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**TRANSACTIONS**  
**of the**  
**NATIONAL ACADEMY OF SCIENCE**  
**AND TECHNOLOGY, PHILIPPINES**

1997  
Volume XIX



Republic of the Philippines  
National Academy of Science and Technology, Philippines  
Philippine Science Heritage Center, DOST Complex  
Bicutan, Taguig, 1631 Metro Manila

**TRANSACTIONS OF THE  
NATIONAL ACADEMY OF SCIENCE AND  
TECHNOLOGY, PHILIPPINES**

# 19th ANNUAL SCIENTIFIC MEETING

## Theme: "Science for Better Health"

held at

The Westin Philippine Plaza  
CCP Complex, Roxas Boulevard  
Pasay City  
9-10 July 1997

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## INTRODUCTION OF THE KEYNOTE SPEAKER

WILLIAM G. PADOLINA

*Secretary*

*Department of Science and Technology*

I am privileged to introduce to you our guest of honor and keynote speaker this morning.

Our distinguished guest belongs to an illustrious clan of lawmakers, educators, and public servants from the province of Batangas. In fact, a random scan of famous names from his family reads like a Who's Who of the late-19th and 20th century Philippine history.

He prepared for his storied political career by completing his elementary education at the Ateneo de Manila Grade School. He finished high school at De La Salle College during the war years. At the University of the Philippines, where our speaker became renowned as a passionate orator and debater, he graduated with an Associate of Arts degree in both pre-med and pre-law before capping his collegiate career with a Bachelor of Laws degree in 1952. He then went to Yale University, where he obtained his Master of Laws in 1953 and Doctor of Juridical Science degree in 1960.

Our distinguished guest applied his extension knowledge on civil libertarianism and judicial reform into good use at the Senate floor. Among his more significant achievements are the passing of the Government Reorganization Act (1968-1970) and the amendments to the Land Reform Code (1971). He, however, became most acclaimed for passing a series of landmark legislations collectively known as the "Justice for the Poor" laws, in which poor litigants are given top priority in courts, provided free meals, lodging, and transcripts of their court proceedings, etc. He also wrote extensively on law, labor, foreign relations, and the dynamics of social conflict in the Philippines. For his outstanding performance and contributions, he was named "Outstanding Senator" from 1968 to 1971.

As the Chairman of the Senate Committee on Justice, he was responsible for updating the Filipino people on a wide range of national issues at the time, reporting on the criminal jurisdiction provisions of the RP-US Military Bases Agreement, the state of penal institutions in the country, and violations of civil liberties in the "Golden Buddha" scam of 1971.

## 2 INTRODUCTION

After the imposition of martial law, he became a leading figure in the fight against the dictatorship of Ferdinand E. Marcos. While keeping vigil for democracy's return in the seventies, he was awarded "Most Outstanding Legal Aid Lawyer of the World" by the International Bar Association in rites held at Stockholm in 1976. In the same year, he was also given the "Dangal ng Batangan" Award.

As President of the Nationalista Party, he closed ranks with the united opposition that would ultimately topple the Marcos regime in 1986. After EDSA, he went on to serve as Prime Minister of the Aquino Revolutionary Government, Secretary of Foreign Affairs and, most memorably, as Vice-President of the Republic of the Philippines.

Now Chairman of the National Centennial Commission, it is my privilege to introduce to you the Honorable SALVADOR H. LAUREL.

## KEYNOTE ADDRESS

SALVADOR LAUREL

*Chairman*

*National Centennial Commission*

There is one point I would like to stress at the beginning and that it is natural for our scientists, the best scientific minds, the best academicians to now be involved in the celebration of our centennial. Why? Because our own revolutionary heroes, the finest intellects, or the finest minds of our revolution namely Rizal, an Ateneo product and Mabini, a UST product, wanted to have an industrial revolution during their time. As far back as 1896, 1890's they were already dreaming of being able to duplicate here in our country the industrial revolution that was then taking place in Europe. They wanted an industrial revolution because it was this significant event, which gave rise to democratization movement in France and America reading some of the materials I received you were the founders, the founding fathers of DOST or NAST were similarly inspired by the examples set by the scientific academies in Europe and the US. So there is a close connection between the nationalism of our forebears and your commitment today to make this country abreast to the latest in the global economy now taking place so that we can be more competitive, so that we can sustain our development and maintain our political independence and economic independence. I did not prepare any particular written speech for this morning because I would like to just drive home one point and a keynote should not cover too many things like – who was this who say, it was long enough to cover the important parts but short enough to be interesting, so let this keynote speech be that interesting and that short.

