1 (Acp-1).

Among the 8 presumptive loci observed S (slow), M (moderate) and F (fast) isozymes were noted for Alph-2, Est-2 and ME; only S and F for Acph-2, Alph-1, Est-1 and Est-3, while only S for Acp-1. Only Acph-1 and Est-2 in the upland areas and Acph-1 and Acph-2 in the lowland areas showed a goodness of fit to the Hardy-Weinberg equilibrium.

Genotypes Acph-2 SF, Est-2 MF, Est-3 SF were observed only in Sta Cruz, Alph-2 MM in San Pablo and Anos, Est-1 SF exhibited in Forestry, Est-2 MM and ME MF were unique to Sta Cruz and Anos.

Localities within each area showed very high degree of genetic identity and did not vary much in terms of the types of alleles. The two areas had equal proportion of polymorphic loci (P) however greater average heterozygosity (H) was observed in Laguna de Bay populations. No significant difference in enzyme variability was observed between the pooled upland and lowland populations of Laguna based on P, H, average number of alleles per loci (A), genetic identity (I_s), genetic distance (D) and genotypic similarity (I_u).

Keywords: *Apis cerana* F., honeybee, isozyme, polymorphism, acid phosphatase, alkaline phosphatase, esterase, malic enzyme

BSD No. 26

**SOME STICK AND LEAF INSECTS (PHASMATODEA)
FROM MOUNT MAKILING, LAGUNA**

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The stick and leaf insects (Phasmatodea) are among the most unique and important features of terrestrial arthropod biodiversity in the tropics. This preliminary study was conducted to gain initial knowledge on the phasmatodean fauna of Mount Makiling with a view to expand later to more extensive faunistic/taxonomic studies of Philippine Phasmatodea. Four stick insects and two leaf insects are identified from specimens gathered in limited initial field work and as well as collections available at the Entomology Section of the UPLB Museum of Natural History. They are *Lonchodes mindanaense*, *L. nodulosus*, *Orthomeria pandora* *Pharnacia ponderosa*, *Phyllium sp. nr celebicum* and *Phyllium sp.* In addition, there are two undetermined stick insects belonging to the subfamily Platycraninae of Phasmatidae and Necrosiinae of Heteronemiidae. The nocturnal habit of these insects as well as the limited time and funds available have not favored more extensive collections and field work. However, despite the limitations, these initial results suggest that further and bigger studies on the Phasmatodea of Mount Makiling in particular and of the Philippines in general are worth pursuing and that more new species and new records await discovery.

Keywords: stick insects, leaf insects, Phasmatodea, Mount Makiling, terrestrial arthropod biodiversity

BSD No. 27

**TAXONOMY OF PHILIPPINE LAC INSECTS
(KERRIIDAE, COCCOIDEA, HEMIPTERA)**

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Lac insects are sources of commercial shellac, the raw material for varnish and other industrial products. They are not very common in the Philippines, there being hitherto only two known species that are difficult to recognize based on available antiquated and inadequate descriptions and illustrations. This taxonomic study was therefore conducted to establish their validity as species and facilitate their identification. *Kerria* (*Chamberliniella*) *greeni* (Chamberlin) and *Paratachardina minuta* (Morrison) are hence redescribed from specimens loaned from American and British natural history museums. These two have not been recollected since they were described some 80 years ago. *K. greeni* was originally described from specimens collected on *Ficus ulmifolia* on Mount Makiling whereas *P. minuta* was originally described from leaves of *Mangifera indica* on Basilan Island and was listed as a 'pest' of mangoes.. A third species belonging to the genus *Paratachardina* was collected from *Ficus* sp. from Imugan, Sta. Fe, Nueva Vizcaya and is also described as new to science. Scientific illustrations and a key to facilitate identification of adult females are provided and their possible conservation status is also noted.

Keywords: lac insects, Hemiptera, Kerriidae, Coccoidea, *Kerria*, *Paratachardina*

BSD No. 28

***Coniothyrium zuluense*: FIRST REPORT ON LEAF AND STEM
CANKER OF *Eucalyptus camaldulensis* IN THE PHILIPPINES**

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This study attempted to isolate and identify the causal agent of a leaf and stem canker disease observed in *Eucalyptus camaldulensis*. Distinguishing morphological characteristics were investigated using a scanning electron microscope (SEM). A fungal organism consistently isolated from infected tissues as well as in pathogenicity testing was confirmed by SEM observations to be *Coniothyrium zuluense* of Class Deuteromycetes (imperfect fungi), Order Sphaeropsidales. It can be cultured, producing pycnidia in artificial media. Symptoms initially appeared as measles-like spots, transforming into canker appearance and eventually progressing into a necrotic lesion. Cracking of the main stem was observed in severely damaged tree. This is the first recorded fungal pathogen of *Eucalyptus camaldulensis* in the Philippines. It is one of the most serious threats to the species.

Keywords: leaf and stem canker, *Eucalyptus camaldulensis*, *Coniothyrium zuluense*

BSD No. 29

**THE ROLE OF PLANTS IN THE CULTURAL PRACTICES
OF THE KALANGUYAS IN TINOC, IFUGAO,
CORDILLERA REGION, PHILIPPINES**

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The diversity of plant species in the Cordillera Region, the northernmost part of the Philippines is matched by an equally rich cultural diversity. However, both have not been systematically recorded and documented. This paper presents the relationship between humans and plants with the integration of the cultural practices regarding plant use among the *kalanguyas* in Tinoc, Ifugao Province.

The *kalanguyas*, who dominate the towering mountains of Tinoc, one of the Municipalities of Ifugao Province exhibit unique cultural practices. One aspect, which is the focus of this study, is plant utilization. The methods used were individual interviews, focused group discussions, field research and direct observation. Key informants were identified and participants included the senior citizens and mabakis (pagan priests). Taxonomic method was used in the collection and processing of useful plants. Voucher specimens served as materials for identification and validation.

There are 293 identified useful plant species in Tinoc, Ifugao. These are distributed to 96 families, which include the major plant groups: ferns, gymnosperms and angiosperms. Indigenous uses of plants among the *kalanguyas* are integrated into every facet of their daily lives. These uses ranges from the most basic use to a variety of uses such as food, clothing, shelter, adornment, cordage, dyes, toys, rituals, basketry, medicines, musical instruments, cosmetics, poisons, tools, transportation, weapons, soil and water conservation, ornamentals and many more. The list is endless. To this indigenous group, the forests served as their natural grocery store, the pharmacy and the hardware store. This indigenous knowledge of plant use by the *kalanguyas* has evolved for thousands of years. How they utilize plants can also help define the basic elements of their earlier culture.

The distribution of traditional knowledge on plant use indicated that the more aged segment of this indigenous group held much of the information hence there is a need to capture the indigenous knowledge before it is irretrievably lost to future generations.

Keywords: *kalanguyas*, indigenous practices, *mabaki*, Tinoc, Ifugao, ritual plants.

BSD No. 30

OUTSTANDING ENDEMIC HOYAS OF THE PHILIPPINES

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The Philippines possesses one of the richest and most diverse range of hoyas species in the world. Hoya belongs to the Asclepiadaceae family. The plant is characterized by shiny waxy leaves, hence the common name "wax plant". Most of the hoyas species have a climbing or viny habit, however some species are short and bushy. The flowers are so striking and beautiful resembling a star with sweet lemony fragrance at night.

In the Philippines, hoyas can be found all over the islands at all altitudes. To date there are about 51 species which have been identified. It is sad to note, however, that it is one of our most outstanding endemic ornamental plant which have been relegated to the background and was not given much interest and attention in terms of research and media mileage. Most of these species are now in private collections especially those avid hoyas enthusiasts and rare ornamental collectors most of whom are foreigners. Their number is rapidly depleting and most of these rare hoyas have found their way to other countries. Thus, other countries which are less endowed in terms genetic resources continue to forge ahead and profit from a global hoyas industry to the detriment of our national heritage and economy. Active measures like recollecting, preserving and utilizing these valuable germplasm have to be done now.

Keywords: hoyas, endemic

BSD No. 31a

**SCANNING ELECTRON MICROSCOPIC STUDIES OF THE LEAF
EPIDERMAL FEATURES OF FOUR PHILIPPINE PLANTS
AFFECTED BY CEMENT DUST POLLUTION**

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The cement industry is considered one of the polluting enterprises in the Philippines. Dust from cement factory kilns, which is injurious to plant life, has a very high air pollution potential. The intensity of cement pollution in an area can be gauged via the degree of injury imparted on the morphological and physiological characteristics particularly the modifiable leaf epidermal characters.

In this study, leaves of four plant species viz., *Bougainvillea spectabilis* Willd., *Hibiscus rosa-sinensis* Linn., *Mangifera indica* Linn. and *Psidium guajava* Linn. from the vicinity of three cement factories (Central Cement and Hi-Cement in Bulacan; Rizal Cement in Rizal) and from presumably relatively unpolluted areas (Montalban, Rizal and Pulilan, Bulacan) were compared in terms of four features viz., (1) stomatal density, (2) stomatal size, (3) trichome density and (4) trichome length. This was done to determine their potentials as bioindicators of pollution. Quantitative measurements and statistical analysis using the MANOVA and Waller-Duncan K-ratio T-test showed significant differences between plants from cement factory sites and control sites. Stomatal density and trichome length significantly changed for *Psidium* while variation in stomatal density was highly significant in *Hibiscus*. There were no significant changes in the four features for *Bougainvillea* and *Mangifera*. Scanning electron microscopy showed that epicuticular wax is abundant in *Bougainvillea* and *Mangifera* leaves from cement factory areas in contrast to those from the control areas. Wax nearly completely occluded the chambers of most stomata of *Hibiscus* leaves from cement-polluted areas while it partially occluded the stomata of leaves from the control areas.

Keywords: bioindicators, cement, leaf, pollution, scanning electron microscopy, stomata

BSD No. 31b

MORPHO-ANATOMICAL RESPONSES OF *Blumea balsamifera* (L.)DC. (SAMBONG) TO DIFFERENT LIGHT ENVIRONMENTS

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Blumea balsamifera, an aromatic weed found in open and partly shaded areas was grown under three different light environments designated as low light (LL), medium light (ML) and high light (HL) to determine any morpho-anatomical variations in response to different light intensities for a period of two months. A fourth group was grown under greenhouse conditions as control. Plant height, stem thickness, specific leaf weight (SLW) and leaf surface area were noted. The anatomical features measured were total blade thickness, thickness of upper and lower epidermis, and mesophyll thickness. Results show that among the morphological attributes measured, only leaf surface area was significantly altered by exposure to the HL set-up. However, anatomical measurements show an increase in leaf thickness in both ML and HL set-ups as compared to LL and the control. The differences in leaf thickness was due to changes in the thickness of the mesophyll, brought about by concomitant increases of either the size of the cells or the number of mesophyll layers or both. The leaf anatomy was also characterized according to the size and shape of epidermal cells and the number of epidermal layers, presence of cuticle, location of stomata, the presence and type of crystals and trichomes, and the arrangement of vascular tissues in the midrib. The mesophyll of *B. balsamifera* is undifferentiated, although the layers comprising the mesophyll have distinct characteristics resembling palisade and spongy features. The results of this study will be useful in finding the optimum light conditions for large scale cultivation and effective management of this medicinal plant.

Keywords: crystals, trichomes, mesophyll, epidermis, vascular tissues

BSD No. 32a

**IDENTIFICATION OF MOLECULAR MARKERS FOR MUNGBEAN
(*Vigna radiata* (L.) WILCZEK) BRUCHID (*Callosobruchuschinesis* L.)
RESISTANCE BY RANDOM AMPLIFICATION OF
BULKED GENOMIC DNA SAMPLES**

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To identify Random Amplified Polymorphic DNA (RAPD) markers that are potentially linked to the bruchid resistance gene, 200 recombinant inbred lines from a cross between NM 92 (cultivated variety) and TC 1966 (a *V. radiata* var. sublobata accession resistant to bruchid) were bioassayed and the DNA were extracted. Results from the bioassayed revealed that 44 were highly resistant (seed damage= 0%), while 38 were highly susceptible (seed damage< 80%). The DNA of resistant individuals were bulked into two (R1 and R2) and so were the DNA from susceptible individuals (S1 and S2). Both R1 and R2 were composed of 22 individuals while s1 and S2 were composed of 20 and 18 individuals, respectively. Parental DNA was screened using 240 Operon primers and 379 UBC primers for polymorphism. A total of 158 Operon primers and 204 UBC primers were able to distinguish polymorphism between the parents and these primers were subsequently used in screening the bulk samples and the nearly isogenic lines (NILs). From a total of 360 primers used in the screening of bulk samples, four primers (Operon T 16, Operon V02, UBC 193 and UBC 313) produced fragments unique to the resistant samples including the resistant NIL. Thus, there is a possibility that these fragments are linked in cis with the bruchid resistance gene for they cosegregate.

Keywords: RAPD, bruchid resistance, *Vigna radiata*, genomic, DNA, molecular markers

BSD No. 32b

**QUANTITATIVE AND QUALITATIVE ANALYSES OF
CORN (*Zea mays* L. IPB VAR 911) SEEDLINGS
TISSUES EXPOSED TO LEAD NITRATE**

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Responses of plants to toxic and essential substances are manifested in various levels of organization. Cellular aberrations may be present but are not reflected in the gross morphology of plants. In this study, corn seedling grown in potted soil were subjected to various concentrations of lead nitrate [$\text{Pb}(\text{NO}_3)_2$]. After 21 days of treatment, harvested plant samples were processed using the Paraffin Microtechnique procedures. Prepared slides were analyzed using quantitative and qualitative anatomical parameters. Results revealed significant ($P < 0.05$) enlargement of root pith, root cortex, stem ground parenchyma, stem vascular bundle, leaf vascular bundle and diameter of root metaxylem at the higher treatments [HT-2000 and 5000 mg kg⁻¹ Pb(NO₃)₂]. A similar increase in the number of root metaxylem cell at 500 and 5000 mg kg⁻¹ treatments was also noted. However, remarkable decreases in the number of root metaxylem cell, widths of root pith, root cortex at 100 mg kg⁻¹ treatment and reduction of width of stem ground parenchyma and stem vascular bundles at 500 mg kg⁻¹ treatment were obtained, respectively. Photomicrographs of root cortical tissue exposed to HT showed disrupted cell walls. Likewise, there was an apparent damage of root metaxylems in the treated samples. Distortion of root cortex was observed at 2000 and 5000 mg kg⁻¹ treatments, while aberration of stelar area was noticed at 500 mg kg⁻¹ treatment. Results suggest that the nitrate counter ion brought the positive growth of tissues, however, the negative effects of Pb on cells and tissues were still apparent.

Keywords: corn, anatomy, nitrate, toxic heavy metal, Pb, analysis

BSD No. 33

**LIGHT MICROSCOPY STUDIES ON THE PATTERN AND
DISTRIBUTION OF CALCIUM OXALATE CRYSTALS
IN *Azadirachta indica* L. (NEEM)**

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Plants accumulate crystals of calcium oxalate and calcium carbonate in a variety of shapes, sizes, amounts, and locations. To gain insight into this phenomenon, the morphology and distribution of these crystals in mature leaves of *Azadirachta indica* L. (Neem) were studied using light microscopy. *Azadirachta indica* L. commonly known as Neem is considered as one of the most promising trees of the 21st century. It has great potential in the fields of pest management, in environment protection, in helping the control of diseases like malaria and AIDS, in combating desertification and deforestation, in reducing excessive global temperature, and even contribute to population control. Styloids and raphide crystals, some with peculiar forms, were observed in the epidermal cells of cleared leaves. Cross sections of the leaves also showed druse crystals located in the mesophyll layers. These three types of crystal forms found in different tissues indicate that calcium oxalate crystallization, a biomineralization process, does not occur at random but are always located in specific tissues.

Keywords: crystal, calcium oxalate, raphide, druse, neem, biomineralization

BSD No. 34

**ESTROGENIC ACTIVITY SELECTED PLANT CRUDE EXTRACTS ON
THE MAMMARY GLAND OF SARCOMA-INOCULATED FEMALE MICE**

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This is a study on the estrogen receptor in mammary gland of sarcoma-treated ICR mice. The plants used have been identified to contain phytoestrogen – chemical which have similar structure to estrogen. They are implicated in the reduction of hormone-related cancers by competitively inhibiting

the binding of endogenous oestrogens and xenoestrogens to receptor sites.

Female ICR mice, weighing 25-30 were given subcutaneous injection of 10 million sarcoma T 180 cells. The experimental group were treated with either garlic, ginger or onion crude extract, dose of 7mg/30 g body weight at three different schedules: 3 days before sarcoma injection, 3 days after sarcoma injection and simultaneous with sarcoma injection. Thereafter, each group was injected twice a week with crude until day 30 past sarcoma treatment.

Mammary glands were excised, fixed in 4% formalin and proceed by light microscopy. Immunohisto-chemical reactions were performed using mouse estrogen monoclonal antibodies. Qualification of estrogen receptor was evaluated at 400x following the protocol of Zava et. al., (1977)

Among the three extracts, garlic and ginger showed estrogen receptors.

Keywords: estrogen, sarcoma, mammary gland

BSD No. 35

**EFFECTS OF *Carica papaya* L., *Hippobroma longiflora* (L.)
G. DON., *Strophanthus cumingii* A. DC., AND *Vitex negundo* L.
IN VERTEBRATE NERVE, SKELETAL AND HEART MUSCLES**

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Bioactive substances in plants, presumably, the defenses of the plants against their natural enemies affect animals in various ways. In this study, the effects of *Carica papaya* L. (papaya) *Hippobroma longiflora* (L.) G Don (estrella), *Strophanthus cumingii* A. DC., and *Vitex negundo* L. (lagundi) were tested on the toad heart *in situ*, toad sciatic nerve-gastrocnemius muscle preparation, and the isolated guinea pig atrium. The commercialized tablet of lagundi (Ascof) was also tested. The results of the experiment could be useful in the evaluation of the plants for their potential as source of drugs or in the case of *V. negundo* and *C. papaya* for their more effective management as herbal medicines. Ethanol

extracts of various parts of the plants at varying doses were applied either by immersion of the tissues in baths containing them or parenterally, as for the experiments with the toad heart *in situ*. The monitoring of responses to the treatments were done electronically. Notable results were the (1) 62% reduction of skeletal muscle activity by papaya seed (5%) with recovery to maximum 87% after washing, (2) significant neurotoxicity of estrella leaves and stems causing reduction of muscle activity, respectively, of 61.4% and 40.2%, (3) negative chronotropic effects of papaya seed (5% and 10%), (4) on the toad heart *in situ*, positive inotropic effects of *S. cumingii* young stems and flowers (5% and 10%) but negative inotropic effects at 20% and toxicity at 40%, (5) on the isolated guinea pig atrium, negative chronotropic and negative inotropic effects of lagundi leaves and tablet with initial positive inotropic effect only at low dose tablet, cardiotoxicity of 0.6% leaf and 0.4% flower extract, and (6) the inaction of lagundi on the muscarinic receptors of the heart. The data were analyzed by Student's T-test, analysis of variance, and Tukey's Test.

