# **BIOLOGICAL SCIENCES**

#### 1. GLUCOSIDASE AND PROTEASE FROM Acanthamoeba spp.

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A highly active glucosidase enzyme of Acanthamoeba sp. (H-1) isolated from a keratitis patient was detected. The glucosidase was able to digest  $\beta$ -Dglucopyranoside labeled with 4-methyl umbelliferyl. This was isolated from a crude cytoplasmic lysate from trophozoites successfully grown in Proteose Peptone Yeast Glucose medium. The protein was identified as a high molecular weight band visualized by UV light on 7% nondenaturing polyacrylamide gel electrophoresis (PAGE). This Acanthamoeba glucosidase has a slightly lower size compared with the Aspergillus niger cellulase enzyme which was used as control. It may be postulated that the Acanthamoeba indeed contains a cellulase enzyme and its role in understanding the properties of protozoan glycosidases is indeed significant with the advent of glycobiology. Moreover, the cloning of the cellulase gene for biotechnology has been a difficult pursuit in the degradation of massive cellulosic materials from plants. Acanthamoeba as an ubiquitous free-living and pathogenic organism contains many bioactive proteins like proteases which were shown to be present by their ability to hemolyze 1.5% rabbit, horse, as well as human, erythrocytes. These putative proteases were also found to have antimicrobial properties as demonstrated by a large zone of inhibition against Bacillus subtilis. Partial purification of these proteolytic enzymes was performed using Sephadex G200 size exclusion column chromatography. Sample fractions with hemolytic activity were shown to contain a high amount of low molecular weight protein subunits at the 50-60 kDa range in 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

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## 2. HISTOARCHITECTURE OF THE INTESTINES OF WILD AND LABORATORY ACCLIMATED MOSQUITOFISH, Gambusia affinis affinis, FROM THE MALABON RIVER

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Anterior intestines of wild *Gambusia affinis affinis* from three tributaries of the Malabon-Tullahan River were compared with those that were laboratory-acclimated for 30 days. Irregular shape of the intestinal folds, mucosal fold fusion, and presence of globular structures in the mucosa and in the lumen were characteristics of the wild fish that disappapeared upon becoming lab-acclimated. A comparative study was also done between populations of the fish in the Malabon River and those in the College of Science pond, UP Diliman. The anterior intestines of the lab-acclimated fish closely resembled the anterior intestines of *Gambusia affinis affinis* from the College of Science pond.

The compensatory mechanisms of this fish in a harsh environment account for its unabated proliferation in the Malabon River.

## 3. IN VITRO CHEMICAL PROTECTION AGAINST IONIZING RADIATION ON C6 NEUROGLIAL CELLS: DEVELOPMENT OF A RAPID MICROCOLORIMETRIC ASSAY FOR RADIOPROTECTORS

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An in vitro assay for radioprotectors was developed based on the standard MTT assay applicable to screening and testing of drugs with radiomodifying effects for combined modality treatment of cancer. Assay conditions were tested based on the following parameters: type of radioprotector (L-cysteine,  $\alpha$ -tocopherol, and *Panax ginseng* extract), concentration of radioprotector (0, 5, 10, and 50 µg/ml), radiation dose (0, 1, 5, 10, and 50 Gy) and post-irradiation recovery period (24 and 42 hours). Radiation protection was demonstrated at all concentrations of the radioprotectors. Optimal assay conditions are at 10Gy radiation exposure and 42 hours recovery period. A standard 30-day mouse assay was also performed for comparison with the in vitro assay.

### 4. RESPONSE OF Vigna radiata WILCZEK VARIETY MGO7 γ-IRRADIATED AT SUBLETHAL DOSES

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Dried seeds of Vigna radiata Wilczek variety MGO7 were  $\gamma$ -irradiated at 200 and 400 Gy and cultivated in vitro. Direct regeneration of the cotyledons was performed. Decreasing capacity for regeneration was noted with increasing doses. Stem lengths markedly increased for plants whose seeds were irradiated at 200 Gy (19.4 ± 1.44 cm) but decreased at 400 Gy (15.70 ± 1.11 cm) and 0 Gy (13.30 ± 0.74 cm) exhibiting "radiation-dependent reversed phenotypes". Other epigenic effects noted include the relative thickness of the stems, the elaborations of the roots, directions of stem growth, and the relative sizes of the leaves.