When President Ramos asked me to take over the Chairmanship of the Centennial Commission, I accepted without hesitation but I asked him two conditions. "Mr. President," I said, "may I just ask for two things as a condition?" "What's that?" he said over the phone. "I would like to serve for free. Please do not pay me a single centavo as compensation. I want this centennial celebration of our country to be above politics. And he readily agreed and that's why I believe that the scientists of our country who are above politics will feel at home and comfortable in the celebration of our centennial. I could speak to you freely because I feel comfortable here because I know that some people regard science as something. The group of academicians and scientists are supposed to be an elite group but your response to this appeal to get involved in the club of our centennial shows

that you are not detached from items that really matter like the celebration of our 100 anniversary.

The objective of all these celebrations is to bring about a renaissance of the Filipino spirit. We will have the hoopla, we will have the parades, the speeches, the singing, the dancing, the banquets, the fireworks. Yes, we will have all that and we will try to make it as eventful and as dramatic and as memorable as possible. A song after being sung is nothing. A speech after it has been delivered is nothing. After the parade, the fireworks, it's gone. What we want is to have something that will remain and be passed on to the next generation. And what is that? that is the spirit of our race. The spirit that enabled our forefathers to make that quantum leap and enable them to make this country become the first constitutional democracy in Asia. That is something that they were able to do at a time when no other country in Asia fought for freedom. We fought for freedom. What made our forefathers garner the courage to fight for our freedom. That is what we want to review. And how do we intend to do that? How do we intend to review the Filipino spirit? By the Centennial Movement, by becoming members of the Centennial Movement.

What we want to achieve through the movement is the passing of the torch to the next generation. Every generation has a responsibility to pass on what he knows about the past to the next generation. The collective memory of a people is what keeps us together and strong. The collective memory of the people is what our generation passed on to the next generation. Nowadays, as we celebrate the celebration of our centennial, we ask, "What did our forefathers do in their time? And the answer came, and they're magnificent. They did brilliantly. They contributed so much that made us a nation. But a hundred years from now, another generation will ask the same questions. Yes, they will also ask what Rizal and Mabini and their fathers did for our country during their time. They will ask, "What did the generation of 1998 did in their time? We are in that generation. The answers to the questions that our great grandchildren will ask a 100 years from now is what you are going to do now. And so that we are sure that they know that you participated in the celebration of our centennial, we are going to place a golden roll of honor the names of all those who joined the Centennial Movement today. It will be listed and placed in a time capsule and dropped in the Center of Clark field on June 12, 1998 and it would be vacuum sealed and it will not be opened until June 12, 2098. It would be your grandchildren who will open them and it would be good if they see the list of their grandfathers listed there as having given your share. Let me take advantage of this opportunity to tell you about one particular success that we attained so far. It will give you an idea of the magnitude of what we are trying to do on this first centennial. We're trying to involve in our centennial the countries that influenced us in our ways. Well, one of the first countries I asked to participate in our centennial was Spain. Spain had been with us for 350 years or more and so the first country I approached to participate was Spain. And when I went to the Ambassador of Spain to ask them to participate, to ask them to put up a status of Rizal in Madrid, they were non-committant. They would not respond, they were