Keywords: *Carica papaya*, papaya, *Hippobroma longiflora*, estrella, *Strophantus cumingii*, *Vitex negundo*, lagundi, neurotoxin, neuromuscular toxin, chronotropic effect, inotropic effect

BSD No. 36

**THE PROTECTIVE ACTION OF THE LEAF EXTRACTS OF
Mangifera indica Linn., *Premna odorata* Blanco,
AND *Psidium guajava* Linn. AGAINST ETHANOL-INDUCED
LIVER LIPID PEROXIDATION**

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Studies have indicated association between health promoting properties of plant foods and medicines with active phytochemicals including antioxidants. In the present study, the antioxidant activities of the leaves of three plants namely

the mango (*Mangifera indica* var. *indica* Linn.), "alagao" (*Premna odorata* Blanco), and guava (*Psidium guajava* Linn.) were assessed. Ethanol extracts of young tender leaves, young mature leaves, and old mature leaves, were tested for their abilities to inhibit the lipid peroxidation that can happen in the liver of mice that were administered ethanol or that which can happen spontaneously. For a period of 7 days prior to the administration of alcohol (3g/kg) *per os* or distilled water, leaf extracts (1.5g/kg) were fed daily to the experimental animals. These tests were done alongside both positive and negative controls that were not given the plant extracts but treated respectively with ethanol and water on the eighth day following administration of water for 7 days. During the experiments, the animals had free access to pellet food and drink. Four hours after the last treatment, the mice were sacrificed, the livers excised and assayed for lipid peroxidation product malondialdehyde using the thiobarbituric acid reactive substances (TBARS) assay procedure. The results showed the significant inhibition of spontaneous lipid peroxidation by all types of leaves of *P. odorata* (28-40%) and the young tender leaves of *M. indica* (7%) and the significant inhibition of ethanol-induced liver lipid peroxidation of all types of leaves of *P. odorata* (102-124%), *M. indica* (93-111%), and *P. guajava* (97-111%). The lipid peroxidation in plant and alcohol-treated mice could go lower than negative control levels. Maximum protection was offered by the young tender leaves.

Keywords: *Mangifera indica*, mango, *Premna odorata*, alagao, *Psidium guajava*, guava antioxidant, lipid peroxidation, herbal medicines, TBARS assay

**CHEMICAL MATHEMATICAL AND PHYSICAL SCIENCES
DIVISION**

CMPSD No. 1

SUBDIVISION NUMBERS OF THE CLOSURE OF SOME GRAPHS

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The *closure* $c(G)$ of a graph G of order p is the graph obtained from G by recursively joining pairs of non-adjacent vertices whose degree sum is at least p until no such pairs remain. A *unit graph* in the Euclidean n -space R^n is a graph whose vertices are points in R^n and every pair of adjacent vertices $|x - y| = 1$, satisfy, where $|x - y|$ denotes the Euclidean distance between x and y .

The *subdivision number* of a graph G , denoted by $sd(G)$, is the minimum number of vertices to be inserted into the edges of G to make it isomorphic to a unit graph in R^n . In 1998, Gervacio and Maehara found the subdivision numbers of the complete graph and the complete bipartite graph. The subdivision numbers of the closure of some graphs are determined in this study.

For the closure of the cycle, the wheel, and the fan, we obtain the following results:

$$i) \quad sd(c(C_n)) = \begin{cases} 0 & \text{if } n \neq 4, \\ 2 & \text{if } n = 4. \end{cases}$$

$$ii) \quad sd(c(W_n)) = sd(c(F_n)) = \begin{cases} 2 & \text{if } n = 3, \\ 4 & \text{if } n = 4, \\ 8 & \text{if } n = 5, \\ 0 & \text{if } n = 6. \end{cases}$$

$$iii) \quad \text{for } n \geq 7, \quad sd(c(W_n)) = \left\lfloor \frac{n}{6} \right\rfloor \quad \text{and} \quad sd(c(F_n)) = \left\lfloor \frac{n-1}{6} \right\rfloor$$

Keywords: closure, unit graph, subdivision number

A BER EXPRESSION FOR A CHANNEL WITH
AWGN AND ACI

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Abstract

We present a closed form expression for the average bit error rate (BER) for a binary phase-shift keying (BPSK) modulated signal corrupted with additive white Gaussian noise (AWGN) with adjacent channel interference. The simplest mathematical model for a communication system is the additive white Gaussian noise channel, where the transmitted signal is corrupted by additive random process $n(t)$, resulting in a received signal in the form $r(t) = s(t) + n(t)$, where $n(t)$ is a random process with zero mean and variance $N_0/2$. If ACI is added into the model, the received signal is $r(t) = s(t) + n(t) + a(t)$, where $a(t)$ is ACI component. We consider two cases: we first assume that noise from the adjacent channel is constant, i.e., $a(t) = \alpha$, and also consider the case when $a(t)$ is a random process. To create a closed form BER for the two cases, we take the convolution of the distributions of $n(t)$ and $a(t)$, and we identify the conditional pdfs corresponding to each BPSK signal. For the constant case, the average BER was found to be

$$\bar{\epsilon} = Q(2\rho),$$

which is the theoretical BER for AWGN with no ACI (here, Q is Q -function). For the case where $a(t)$ is a random process, we assume a trigonometric probability distribution given by

$$f_a(z) = \begin{cases} \frac{1}{\pi\sqrt{\alpha^2 - z^2}}, & |z| < \alpha, \\ 0, & |z| \geq \alpha, \end{cases}$$

where we let z be the random ACI variable with magnitude α . The BER

obtained for this case is

$$\bar{\varepsilon} = \frac{1}{2\sqrt{\pi^3 N_0}} \left[\int_{-\infty}^0 \int_{-\alpha}^{\alpha} \frac{e^{-\frac{(z-\sqrt{E_b}-zt)^2}{N_0}}}{\sqrt{\alpha^2 - \xi^2}} dz d\xi + \int_0^{\infty} \int_{-\alpha}^{\alpha} \frac{e^{-\frac{(z+\sqrt{E_b}-zt)^2}{N_0}}}{\sqrt{\alpha^2 - \xi^2}} dz d\xi \right],$$

where ξ is the random Gaussian variable and E_b is the BPSK signal energy.

Keywords: BPSK, BER, AWGN, convolution

CMPSD No. 3

CHARACTERIZATION OF SEMI-CONTINUITY IN THE CARTESIAN PRODUCT

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N. Levine introduced the concepts such as semi-open set and semi-continuity in topological spaces. The class of all semi-open sets in a topological space includes all open sets. Although an arbitrary unions of semi-open sets is semi-open, the class does not always form a topology on the underlying set.

Now, given a family of topological spaces $\{Y_\alpha : \alpha \in \Omega\}$ and a function f from a topological space X into the Cartesian product Y of the spaces Y_α with the Tychonoff topology, it is well known that f is continuous if and only if each coordinate function $p_\alpha \circ f$ is continuous. In this paper, we give a necessary and sufficient condition for function f to be semi-continuous. More precisely, the results obtained are as follows:

- (1) If O is a non-empty semi-open set in the Cartesian product space Y , then $p_\alpha(O) = Y_\alpha$ for all but at most finitely many α and $p_\alpha(O)$ is semi-open for every $\alpha \in \Omega$.
- (2) Let $S = \{\alpha_1, \alpha_2, \dots, \alpha_t\}$ be a finite subset of Ω and $O_\alpha \subseteq Y_\alpha$ for each $\alpha_i \in S$. Then $\langle O_{\alpha_1}, O_{\alpha_2}, \dots, O_{\alpha_t} \rangle$ is semi-open in Y if and only if each O_{α_i} is semi-open in Y_{α_i} .
- (3) Let X be an arbitrary space and Y the product space. A function $f: X \rightarrow Y$ is semi-continuous on X if and only if each coordinate function $p_\alpha \circ f$ is semi-

continuous on X .

(4) Let X and Y be product spaces of the families $\{X_\alpha : \alpha \in \Omega\}$ and $\{Y_\alpha : \alpha \in \Omega\}$ respectively. For each $\alpha \in \Omega$, let $f_\alpha : X_\alpha \rightarrow Y_\alpha$ be a function. If each f_α is semi-continuous on X_α , then the function $f : X \rightarrow Y$ defined by $f = \{f_\alpha\}$ is semi-continuous on X .

Keywords: topology, subbase, base, open set, semi-open set, continuous, semi-continuous, Tychonoff topology, product space

CMPSD No. 4

NUMERICAL COMPUTATIONS FOR PARAMETER ESTIMATION IN A SMART BEAM STRUCTURE

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We present a method to estimate the parameters of a smart beam structure. The data consists of beam displacements taken at different time instances but due to unavailability of an actual experimental setup, we will use numerically simulated data. The model used is the Euler-Bernoulli equation modified to include internal damping and passive actuator contributions. Piezoceramic patches were used as the smart materials. The parameters we estimate are the density, stiffness and damping of both the beam and patches, and also the dielectric constant of the patches. The first step is to numerically discretize the PDE describing the vibrations of the beam. The Galerkin approximation method using cubic splines as basis functions is used. Then, numerically simulated data is collected by simulating the PDE and recording numerical displacements at one point on the beam and at different time instances. The parameters used in simulating data will be the "true" parameters of the system. Then we formulate the cost function that returns the difference between data and numerical displacements. Finally, the Nelder-Mead optimization algorithm is used to obtain the minimizer of the cost function. Numerical results show that the method can obtain the "true" or "estimated" parameters of the system even if noise is added onto the data. We were also able to determine that the initial guess supplied to the numerical optimizer can have an error (i.e., difference from the "true" parameters) of up to 10% and still

the method can obtain the optimal parameters. We will present the true and estimated parameters for different data noise and different initial guess.

Keywords: smart materials, parameter estimation, optimization

CMPSD No. 5

**ON CONNECTED GRAPHS THAT INDUCE THE INDISCRETE
AND THE DISCRETE TOPOLOGIES**

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There are various ways of constructing a topological space from a given graph $G = (V(G), E(G))$. Indeed, depending on the conditions imposed on some sets, one can generate a topological space from a given graph $G = (V, E)$. Diesto and Gervacio have successfully given one construction of a topological graph. Their construction was further investigated by Guerrero, Canoy, and Lemence.

In this paper, we present a way of constructing a topology $T(G)$ from a connected graph G . In this type of construction, $T(G)$ is the indiscrete topology on $V(G)$ if and only if G is the trivial graph. We also characterized those connected graphs which induce the discrete topology. Specifically, we have obtained the following major results:

- (1) Let $G = (V(G), E(G))$ be a connected graph. Then $T(G)$ is the indiscrete topology on $V(G)$ if and only if $G = K_1$.
- (2) Let $G = (V(G), E(G))$ be a connected graph. Then $T(G)$ is the discrete topology on $V(G)$ if and only if for every $a \in V(G)$ such that $D_1(a) \neq \emptyset$, the set $D_2(x) \setminus [D_2(a) \cup \{a\}] \neq \emptyset$ for every $x \in D_2(a)$, where $D_2(a) = \{x \in V(G) : d(x, a) = 2\}$.
- (3) If K_n is the complete graph of order $n > 1$, then $T(K_n)$ is the discrete topology on $V(G)$.
- (4) If W_n is the wheel of order $n + 1$, where $n \geq 5$, then $T(W_n)$ is the discrete topology on $V(G)$.

(4) Let C_n is the cycle of order $n \geq 3$. Then $T(C_n)$ is the discrete topology on $V(C_n)$ if and only if $n \in \{4,6\}$.

Keywords: connected graph, topology, base, indiscrete, discrete, complete graph, wheel, cycle

CMPSD No. 6a

**SHADOWABILITY OF STATISTICAL AVERAGES IN A NONHYPERBOLIC
CHAOTIC DYNAMICAL SYSTEM WITH UNSTABLE DIMENSION VARI-
ABILITY: A SIMULATION STUDY**

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Results of the study of Lai (1999) showed that the natural measure of a nonhyperbolic chaotic system can be related to the dynamical properties of all unstable periodic orbits embedded in part of a chaotic set contained in that region. Furthermore, he showed that at blowout bifurcation point there is a change in the transverse stability of an infinite number of unstable periodic orbits embedded in the chaotic attractor in the invariant subspace. Thus, for this system, the chaotic attractor becomes transversely unstable at this point. In effect, the natural measure of the chaotic attractor is unstable.

Motivated by this result, this research study investigated through simulation the stabilities and dynamics of statistical averages at blowout bifurcation point, a phenomenon in nonhyperbolic chaotic dynamical system with unstable dimension variability (UDV), which occurs when a chaotic attractor lying in some invariant subspace, becomes transversely unstable. In particular, the sensitivity of the mean and variance under various perturbations for the Henon type model was explored at the blowout bifurcation point.

The main conclusion of this paper is that systems having UDV are not statistically shadowable. The instability of statistical averages has deep conse-

quences particularly in the validity of the model. As a result, there is a wide parameter regime for which the model does not accurately represent the deterministic evolution and statistical properties of the real system. Thus, no reliable information, deterministic or statistical, can be obtained.

Keywords: nonhyperbolic chaotic dynamical system, unstable dimension variability, natural measure, shadowability, blowout bifurcation point

CMPSD No. 6b

SPAN OF PARTITE GRAPHS

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A unit graph in the Euclidean n -space R^n is a graph whose vertices can be represented by points in R^n such that the distance between points representing adjacent vertices is equal to unity. If G is a unit graph in R^n and \hat{G} is one such representation, then \hat{G} is called a unit representation of G in R^n . If \hat{G} is a unit representation of a graph G , then there is a smallest ball containing \hat{G} . The infimum of the diameters of these balls taken over all unit representations of G in R^n is called the span of G , denoted by $span_n G$.

For an n -partite graph G , $span_n G \leq \sqrt{2(n-1)/n}$. If G is not empty, $span_n G$ for each $n \geq 4$. For the complete n -partite graph G , $span_n G \leq \sqrt{2(n-1)/n}$. For the complete bipartite graph $K(r, s)$, $span_n K(r, s)$ is 1, 2, or $\sqrt{2}$ depending on n, r , and s .

Keywords: Euclidean space, unit graph, unit representation, ball, infimum, span, n -partite graph, complete n -partite graph

CMPSD No. 7a

ON THE HULL NUMBER OF THE COMPOSITION OF GRAPHS

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Given a connected graph $G = (V(G), E(G))$, the couple $(V(G), d)$, where $d(u, v)$ is the length of a shortest path connecting vertices u and v in G is a metric space on $V(G)$. Any u - v path of length $d(u, v)$ is called a u - v geodesic. A subset C of $V(G)$ is convex if for every two vertices u and v in C , the vertex set of every u - v geodesic is contained in C .

If u and v vertices of a graph G then the set $I[u, v]$ is the closed interval consisting of u and v and all vertices lying on a u - v geodesic of G . If S then $I[S]$ is the union of $I[u, v]$, where u and v range over all elements of S . The convex hull $[S]$ of S is the smallest convex set containing S . It can be formed from the sequence $\{I^p[S]\}$, where p is a nonnegative integer, $I^0[S] = S$, $I^1[S] = [S]$, and $I^p[S] = I^{p-1}[S]$ for $p > 2$. For some p , we must have $I^q[S] = I^p[S]$ for all $q \geq p$. Further, if p is the smallest nonnegative integer such that $I^q[S] = I^p[S]$ for all $q \geq p$, then $I^p[S] = [S]$. A set S of vertices of G is called a hull set in G if $[S] = V(G)$ and a hull set of minimum cardinality is called a minimum hull set. The hull number of G , denoted by $h(G)$, is the cardinality of a minimum hull set in G .

In this paper, we give the hull number of the composition of two connected graphs. Among others, we obtained the following major results:

- (1) Let G and H be connected graphs. If H is non-complete, then $h(G[H]) = h(H)$ if G is the trivial graph and $h(G[H]) = 2$ if otherwise.
- (2) Let G be a connected graph and K_m be the complete graph of order m . Then $h(G[K_m]) = h(G) + (m-1)|A_e|$, where A_e is the set of extreme vertices of G .

- (3) Let G be a connected graph of order $n > 3$ and K_m be the complete graph of order m . If G has no extreme vertices, then $h(G[K_m]) = h(G)$.
- (4) Let G be a connected graph of order n and K_m be the complete graph of order m . If A_e (the set of extreme vertices of G) is a hull set in G , then $h(G[K_m]) = m|A_e|$.

Keywords: graph, geodesic, convex, convex hull, hull set, minimum hull set, hull number, composition of graphs

CMPSD No. 7b

**ON TESTS OF MULTIVARIATE HYPOTHESES BASED ON THE ROOTS
OF CERTAIN DETERMINANTAL EQUATIONS**

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Multivariate tests in normal samples based on the roots of its associated matrix sometimes result in different tests. Quite a number of tests of hypothesis using maximum likelihood estimation (m.l.e.) and Union-Intersection (UI) approaches often result to the same test, but there are cases when they lead to different tests. From the nature of the determinantal equations resulting from both approaches, it is clear that functions of the roots of these equations are being used to represent the univariate analogue of variance 2; that is, $\det(*)$ and $\text{tr}(*)$, the determinant and trace of the associated matrix (*). The difference thus arises due to the difference in the invariant measures of the matrix "structure" or configuration under test. In addition, both tests would be inadequate or insufficient in completely describing the structure. This paper proposes the use of the set of e.s.f (or trace statistics) tests to complete the structural tests on the associated matrix. Relevant distributional issues are discussed.