Protein analysis of  $\gamma$ -irradiated intact plants was performed. Whole plants irradiated at 200 and 400 Gy and allowed to recover for 4 and 25 hours, were homogenized and the water-soluble proteins were extracted and electrophoresed in denaturing gel. Changes in protein profiles at various doses (200 and 400 Gy) and recovery periods (4 and 24 hours post-irradiation) were evident. The possibility that these radiosensitive proteins may act as biomolecular markers for irradiated plants (i.e., during mutational breeding and food irradiation) for the determination of the extent of radiation damage may be explored.

## 5. TOXICITY LEVEL OF POLLUTED SOILS IN BELGIUM USING THE EARTHWORM, Eisenia fetida, AS INDICATOR ORGANISM

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The acute toxicity test using the earthworm, *Eisenia fetida*, was used to evaluate the toxically of polluted soils from 12 different industrial sites of Belgium. Four sites were identified as non-acutely toxic, two toxic, four highly toxic, and two extremely toxic. The identified toxicants were petroleum ether- and chloroform-extractable organic halogens like chlorinated pesticides and heavy metals. There was no significant relationship between the  $LC_{50}$  values and the concentration of each

chemical present, hence it was not possible to single out any individual chemical responsible for toxicity.

The *E. fetida* acute toxicity test showed a significant relationship (r = 0.90) with the result of the rotifer, *Brachionus calyciflorus*, acute toxicity test using the same soil samples. The chemicals present had the same effect on earthworm and rotifier.

## 6. SOURCES AND LEVELS OF RADIOACTIVITY IN SPECIFIC MARINE AREAS AND THEIR RELATIVE CONTRIBUTIONS TO OVERALL ASSESSMENT FROM MARINE RADIOACTIVITY

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The Philippines, designated as FAO Area 71 (Western Central Pacific), is an archipelago of islands with over 17,000 km of coastline. Fish and other seafood constitute a major portion of the daily diet among a large number of the population. The study to be reported here was undertaken to assess the dose received by the Philippine population – due to consumption of marine products – as part of the Coordinated Research Programme of the International Atomic Energy Agency. Two radionuclides, <sup>137</sup>Cs and <sup>210</sup>Po activities in fish, molluscs, crustaceans, and seawater in FAO Area 71 are analyzed in thirteen predominant specific groups and species of fish present in the Filipino diet. Concentration factors of <sup>137</sup>Cs and <sup>210</sup>Po in

fish, molluscs, and crustaceans are computed based on the mean activity concentrations of these radionuclides in marine biota and surface seawater. Concentration factors of <sup>137</sup>Cs in fish and shellfish FAO Area 61 are analyzed and reported from Japanese data on <sup>137</sup>Cs activity concentrations in fish, shellfish, and seawater available in published literature. Total marine catch, total daily consumption of marine products in FAO Areas 71 (Western Central Pacific) and 61 (North Western Pacific), and time delays from catch landing to consumption of fish and fish products in FAO Area 71 (Philippines) are presented.

### 7. METAZOAN PARASITES OF SOME FISHES FROM TAAL LAKE

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Six species of fishes collected from Lake Taal, Batangas Province, Philippines from June 1993 to June 1994 were examined for metazoan parasites. Eleven species of parasites were recovered: four Monogenea, three Digenea, one Nematoda, two Acanthocephala, and one Crustacea. No parasites were recovered from *Sardinella tawilis* (Herre). Two digenetic trematodes, *Neochasmus microvatus* (Tubangui 1928; Tubangui and Masilungan, 1944), and *Opegaster minima* (Tubangui, 1928; Yamaguti, 1934), and a nematode were found in *Glossogobius guirus* (Hamilton-Buchanan).

Opegaster minima and the isopod, Alitropus typus Edwards, 1840 were recovered from Ophiocara aporos Bleeker and Apogon thermalis Cuvier and Valenciennes. Oreochromis niloticus (L.) was infested with Monogenea belonging to three species of Cichlidogyrus (C. tiberianus Paperna, 1960, C. sclerosus Paperna and Thurston, 1969, and C. halli Price and Kirk, 1967). An Acanthocephala and Alitropus typus were also recovered from O. niloticus. Therapon plumbeus (Kner) harbored the monogenetic fluke, Diplectanum sp. Diesing, 1858, the Digenea, Paropecoelus sp. Pritchard, 1966, an Acanthocephala, and Alitropus typus. Prevalances (%) and intensities of infection are presented for the different species of parasites.