polite but they would not say "yes" but they would not also say "no", and so after three months of waiting, I became weary and I told the President "Mr. President, I think we have to go to Spain. Why? Because the ambassador here in the Philippines was not giving any definite commitment. Let's go to Spain and talk to the highest official there and tell that this is very important for us, for our people, and for our relationship – Spain and the Philippines." He agreed and so we went. We arrived there September of 1995 and we met with the Highest officials. And when we were there gathered in a long table with the top officials of Spain, they first gave us brandy and we drunk after the toast. And then they gave us another round of another brandy. And we have to be polite so they poured another half glass and we drank. They asked somebody who's fluent in English to talk to us because they know that President Ramos and I were not proficient anymore in Spanish and they said, "Excellencies, we have a problem. We want to participate in your centennial but we have a big problem. You see what you are celebrating, the centennial of your declaration of independence, was our defeat. Your victory was our disaster. 1898 was the year we lost not only the Philippines but we lost Cuba, we lost Puerto Rico, we lost the Marianas, we lost the Carolines, we lost Guam and the American. So how can we celebrate our disaster? Tell us, help us so we can explain and defend that to our people.

I said, "I agree with you, Excellencies, you are correct and you are very human to be hesitant about celebrating your or your defeat. That's very human." And so they were nodding. "But you forget two things. You forget two very important things. First you are forgetting that Spain produced, Spain educated Rizal. You allowed him to study in the best schools in the Philippines, at the Ateneo de Manila, he was one of our best students there, at the University of Sto. Tomas. You allowed him to study in Madrid and because of that he mastered the Spanish language and wrote the most beautiful novel "Mi Ultimo Adios" which inspired our people to rise for our freedom, so you have to pay credit also for Rizal. You cannot be hands-off on Rizal because you produced our national hero. They were very quiet. And then I said, "The second point you are forgetting – the nobility of the Spanish people. Spain is noble. The Spanish said I saw the Statue of Senor Bolivar at the Heart of Parque dela Huerte. A beautiful monument with Senor Bolivar riding on a horse and a big name there says Senor Bolivar, a grand liberator. You are honoring a man who used the sword to fight against Spain and liberate the South American colonists of Spain to become independent but because of Spanish nobility, you erect a monument to honor Senor Bolivar who used the sword and shed so much blood. On the Spanish people, Rizal did the same thing only he did not use the sword. He used the pluma; he just used a pen. He did not shed a single drop of Spanish blood. Yes, you had him executed, you have him court martialled, you had him shot by firing squad as a traitor to heal to Spain but that was 100 years ago. Time have a tendency to heal all wounds and make the real nature of a people rise above the dim and that is the nobility of Spain. There is a difference between what Rizal and Bolivar did. They both tried to gain the freedom of their

country. And now you are honoring Bolivar but you are not honoring Rizal. I hope it is not because Rizal's skin was darker. After a few seconds, the Prime Minister, the foreign minister, the cultural minister said, "With your permission, may we be allowed to withdraw. We would like to talk among ourselves because this is a very important point." And they withdrew. After 20 minutes, they came out again and made an announcement. "Excellencies, we have reached a decision. We have decided to claim Rizal as our product. We will honor Rizal. We will give him the same honor we gave Bolivar. We are going to give him a better place, a more beautiful park in Parque de San Tander. We will give you 800 sq. m. and we will put up the pedestal, you just take care of the statue." And then I said, "What statue, Excellencies? Rizal was so many things. He was a linguist, a doctor, a novelist, a marksman, a swordsman, a painter, a sculptor. Without hesitation they said, we will put up "the exact replica of the monument of Rizal at the Luneta." And then I looked at the President and he gave the signal. I said, "We will deliver it to you." I shook their hands, and it is agreed, it is going to weigh about 8 tons but we will send it by plane part by part. One thing more beautiful there is, it is located at the corner of the Parque de San Tander and fronting Avenida de las Filipinas – in front of it will be the Avenue of the Philippine Islands and they were planning to name the other street adjoining it as Avenida Rizal and it is close to Canal de Isabelo Catolico. At night if you see it, if you have the chance in going to Spain, don't miss going to Parque de San Tander. You will see the monument of Rizal there, in its full beauty, lighted and at the left side of the statue if you're facing it you will see the Spanish text of "Mi Ultimo Adios" so that those passers by, those who speak Spanish only can read the Spanish and they are now admiring Rizal more for that. And our Filipinos there, our OCWs there are also able to read "Mi Ultimo Adios" which is in Filipino. That gives you an idea of what we are trying to do in our time. Rizal was executed 100 years ago in 1897 and from the time he was executed as a traitor to Spain, his picture, his bust, his statues were all banned for 100 years. But now, even Spain has turned around. No longer it regards Rizal as a traitor, he is now considered a great man, a great champion of democracy and freedom and you can be proud of that.