Keywords: multivariate test, associated matrix, determinantal equations

CMPSD No. 8a

ALTERNATIVE METHODS FOR SOLVING
A TWO-DIMENSIONAL CARTESIAN TSUNAMI

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Abstract

In this paper, we formulate two alternative finite-difference methods for solving the system of partial differential equations describing a Cartesian tsunami in two-dimensions. In both methods, we will employ the explicit method for solving the parabolic partial differential equation describing the variation of the vorticity with time. Furthermore we will use the Gauss-Seidel method for solving the elliptical partial differential equation describing the stream function. The only difference between the two methods is that one will use a static grid and the other will use a dynamic grid. The formulation for the static grid with increments Δx and Δz (which correspond to the grid point coordinates x_i and z_j , respectively) do not vary in time. On the other hand, the formulation for the dynamic grid with increment Δx (corresponding to grid point coordinates x_i) do not vary in time; however, the increment Δz (corresponding to the grid point coordinates z_j) do vary in time. If we denote the velocity in the x and z directions by u and w , respectively, denote the pressure by p , and if we denote the density of the fluid by ρ , and viscosity by μ , then the laws of conservation of mass and momentum are given by:

The equation of continuity within the hydrosphere:

$$\frac{\partial u}{\partial x} + \frac{\partial w}{\partial z} = 0$$

The equation of x -directed motion within the hydrosphere:

$$\rho \left[\frac{\partial u}{\partial t} + u \frac{\partial u}{\partial x} + w \frac{\partial u}{\partial z} \right] = - \frac{\partial p}{\partial x} + \mu \left[\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial z^2} \right]$$

The equation of z -directed motion within the hydrosphere:

$$\rho \left[\frac{\partial w}{\partial t} + u \frac{\partial w}{\partial x} + w \frac{\partial w}{\partial z} \right] = - \frac{\partial p}{\partial z} + \mu \left[\frac{\partial^2 w}{\partial x^2} + \frac{\partial^2 w}{\partial z^2} \right] - \rho g$$

The equation of continuity and motion at the ocean surface

$$\frac{\partial h}{\partial t} = w_n .$$

We will derive the governing equations given above, and also determine which of the two (alternative) methods yields better results.

Keywords: tsunami, finite difference, partial differential equations, law of conservation

CMPSD No. 8b

VERTEX COVER OF GRAPHS

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Let G be a graph. A set U of vertices in G is a vertex cover of G if every edge in G is incident with a vertex in U . The vertex covering number of G , denoted by $\alpha(G)$, is given by $\alpha(G) = \min\{|U|; U \text{ is a vertex cover of } G\}$. If H is a subgraph of G , then $\alpha(H) \leq \alpha(G)$. For path P_n , cycle C_n , complete graph K_n , complete bipartite graph $K_{m,n}$, and Petersen graph P , we have, $\alpha(P_n) = \lfloor n/2 \rfloor$, $\alpha(C_n) = \lfloor n/2 \rfloor$, $\alpha(K_n) = n-1$, $\alpha(K_{m,n}) = \min\{m, n\}$, and $\alpha(P) = 6$.

Let \bar{K}_n be the empty graph of order n . Then $\alpha(K_1 + G) = 1 + \alpha(G)$ and $\alpha(\bar{K}_n + G) = |V(G)|$ if $n \geq |V(G)|$. Moreover, $\alpha(\bar{K}_n + G) \leq \min\{n + \alpha(G), |V(G)|\}$. It follows that $\alpha(\bar{K}_n + K_n) = n$, and $\alpha(F_{n,m}) \leq \min\{n, m + \lfloor n/2 \rfloor\}$, where $F_{n,m}$ is the generalized fan and $W_{n,m}$ is the generalized wheel.

The following characterizations were also obtained: (a) characterization of vertex cover in terms of vertex independent set, (b) characterization of all graphs with vertex covering number equal to 1, and (c) characterization of hamiltonian complete bipartite graphs in terms of order and vertex covering number.

Keywords: graph, vertex cover, vertex covering number, independent vertices, hamiltonian graph

CMPSD No. 9a

NUMERICAL COMPUTATIONS OF TSUNAMIS THAT CAN BE GENERATED BY MANILA TRENCH EARTHQUAKES

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This paper presents numerical computations of possible tsunamis triggered by earthquakes in the Manila trench. In the study, different earthquake scenarios were created at different points on the Manila trench and with varying fault parameters (such as magnitude, rupture direction of the fault plane, etc.) of an earthquake source model then compute the resulting tsunami amplitude for each scenario. This set of different scenarios were considered as a basis for the sensitivity analysis of tsunami wave characteristics near coastlines to identify earthquake parameters that are most influential in tsunami generation from the Manila trench.

Linear and nonlinear conservation of momentum equations were used in the computations. The nonlinear terms can be neglected for propagation in deep ocean but should be retained for tsunami amplitude computation near the coast. The linear computation results for points near the coast is presented for comparison purposes.

This paper presents a preliminary investigation on the use of (linear and nonlinear) shallow water equations and a certain finite difference scheme in simulating tsunamis around the Manila trench/Manila bay area, we will not at this point concentrate on the applicability of numerical results to physical scenarios and cannot be used for emergency or mitigation planning. We believe this is the first attempt to numerically simulate earthquake scenarios on this domain.

One limitation of this study is the unavailability of detailed bathymetry (ocean depth) data for the computational domain. We used the freely available Sandwell / Smith bathymetry from the internet. It has a 2-minute resolution (approximately 4km) and this is not enough for nonlinear computation near the shore.

Keywords: Tsunami, numerical computation, finite difference method, Manila Trench

CMPSD No. 9b

CONVEXITY AND A NON-CONNECTIVITY CONCEPT

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It is well known that the couple $(V(G), d)$, where $V(G)$ is the vertex set of a connected graph G and $d(x, y)$ is the length of a shortest path connecting vertices x and y in G , is a metric space. Any x - y path of length $d(x, y)$ is called an x - y geodesic. With this terminology, we say that a subset C of $V(G)$, where G is a connected graph, is convex if for every two vertices $x, y \in C$, the vertex set of every x - y geodesic is contained in C .

We say that a nonempty subset S of $V(G)$ is a non-connecting set in G if it satisfies the following condition: For every pair of vertices $u, v \in V(G) \setminus S$ such that $d(u, v) = 2$, $N(u) \cap N(v) \cap S = \emptyset$. A non-connecting set with minimum cardinality is called a minimum non-connecting set. In this paper, we showed that if a set is convex set then its complement is a non-connecting set. Also, we characterized those subsets of $V(G)$ that yield convex complements in $V(G)$ using this non-connectivity concept. The main results obtained in this study are the following:

- (1) Let G be a connected nontrivial graph. Then a minimum non-connecting set S in G is a singleton if and only if G has an extreme vertex.
- (2) Let G be a connected graph and S a nonempty subset of $V(G)$. If $V(G) \setminus S$ is convex in G , then S is a non-connecting set in G .
- (3) Let G be a connected graph of order $n \geq 2$. Then there is a convex set in G of order $n - 1$ if and only if a minimum non-connecting set in G is a singleton.
- (4) Let G be a connected graph and S a nonempty subset of $V(G)$. Then $V(G) \setminus S$ is convex in G if and only if S is a non-connecting set in G satisfying the following property: (NN) For every nonempty non-singleton $S^* \subseteq S$ and for any $x, y \in V(G) \setminus S$, S^* is not contained in the vertex set of some x - y geodesic.

Keywords: graph, convex, geodesic, extreme vertex, non-connecting set, minimum non-connecting

CMPSD No. 10a

FOLDING THE SUM OF TWO GRAPHS

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The graph G' obtained from a connected graph G by identifying two nonadjacent vertices in G having at least one common neighbor is called a 1-folding of G . A sequence $G_0, G_1, G_2, \dots, G_k$ of graphs such that $G_0 = G$ and G_i is a 1-folding of G_{i-1} for each $i = 1, 2, \dots, k$ is called a k -folding of G . If G is not a complete graph, then it always has two nonadjacent vertices that have a common neighbor. Thus G can undergo a sequence of folding until a complete graph is obtained. Denote by $F(G)$ the set of all non-isomorphic complete graphs that can be obtained from G by a sequence of folding.

This study attempts how to determine the smallest and largest element of $F(G + H)$. It also tries to find a relationship between $F(G + H)$ and $F(G) + F(H)$. This study came up with the following conclusions:

1. Let G and H be two bipartite graphs. Then $K_n \in F(G + H)$ and $K_n = F(G) + F(H)$. Hence, $F(G + H) \supseteq F(G) + F(H)$.
2. For any two graphs G and H each of diameter 2,
$$K_{c_{(G+H)}} \in [F(G + H) \cap (F(G) + F(H))].$$
3. For any graphs G and H , $F(H) + F(G) \in F(H + G)$.
4. For any graphs G and H , (a) $\max\{s \mid K_s \in F(G + H)\} = q(G) + q(H)$, where $q(G)$ and $q(H)$ denote the maximum number into which $V(G)$ and $V(H)$ can be partitioned into independent and pairwise linked sets, respectively; and (b) $\min\{r \mid K_r \in F(G + H)\} = p(G) + p(H)$, where $p(G)$ and $p(H)$ denote the minimum number into which $V(G)$ and $V(H)$ can be partitioned into independent and pairwise linked sets, respectively.

Keywords: 1-folding, k -folding, non-isomorphic, complete graphs, sum of two graphs, diameter, bipartite, independent, pairwise linked.

CMPSD No. 10b

**ELECTRICAL CHARACTERISTICS OF POLYANILINE FILM AND THE n
AND p-TYPE GERMANIUM CRYSTALS**

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Numerous semiconductors already exist nowadays, may they be inorganic e.g. Ge and Si, or organic e.g. polyaniline and polythiophene semiconductors. The latter materials are of greater interests now because of their low cost synthesis and environmental stability. What signals their fundamental and practical applications are their magnetic, electronic and electrical properties that they exhibit in different temperature configurations especially at room temperature where most electrical and radiation devices such as rectifier diodes and EM wave absorbers, are operated.

In this study, electrical characteristics of n and p-type germanium crystals as well as the chemically prepared polyaniline films, an organic polymer, are investigated. Hall Effect experiments in conjunction with conductivity measurements via four – probe method are performed to measure the resistivity, charge carrier density and the hall mobility of each sample at room temperature. The respective resistivities of n and p-type germanium crystals are $8.10 \text{ } \Omega \cdot \text{cm}$ and $20.1 \text{ } \Omega \cdot \text{cm}$ with their corresponding charge carrier densities of $3.1 \times 10^{14} \text{ cm}^{-3}$ and $5.9 \times 10^{15} \text{ cm}^{-3}$. The n – type shows a mobility of $2,477 \text{ cm}^2 \text{ volt}^{-1} \cdot \text{sec}^{-1}$ and $1,825 \text{ cm}^2 \text{ volt}^{-1} \cdot \text{sec}^{-1}$ for the p –type. On the other hand, the resistivity, charge carrier density and the mobility of the polyaniline samples are $3.41 \text{ } \Omega \cdot \text{cm}$, $6.7 \times 10^{14} \text{ cm}^{-3}$, $2,727 \text{ cm}^2 \text{ volt}^{-1} \cdot \text{sec}^{-1}$, respectively.

Ohmicity of the current –voltage curve for all the samples is observed. The hall voltage of the germanium samples exhibit magnetic field dependence which is not observed in polyaniline samples. Applications of these samples such as radiation absorbers and sensors, particularly the polyaniline films, are under study.

Keywords: conducting polymer, polyaniline, resistivity, charge carrier density, mobility

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CMPSD No. 11

PREPARATION AND OPTIMIZATION OF ELECTRICALLY CONDUCTIVE FILMS: POLYPYRROLE OR POLYANILINE IN POLY(VINYL CHLORIDE) AND POLYSTYRENE

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Conducting polymers such as polyaniline (PAn) and polypyrrole (PPy) have poor physical properties, environmentally unstable and poor processibility. To obtain a mechanically stable film with high conductivity, conducting polymers are imbedded into the host polymers, poly(vinyl chloride) (PVC) and polystyrene (PS). This study aims to attain the optimum conditions to determine the highest possible conductivity with high mechanical strength which can serve as alternatives for metals used in aerospace application such as electromagnetic interference (EMI), shielding enclosures and spacecraft grounding.

Three composite films, PPy-PS, PPy-PVC and PAn-PVC, were studied which involves varying working conditions namely, the soaking time in the diffusion medium, the oxidation time, oxidant concentration and temperature. The monomers, pyrrole (Py) and aniline (An), were diffused into the base polymer matrices in the swelling medium of n-hexane and acetone mixture. The diffused monomer was oxidatively polymerized in a binary solvent system of acetonitrile and methanol in FeCl_3 for Py and $(\text{NH}_4)_2\text{S}_2\text{O}_8$ in HCl for An. The conductivity was measured using the four-point probe technique. Likewise, the tensile strength and the surface morphology via SEM were also conducted for further characterization.

The highest conductivity obtained was $1.33 \times 10^{-2} \text{ Scm}^{-1}$ for PPy-PVC (1 h monomer soaking, 0.8 M FeCl_3 , 2.0 h oxidation at room temp), $1.75 \times 10^{-3} \text{ Scm}^{-1}$ for PPy-PS (8.0 min. monomer soaking, 1.0 M FeCl_3 , 4.0 h oxidation at room temp) and $6.44 \times 10^{-5} \text{ Scm}^{-1}$ for PAn-PVC (10 min monomer soaking, 1.0 M $(\text{NH}_4)_2\text{S}_2\text{O}_8$, 2.0 h oxidation at room temp). The SEM analysis and the tensile strength tests are currently being undertaken.

Key words: conducting polymers, host polymers, polypyrrole, polyaniline, composite films

CMPSD No. 12

POLYANILINE FILM: PRODUCTION AND CHARACTERIZATION

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Conducting polymers are being extensively researched for their application in several new technologies. There is a lot of literature concerning the synthesis and characterization of conducting polymers. Examples of conducting polymers are polyaniline, polyacetylene, polypyrrole, and polythiophene. They have potential applications in electronic displays, as electrode materials in batteries, as molecular electronic circuit elements, in restoration of data, as indicators of gasometers and in biochemical analysis.

This study is on the production of a polyaniline film using the electrochemical polymerization method under potentiostatic conditions at room temperature. This method is done by supplying a constant voltage across the two electrodes (Indium Tin Oxide (ITO) and Platinum) that are immersed in an electrolytic solution containing aniline (monomer), hydrochloric acid (dopant), and distilled water (solvent). The resulting film is then characterized by measuring its conductivity and hall mobility using the Four Probe and Hall Effect apparatus. The current-voltage (I-V) characteristic curve of the film is also determined. In addition, the film produced is analyzed using the NIM and CAMAC instruments for performance testing.

Keywords: Conducting polymers, Polyaniline film, electrochemical polymerization, Nuclear Instrumentation Module (NIM), Computer Automated Measurement and Control (CAMAC), aniline, monomer, Four Probe and Hall Effect apparatus

CMPSD No. 13

**PIEZOELECTRIC BIOMIMETIC SENSOR FOR DETERGENT
BASED ON MOLECULARLY IMPRINTED POLYMER
ELECTROSYNTHESIZED POLYPYRROLE**

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Surfactants impose a great threat in the environment. There is therefore a need to monitor its concentration in water systems. Presently, the analytical methods used for the measurement of detergent require specialized personnel skill and expensive instruments. In this paper, we describe an alternative low cost and reliable method based on a piezoelectric biomimetic sensor.

The sensor involved a piezoelectric quartz crystal coated with a molecularly imprinted polypyrrole reagent phase. The reagent phase was electrosynthesized in the presence of pyrrole monomer and sodium dodecylsulfonate (SDS). In the presence of an applied potential, the monomer underwent polymerization and entrapped SDS molecules in its matrix. Equilibration with a buffer system resulted in the extraction of SDS, leaving behind cavities having a shape complementary to that of the template molecule. Upon exposure to a solution of SDS, the reagent phase re-binds template molecules within the cavities in its matrix.

The rebinding of SDS is monitored through measurement of the oscillation frequency of the quartz crystal. An instrumentation system was assembled based on a Pierce oscillator and a frequency counter. The preparation of the reagent phase was optimized by studying electropolymerization parameters such as time of polymerization, pH of buffer and current density. The resulting sensor exhibited a linear response to buffered SDS solutions containing from 10^{-7} M to 10^{-4} M SDS. It had an average response time of 2 minutes and showed a sensitivity of 38.223Hz/logM. The development of this biomimetic sensor, which uses artificial guest-host recognition systems, could provide an alternative strategy for anionic surfactant detection with consideration to cost, sensitivity, selectivity and ease of handling.

Keywords: Piezoelectric quartz crystal, molecularly imprinted polymer, sodium dodecyl sulfate, electropolymerization

CMPSD No. 14

**MOLECULAR DESIGN OF MULTIVALENT SYNTHETIC
VACCINES AGAINST BIOLOGICAL WARFARE (BW) AGENTS**

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The new reality of biologic terrorism and warfare has ignited interests in the development, mass-production as well as dissemination of vaccines against BW agents to the general population, as the case of anthrax. One particular type of vaccine that possesses a strategic anti-terrorist advantage is the synthetic peptide vaccine. As an alternative to conventional vaccines, they are safe, they can be designed to induce defined immune responses and they can be synthesized in large quantities in high purity in a short span of time. In this study, we describe the molecular design of several candidate synthetic vaccines against the listed top BW agents of highest likelihood of rogue use, namely *Bacillus anthracis* (anthrax), smallpox, *Francisella tularensis* (tularemia), *Coxiella burnetii* (Q fever), *Yersinia pestis* (plague) and *Brucella melitensis* (brucellosis). Tapping into the huge genomic information available in the world wide web, candidate immunogenic peptides were engineered to create concatenated epitopes based on defined molecular criteria for T- and B-epitope prediction: proteosomal size and structural stability. The development of these BW vaccines represents a useful paradigm for the application of molecular bioinformatics and immunology into the defense arena.