### 8. STRUCTURAL CHANGES IN THE HEPATOPANCREAS OF Pomacea canaliculata AFTER EXPOSURE TO MALATHION

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Young (one month old) and adult (two and a half months old) *Pomacea* canaliculata were directly and indirectly treated with malathion. For the direct treatment, 2 sublethal concentrations, (10 and 12 ul) for the young and (15 and 17 ul) for the adults were used. With the 15-day treatment, the solution was changed every five days. For the indirect treatment, the snails were fed with lettuce immersed for 24 hours in sublethal concentrations double the concentrations used for

the direct treatments. After 15 days of direct and indirect treatments, the hepatopancreas of both the young and the adult were dissected out and processed for histological studies under both light and electron microscopes.

The presence of digestive cells was observed in all treated snails. The hepatopancreas showed non-compact digestive cells because of the destroyed connective tissues that bind them. This observation was true in all treated snails, whether young or adult. There were calcium cells that could be seen in the hepatopancreas and this effect of malathion in the calcium cells has not been previously reported. The changes in the histology of hepatopancreas are evidences of the effect of malathion.

## 9. ULTRASTRUCTURAL CHANGES IN THE DIGESTIVE SYSTEM OF Achatina fulica FERRUSAC INDUCED BY THE MOLLUSCICIDE BAYLUSCIDE

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Achatina fulica Ferrusac, known as the giant African snail, is a pulmonate terrestrial gastropod of economic and medical importance.

The study determined the effect and the extent of the cell damage caused by Bayluscide on the digestive tract and digestive glands of *Achatina fulica* Ferussac, using the paraffin and resin method. The sectioned tissues were viewed under the light and electron microscopes.

The macroscopic reactions of the *Achatina fulica* Ferussac correspond to the reactions described as typical for carbamate or metaldehyde intoxication. Thirty minutes after ingestion of Bayluscide-soaked shredded lettuce leaves, the animal shows violent muscle convulsions. The anterior body begins to swell while the posterior part flattens. The tentacles are relaxed, the animals release a lucid mucus and take up liquid from the environment. Hours after treatment, the snails lie almost motionless but recovery is possible. The animals were observed to lose much more slime after the application of pesticide.

### 10. CHARACTERIZATION AND IDENTIFICATION OF PHB-PRODUCING BACTERIA\*

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A study was conducted to characterize and identify local bacterial isolates which produce poly- $\beta$ -hydroxybutyrate (PHB), a biodegradable plastic, as an alternative to conventional plastics.

Ten selected isolates were characterized morphologically, culturally, and physiologically using a combination of tests including the use of a commercially available identification kit. The kit was used to obtain the biochemical profile of the organism and to identify it at the species level.

Results of morphological characterization showed that all isolates were rodshaped Gram-positive sporeforming bacteria belonging to the genus *Bacillus*. Further characterization showed a generally homogeneous grouping of isolates based on the utilization of 49 carbohydrates. The isolates however were found to belong to either one of the two *Bacillus* species having very similar carbohydrate utilization patterns, i.e., *B. megaterium* and *B. circulans*. Nevertheless, the morphological and cultural characteristics strongly indicated that the isolates belong to *B. megaterium*.

### 11. CULTURE AND STOCK MANAGEMENT OF COMMON NON-TRADITIONAL EDIBLE BIVALVES OF BICOL WATERS

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The growth of blood cockle (*Anadara granosa*) in Mambulao Bay, Jose Panganiban, Camarines Norte, and that transplanted from the bay to the muddy bottoms of Cagraray Island, Bacacay, Albay, were studied comparatively to determine success of transplantation. Von Bertalanffy Growth Function curves were

<sup>\*</sup>Best poster paper award in the Biological Sciences Division.

fitted to the shell length frequencies of both populations collected over a 12-month period to estimate their asymptotic length  $L_{00}$  (in mm) and growth constant K (/yr).

Optimum estimates of the growth paramaters were 54.5 mm and 1.04/yr in the natural stock and 50.3 mm and 0.93/yr in the pen-cultured stock. The close  $L_{00}$  and K values indicate comparable growth of the bivalve in the two areas. The slightly lower estimates for the captive stock may suggest some depression of its growth due to the increase in culture density. However, production in the cultured stock is significantly higher than that estimated for the wild stock.

The growth and mortality parameters of blood cockle in the bay were used to assess the exploitation level and yield-per-recruit of the stock. Resource conservation and protection schemes for the mollusc fisheries of the bay have been outlined to provide options towards a community-based coastal resource management.