I extend my warmest congratulations to NAST for successfully holding this 19th ASM a great part of the celebration of the Philippine Centenary. Rizal wrote,

"Genius knows no country; genius sprouts everywhere; genius is like light, air, the patrimony of everybody, cosmopolitan like space, like life, like God."

Those words suit well with our prominent Academicians and beloved National Scientists. You are a few invaluable treasures this country has had today. I hope your scientific legacy will go on as we make our history till the next Centenary.

Thank you and good day.



## PLENARY SESSION I

### A CENTURY OF PUBLIC HEALTH IN THE PHILIPPINES

TEODORA V. TIGLAO  
*Professor Emeritus*  
*Institute of Public Health*  
*University of the Philippines, 1000 Manila*

#### **What is Public Health**

*“Public Health is ecological in perspective, multisectoral in scope and collaborative in strategy. It aims to improve the health of communities through an organized community effort:*

*Public health infrastructures need to reflect that it is an interdisciplinary pursuit with a commitment to equity, public participation, sustainable development and freedom from war. As such it is part of a global commitment and strategy”.*

– Ilona Kickbush

Let us first have a bird’s eye-view of the current health scenario in the Philippines. If you look back, as the year 1997 dawned on us, we were greeted by such dismal news as the measles outbreak, typhoid in Manila jail, lead in our water, increasing number of HIV/AIDS cases, hepatitis outbreak at U.S.T., ebola virus in monkeys, the poor condition of the water supply, TB on the upsurge, etc. This grim picture seems to dominate the public health scenario today. Have we really not made any headway in the status of public health in the Philippines? Allow me to take you on a flight to the past, “back to the future” to view how the real situation is. Our first stop is the pre-American occupation.

### Pre-American Occupation

The Americans did not establish public health in a vacuum. When Spain came to introduce occidental civilization in the country, she had withal to make use of the type of hygiene and preventive medicine then understood and practiced in the Iberian peninsula. She undeliberately prepared the grounds on which the Americans later launched the public health program and it would be unpardonable to ignore her contributions. As historian E.G. Bourne stated in PHILIPPINE ISLANDS 1494-1898, the Philippines and Manila in particular were ahead of other English colonies with regard to provision for the sick and invalids.

Public health work during the Spanish regime inauspiciously began at the *portera* of the old Franciscan convent in Intramuros where a friar Fr. Juan Clemente put up a dispensary in 1577 for treating the indigents in Manila. This eventually became the San Juan de Dios Hospital (1659). When the Americans occupied the Philippines, there were already in operation:

#### Five General Hospitals

- \* The San Juan de Dios Hospital (1659)
- \* Chinese General Hospital (1891)
- \* Hospicio de San Jose in Cavite (1611)
- \* Casa de la Caridad in Cebu
- \* Infirmaries de Sta. Cruz in Laguna (1870)

#### Four Contagious Hospitals

- \* San Lazaro Hospital (1577)
- \* Hospital de Palestina in Camarines Sur
- \* Hospital de Leprosos in Cebu
- \* Hospital de Argencina in Manila for smallpox and cholera

#### Two Military Hospitals

- \* Hospital Militar de Manila
- \* Hospital Militar de Zamboanga

#### Two Naval Hospitals

- \* Hospital de la Marie in Canacao, Cavite
- \* Hospital de Basilan in Basilan

#### Other Hospitals/Asylum

- \* Hospicio de San Pascual Baylon in Manila
- \* Asylum of St. Vincent de Paul in Manila for poor girls (1885)
- \* Founding Hospital of San Jose for orphaned children and mentally ill (1782)

Real public health work started with the creation of the Board of Vaccinators in 1806 to prevent smallpox. Later, the office of "Medicos titulares" (health officers) was created together with a Board of Health with a priest as president. Maritime quarantine was instituted (1885). Spain can also be credited for the construction of the Carriedo waterworks (1876); for founding the first medical school, University of Santo Tomas (1872); a school of midwifery in 1879, a public health laboratory (1883) and "medicos forense" (forensic medicine) in 1892.