Keywords: synthetic vaccines, biological warfare agents, protein engineering, epitopes

CMPSD No. 15

MOLECULAR ORBITAL CALCULATIONS ON THE MAILLARD REACTION: PROBABLE MECHANISM AND STRUCTURES OF INTERMEDIATES AND PRODUCTS

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The detailed mechanism of the Maillard reaction of sugars and amines leading to dark-colored polymeric (melanoidin) products has not been elucidated so far despite extensive kinetic and structural studies worldwide. Molecular orbital (MO) computation serves as a valuable tool in studying unstable reaction intermediates and in interpreting kinetic data, as well as in evaluating probable reaction mechanisms.

The present study deals with MO calculations on six Maillard reaction model systems consisting of glucose, fructose or xylose as sugar reactant and glycine or butylamine as amine reactant. The PM3 semi-empirical MO method was used to (a) calculate reactivities of reactants and probable intermediates, (b) evaluate molecular structures of polymeric products which have been suggested in the literature and (c) propose a polymerization pathway leading to melanoidin.

Ab initio, DFT and PM3 computations were performed on the sugar and amine reactants. Heats of formation (ΔH_f°) calculated using the PM3 method were closer to experimental values compared to ab initio and DFT results. Only PM3 computations were performed on molecular systems larger than the sugar or amine reactants. A computational level of accuracy of approximately 2.4 kcal/mol (0.02 kcal/g) was obtained using twenty organic compounds of known ΔH_f° values. All computations simulated molecular systems in vacuum. The computed HOMO-LUMO energy differences indicated that nucleophilic addition of two Amadori/Heyns rearrangement products is favored over combination with a sugar or amine.

The Yaylayan-Kaminsky (1998) polymeric product for the six model systems consistently gave the most negative computed values of ΔH_f° compared to the Kato-Tsuchida (1952) and Cammerer-Kroh (1995) products; the differences in ΔH_f° values among the proposed structures were greater than the estimated computational error. The computational results imply that the Yaylayan-Kaminsky mechanism is more probable than the Cammerer-Kroh and Kato-Tsuchida pathways.

Keywords: Maillard reaction, M.O. calculations, melanoidin, PM3

CMPSD No. 16

MOLECULARLY IMPRINTED ELECTRO-SYNTHESIZED POLY(O-PHENYLENEDIAMINE) BASED CAFFEINE SENSOR

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An investigation on the possibility of combining poly(o-phenylenediamine) as a sensing layer with molecular recognition capability for caffeine and piezoelectric quartz crystal was undertaken. The measurement of caffeine is necessary because of its occurrence in some of the food we eat and could pose as health hazards when taken in excessive amount.

The caffeine imprinted polymer was prepared using galvanostatic electropolymerization of o-phenylenediamine monomer directly onto one of the gold electrodes of a 9 MHz AT-cut quartz crystal. The optimum conditions including polymerization time, monomer to template ratio, current density and concentration of polymerizing solution during electro-synthesis of the reagent phase were considered. Extraction of the template caffeine from the polymer matrix was done by washing the polymer with water.

The instrumentation system for the caffeine sensor consists of a coated-quartz encased in a Teflon cell with its electrodes connected to Pierce-based oscillator circuitry and a frequency counter. Monitoring of the resonant frequency of the quartz crystal as it comes in contact with the caffeine solution was done in a stopped flow mode. A steady state response was achieved in about 10 min. The sensor exhibited a linear relationship between the frequency shift and caffeine concentration in the range of 0.1 to 10 mg/mL (correlation coefficient, $r = 0.9923$). It revealed a good sensitivity of about 130 Hz/ln conc.(mg/mL) and good repeatability, $rsd = 10.6$ ($n=7$) for 0.5 mg/mL caffeine solution.

Surface examination of the sensor using scanning electron microscopy and x-ray photoelectron spectroscopy were also performed to have a better understanding of the sensor behavior and the imprinting process. The developed sensor can be used as a potential inexpensive option for measuring caffeine.

Keywords: Piezoelectric quartz crystal, molecularly imprinted polymer, caffeine, o-phenylenediamine

CMPSD No. 17

**PREDICTION OF PROTEIN SECONDARY STRUCTURE
USING TWO-LAYERED NEURAL NETWORKS**

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In this study, prediction of protein secondary structure using two-layered neural network architectures was conducted. Network training was performed using a data set of 126 globular proteins, which was in turn used for testing the 3-state (helix, sheet, and coil) predictive accuracy. To determine the network parameters at which optimum 3-state prediction occurs, predictive accuracy for a one-output node network was tested depending on the state threshold, the number of inputs used, and the number of hidden nodes used. Helix and sheet states were predicted most accurately (75% and 49%, respectively) with thresholds of 0.10 and -0.10, respectively, while for the coil state (99.9%) these were at 0.90 and -0.90, respectively. Helix and sheet predictions were found to generally increase with increasing number of inputs (76% and 53%, respectively) and hidden nodes (75% and 52%, respectively) used, while the opposite was true for coil prediction (26%). In addition, predictive accuracy for networks with one-output, two-output, and three-output nodes was compared. Overall accuracy increased with an increasing number of output nodes used (56% for the three-output network), but both helix and sheet prediction were better for the one-output node network (76% and 53%, respectively) while coil prediction was better for the two-output network (86%).

Keywords: neural network, secondary structure

CMPSD No. 18**A NEW ANALYSIS OF 6-JET HIGGS STRAHLUNG CHANNEL AT JLC**

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The Higgs boson H is a particle required by the Standard Model (SM) to explain many unanswered phenomena in Physics. At present, it is the only SM particle that has yet to be discovered by actual experiments, either directly or indirectly. This particle is known to be responsible for the mass generation of other particles through the process called Higgs Mechanism. None has been known exactly of this particle, not even its mass. In this paper, the decay width, $\Gamma(H \rightarrow X)$, or the standard deviation of the Higgs boson mass is studied by computer simulation of e^+e^- collisions in the proposed Asian Joint Linear Collider (JLC). JLC Study Framework (JSF) is employed for this experiment. JSF is a software library for the analysis of high energy physics data, based on the ROOT Data Analysis Framework. CERNLIB, ROOT, LCLIB libraries are also installed to be able to run JSF. The mass of the Higgs is assumed to be within the theoretical range of the SM. With this assumption of the Higgs mass, events of colliding electrons and positrons and their by-products can be generated using the PYTHIA Event Generator. Not only the target signal, $e^+e^- \rightarrow ZH \rightarrow q\bar{q}WW^* \rightarrow q\bar{q}q\bar{q}q\bar{q}$, but also the background signals which may mimic the desired signals and with considerable contributions, were also generated to determine the error of the measured quantity. Then the simulation of these events follows using the JSF Quick Simulator. The default configurations of the JLC detectors such as the different vertex detectors, central drift chamber, calorimeter, muon detector, and superconducting solenoid magnet are set before simulation. In the analysis of the data, the tracks found in the main detector are clustered to obtain the needed signals and then by formulating an event selection criteria further discrimination of unnecessary signals is done. So far obtained, we have calculated the relative error in Γ_{total} to be $\Delta\Gamma_{total}/\Gamma_{total} \approx 12$ percent. This is approximately equal to the square root of the target and background signals all over the target signals $\sqrt{S+B}/S$, from this we calculate the error. The discovery or non-discovery of the Higgs boson in a linear collider will change the course of our understanding of Physics. And if discovered, some theoretical predictions will be wiped out and some may

be strengthened by the determination of its mass alone. In fact, the existence of Higgs bosons might explain the possible beginnings of the universe and possibly uncover nano-technologies.

Keywords: Higgs boson, Standard Model, decay width, computer simulation, JLC Study Framework (JSF), Joint Linear Collider (JLC), CERNLIB, ROOT, LCLIB, PYTHIA Event Generator, JSF Quick Simulator

CMPSD No. 19

**OPTIMIZATION OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION
OF THE ESSENTIAL OIL OF PHILIPPINE
Cananga odorata Hook Fil et. Thomson
FLOWERS BY RESPONSE SURFACE METHODOLOGY**

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ABSTRACT

Essential oil was extracted from the flowers of Philippine *Cananga odorata* var. *genuina* grown in Pala-o, Iligan City by supercritical carbon dioxide (SC-CO₂).

A statistical experimental design, first-order 2³ factorial, was used to investigate the effects of three independent variables (pressure, temperature, and flow rate of CO₂) on % oil yield (w/w), % linalool (v/v), and % benzyl benzoate (v/v) on the extracted oil. Three corresponding response equations have been generated for values of pressure (80-100 bar), temperature (35-50 °C) and flow rate of CO₂ (1-4 mL/min). The pressure, temperature and flow rate of the SC-CO₂ extraction conditions were 98.61 bar, 39.58 °C, and 2.99 mL/min, respectively. Gas chromatography was performed on the ilang-ilang oil extracted by SC-CO₂, laboratory and commercial scale hydrosteam distillation. Two constituents of the

different ilang-ilang oils (linalool and benzyl benzoate) were evaluated with reference to the nature of the starting material, the extraction technique and source of plant material.

An optimum oil yield of 8.479 % (w/w) was obtained under the SC-CO₂ extraction operating conditions. This oil yield is much higher compared to the oil yield from hydrosteam distillation which is 2-2.25 % (v/w). Degradation products were observed in hydrosteam distillation. On the basis of linalool to benzyl benzoate ratio, the SC-CO₂ extraction of fresh flower (0.583) is much higher as compared to the SC-CO₂ extraction of freeze-dried flower (0.009). The SC-CO₂ extraction and hydrosteam distillation of freeze-dried sample gave a ratio of 0.009 and 0.001, respectively. The oil quality in Anao, Tarlac (0.658) is more superior than in Pala-o, Iligan City (0.583) due to the agroclimatic origin of the plant trees.

Keywords: benzyl benzoate; ilang-ilang oil; linalool; SC-CO₂ extraction

CMPSD No. 20

**GAMMA RAY SURVEYS FOR GEOLOGICAL STUDIES AND
ENVIRONMENTAL MONITORING: EXPERIENCES AT
THE PHILIPPINE NUCLEAR RESEARCH INSTITUTE**

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ABSTRACT

The Philippine Nuclear Research Institute, with the assistance of the International Atomic Energy Agency, initiated the use of airborne and ground gamma ray survey techniques in lieu of the very expensive airborne survey. The objectives of this project were to establish environmental baseline information on the natural radioactivity of the entire country and to generate radioelement maps

for geological mapping and mineral resource assessment. In preparation of the planned nationwide survey, a regional survey was conducted over the small island of Marinduque (989 km²) and a detailed survey was carried out at the San Antonio porphyry copper deposit in Sta. Cruz, Marinduque. Highlight of this study is the production of the first natural background radioactivity maps in the country. The radioelement maps in the regional survey showed good correlation with the local geology of Marinduque Island. Radiometric patterns in the detailed survey showing the combination of K and K/Th highs, including U and U/Th highs, if present, can be good radiometric-based indicators in the exploration for porphyry copper mineralization.

Carborne gamma ray spectrometric surveys were likewise undertaken at the former Subic US naval base and Clark US airforce base. This was due to mounting public concern over the presence of possible radioactive materials left behind by the US military forces in these bases. Using the gamma-ray spectrum ratio technique, results indicated the absence of radioactive sources in areas monitored within the two bases.

A sizeable part of Metro Manila was also covered by the carborne survey. Results discovered an area with high measurements of thorium up to 246.18 ppm. If converted to radiation dose would yield 5.88 mSv/y. This amount slightly exceeds the recommended maximum allowable radiation dose of 5 mSv/y that may be received by any individual. The radiation source comes from an establishment that produces mantles coated with thorium nitrate, a radioactive substance.

The surveys have demonstrated that the carborne and ground gamma ray spectrometric survey techniques are rapid and cost-effective.

Keywords: Environmental monitoring, carborne gamma ray survey, ground gamma ray survey, natural radioactivity, porphyry copper, radioelement

CMPSD No. 21

**REMOVAL OF BASIC RHODAMINE B
FROM AQUEOUS SOLUTION USING RICE STRAW AS ADSORBENT**

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Rice straw, the leftover in rice grain harvesting, is a major agricultural problem because rice straws have poor biodegradability, and are generated in large amounts, 16 to 19 million metric tons in the year 2000. While some of the rice straws are used in mulching, as padding for egg transport, and as additive to animal feeds, a greater portion of rice straws is burned on the field.

In this study, we investigated the feasibility of rice straw as low-cost adsorbent for color removal of effluent from dyeing and textile finishing. The study focused on the removal of Basic Rhodamine B (BRB) from aqueous solution using rice straw as an adsorbent.

Results from batch equilibrium studies showed maximum sorptive capacities of rice straw for BRB are 22.5 mg/g and 15.5 mg/g at pH 2 and 12, respectively. Using the NaOH-H₂O₂ treated rice straw, the maximum sorptive capacities are 7.2 mg/g and 5.6 mg/g at pH 2 and 12, respectively. Delignification of the rice straw resulted in lower sorptive capacity for the basic or cationic dye such as BRB. This indicates that not only cellulose but also lignin and hemicellulose may provide significant active sites or functional groups that bind or adsorb cations from solution.

Results from column studies are consistent with the results observed in batch equilibrium studies. The bed reached its breakthrough and exhaustion time slightly earlier at pH 12 (compared to that of pH 2) and for NaOH-H₂O₂ treated rice straw (compared to that of untreated rice straw). The adsorption zone (D), bed sorptive capacity (N), and adsorption rate constant (K) were determined from the breakthrough profile. Results also showed that the initial dye concentrations and feed flowrates have significant effects on the adsorption behavior of BRB on the fixed bed of rice straws. The results from these studies will be useful for further researches in utilizing rice straw as an alternative adsorbent.

Keywords: rice straw, adsorption, Basic Rhodamine B, breakthrough curve

CMPSD No. 22

**PARAMETRIC STUDY ON THE ADSORPTION OF BASIC RHODAMINE B
ON SODIUM HYDROXIDE-PRETREATED CORN PITH**

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Industries involved in dyeing operations discharge colored effluents. The color inhibits the penetration of sunlight through the surface of the receiving body of water, thus inhibiting the growth of photosynthetic organisms. In addition, some dycstuffs are potentially toxic to living organisms. In this study, we explored the potential of sodium hydroxide (NaOH) -pretreated corn pith as an adsorbent to remove Basic Rhodamine B (BRB) from aqueous solution. Corn pith is the soft and porous cellulosic part of cornstalk that is primarily used as animal feed in areas where there is extensive corn production.

Batch equilibrium experiments were conducted to investigate the adsorption of BRB from aqueous solutions on pretreated corn pith. Initially, the corn pith was pretreated at different levels of NaOH concentration (0.1M, 0.01M and 0.001M), pretreatment time (3 hours, 1 hour and 15 minutes), and pretreatment temperature (room temperature and 80°C).

The data were fitted to four different adsorption isotherm models (Langmuir, Freundlich, Redlich-Peterson and Brunauer-Emmet-Teller (BET)). The isotherms, which had average correlation coefficients greater than 0.97 were Langmuir, Freundlich and Redlich-Peterson respectively. The BET model had correlation coefficients less than 0.6.

Results indicated that the pretreatment of the corn pith resulted in the improvement of its adsorptive capacity for BRB. The maximum adsorptive capacities (Q_{max}) from the Langmuir model were determined for the different pretreatment conditions. Results showed that the Q_{max} of the pretreated corn pith ranges from 72.5- 105.0 mg/g. Pretreating the corn pith with 0.1 M NaOH at 80°C for 1 hour resulted to the highest improvement in the adsorptive capacity of corn pith, i.e., a 55.5% increase compared to that of untreated corn pith. On the other hand, the lowest in improvement was observed at pretreatment of corn pith with 0.001M NaOH at room temperature for 15 minutes i.e., a 7.25% increase compared to that of untreated corn pith. Statistical analysis of the data indicated that the effect of NaOH concentration, pretreatment time and temperature were significant ($\alpha = 0.05$ level of significance). Results from this study will be useful for further optimization of parameters to maximize the adsorptive capacity of the pretreated corn pith.

Keywords: NaOH-pretreated corn pith, Basic Rhodamine B, adsorption isotherm

CMPSD No. 23

**INVENTORY OF DIOXINS, FURANS AND
DIOXIN-LIKE PCBs IN THE PHILIPPINES**

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Emissions from polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and dioxin-like PCBs which are formed unintentionally in industrial and combustion processes, were estimated through a Toolkit developed by United Nations Environment Program (UNEP) Chemicals. The Toolkit includes information on relevant industrial and non-industrial processes releasing PCDD/PCDF and detailed database of emission factors with default data representative of process classes. Screening matrix was applied to identify the main categories/subcategories of existing activities and sources in the country. Detailed information on the processes were gathered and classified. Releases were quantified using default/measured emission factors and estimates of the average annual release to air, water, land, products and residues were calculated by this basic equation: Source Strength (Dioxin emissions/year) = Emission Factor x "Activity Rate", presented in grams of toxic equivalents (TEQ) per annum (gTEQ/a).

Compiled PCDD/PCDF inventory yielded 530.70 gTEQ/a as total annual released to all environmental compartments. Uncontrolled combustion processes ranked 1st with 187.05 gTEQ/a followed by power generation/cooking at 157.23 gTEQ/a and production of chemicals and consumer goods at 91.56 gTEQ/a. Air had the highest PCDD/PCDF contamination with 327.60 gTEQ/a, product and land trailed with 77.64 and 46.86 gTEQ/a, respectively. Major source of PCDD/PCDF contamination for air and land was uncontrolled combustion of agricultural residues. This was in congruence with the study by United States Environmental Protection Agency (USEPA) that PCDD/PCDF released from open burning was higher compared to municipal solid waste incinerators. The Philippine waste incineration activity contributed only 6.7% of the total PCDD/PCDF released to the environment. Other major sources of PCDD/PCDF released

to products, water and residues were leather plants, open water dumping and household biomass cooking.

The Inventory could serve as guide in the formulation of National Action Plan for Persistent Organic Pollutants (POPs) as part of the country's obligation to the Stockholm Convention and basis for future exposure studies in PCDD / PCDF and dioxin-like PCBs.

Keywords: dioxins, furans, dioxin-like PCBs, national inventory, UNEP toolkit, TEQ

CMPSD No. 24

ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF FRUITS AND VEGETABLES COMMONLY FOUND IN THE FILIPINO DIET

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Edible portions of 25 commonly eaten fresh fruits and vegetables in the Philippines listed into four groups namely, a) green leafy vegetables, b) other vegetables, c) root crops, and d) fruits, were analyzed for their antioxidant activity and total phenolic content. The antioxidant activity varied from 0 – 76%. Among the plant groups studied, root crops had the highest antioxidant activity, specifically 68.51% on the average. This is equivalent to more than 43.28-mg/g antioxidant activity of a - tocopherol. The total phenolic content of the fruits and vegetables studied range from 0 to 2015 mg catechin equivalents (CE)/100g sample.

Among the green leafy vegetable, kamote (*Ipomea batatas*) had the highest total phenolic concentration at 2015 mg CE/100g sample. Unripe jackfruit (*Artocarpus heterophyllus*), ubi (*Dioscorea alata*) and starapple (*Chrysophyllum cainito*) were the only plant foods in their group that showed considerable amounts of total phenolics with 807.5, 450, 502.5 mg CE/100g, respectively. Antioxidant activity and total phenolic content of the samples were not linearly correlated.

However, a strong significant correlation was observed when the samples were considered within a group.

The effect of processing and cooking, specifically boiling, on the antioxidant activity and phenolic content of the samples was also determined. Significant decreases in antioxidant activity were observed for jackfruit and gabi (*Colocasia esculenta*) at 41% and 37%, respectively. The total phenolic content of samples likewise decreased significantly. Boiling of root crops resulted in an average decreased of 30.6% in its antioxidant activity. On the other hand, the total phenolic content of both kamote and gabi was totally lost; but for ubi, a 22.9% decreased was observed. The activities of processed mango (*Mangifera indica*) and pineapple (*Ananas comosus*) were significantly greater than the fresh fruits. Blanching green leafy vegetables generally resulted in a significant decreased in antioxidant activity and total phenolic content.

Keywords: antioxidant activity, total phenols

CMPSD No. 25

**ALKALOIDS FROM THE ANTITUBERCULAR FRACTION OF
ALSTONIA SCHOLARIS (LINN.) R. BROWN LEAVES**

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Tuberculosis is becoming a serious concern due to an upsurge of infected persons caused by the reemergence of multi-drug resistant strain of the causative agent, *Mycobacterium tuberculosis*. In the course of our study on the secondary metabolites from the antitubercular extracts of *Alstonia scholaris* (Linn.) R. Brown leaves, several indole alkaloids were isolated from the bioactive fraction of the alkaloid extract obtained at pH 5. This fraction exhibits a 99% inhibition to *Mycobacterium tuberculosis* H37Rv at 50 ug/mL using the Microplate Alamar

Blue Assay (MABA). The alkaloids were elucidated on the basis of the data obtained from UV, IR, mass spectrometry (LR-EIMS, LR-ESIMS, HR-ESIMS) and nuclear magnetic resonance spectroscopy (^1H , selective NOE, ^{13}C , APT, DEPT-90, DEPT-135, ^1H - ^1H COSY, HMQC, HMBC, COLOC, ROESY). These were identified as, 19S-tubotaiwine, 6,7-secoangustilone B and a new vallesamine indole alkaloid.

Keywords: *Alstonia scholaris*, indole alkaloids, *Mycobacterium tuberculosis* H37Rv, MABA

CMPSD No. 26

**HYDROLYTIC ENZYMES FROM GERMINATING MUSTARD SEEDS
(*Brassica juncea*): PURIFICATION, CHARACTERIZATION
AND ENANTIOSELECTIVITY IN THE KINETIC RESOLUTION OF
RACEMIC α -ARYLPROPIONIC ESTER**

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Hydrolases from germinating mustard (*Brassica juncea*) seeds were extracted with 0.1M Tris buffer containing 1 mM dithiothreitol. This was subjected to ammonium sulfate fractionation where the hydrolytic activity was found to be concentrated in the supernatant at 100% saturation; this fraction was passed through a hydrophobic interaction chromatographic column yielding a fraction with a purification factor of 185. The same fraction when passed through gel filtration column yielded a sample with 1370-fold increase in purification. Analysis through SDS-PAGE of active fractions revealed a common protein band at 41.2 kD.

The crude extract, the supernatant at 100% ammonium precipitation and the gel lipase active fractions were each used in the kinetic resolution of the racemic ester of an α -arylpropionic acid. All three fractions were found to be S (+) enantioselective where the product has 100% enantiomeric excess. Varietal difference indicated that the seeds from several sources exhibited distinct properties.

Keywords: hydrolases, enantioselectivity, mustard, *Brassica juncea*

CMPSD No. 27

**PRODUCTION OF HIGH VALUE CHROMATOGRAPHIC SUPPORTS
FROM LOCALLY AVAILABLE MATERIALS**

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Chromatographic supports are of diverse nature depending on their intended application. These are made from an inert solid material to which is anchored active for resolution, catalysis and other uses. These supports are usually purchased abroad, are not manufactured locally and are therefore very expensive. This project hopes to circumvent this difficulty by producing chromatographic supports in the Philippines using locally available materials.

The Philippines, being a prime producer of sugar (sucrose) from sugar cane may increase the economic value of its products by converting sucrose into invert sugar. Invert sugar is 1.4 times sweeter than sucrose and is of great demand in the food and beverage industry. The suitable enzyme for conversion is invertase which may be immobilized on solid supports. Immobilization of the enzyme facilitates its conversion to a shelf reagent which is stable and reusable.

Chromatographic supports were prepared from locally available materials. Volcanic ejecta (lahar), chitosan, and nata de coco were used as solid support for the immobilization of yeast invertase and utilized for the conversion of sucrose to invert sugar. Some of the raw materials were converted to chemically active materials. The enzyme was immobilized by covalent bonding or adsorption on the solid support. Optimization of the immobilization procedures was conducted. The immobilized enzyme and solid supports produced were characterized to assess their performance. Experimental results indicated the efficiency and suitability of these materials as solid support. These studies could serve as ground work for the synthesis of chromatographic supports from locally derived materials for academic and industrial applications.

Keywords: chromatographic support, lahar, chitosan, nata de coco

CMPSD No. 28

**THE PURIFICATION OF A PROTEASE FROM JACKFRUIT
(*Artocarpus heterophyllus*) LATEX: ENANTIOSELECTIVITY
OF ITS CATALYTIC ACTION IN THE HYDROLYSIS OF A METHYL
ESTER OF A RACEMIC ARYLPROPIONIC ACID**

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A protease was extracted from jackfruit (*Artocarpus heterophyllus*) latex and purified to determine its potential as catalyst for the hydrolysis of 2-arylpropionic acid esters. The protein was localized in the precipitate of the 45% ammonium sulfate fraction (45 P). The fraction was desalted and subjected to ion exchange chromatography. A fraction was identified to be active and after SDS - PAGE analysis revealed the presence of a 22 and 27 kD protein bands.

The 45 P fraction was used to hydrolyze racemic ibuprofen methyl ester. HPLCX analysis using a chiral column indicated that the enzyme was at least 81% enantioselective for the hydrolysis of the R (-) methyl ester. This study demonstrated that the protease from jackfruit latex could be used for the preparation of R (-)-2 - arylpropionic acids.

Keywords: protease, enantioselective, jackfruit, *Artocarpus heterophyllus*

ENGINEERING SCIENCES AND TECHNOLOGY

ESTD No. 1

PURIFICATION OF CITRONELLAL FROM CITRONELLA OIL THROUGH PRECIPITATION AND DISSOLUTION

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Essential oils are widely used in soap and cosmetic production as perfumes, in food technology as flavorings, in medicine, and in rubber and paint industry as masking agent to hide objectionable odor.

In the Philippines, oils of peppermint, spearmint, lemon, pachouli, and eucalyptus are being exported. This study aimed to isolate and purify the citronella component of citronella oil through precipitation and dissolution.

Citronellal, an aldehyde, was precipitated using sodium bisulfite by mixing 25 ml of citronella oil and 50 ml of concentrated sodium bisulfite. The precipitate formed was washed with 50 ml of hexane. After washing, the solid was allowed to dissolve in aqueous Na_2CO_3 and the oil obtained after separation was analyzed through gas chromatography-mass spectrometry.

Effects of Na_2CO_3 concentration and its temperature before dissolution were determined. Ratios of Na_2CO_3 (g) to water (ml) studied were: 5:75, 15:75, and 25:75, while temperatures of Na_2CO_3 used were 60°C, 70°C and 80°C.

Results showed that at a ratio of 5:75 the oil obtained contained 52.7% of citronellal and recovery was about 29%. Using 15:75 ratio, recovered oil has 61.3% citronellal and percent recovery of citronellal was calculated to be 50.6. While dissolving the precipitate in 25:75 ratio of Na_2CO_3 (g) to water (ml), obtained an oil with 64.8% citronellal and recovery was computed to be 69.21%.

At 60°C, the produced oil after dissolution contained 53.3% citronellal and recovery was equal to 34.4%. Sodium carbonate heated up to 70°C obtained

an oil with 53.8% citronellal and recovery was 47.25% citronellal. Heating aqueous Na_2CO_3 to 80°C before dissolution, recovered an oil with 64.8% citronellal and a recovery of 69.21%.

Increasing the concentration of Na_2CO_3 and the temperature of Na_2CO_3 before dissolution also increased the yield.

Keywords: citronellal, citronella oil, essential oils

ESTD No. 2

UTILIZATION OF RICE HULL ASH AND SOLSONA WHITE CLAY FOR THE MANUFACTURE OF CERAMIC WATER FILTERS

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Various types of filter elements ranging from organic materials, to metals and to ceramics are used to make water potable. Ceramics are preferred because these materials are inert, heat resistant, lightweight and porous. Its porosity can be designed to different sizes; hence, it can be used for various applications.

The use of locally available raw materials has been evaluated in the manufacture of ceramic filters. The materials were the following: calcined rice hull ash, which is highly siliceous and used as the primary raw material; beneficiated Solsona white clay, used to increase the plasticity and workability of the mixture; and coconut shell charcoal as a pore forming agent. The materials were mixed at different combinations. The formulations were pressed using a hydraulic press and dried in an oven for 110°C. The formed filters were fired at 1000°C to 1200°C.

Results of the evaluation indicate the filters have higher water absorption and lower apparent porosity (60.65 to 64.89%) compared to diatoms and the commercial filters. The bulk density (0.55 to 0.56 g/cc) was also lower. These physical properties of the locally formulated filters are in accordance with the properties of a workable water filter. The utilization of indigenous local materials such as rice hull ash, Solsona white clay and charcoal were found to be promising for the manufacture of ceramic filters.

Keywords: ceramic, clay, coconut shell charcoal, filters, porous, rice hull ash, water.

ESTD No. 3

**EFFECTS ON THE MICROSTRUCTURE OF COMMERCIAL BRICKS IN
ILOCOS NORTE USING DIFFERENT FIRING METHODS**

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A JSM-35C Scanning Electron Microscope was employed to determine the morphology of commercial structural clay bricks (SCB) in Ilocos Norte.

Bulk commercial SCB was prepared and formed using the existing traditional way of manufacturing process with clay sand composition ratio of 100% clay and 85%:15% clay sand ratio respectively.

The commercial SCB sample was fired inside the kiln in controlled temperature on set at 950°C. The same sample was fired but in an open field firing (Open firing). The effect on the microstructure of commercial SCB using different firing methods was evaluated.

Results show that the presence of open pores and microcracks at varying locations in the system are evident in an open field firing (open firing) at both 100% clay and 85%:15% clay-sand ratio. More compact, dense, uniform texture and even distribution of particles are evident in commercial SCB fired in controlled temperature at 950°C was both 100% and 85%:15% Clay Sand ratio.

Keywords: microstructure, commercial bricks, open firing, controlled temperature

ESTD No. 4

**PHYSICAL ANALYSIS OF STRUCTURAL CLAY
BRICKS IN ILOCOS NORTE**

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Physical analysis of commercial clay bricks in Ilocos Norte from local manufacturer was conducted and compared to the standard set by the American Standard for Testing Materials (ASTM).

On-site and laboratory experiment were conducted and the results were compared. Both commercial, on site and laboratory experiment correlated with ASTM standards.

Results show that laboratory formulations passed the ASTM standard in terms of water of plasticity, drying shrinkage, firing shrinkage, apparent porosity, water absorption, modulus of rupture and compressive strength.

The correlation analysis of the chosen best body formulation against the standards for bricks set by the ASTM give positive remarks in all properties tested. Comparative analysis with the best body formulation and locally produced commercial structural clay bricks indicate that the chosen best laboratory formulation is more superior than the existing locally produced commercial clay bricks.

Keywords: structural clay bricks, physical analysis, ASTM standard for bricks

ESTD No. 5

**PERFORMANCE AND EMISSION TESTING
OF A PROTOTYPE C³ LOG BURNER HEATING APPLIANCE**

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A prototype C³ (Caves Clean Combustion) woodburner was tested on the basis of burn rate, efficiency, power output and particulate emission based on the flue gas composition and airflow measurement. The procedure involves measurement of parameters: wood type and log size to test the sensitivity of the test method.

Results showed that hardwoods, eucalyptus gum, have a lower emission than softwoods with a solid particulate level of 0.046g/m³ compared to 0.059g/m³, 0.065g/m³, for radiata pine and larch. The efficiency of the burner does not vary with wood type. The irregular-shaped radiata pine produced 0.047g/m³ compared to the regular-shaped of 0.059g/m³. The unsteady state condition emission yielded 0.243g/m³.

The over-all efficiency of the C³ Log Burner is between 75-79% with emission ranging between 0.046 to 0.065g/m³ (0.206 to 0.700g/kg oven-dry wood) on a steady state condition. Based on the test method used, the C³ log Burner passes the 1.5g/kg limit producing an emission level 10 times lower than the limit set by the Canterbury Regional Council.

Keywords: woodburner, particulate emission, eucalyptus gum, Radiata pine, larch

ESTD No. 6

**FORMULATION OF LEAD GLAZE USING LOCAL RAW
MATERIAL FOR RED CLAY BODY**

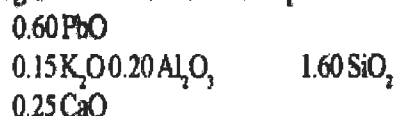
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Lead has a long history of being essential to many low maturing glazes. Its contribution to the formulation of good, acid resistant glaze is known.

The study was focused on the formulation of lead glaze utilizing locally available raw materials such as calcined rice hull ash, Solsona white clay with the addition of commercial oxide, to determine the suitability of the materials as alternative replacement for the imported glaze materials.

The lead glaze was based on the empirical formula:



and the body composed of 70% red clay (Baligat red clay in Batac, Ilocos Norte) combined with 30% sand dunes as filler were developed. Forming by slabbing, glaze application by dip method. The prepared lead glaze was fired (monofiring – body/glaze) at 1050°C.

Results show that all the formulated glaze specimens exhibit a transparent appearance especially formulation A. Some defects were evident in the test samples such as pinholes but for decorative and facing red tiles this are minors defects.

Keywords: slip glaze, local red clays, slabbing, lead glaze formulation, monofiring

ESTD No. 7

**ADSORPTION OF DISPERSE MIKETON BLUE, REACTIVE PROCION
BLUE AND BASIC BLUE GRL DYES FROM SYNTHETIC
TEXTILE MILL EFFLUENT USING CHARRED AND
ACTIVATED CARBON FROM RICE HULL**

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Rice hull is a potential material for activated carbon. Rice hull, the covering structure of the rice grain, constitutes about 18 to 28% of the rough rice weight.

The general objective of the study was to compare the percent dye removal of rice hull, in its charred and activated form.

The experiment investigated the kinetics of adsorption and adsorption isotherms of Disperse Miketon Blue (DMB), Reactive Procion Blue (RPB) and Basic Blue GRL (BB) on charred and activated carbon (AC) from rice hulls. The adsorptive capacity of charred and AC from rice hulls was also determined. Results revealed that the order of adsorption using charred and activated carbon (at 800°C) from rice hull for DMB, RPB and BB were 1.14, 0.93, 1.40, 2.10, 2.33 and 2.37 respectively. The adsorptive capacity of charred and AC as adsorbent for DMB, RPB and BB were 141.71, 117.33, 128.25, 273.14, 246.67, and 145.01 mg/g, respectively. The adsorption isotherm of the dyes fitted the Freundlich isotherm model.

Statistical analysis using analysis of variance (ANOVA) showed that adsorptive capacity and percent of removal of the three dyes on charred and activated carbon was significant at 5 % level of confidence.

Keywords: rice hull, adsorptive capacity, adsorption kinetics, adsorption isotherms, activated charcoal

ESTD No. 8

**ENTHALPY, ENTROPY AND GIBBS' FREE ENERGY OF THE
ADSORPTION OF BASIC DYES FROM SYNTHETIC
TEXTILE WASTEWATER USING
SUGARCANE BAGASSE PITH AS ADSORBENT**

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Present technology uses activated carbon as adsorbent for the removal of color, taste, odor and many other organic contaminants, specifically those that are biologically resistant. Others use agricultural wastes as adsorbents. Sugarcane bagasse is a potential adsorbent for color removal. Generally, bagasse is used as fuel in sugar factories. Depithing bagasse is a good practice of separating pith (a good adsorbent) from about 5.5 million tons bagasse produced annually by the sugar industry.

The general objective of this study was to determine the enthalpy, entropy and Gibbs' free energy on the adsorption of basic dyes from synthetic textile wastewater using sugarcane bagasse pith as adsorbent.

The ability of sugarcane bagasse pith to adsorb basic dyes, namely Basic Rhodamine Blue (RBR), Basic Auramine Orange (BAO) and Basic Malachite Green (BMG) from synthetic textile wastewater was investigated.

The kinetic parameters for the three dyes were determined. For Basic Rhodamine Blue Dye, the adsorption was of 4.81th, 4.82th, 4.90th, and 4.83th order for room temperature, 40°C, 50°C and 60°C, respectively. For Basic Auramine Orange Dye, the adsorption was of 5.45th, 5.40th, 5.53th, and 5.25th order for room temperature, 40°C, 50°C and 60°C, respectively. Finally, for Basic Malachite Green Dye, the adsorption was of 5.50th, 5.52th, 5.60th, and 5.70th order for room temperature, 40°C, 50°C and 60°C, respectively.