It may be worth mentioning the noble figure of Don Francisco de Carriedo as a benefactor of the City of Manila. He willed the amount of P10,000 to the city in 1733 to be invested in gainful trade and when the accumulated capital was sufficient, to employ it for the construction of waterworks. This provided Manila with piped water which served as an effective prevention of water borne diseases.

### **The American Military Government (1895-1907)**

Our next stop is the American occupation in 1898 during the Military and Commission form of Government (1898-1907). At the outset, the unsettled state of affairs demanded the attention of the Commission in areas deserving priorities other than public health. However, a Board of Health, was established and Dr. Guy Edi was appointed as first Commissioner. He was unable to report immediately as he contracted typhoid.

The early pre-occupation of the Americans witnessed the control of epidemics - cholera, smallpox and plagues; the fight against other communicable diseases - leprosy, diarrhea, malaria as well as beri-beri; the establishment of a health organization and administration and general sanitation. These activities were undertaken during this period that underscores these concerns:

- \* establishment of garbage crematory (1899)
- \* first sanitary ordinance and rat control (1901)
- \* established technologically that contaminated water and unclean vegetables are important factors in the control of amoebic dysentery while *Anopheles minimus* was first pointed out as the vector of malaria (1904)
- \* cholera vaccine was first tried (1905) followed by compulsory vaccination of school children
- \* confirmation of the theory that plague in man comes from infected rats (1905)
- \* opening of the leper colony in Culion and compulsory detection of lepers (1906)
- \* founding of the Manila Medical Society and the Philippine Island Medical Association (1902 & 1903).
- \* opening of the College of Medicine U.P. with Preventive Medicine as one of the departments (1907)

Medical education was one of the concerns of the Insular Board of Health not only because of the intimate relationship of medicine to sanitation but because of the scarcity of local physicians. The only existing medical school was the University of Sto. Tomas.

Of outstanding importance to public health was the establishment of the Bureau of Science in 1905. Working in close collaboration with the Philippine General Hospital and the University of the Philippines, it became an active center for scientific instruction and research and made valuable contributions to public health.

### **Philippine Assembly (1907-1916)**

We arrive at our next stop, the period of the Philippine Assembly (1907-1916), a step towards Filipinization. While most of the executive departments were placed under Filipinos, the Bureau of Health remained under American administration. The public health program started to take off.

Major developments in public health took place. The period was marked with several "first".

#### **1908**

- \* first institution of the search for germ carriers
- \* the new waterworks in Manila was inaugurated first using general chemical disinfection as emergency measure against cholera
- \* first recruitment of nursing students who began studies at the Philippine Normal School sponsored by business firms in Manila
- \* passing of the Employers Liability Law which made employers liable for injury and death of employees

#### **1909**

- \* inclusion of Hygiene and Physiology in the curriculum of public elementary schools
- \* start of anti-tuberculosis campaign with P35,000 appropriation from the government
- \* conduct of first sanitary survey of rural community
- \* dissemination of the results of first nutrition survey

#### **1910**

- \* organization of the Philippine Tuberculosis Society
- \* opening of Pasteur Prophylaxis treatment against rabies
- \* recognizing that Beri-beri as associated with eating white polished rice
- \* opening of the Philippine General Hospital with the P1,000,000 appropriation from the government

**1911**

- \* First demonstrated the eradication of beri-beri among Philippine scouts by means of simple change in the diet.