The eight-hour concentration-decay curves of each dye at different temperatures show that elevated temperatures increased the overall rate of adsorption of dyes onto an adsorbent.

The eight-hour adsorption isotherms of each dye at different

temperatures were constructed. The adsorption isotherms of the three dyes were of the Langmuir Class. For Basic Rhodamine Blue Dye, it was of Type L-2. For Basic Auramine Orange and Basic Malachite Green Dye, it was of Type L-4.

The eight-hour maximum adsorptive capacities of bagasse pith for the three dyes at different temperatures were determined. The eight-hour maximum adsorptive capacities of bagasse for the three dyes increase with an increase in temperature.

The affinity of dyes onto an adsorbent can be described using the three thermodynamic properties computed. The higher the isosteric heat of adsorption, the higher the entropy of adsorption, the lower the Gibbs' free energy, the higher is the affinity of the dye onto the adsorbent.

Keywords: bagasse, adsorbent, dyes, adsorption isotherms

ESTD No. 9

**FLOCCULATION OF COPPER (II) FROM WASTEWATER
BY *Rhizobium* sp. EXOPOLYSACCHARIDE (EPS) BIOPOLYMER**

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Biopolymers such as exopolysaccharides (EPS), exhibit heavy metal-binding property and are becoming an interesting option for the sequestration of heavy metal in wastewater treatment. Aside from the low cost of production, exopolysaccharides produced by microorganisms are considered more environmental friendly than their synthetic polyelectrolyte counterpart.

In this work, the potential of *Rhizobium* sp. (BJVR-12) exopolysaccharide (EPS) in combination with cationic polyelectrolytes such as polyethyleneimine (PEI) and chitosan (CHI) to flocculate cupric ions from aqueous solutions was investigated. Qualitative evaluation of the process revealed that the presence of any of the cationic polyelectrolytes enhanced the flocculation of EPS-heavy metal

complex. Results showed that heavy metal removal was influenced by the EPS-polycation ratio, pH, and type of the polycation used. The optimum [EPS]/[polycation] ratios were both 15 for PEI and chitosan respectively. At optimum ratio, residual EPS and cationic polymers of the filtrate was found minimum. Also, at optimum [EPS]/[polycation] ratio, the Cu^{2+} removal was found to increase as the EPS concentration increased. The EPS-PEI flocculant system was found effective at a wide range of pH for so that even at a solution pH of 2, more than 50 % Cu^{2+} removal was achieved. EPS-Chitosan flocculant system was found effective at above pH 4. Both systems were able to reduce Cu^{2+} concentration of synthetic Cu^{2+} solutions to effluent standard.

Copper (II) removal by EPS-PEI flocculant system from a semiconductor company wastewater was further studied. The optimum EPS/PEI mass ratio for such wastewater was found to be 26.67. Under this condition, clear filtrates and readily separable flocs were obtained. The Cu^{2+} concentration of the treated wastewater was also reduced beyond regulatory standards. A capacity of around 31.120 mg Cu/ g EPS-PEI was achieved.

Keywords: Copper(II); exopolysaccharide (EPS); flocculation; polyelectrolyte; wastewater treatment

ESTD No. 10

DESIGN AND DEVELOPMENT OF CROP GATHERING ATTACHMENT TO PHILRICE-JICA ROTARY REAPER

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Mechanically reaped and windrowed crop still requires 8 persons to gather in a day. To further increase mechanical reaping efficiency, a crop gathering device was conceptualized as an attachment to a commercial rotary reaper.

Three different designs, namely: 1) a vertical gatherer, 2) a horizontal collector with scraper, and 3) a rake-type gatherer were fabricated and tested. The rake-type gatherer was found to perform better and was simpler in construction and could be easily attached to and detached from the reaper. It consisted of three

major parts, namely: the raking fork assembly, driving mechanism and main frame.

Field tests of the reaper-gatherer showed that the cut crop could be gathered into small bunches similar to what is done in manual reaping. The average distance between adjacent bunches was 2.6 m while the straw bunch was measured 33 cm in diameter. With the use of this device, the labor requirement for gathering a hectare of mechanically reaped rice crop was reduced by 25%, i.e. from 8 to 6 persons.

Keywords: Windrow, Crop gathering attachment, Vertical gatherer, Rake-type gatherer, Horizontal collector with scraper, Reaper-gatherer.

HEALTH SCIENCES

HSD No. 1

PROPHYLACTIC AND ANTIMICROBIAL PROPERTIES OF BREAST MILK: FACT OR MYTH?

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Breast milk, once the only known milk for infants has largely been replaced by a great variety of commercial milk formulas. Reports from various health centers finally prompted the World Health Organization (WHO) to urge mothers to go back to breast-feeding. To contribute to the Philippine government's efforts to this end, we did both laboratory experiments and surveys to obtain concrete indicators supporting the health claims concerning breast milk.

Samples of human breast milk were collected and tested for antimicrobial properties against four potentially pathogenic bacterial isolates. A replicated disc diffusion assay was used to evaluate the extent of the antimicrobial activity of the milk samples. Questionnaires with 30 carefully crafted questions were randomly distributed to 37 respondents, mothers with at least one child either breast-fed or bottle-fed for at least the first six months of life. The prophylactic values of breast milk and milk formula were compared using five commonly encountered childhood

diseases as indicators. The index of prophylactic value of breast milk was also computed for each of the five diseases.

All milk samples significantly showed antimicrobial action on all test organisms. Breast milk also showed a higher prophylactic value either specifically for each disease, or generally when taken as a whole, compared to milk formula. Computed indices of prophylactic value revealed that breast milk surpassed milk formula the most in preventing the occurrence of diarrhea.

Based on our results, we conclude that the general health claims concerning breast milk can be scientifically supported. Breast milk has both desirable antimicrobial activities and prophylactic properties against a number of infections commonly affecting children. The lesser incidence of breast cancer in breast-feeding mothers should be an added incentive to all nursing women.

Keywords: breast milk, antimicrobial, prophylactic value, index of prophylactic value, diarrhea, breast cancer

HSD No. 2

BACTERIOLOGICAL EXAMINATION OF LOCAL DRINKS SOLD IN THE STREETS OF LOS BAÑOS, LAGUNA

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The bacteriology of local drinks ("palamig") sold at selected sites in the streets of Los Baños, Laguna was studied, thirteen samples obtained from 5 types of drinks (namely, buko, melon, sago-gulaman, pineapple and nata-gata) were analyzed for coliforms, total viable bacterial count under mesophilic conditions and for the constituent bacterial microflora.

All of the samples were found to contain coliforms at 37°C; 78% had

detectable coliforms even at 45°C. Mesophilic bacterial counts ranged from 4.4×10^4 to 2.3×10^6 colony forming units (cfu) per ml. Except for the pineapple juice samples which had the lowest counts, almost all the drinks had high bacterial counts: 10^3 or 10^6 cfu/ml.

A total of 6 (15 gram-positive and 48 Gram-negative) pure bacterial isolates were obtained from the different local drinks samples. Of the 15 Gram-positive isolates, 12 were *Staphylococcus* spp., 2 were *Micrococcus* spp. And one was an unidentified catalase-positive, oxidase-negative, non-sporeforming rod. Majority of the Gram-negative bacterial isolates (34) belonged to Family Enterobacteriaceae in particular, to the following genera: *Serratia*, *Escherichia*, *Enterobacter*, *Salmonella* and *Providencia/Citrobacter*. Fourteen non-enteric bacteria were identified to be *Pseudomonas*, *Alcaligenes/Agrobacterium*, *Aeromonas* and *Vibrio* spp. Three were very similar to *Acinetobacter*.

The bacteria most commonly found in the street-sold drinks sampled were: *Staphylococcus*, *Providencia/Citrobacter*, *Serratia* and *Enterobacter*.

Keywords: bacterial microflora, street-sold drinks, coliforms, mesophilic counts

HSD No. 3

PATHOGENIC MICROORGANISMS IN COCKROACHES FROM ILIGAN CITY

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Pathogenic organisms were isolated from cockroaches collected from different households in Iligan City. The most prominent of which is *Vibrio cholerae*. Other microorganisms isolated were bacterial families belonging to Enterobacteriaceae and Pseudomonaceae depending on the type of environment the cockroaches inhabit. Bacteria associated with cockroaches were also isolated from individuals belonging to the genus *Leucophaea* and *Periplaneta*. These associates were tested against the test bacterial organisms *Escherichia coli*, *pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* as well

as the fungi *Sacchromyces cerevisciae* and *Aspergillus niger*. Results showed that one isolate exhibited antibacterial activity against all test organisms. Implications of the study in disease transmission based on survey conducted on different households are also presented.

Keywords: pathogen, cockroach, *Vibrio cholerae*

HSD No. 4

**ISOLATION, CHARACTERIZATION AND DETERMINATION
OF BIPHENYL-DEGRADING ACTIVITY OF BACTERIA
FROM CONTAMINATED SOIL AND WATER**

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Bacteria were isolated from wastewater of a solid waste treatment facility and machine oil-contaminated soil on the basis of their ability to grow on mineral medium supplemented with naphthalene or biphenyl as the sole carbon source. Diluted wastewater and soil slurries were lawned onto non-nutrient agar plates supplemented with naphthalene or biphenyl, and individual colonies were picked and transferred into M9 mineral broth with 0.01% naphthalene or biphenyl. Some isolates were observed to turn the colorless M9 medium into bright green or yellow after incubating overnight at 37°C. Genomic DNA was extracted and the isolates were screened by PCR for the presence of *bph* genes (A1, A2, A3, A4, B and C) using primers designed from the *Pseudomonas pseudoalcaligenes* KF707 *bph* operon. Isolates that grow in the selection media and that have any of the *bphA* genes were further purified and re-screened for *bphA* genes. These were then identified using the Crystal ID system from BBL™. All isolates identified so far were gram negative bacilli or cocci belonging to the genera *Pseudomonas*, *Klebsiella*, *Burkholderia*, *Acinetobacter*, *Citrobacter*, and *Serratia*.

Different concentrations of biphenyl in absolute ethanol were scanned

using a luminescence spectrometer (Perkin Elmer LS55) to determine the excitation and emission wavelengths specific for biphenyl. Biphenyl can be detected by this system at a concentration range of 0.001 ug/mL to 0.2 ug/mL. To trace the degradation of biphenyl by the bacterial isolates, tubes containing 100 ug/mL biphenyl in M9 mineral medium were inoculated with selected isolates and incubated at 37 °C. Biphenyl was extracted with an equal volume of chloroform. The chloroform was evaporated under a steady stream of nitrogen gas and the resulting residue was dissolved in an equal volume of absolute ethanol and the samples were read in the luminescence spectrometer. Four strains (*Pseudomonas* D12e, *Pseudomonas* I5c, *Burkholderia* I6b, and *Acinetobacter* Gr2 1a) were chosen for their ability to degrade biphenyl from 100 ug/mL to almost zero within 24 hr. The biphenyl-degrading bacteria were characterized as to generation time, rate of biphenyl degradation and growth rate in the presence of high amounts (1000 ug/mL) of biphenyl.

Keywords: biphenyl-degradation, environmental biotechnology, bioremediation

HSD No. 5

RAPID SPECTROFLUOROMETRIC DETECTION OF GALACTOSEMIA

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Galactosemia is a metabolic disorder characterized by the absence of the enzyme galactose-1-phosphate uridyl transferase (GALT) which is responsible for the transformation of galactose-1-phosphate into glucose-1-phosphate. If undiagnosed and untreated, it could lead to retardation and even death in newborns.

The enzymatic method by Beutler and Baluda was adopted and modified for the assay of GALT. Blood samples from infants were collected on a special filter paper. A blood spot from each sample was obtained and placed in a black wellplate. GALT substrate was added and the blood spot was incubated at 37 °C. After addition of ethanol, the samples were analyzed using the LS55 Perkin Elmer

Luminescence Spectrofluorometer with an excitation wavelength of 355 nm and emission wavelength of 460 nm. With the use of a well plate reader both the calibrators and samples could be simultaneously analyzed unlike in the conventional spectrofluorometer using a one-cell holder in which fluorescent intensity is read one at a time. Linear response is obtained for the concentration range of 2.4 to 16.5 units GALT/ g Hb.

The method was tested using normal and abnormal controls. Thirty blood samples from newborns, 10 blood samples from children and 17 blood samples from adults were analyzed. All samples were found to be negative for galactosemia.

This sensitive and rapid assay requires a very small amount of sample and lesser amount of reagents compared to the classical spectrofluorometric method. It is more reliable than the diagnostic kits. This technique is thus very suited for routine detection of GALT.

Keywords: Galactosemia, galactose 1-phosphate uridyl transferase, spectrofluorometer, well plate reader

HSD No. 6

DETECTION OF TELOMERASE ACTIVITY IN ADULT LEUKEMIA USING TELOMERIC REPEAT AMPLIFICATION PROTOCOL (TRAP) ASSAY

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Telomeres are the protein DNA structures at the end of eukaryotic chromosomes. It is essential in eukaryotic chromosomes because it protects the chromosomes from degradation and end-to-end recombination. Telomeres are usually replicated by telomerase, a ribonucleoprotein enzyme complex that stabilizes telomere length by adding hexameric TTAGGG repeats. Telomerase is repressed in most somatic cells but is active in germ cells, proliferative renewed tissues and in most cancer cells. Several studies have reported that an up-

regulation of telomerase activity suggests progression from chronic to blast phase in most types of leukemia. In this study, we used a PCR-based assay, the Telomeric Repeat Amplification Protocol (TRAP) to detect telomerase activity. TRAP assay, unlike most PCR-based applications, which measure a fixed amount of nucleic acid target in a sample, a technique that measures an enzymatic activity where the amount of target is dependent upon the biochemical activity of the enzyme. In this study, TRAP assay was performed on bone marrow aspirates from 28 adult Filipino patients clinically diagnosed with leukemia. The assay showed that 20 out of the 28 samples have an up-regulation of the regulation of the telomerase activity.

Keywords: telomere, telomerase, leukemia

HSD No. 7

**A COMPARATIVE STUDY BETWEEN THE ST. LUKE'S
IgM-CAPTURE ELISA AND A COMMERCIALY AVAILABLE
IgM ELISA KIT FOR CONFIRMATION OF DENGUE INFECTIONS**

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We developed an in-house IgM-capture Elisa for diagnosis of dengue infection at the Research and Biotechnology Division, St. Luke's Medical Center. The procedure makes use of locally isolated dengue viruses as assay antigen and horseradish peroxidase (HRPO) conjugated IgG to Flaviviruses from human high titer pooled serum. We evaluated the performance of this assay by comparing with the imported dengue IgM-capture ELISA kit (PanBio, Australia) which is commercially available in the Philippines. Forty-one (41) IgM positive human serum samples and 44 IgM negative samples were selected from the St. Luke's Dengue Serum Bank.

The sensitivity and specificity of our in-house dengue IgM-capture ELISA were 100% (27/27) and 80% (44-55), respectively. The positive predictive value and the negative predictive value were 65.9% (27/41) and 100% (44/44), respectively. Statistical analysis showed that the agreement rate between the St. Luke's dengue IgM-capture ELISA and PanBio kit was good: κ (kappa) value was 0.725 (significant at $\alpha < 0.001$). The correlation between the two assays was also good, with correlation coefficient of 0.900 (significant at the 0.01 level, 2-tailed). The St. Luke's Assay can test 45 samples in duplicate per run (including duplicates of positive control, negative control and blank) and costs P1,250.00, while the PanBio kit costs P24,090.00 for similar format. The locally developed kit is more cost effective although it takes two hours longer. However, the additional two hours is insignificant because the results are also obtained within the same day.

Keywords: dengue infection, IgM-capture ELISA, PanBio kit, dengue confirmation

HSD No. 8

CHARACTERIZATION OF *Candida* spp. ISOLATED FROM PAPSMEAR-TESTED WOMEN IN LOS BAÑOS, LAGUNA

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Vaginal swab samples were taken from women who consulted for pap smear-testing in participating clinics in Los Baños, Laguna. The objectives were to isolate *Candida*-like fungi from the pap smear samples, characterize the isolates culturally, morphologically and biochemically and estimate the prevalence of *Candida* species among the subjects.

Twenty-six yeast-like fungi were isolated and purified from 95 vaginal swab specimens but only 20 characteristically produced blastospores and pseudohyphae on Corn meal Agar. Each of these 20 suspected *Candida* isolates was then further characterized for identification based on: colony characteristics on Saboraud Dextrose Agar (SDA) and Broth (SDB), wet mount preparations of a

3day old-culture grown on SDA, observations of a 2-day old-plate culture and a 4-5 day old-agar slide culture growing on Corn Meal Agar, and fermentation of the sugars dextrose, maltose, sucrose and lactose.

Five of the *Candida* isolates were identified to be *C. albicans*, exhibiting creamy, soft, smooth growth on SDA, forming chlamydo spores, pseudohyphae and blastospores on Corn Meal Agar, producing no surface growth on SDB and fermenting dextrose, maltose and sucrose but not lactose. Five other isolates were very similar to *Candida tropicalis*, three resembled *C. stellatoidea* while seven remained unspciated due to the need for additional tests.

The estimated prevalence of *Candida* species in the study population was 21.2% and its was found to be relatively higher among past users of oral contraceptives, or OC (35.5%) compared to those who never used OC (14.8%). It was also higher among pregnant (42.9%) than among non-pregnant study subjects (19.3%).

Keywords: *Candida* species, vaginal swabs, pap smear-testing, chlamydo spores, pseudohyphae

HSD No. 9

GENOTYPIC ANALYSIS OF CITRIC ACID-PRODUCING FUNGI USING SINGLE STRAND CONFORMATION POLYMORPHISMS (SSCP)

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The study made use of single strand conformation polymorphisms (SSCP) and the ITS1-5.8S-ITSII (ITS: internal transcribed spacer) region of rDNA in differentiating fungal strains. Citric acid-producing fungi isolated from rotten fruits (*Mangifera indica* and *Citrus reticulata*) were used. Fungal isolation was

done by serial dilution and plating of macerated rotten fruits. Citric acid production of purified isolates was determined by titrimetric and colorimetric assays. Genomic DNA was extracted from fresh mycelial pellets using the CTAB method. PCR was performed using the ITS1-5.8S-ITSII region of rDNA as the target. Four primers, namely, ITS1, ITS2, ITS86, and ITS4, were used to obtain 3 differently sized fragments. PCR products were mixed with formamide loading dye, denatured for 10 min at 95°C, snap chilled on wet ice, and then ran on 6% polyacrylamide gel at a constant current of 5mA.