The first epidemic of beri-beri was described by Koeniger in 1882. It was claimed that 60% of infant mortality was due to infantile beri-beri. The results of the researches of Fraser and Stanton, Aron and Hocson, Cromwell and Concepcion advanced the theory that the etiology of beri-beri is food deficiency especially diet consisting mainly of rice. This theory was endorsed by the Far Eastern Association of Tropical Medicine in 1910. The credit for the discovery of a specific treatment for the disease, tiki-tiki extract belongs to Chamberlain of the Army Board for the study of Tropical Diseases and two Filipino physicians: Dr. Joaquin Quintos and Dr. Manuel S. Guerrero. In 1914, Congress passed a law allowing free distribution of tiki-tiki to indigent mothers.

The control and prevention of beri-beri is a distinct triumph of modern medicine. Some practitioners, however, discount the existence of the disease.

**1912**

- \* initial use of anti-typhoid vaccine
- \* use of hypochlorite of lime for the first time for treating Manila water supply
- \* initial study on vitamin deficiency as cause of beri-beri which ultimately culminated in the isolate of Vitamin Biothiamine

**1913**

- \* use of etiology of amoebic dysentery in the detection of carriers and mild cases
- \* first use of dry vaccine against smallpox with successful results with potency for two months versus one week of glycerinated lymph vaccine
- \* first offering of graduate courses in hygiene and tropical medicine at the UP College of Medicine.

**1914**

- \* manufacture and free distribution of tiki-tiki for treatment of beri-beri
- \* inauguration of first "clean up week"

Rise of non-government health organizations, such as: Gota de Leche, later named La Protection de la Infancia for the protection and care of infants; La Liga Nacional Filipinos Para la Proteccion de la Infancia; Asociacion de Damas Filipinas to help poor women and children; Settlement House - for the temporary shelter of destitute women and children; and Women's clubs.

Definitely, public health has gained momentum and one could view the important cornerstones upon which the public health program could be erected. Technological advances were visible and the epidemiology of common communicable diseases- tuberculosis, rabies, typhoid and beri-beri had been defined and control measures instituted such as immunization, environmental sanitation and proper nutrition, smallpox, cholera and plague were gradually brought under control. These measures were supported by the education of health personnel, budgetary allocation, and partnership with non-government organizations, foremost among which is the Philippine Tuberculosis Society that pioneered in TB work.

### **The Jones Law (1916-1936)**

The next leg of our flight is the period from 1916-1936 covering three decades from the enactment of the Jones law until the Philippine Commonwealth. The take off was characterized by some turbulence but it was able to recover altitude and fly smoothly later on. There was a shift in administration in Washington. The Democrats were able to wrest control from the Republicans. The appointment of Francis Burton Harrison as Governor General led to the enactment of the Jones Law which promoted further Filipinization. This was done rather hurriedly resulting in the Civil Service being increasingly riddled with politics. However, the Department of Public Instruction where education and the health services were located, still remained under an American leadership, while the rest of the departments became nationalized.

Hayden reports that the Harrison administration was one retrogression rather than progress in so far as health is concerned. Crude death rate and infant mortality rate rose. Deaths from smallpox, cholera, typhoid, malaria, beri-beri and tuberculosis increased. Even Governor Harrison admitted that the health record of 1919 was a disappointment.

By 1921, the Republicans regained control of the White House and Governor General Leonard Wood, a physician, an administrator, a sanitarian and a humanitarian assumed office. Governor Wood had a militaristic orientation. He re-energized the health service and encouraged effective supervision. He had a profound effect on the public health service. It was recognized however, that whatever accomplishments the Wood administration made could not be attributed solely to him. A good deal was contributed by the Filipinos who manned the health services supported by necessary legislation and budgetary outlay.

Governor Wood was succeeded by four other governors until the Commonwealth: Henry Stimson (1928-29); Dwight F. Davies (1929-32); Theodore Roosevelt (1932-33); and Frank Murphy (1933-35). To the Roosevelt administration could be attributed improvement in the organization of the government. The semi-militarized Philippine Health Service was transformed again into a Bureau of Health and together with the Bureau of Public Welfare formed the Office of the Commission of Health and Welfare (Reorganization Act of 1932).