Six isolates were obtained and were putatively identified as *Aspergillus* strains based on morphology and cultural characteristics. The sizes of the amplicons are 600, 270, and 300 bp for ITS1-5.8S-ITSII, ITS1, and ITSII fragments, respectively. SSCP results show that the isolates have very similar, if not identical, banding patterns with *Aspergillus niger* compared with other control strains under the same genus. This indicates that the isolates belong to the same species of *Aspergillus* and this is corroborated by data on morphological and cultural characteristics. SSCP analysis is generally useful for fragments of shorter lengths (300 bp or less). However, this study has shown that it can also be useful for fragments up to 600 bp. Using the appropriate control strains, SSCP can be applied in identifying isolates even up to species level.

Keywords: SSCP, *Aspergillus*, rDNA, ITS

HSD No. 10a

**SEROTYPES OF DENGUE VIRUSES ISOLATED
FROM PATIENTS ADMITTED AT ST. LUKE'S MEDICAL CENTER,
QUEZON CITY IN THE YEAR 2001**

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Dengue infection is a major health problem in the Philippines and it occurs every year all year round with peaks during the rainy season. The caus-

ative agent of the disease is the mosquito-borne dengue virus (family Flaviviridae) of which there are 4 serotypes: DEN-1, DEN-2, DEN-3, and DEN-4. In the year 2001, more than 25,000 patients were admitted in hospitals all over the country. Serum samples from 461 patients were sent to the Research and Biotechnology Division (RBD) of St. Luke's Medical Center (SLMC) for laboratory confirmation of the disease through IgM-capture ELISA, a technique that detects the presence of IgM antibodies against dengue. The samples were also used as inoculum to infect the C6/36 mosquito cell line for virus isolation. Two hundred two (202) out of the 449 samples (45%) were found positive for the presence of flavivirus in the infected culture fluid by focus-formation assay. Reverse transcription polymerase chain reaction (RT-PCR) of the positive infected culture fluid showed that 146 samples were positive for dengue. The most prevalent serotype for 2001 was DEN-2 (112 samples), followed by DEN-1 (18 samples), DEN-3 (3 samples) and DEN-4 (2 samples). Some samples showed co-infections of more than one serotype: 6 samples were positive for both DEN-1 and DEN-2, 2 samples for DEN-1 and DEN-3, 2 samples for DEN-2 and DEN-3, and one sample for DEN-2 and DEN-4.

Keywords: dengue virus, flavivirus, IgM-capture ELISA, focus-formation assay, reverse transcription polymerase chain reaction

HSD No. 10b

**PLASMID CONSTRUCTION AND POLYHISTIDINE-TAGGED
DENGUE-2 VIRUS ENVELOPE PROTEIN EXPRESSION**

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A more rapid and efficient site-specific recombination reaction, instead of the conventional restriction enzyme digestion and ligation protocol, has been employed in this experiment using the new Gateway™ Cloning Technology (Life Technologies). In this method, the previously sequenced SLMC 179 dengue-2 virus envelope gene was rescued from virus culture cDNA and modified

through high-fidelity PCR to generate and amplify a Gateway-compatible amplicon. Subsequent BP and LR recombination reactions produced entry and pDest17-based expression constructs, respectively. BL21-S1 *E. coli* competent cells were then transformed with the plasmid construct. Expression of the recombinant amino terminal (His)₆-tagged envelope protein was facilitated through induction with 0.3 M NaCl for 5 hours. Differential expression of total cellular protein and western blotting using mouse anti-6xHis monoclonal antibody verified the presence of the desired protein product. In forthcoming studies, this recombinant envelope protein will be purified by immobilized metal affinity chromatography and renatured to be used as an antigen for ELISA-based diagnosis of dengue virus and for the production of dengue-2 envelope-specific monoclonal antibodies. Furthermore, this could serve as a precursor in the development of candidate dengue sub-unit vaccine.

Keywords: recombination, dengue-2 virus envelope gene, high-fidelity PCR, expression, western blot

HSD No. 11

**CHARACTERIZATION OF THE MANILA FAMILY OF
MYCOBACTERIUM TUBERCULOSIS IN FILIPINO
PATIENTS WITH PULMONARY TUBERCULOSIS**

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Based on DNA fingerprinting, the population structure of *M. tuberculosis* has been found to vary from region to region. Notable among these are the Beijing-, Haarlem- and African Genotype families. Additional DNA technologies also confirm the family groupings of *M. tuberculosis* such as spoligotyping analysis, analysis of

pseudogene *oxyR*, polymorphic GC risk sequences (PGRS) Restriction Fragment Length Polymorphism and variable numbers of tandem repeats (VNTR) typing. No study has yet been done to identify the genotype family of *M. tuberculosis* isolates in the Philippines.

Forty *M. tuberculosis* isolates were analyzed from individual pulmonary TB cases included in a regional prevalence survey of tuberculosis conducted by the University of the Philippines College of Medicine and the Research Institute for Tropical Medicine from December 1995 to August 1996. These *M. tuberculosis* isolates were heat-killed at 80 C in TE buffer and then transported to the University of Hawaii in Honolulu for DNA analysis employing different techniques.

Comparison of the IS6110 RFLP and the spoligopatterns were performed using the Compar software at the RIVM in Bilthoven, the Netherlands. The results were compared with the international database of IS6110 RFLP patterns consisting of 5906 patterns from 30 countries and the international database on spoligopatterns consisting of 3575 patterns from 55 countries. Comparisons were done using the Dice coefficient for calculation of similarities.

The IS6110 RFLP pattern of the *M. tuberculosis* isolates from the Philippines exhibited a high degree of similarity, yet none of them were identical. 38 (95% of the 40 isolates showed a similarity of 80% or greater between the patterns. We designated these 38 isolates the "Manila Family."

It is clear that the high frequency of occurrence, restricted geographical range and similarities of the IS6110 RFLP patterns of the Philippine isolates of *M. tuberculosis* indicate a unique family of *M. tuberculosis*. These findings may be valuable in providing the international database and reference for future studies that will identify geographic distribution of these *M. tuberculosis* isolates as well as studies in identifying modes of transmission among high risk groups that may impact on public health policy and programs on TB Control.

Keywords: DNA fingerprinting, Restriction Fragment Length Polymorphism (RFLP), Spoligotyping, *Mycobacterium tuberculosis*

HSD No. 12

HCV GENOTYPES IN THE PHILIPPINE POPULATION

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Hepatitis C virus (HCV) is recognized as the major etiologic agent of most cases of acute and chronic non-A non-B liver diseases and infects around 1% of the general population worldwide. At least nine major genotypes have been documented and a significant number of data indicate that correlation exists between HCV genotypes with clinical course, virulence and response to interferon therapy. This study aims to identify existing genotypes in the Filipino population by restriction endonuclease cleavage of the RT-PCR amplified 5' non-coding region of the HCV genome. Patients undergoing hemodialysis and blood transfusion, renal transplant patients and blood donors found to have HCV infection by second generation EIA are included in the study. RNA from serum is extracted and a reverse transcription PCR is performed using nested primers from the highly conserved 5' non-coding region of the HCV genome. Samples positive for HCV RNA are genotyped by Restriction Fragment Length Polymorphism (RFLP). Out of the 99 serum samples reactive with anti-HCV by ELISA, 52 were confirmed positive by PCR. Of the 52 samples, 34 were genotype 1a, 9 genotype 1b, 3 genotype 2a, genotype 2b and 1 genotype 4 (Simmond's classification).

Keywords: reverse transcription PCR, Hepatitis C virus, restriction fragment length polymorphism, ELISA

HSD No. 13

**FIVE-YEAR ANTIBIOTIC SENSITIVITY PROFILE OF LOWER
RESPIRATORY TRACT PATHOGENS FROM HOSPITALIZED PATIENTS**

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One thousand two hundred twelve (1,212) lower respiratory tract pathogens commonly isolated from hospitalized cases were collected over a period of five years (1998-2002) from three Metro Manila hospitals and tested for susceptibility to various antibiotics to determine resistance trends. Specimen included sputa, endotracheal aspirates, and bronchial washings. The isolates were identified and tested for susceptibility using the Vitek 60 (Biomérieux). Four hundred sixty-six (38.4%) were identified as *Klebsiella* spp, 480 (39.6%) as *Pseudomonas* spp, 166 (13.4%) as *Enterobacter*, and 100 (8.2%) as *E. coli*. The study employed nine antibiotics representing 4 classes: beta-lactams (ampicillin-AMP, cefazolin-CZ, cefuroxime-ROX) and beta-lactam/b-lactamase-inhibitor (ticarcillin/clavulanic acid- TCC) combination, aminoglycosides (gentamicin- GM, tobramycin- TOB), fluoroquinolones (ciprofloxacin- CIP), and a sulfonamide-trimethoprim combination (trimethoprim/sulfamethoxazole- SXT).

Results indicate that the beta-lactams tested only CZ remains effective to *E. coli* and *Klebsiella*. Ampicillin is exhibiting an increasing efficacy against *E. coli* over the last 3 years. *Enterobacter* spp has shown a consistently increasing resistance to ROX, while *Pseudomonas* spp showed resistance to all the beta-lactams tested. Of the two drug combinations tested, TCC exhibited a consistently better activity over SXT against all the isolates tested. The aminoglycosides have been effective against the isolates over the 5-year period. An upward trend has been noted for ciprofloxacin against *E. coli* and *Pseudomonas* spp. but a gradual decreasing sensitivity has been indicated for *Klebsiella*.

Although sampling size is limited, the study reveals an insight to the responses of the top pathogens commonly isolated from hospitals, and results indicate that the organisms are developing resistance to antibiotics. It is recommended, therefore, that antibiotic prescription be always preceded by susceptibility tests, and that appropriate guidelines be followed strictly by both patients and medical practitioners to control the emergence of antimicrobial resistance.

Keywords: antibiotic sensitivity, lower respiratory tract, pathogens

HSD No. 14

**CLARITHROMYCIN RESISTANCE IN HELICOBACTER PYLORI
ISOLATED FROM FILIPINO PATIENTS: DETECTION BY PCR-RFLP**

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Helicobacter pylori is a microaerophilic bacterium known to cause gastritis, peptic ulcer, and gastric carcinoma. A major problem in *H. pylori* treatment is resistance to clarithromycin, which is a component of the widely used therapy in the eradication of the organism. Resistance of *H. pylori* to clarithromycin is associated with point mutations in the 23S rRNA gene, mainly at positions 2142 and 2143. In this study, A2143G mutation in the 23S rRNA gene was examined by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) in five clarithromycin resistant, one intermediate, and three sensitive *H. pylori* isolates from Filipino patients. Epsilometer test was used to determine clarithromycin susceptibility of the isolates. PCR-RFLP using primers CLA 18 and CLA 21, as well as restriction enzyme *Bsa*I was employed. The clarithromycin sensitive and intermediate isolates included in the study were wild type by PCR-RFLP. In the RFLP patterns obtained, none of the resistant isolates (5/5) exhibited A2143G mutation commonly associated with clarithromycin resistance.

Keywords: *Helicobacter pylori*, 23S rRNA gene, clarithromycin resistance, point mutation

HSD No. 15

***vacA*, *cagA*, AND *iceA*, GENES: CORRELATION WITH
GASTRODUODENAL DISEASES IN *Helicobacter pylori* INFECTION**

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H. pylori infection is strongly associated with chronic gastritis, peptic ulcer diseases and in the subsequent development of gastric adenocarcinoma. Three genes, the vacuolating cytotoxin (*vacA*), the pathogenicity island (*cagPAI*) gene, and the *iceA* (induced by contact with epithelium) are at present the main well established virulence factors of *Helicobacter pylori*. Their presence is among the many other bacterial factors responsible for the successful colonization and establishment of persistent infections.

This study aimed to assess which of these virulent factors could be correlated with the clinical outcome of gastroduodenal diseases. We studied 64 gastric biopsy samples taken from 16 cases of duodenal ulcer, 16 cases of gastric ulcer and 32 cases of gastritis, for the presence of *cagA*, *vacA*, and *iceA* genes by Polymerase Chain Reaction (PCR) detection using specific primers. All 64 samples were completely genotyped by PCR. The *sla m1* genotype was most common in gastritis (62.5%) and gastric ulcer (37.5%) and the *sla m2* genotype occurred in 31.5% of duodenal ulcer. The *cagA* gene predominates in duodenal ulcer (62.5%), followed by gastritis (59.37%) and gastric ulcer (56.25%). The *iceA2-1* gene was detectable in 50% of duodenal ulcer, and 43.75% of gastritis and gastric ulcer, while the *iceA1* gene was present in 43.75% of gastric ulcer, 37.5% of duodenal ulcer and in 9.38% of gastritis. Of the three genes, the *vacA* genotypes were better correlated with the clinical outcome of gastroduodenal disease

Keywords: *Helicobacter pylori*, *vacA*, *cagA*, *iceA*, Gastroduodenal diseases, PCR

HSD No. 16

**INSULIN AND RETINOIC ACID INHIBIT BRANCHING
MORPHOGENESIS OF EMBRYONIC LUNG BUDS IN ORGAN CULTURE**

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The embryonic lung has been shown to develop through epithelial-mesenchymal interaction undergoing dichotomous branching to form an intricate tree-like structure. Various studies *in vivo* and *in vitro* have elucidated the role of several genes involved in lung function and development and these have helped define the mechanisms involved in branching morphogenesis. Knowing important mechanisms in lung development can help create better clinical methods for the cure of lung diseases as well as in devising gene therapeutic strategies for ameliorating lung injury. A better understanding of lung development and mechanism would help provide a more specific approach to individualized treatment of respiratory diseases. In this study, the effects of various substances on lung branching morphogenesis *in vitro* was investigated. We made use of ICR mouse embryo (11.5 dpc) lung bud explants collected in Leibovitz L15 medium and cultured for six days on top of a collagen-coated Nucleopore filter. We investigated the development of the bronchial tree in different culture media such as Leibovitz L15 which does not require CO₂ during culture, Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (D10), D10 supplemented with insulin-transferrin-selenious acid (ITS⁺), and D10 added with retinoic acid (RA). Lung morphogenesis was captured daily using an Olympus digital camera and images were normalized and processed using Adobe Photoshop 5.0. Explants were observed with respect to the complexity of branching which was analyzed using fractal analysis. Among the results obtained, the effect of insulin in the cultures was most notable. Results revealed that when ITS⁺ was added to the cultures, a more proximal differentiation pattern was obtained as opposed to the normally dichotomously branched pattern which increases in complexity as the lung develops distally. The same effect was observed with lung cultures in D10+RA. These results suggest that insulin and RA inhibit distal lung branching morphogenesis.

Keywords: lung branching, insulin, retinoic acid, lung development

HSD No. 17

**PCR-DETECTION OF CEA mRNA IN REGIONAL LYMPH
NODES OF PATIENTS WITH COLORECTAL CANCER**

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The prognostic potential of reverse transcriptase-polymerase chain reaction (RT-PCR) on carcinoembryonic antigen (CEA) determination was investigated and compared with the histopathological method for lymph node status determination in colorectal cancer.

Detection of CEA by RT-PCR was performed on lymph nodes taken from patients with colorectal cancer and benign colorectal diseases. Lymph nodes were resected from pericolic and peritumoral regions and total RNA was extracted from each node separately. Primers specific for the CEA gene were used and the presence of CEA messenger ribonucleic acid (mRNA) in lymph node samples was considered evidence of metastasis.

Thirty-five or 37% of 94 mRNA samples obtained from histologically negative lymph nodes were found positive for CEA by RT-PCR. Lymph nodes from a patient with benign colorectal disease exhibited no CEA mRNA. Overall, 43 (44%) of the total 102 lymph nodes were positive by RT-PCR detection compared with only eight (7%) lymph nodes positive for micrometastases by histopathological analysis.

This study shows that RT-PCR of CEA mRNA in patients with colorectal cancer may provide additive value to histopathological analysis in detecting lymph node micrometastasis and predicting recurrence, especially when micrometastasis spreads out separately from the main tumor to distant lymph nodes.

Keywords: CEA, carcinoembryonic antigen, mRNA, messenger ribonucleic acid, colorectal cancer, pericolic, peritumoral

HSD No. 18

**MULTI-LEVEL DETECTION OF HEMOGLOBINOPATHY
AMONG BLOOD DONORS AT ST. LUKE'S MEDICAL CENTER**

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Hemoglobinopathies are diseases resulting from hereditary abnormalities of the hemoglobin chains. The abnormality (mutation) may alter globin protein, its synthesis or globin development switching. This results in either complete absence or defective synthesis of different hemoglobin types. Thalassemia, a disease resulting from an imbalance of globin-chain synthesis belongs to this group. The pathophysiology of this disease lies in the resulting imbalance (ratio) between the alpha (a) and beta (b) globin gene synthesis. The chain that is produced at the normal rate is in relative excess. In the absence of a complementary chain with which to form a tetramer, the excess normal eventually precipitates in the cell, damaging the membrane and leading to premature blood cell destruction. Clinical manifestation of the disease ranges from mild anemia to hydrops fetalis depending on the degree of mutation.

A multi-level detection of the disorder was conducted on blood donors of St. Luke's Medical Center. The study aims to characterize the thalassemia mutations at the cellular and molecular level and to determine the frequency of hemoglobinopathies among blood donors.

Blood samples were initially screened using complete blood count with red cell indices specifically Mean Corpuscular Volume (MCV) and serum ferritin. Of the 1,468 samples, 70 samples were included based on the inclusion criteria of MCV less than 80 fl and serum ferritin of greater than 12 g/dl. The presence and the amount of the different hemoglobin types were densitometrically measured and analyzed. Four samples with an abnormally high amount of HbA₂, greater than 3%, were observed. Samples with MCV less than 80 fl were consequently run in PCR using primers specific for alpha thalassemia.