Public Health under the American period from 1898-1935 prior to the Commonwealth was marked by the performance of three men who helped advance the frontiers of public health and welfare in the country. The first was Dean C. Worcester (1898-1914), member of the Philippine Commission, later Secretary of Interior under which was the Bureau of Health under Director Victor Heiser. Worcester, a scientist, had the creativity and the driving force that put through the revolutionary health program in the new American colony. The second was Governor Leonard Wood (1921-1927); and the third was Governor General Frank Murphy who launched a public health and social welfare programs well designed to raise the health level of the Filipino people through a comprehensive program including maternal and child health, slums, unemployment, latrine, nutrition, recreation and leprosy. His technical adviser was Major George C. Dunham, U.S. Army Medical Corps, whose personal qualifications are distinctive of a great public health official, having graduated from the School of Hygiene and Public Health, John Hopkins and the London School of Tropical Medicine coupled with a vast experience of public health and welfare work in the Philippines.

The following were the outstanding contributions during the period:

- 1916** - A Committee was appointed to study the cause and prevalence of typhoid fever.
- 1913-34** - The composition, value and vitamin distribution of many Philippine foods were studied. This is part of the drive for better nutrition.
- 1919** - Schick test was first used on a large scale to determine the susceptibility of Filipino children to diphtheria.
- 1921** - Rockefeller Foundation extended cooperation. Government and Rockefeller fellows were sent abroad to develop a corps of competent public health men who could assume leadership roles. Among these were Dr. Jacobo Fajardo, Director of Health, and Dr. Jose Fabella who became first secretary of Health and Welfare. Dr. Hilario Lara, Dean, Institute of Hygiene. Yaws was found controllable. Yaws clinic for administration of salvarsan opened.
- 1922** - Campaign against hookworm was launched. Anti-dysentery vaccine as first tried locally. Role of seafood in transmission of cholera and of pollution of fishing sector to typhoid were studied.
- 1923** - First training course for sanitary inspector Sewage of Manila purified by hypochlorite of lime Women and Child Labor Law - Act No. 3071 was conducted.
- 1924-26** - Mechanism of transmission through Aedes Egypt of dengue fever was studied successfully.
- 1925** - Construction of Novaliches dam started.
- 1926** - Success of the first rapid sand treatment to purify water of swimming pool constructed at state university was attained. Legislation was passed for the establishment of School of Hygiene and Public Health with support from the Rockefeller Foundation.

- 1927 - Conceived in 1927, the National Research Council of the Philippines was officially organized in 1934 to promote comprehensive projects of research in the basic sciences of which health and medical research were areas of concern.
- 1928 - B.S. in Education, major in health education was offered in U.P.
- 1929 - Compulsory notification and inoculation for reportable disease was effected.
- 1930 - Law was enacted to establish civil registry. Tuberculosis Commission was created.
- 1932 - Free Emergency Medical Treatment for Laborers was offered. Industrial Hygiene and Sanitation were initiated. Philippine Public Health Association was organized. Building of School Hygiene and Public Health (Donation of Rockefeller Foundation) was constructed. First Child Health Day observed.
- 1933 - Reorganization Act - consolidating public health and welfare activities under the Commission of Health and Welfare was promulgated.

#### **The Commonwealth (1936-1942)**

Public health has weathered the turbulence after the Jones Law period and is now in the process of gaining and maintaining altitude. The epidemiology of additional life threatening diseases was studied - diphtheria, yaws and dengue. Health research was promoted. The U.P. School of Public Health was established for the training of public health leaders. The consolidation of welfare and public health activities was ensured. The health of laborers was addressed and the sanitation of the environment pursued. However, with the initial phase of the Commonwealth period, some turbulence once more occurred. Later, this was overcome and public health gained headway up to the Japanese Occupation.

The Jones Law ceased to be in operation and the Filipinos came in control of the executive branch of the government. The rallying cry of President Quezon was social justice to ensure the well-being and security of all people.