Keywords: hemoglobinopathy, thalassemia, mean corpuscular volume, serum, ferritin, polymerase chain reaction (PCR)

HSD No. 19

**LOW PROPORTION OF DYSTROPHIN GENE DELETIONS
AMONG FILIPINO DUCHENNE AND BECKER
MUSCULAR DYSTROPHY PATIENTS**

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Duchenne muscular dystrophy (DMD) is an X-linked progressive neuromuscular disorder affecting 1 in 3500 male livebirths. It has a milder allelic form, Becker muscular dystrophy (BMD) which occurs in 1 in 20,000. Both are caused by mutations in the dystrophin gene on the X chromosome. In DMD, affected individuals have little or no functional dystrophin while those with BMD may have a partially functional dystrophin giving rise to its milder clinical manifestations.

Mutations that cause DMD/BMD are deletions of one or more exons of the dystrophin gene, duplications of the exons or point mutations. The proportion of deletions among mutant dystrophin alleles in North American and European studies is 55-65 % [Baumbach et al., 1989; Koenig et al., 1987; Forrest et al., 1988]. Shomrat et al. [1994] described a lower proportion (37%) of dystrophin gene deletions among Israeli DMD and BMD patients. Other Asian populations have likewise shown a lower proportion of deletions among mutant dystrophin: 40-43% in Japan [Sugino et al., 1989; Kitoh et al., 1992]; 45-50% in Chinese populations [Soong et al., 1991; Lau et al., 1992]. A racial difference in the proportion of deletions may exist

We examined DNA samples of 41 unrelated Filipino patients diagnosed with DMD and BMD. Of these patients, 15 have already been included in a previous report [Cutiongco et al., 1995]; this is an extension of that study. Deletions were detected using multiplex DNA amplification procedure which permits the rapid identification of 80-90% of all dystrophin gene deletions [Chamberlain et al., 1988].

Our results show that only 11 out of the 41 patients (26.82%) have deletions in at least one of the 23 exons of the dystrophin gene examined. This proportion of dystrophin gene deletion is the lowest reported so far compared to other populations. This low proportion makes diagnosis using the current PCR techniques particularly difficult. In addition, we found that the deletions among Filipino DMD/BMD patients were more common in the 5' end (63.63%) than in the central rod domain (36.36%) of the dystrophin gene. The findings suggest the presence of genetic variability among DMD/BMD patients in different populations.

Keywords: Duchenne Muscular Dystrophy, DMD, Becker Muscular Dystrophy, BMD, dystrophin gene, X-linked

HSD No. 20

MUTATIONS OF THE STEROID 21-HYDROXYLASE GENE AMONG FILIPINO PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

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Congenital Adrenal Hyperplasia (CAH), an autosomal recessive disorder, is due to defective enzymes involved in adrenal steroidogenesis. Phenotypic manifestations are variable depending on the effects produced by the deficient hormones and by the excess production of steroids unaffected by the enzymatic block. The worldwide incidence of CAH is 1 in 15,000 with ethnic and racial variability. The incidence in France, Italy, Scotland, New Zealand and Japan ranges from 1 in 10,000 to 1 in 23,000. Among Filipinos, the crude incidence of CAH is 1 in 6,747 (Philippine Newborn Screening Update, 2002), which is higher than what is reported in most populations. More than 90% of all cases result from a 21 hydroxylase (cytochrome P450c21) deficiency involving two 21 hydroxylase genes CYP21, the active gene and CYP21P, a pseudogene. Studies have shown that mutations result from unequal crossover during meiosis which leads to complete deletion of the gene, gene conversion events or to point mutations. Major-

ity of these studies have demonstrated differences in the frequency of several gene mutations. Using a previously described method (Lee et al, 1996) of combined differential Polymerase Chain Reaction (PCR) and Amplification Created Restriction Site (ACRS) approach, direct probing for the presence of known mutations in exon 1,3,4,6,7,8 and intron 2 of the CYP21 and CYP21P genes among Filipino patients with CAH was performed. A total of 12 unrelated CAH patients were examined. One of the twelve cases (8%) demonstrated two different mutations in her CYP21 gene; in ten of the twelve cases (83%) only one type of mutation was detected. Majority of these cases had the mutation at nucleotide 656 of intron 2, a premature splicing error. The determination of the most frequent alleles in our population will facilitate rapid screening for detection of mutations in the 21 hydroxylase gene. Establishment of a definitive diagnosis can be also made available which are important in the management and counseling of Filipino CAH cases.

Keywords: congenital adrenal hyperplasia, CAH, CYP21 gene, CYP21P gene, Amplification Created Restriction Site (ACRS)

HSD No. 21

USE OF FLUORESCENCE IN SITU HYBRIDIZATION (FISH) IN THE DIAGNOSIS OF FILIPINO PATIENTS WITH PRADER-WILLI SYNDROME

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Prader-Willi syndrome, or PWS, is a complex disorder, involving multiple systems with many manifestations including infantile hypotonia, developmental delay and mental retardation, behavior problems, obesity, characteristic facial features, hypothalamic hypogonadism, and short stature. The cause of PWS is the absence of normal active paternal genes in the proximal region of chromosome 15q. There are three different types of mutations on chromosome 15 which may

lead to absent normal paternal genes in this region: (1)paternal interstitial deletion, (2)maternal uniparental disomy (UPD), or (3) mutation or abnormality in the imprinting center. Deletions account for 70-75% of the cases, 25-28% have maternal UPD, and <2% have defects in the imprinting center. High resolution G-banding of chromosome 15 has been shown to be an unreliable method to diagnose the deletions while fluorescence in situ hybridization (FISH) with specific DNA probes has proven to be more sensitive in detecting the deletions. The recent availability of this genetic test locally has given us the chance to confirm suspected PWS cases and to characterize the mutations in the Filipino population. Fourteen unrelated Filipino PWS patients were included in the study. Cytogenetic analysis was normal for all patients but the FISH technique detected deletions in 50% of the subjects. The confirmation of the diagnosis will now allow the determination of the recurrence risk for the families concerned.

Key words: Prader-Willi Syndrome, PWS, fluorescence-in-situ hybridization, FISH, microdeletion

HSD No. 22

TWO FILIPINO CASES WITH RARE AUTOSOMAL TRISOMIES

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Trisomies for autosomes other than chromosomes 21, 18, and 13 are noted to have severe consequences and inevitably result to fetal death *in utero*. There are rare instances wherein these other autosomal trisomies survive the neonatal period. We report two such cases. The first is a case of a 1-month old male born premature to a non-consanguineous Filipino couple. This case presented with intrauterine growth retardation, microcephaly, hypertelorism, epicanthic folds, midface hypoplasia, low set ears, multiple ventricular septal

defects and a hypoplastic 5th distal phalanx. The diagnosis of Trisomy 22 was confirmed in all cells examined by chromosomal analysis (G-banding). The second case is a 3-month old female born term to non-consanguineous Filipino parents. She presented with a high arched palate, low set ears, bifrontal hair whorls, unequal palpebral fissures, hypertelorism, limited movement of the left eye, overlapping fingers on the right hand (5th digit over the 4th digit, 2nd digit over the 3rd digit), large flat nipples with a hypopigmented central area of the areola, a sacral dimple, and a wide gap between the first and second toes. Chest radiographs showed eleven pairs of ribs. Chromosomal analysis by G-banding confirmed the diagnosis of Trisomy 11. To our knowledge, there are no other case reports of trisomy 11 in literature. Mosaicism is not discounted as a possible cause for the prolonged survival of our patient. These cases add to the world literature on autosomal trisomies in liveborns.

Keywords: autosomal trisomy, trisomy 11, trisomy 22, multiple congenital anomalies, chromosome 11, chromosome 22

HSD No. 23

INITIAL VERSUS CONFIRMATORY THYROID STIMULATING HORMONE (TSH) LEVELS: IS THERE A CORRELATION?

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Newborn screening for congenital hypothyroidism (CH) in the Philippines began in 1996. The screening method used is the fluorometric assay of the thyroid stimulating hormone (TSH) from the blood impregnated in the Guthrie card. In the past five years (June 1996-September 2001), 176,548 newborns have been screened. Of these, 237 had elevated TSH levels, and approximately 51 (22%) are confirmed to have CH. 146 (61%) had normal TSH levels on confirmatory testing, five (2%) expired; 25 newborns (11%) are lost to follow-up, while 10 (4%) are currently being recalled. 33 out of 51 (65%) CH patients are female. Only 38 of the 51 patient charts were available for data analysis. Thirteen out of 51 CH patients were lost to follow-up after confirmation of the disorder. The mean age at which levothyroxine medication was initiated is 1 ½ months at a modal dosage of

2.5 mcg/tab OD. The initial TSH levels as determined by the Philippine Newborn Screening Laboratory directly correlates with the confirmatory TSH levels done in other endocrine laboratories (Spearman's $\rho=0.57$, P value= 0.0002 , at $\alpha=0.05$). However, the time of heelprick on the newborn is independent of the TSH levels, (Spearman's $\rho= -0.16$, P value= 0.377 at $\alpha=0.05$) hence there is no significant difference with respect to the initial TSH level of blood sample taken at 48 hours, less than one week, one to two weeks, or even more than two weeks after birth (Kruskall Wallis test, P value= 0.064 at $\alpha=0.05$). Using Fisher's exact test, there is no sufficient evidence to say that there is an association between gender and the incidence of CH among screened newborns whose TSH levels are initially elevated ($P_{2-tailed}=0.183$, $P_{1-tailed}=0.113$ at $\alpha=0.05$).

Keywords: newborn screening, TSH, congenital hypothyroidis

SOCIAL SCIENCES

SSD No. 1

POPULATION STRUCTURE OF THE HIGAONON TRIBE OF ROGONGON, ILIGAN CITY

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This study was conducted to learn the population structure of the indigenous peoples of Rogongon, Iligan City – the Higaonons. The group consisted of true-blooded Higaonons and those who are the children of a Higaonon -- Christian/Muslim marriage, the Kulibugans. Survey of nine Mendelian traits, demographic factors, diseases prevalence, and reproductive health of women were conducted. Results show homozygous recessive genotypes are prevalent in the populations of both pure Higaonons and the Kulibugans based on nine traits. Expected heterozygous genotypes were also high in all the

populations of both men and women that served as basis for the high degree of recessives in all the populations. Disorders and susceptibility to diseases like tuberculosis and high infant mortality were also observed in the tribe and pedigree analysis indicates genetic control of the disorders and predisposition of the individuals to the disease. Based on the result of the survey on the reproductive capability of the women where they were observed to produce large number of children, it is feared that the frequency of the disorders will increase based on the high reproductive potential of the women of the populations. It is argued that with a population like this, it is appropriate that something should be done about the plight of this indigenous group

Keywords: population structure, Higaonon tribe, Kulibugan, Mendelian traits

SSD No. 2

ENHANCING FARMERS' CAPACITY TO MANAGE RESOURCES: THE SOCIAL IMPACT OF FARMERS FIELD SCHOOL (FFS) APPROACH IN WESTERN VISAYAS

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Women participation in the season long training program (Farmer Field School) in Dao, Capiz and Zarraga, Iloilo Western Visayas, Philippines increased through time (15% to 45% from 1995-2000). Their role in managing rice, generally increased in both sites in terms of allocating cash for inputs (i.e. quality seeds, fertilizers), decision not to use pesticides in the field and greater influence in deciding how proceeds will be spent/invested. However, diffusion of knowledge by IPM graduates is strongly divided by gender, men diffusing mostly to men and women mostly to women. These findings indicate the importance of gender balance in information dissemination among FFS participants and non-participants taking into consideration equal communication flow between men and women. Also, younger farmers (20%) were not well represented in the training. Generally, older graduates did not generally pass on what they have learned to them. These affected the diffusion pattern within the farming community wherein knowledge is shared.

Keywords: IPM, FFS, communication diffusion, gender

SSD No. 3

**MARKET STRUCTURE, CONDUCT AND PERFORMANCE
OF THE RICE MILLING AND TRADING INDUSTRIES
IN THE PHILIPPINES**

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This study analyzed the market structure, conduct, and performance of the rice milling and trading industries in the Philippines. It used data from the survey of rice millers and traders in Ilocos, Cagayan Valley, Central Luzon, and Southern Tagalog.

The market structure of the rice milling and trading industries was imperfect based on descriptive analysis, four-firm concentration ratio (CR4), Lorenz curve, and gini-coefficients. Except for the rice retailing industry, market concentration increased and a high degree of inequality was observed in the rice milling, wholesaling, and wholesaling-retailing industries. Paddy and rice traded in the market were highly differentiated with no proper grading and standardization systems used. There was a high level of barriers to entry and exit in both industries. Market information was regarded as imperfect because of the lack of credibility of the source and unreliability of the information disseminated among the participants in the market.

Results of the market conduct analysis showed that different price and product policies were used by both industries to adjust and coerce their market opponents. Rice milling and trading industries were price-inefficient because of the weak degree of market integration between market levels brought about by inadequate infrastructure and high transportation costs. Rice mills were underutilized and economies of scale only exist in large-capacity rice mills. The large-scale rice mills generated higher net returns than the small ones. Rice wholesalers received much net return based on volume sold. Big millers and rice traders with enough capital were progressive.

Findings of the study suggested that there is a need to encourage more private traders in the rice market; simplify the licensing procedures; strictly en-

force proper grading and standardization systems; spot check rice adulteration; improve the delivery of market information; reduce transportation costs; improve farm-to-market roads; and increase the capacity utilization of rice mills.

Keywords: rice, marketing, rice pricing efficiency, market integration, rice milling

SSD No. 4

**FOOD-CARRYING AND INCOME-GENERATING CAPACITIES
OF RICE-PRODUCING PROVINCE OF NUEVA ECILJA, PHILIPPINES**

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This study assessed the food-carrying and income-generating capacities of the rice sector of Nueva Ecija. Food-carrying capacity (FCC) is the number of human population that can be supplied with minimum rice requirement that would meet their recommended dietary allowance for energy. Income-generating capacity (IGC), on the other hand, is the profit from producing optimum level of quantity at equilibrium price.

Linear programming model was used in determining the FCC and IGC of the province. The model used the input-output coefficients generated from the cost and return survey. Resource constraints were also provided. Moreover, sensitivity analyses were done to see the effects of different policies on the base model results. The income generated from the model was compared with the regional poverty threshold level to get the IGC ratio. A ratio greater than one indicates that the province has income-generating capacity. Results showed that Nueva Ecija has IGC ratio of 0.40. This means that income of its rice sector cannot support the minimum basic expenditure of its population represented by regional per capita poverty threshold.

On the other hand, FCC ratio was computed by comparing the optimal production of milled rice generated from the model with the total rice requirement of the province. Total rice requirement is per capita consumption multiplied with the province's total population. Computations showed that Nueva Ecija's FCC ratio is 2.99, thus, it can support the rice requirement of its populace.

Sensitivity analysis showed that to improve their capacities they must use yield-enhancing and cost-reducing technologies. It was then recommended that: 1) R & D should focus on cost-minimizing technologies; 2) increase investments in promotion of yield-enhancing technologies such as use of quality seeds; 3) lower price of inputs through efficient marketing; and lastly, 4) development of infrastructure such as irrigation, farm-to-market roads, and access to credit.

Keywords: rice, food-carrying capacity, income-generating capacity, dietary allowance, linear programming, poverty threshold

SSD No. 5

**DECOMPOSITION OF RICE PRODUCTION COSTS AND
MARKETING MARGINS: CASE OF NUEVA ECILJA, PHILIPPINES**

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This study attempts to decompose the costs and margins from rice production to consumption. It sought to identify which stage or activities in the production and marketing process can innovations be done to improve competitiveness in the rice industry. Results for Nueva Ecija showed that on a per unit basis, a rice farmer gets 60 to 65% of the gross margin, while the paddy trader and rice miller get 20% to 25%, and the rice wholesalers and retailers get 15%. During the dry season, farmers get a net profit per unit similar to traders, which is around P4 per kg milled rice equivalent. However, during the wet season, traders are able to maintain their net profit while farmers get less than half at more than P1 per kg.

On the production side, results showed that the major cost items are labor, land rental, fertilizer and seed cost. Labor cost for harvesting and threshing, which is normally imputed based on prevailing rice prices has the highest cost share at around 20%. Seed cost is still high at P0.50 per kg paddy. On the marketing side, bulk of the cost incurred is drying and transportation costs. No sufficient

evidence was found to show that the marketing margins of the market players are excessive on a per unit basis. In general, the high profits of rice traders are largely owing to the large volume handled and swift turnover of transactions.

Simulations showed that when production cost is reduced to P4 per kg through technological advancement, and assuming reasonable mark-ups for farmers and traders, the price of rice would still be higher than the current world price. A strong policy to support R&D, technology promotion and infrastructure development are still the best options for the rice industry to be competitive.

Keywords: rice, production cost, marketing margin, rice trading

SSD No. 6

ECONOMIC ANALYSIS OF HYBRID SEED (AXR) PRODUCTION IN THE PHILIPPINES

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This paper aims to analyze the profitability status of hybrid seed multiplication and compare it with the returns from inbred seed production, which is a more popular alternative activity. The data used in the analysis is obtained from a survey of 25 hybrid and 30 inbred seed growers in Isabela and Davao del Sur, the major hubs of hybrid seed production in the country. Farm-budget analysis, breakeven and sensitivity analysis were employed in the analysis.

Results showed that the average yield for F1 seed production was 0.75 mt/ha. Labor input for hybrid seed multiplication was about 120 person-days/ha, which is 40 person-days higher than the labor requirement of inbred seed multiplication. In contrast, nitrogen application of the former is 23% lower than that of the latter. Chemical application of hybrid seed production amounts to 4 kg/ha of active ingredient, almost double of the amount applied to inbred seeds.

In general, the net income from hybrid seed production is about 30% higher than that of inbred seed production. However if the hybrid seed production support were removed, this net profit advantage would be reduced to 16%. This is because the price of hybrid seeds is eight times of the price of certified inbred seeds. At the current average yield of hybrid seeds, the breakeven price would be Php76.00/kg. However to induce inbred seed growers to shift to hybrid rice production by having a marginal benefit-cost ratio of two, the yield level must be at least 900 kg/ha.

Given the current economic status of hybrid rice production, the introduction of yield enhancing and cost reducing technologies is still needed in order to improve its profitability vis-à-vis inbred seed production.

Keywords: hybrid, inbred, seed production, farm-budget, breakeven, sensitivity, marginal benefit-cost ratio

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