The responsibility of the Commonwealth became the preparation of the Philippines for the difficult task ahead. There were many problems to be hurdled, foremost of which were political, economic, and social security. However, President Quezon gave attention to public health as attested by a gradual increase given to health appropriation. An additional P19,150,000 was made for specific projects for the construction of additional leprosaria, of provincial hospitals, for the construction and rehabilitation of the Philippine General Hospital, and for the drilling of artesian wells, waterworks, public market and slaughter houses. In 1936, the National Assembly appropriated P500,000,000 for the construction of three regional leprosaria in Cebu, Luzon and Culion. Four Filipinos gained international three recognition for their contribution to the scientific study of leprosy.



The Philippine Charity Sweepstakes was equally generous, appropriating around P2 million for the Philippine TB Society for the operation and maintenance of the sanitarium and the construction of the Quezon Institute. The thrust of the period was research in and control of Tuberculosis, Malaria, Leprosy, Yaws and Maternal and Child health.

In 1939, Commonwealth Act No. 430 provided for the creation of the Department of Public Health and Welfare, with Dr. Jose Fabella as the first secretary. Thus, public health administration was removed from the Dept. of Public Instruction to include the Bureau of Quarantine, health departments of chartered cities, provincial, city and municipal hospitals, dispensaries and clinics, public markets, slaughter houses and health resorts and charitable and relief agencies.

The Bureau of Census and Statistics was created in 1940; therefore vital statistics was transferred from the Bureau of Health to this new Bureau.

In spite of the advances made in facilities and manpower, the nagging problem was still the inequitable distribution of health facilities and resources. It was noted that 80% of those who die never received medical attention. Death rate remained high, the terrible havoc of tuberculosis remained unabated; malaria, beri-beri and intestinal disease claimed their undiminished toll.

In response to this situation, the establishment of charity clinics supported by the Philippine Charity Sweepstakes marked one of the significant developments during the period. The plan called for the establishment of charity clinics in towns and cities with less than 8,000 inhabitants where there were no private hospitals or government physicians. These were placed under the Bureau of Health and eventually under the Bureau of Hospitals. This was an attempt to have equitable distribution of health facilities and human resources and to reach the periphery.

Maternal and Child Health, School Health, Health Education of the public, Public Health Nursing, Public Health Dentistry, Hospital and Laboratory services were all strengthened.

By the end of 1939, the state of health was fairly satisfactory. Only one case of smallpox and no cholera case was reported. The Commonwealth government acquitted itself well of its responsibility in public health. Hayden reported "The Filipinos continued the general health and welfare policies and agencies which had been developed during the period when final control lay in American hands. They adhered to and carried forward the modern long-time inclusive program initiated during the last years of the American regime. They kept public health and welfare administration out of politics and in the hands of highly trained permanent personnel.....".

### **Japanese Occupation**

The bright public health landscape that we had viewed suddenly turned into one of gloom.

The Japanese occupation practically paralyzed the activities. Public health and sanitation were set back a quarter of a century. Hunger, disease and epidemic stalked the land and health and other social services were in a large measure a shambles.

The Bureau of Health continued to function but the activities were directed towards the handling of emergencies; the prevention of the occurrence of epidemics especially malaria, which was ravaging the Japanese military; regulations for slaughtering of cattle, hogs and carabaos and the creation of the Board of Nutritional Research in 1943.

There were no available reports on health status during the period.

### **Post World War II To Martial Law (1946-1972)**

After five years of this grueling experience came Liberation. Slowly public health tried to pick up the debris and rise from the ruins. A U.S.P.H.S. post war survey of public health conditions showed that the incidence of TB, VD and malaria had reached a new high; over 5,000 leprosy cases were dispersed; there was widespread evidence of malnutrition and beri-beri; general sanitation had been reduced to a level to constitute a national hazard; there was widespread destruction of quarantine installation and laboratory; and imminent chances of the introduction of cholera, plague, smallpox and other epidemics. A one million dollar emergency measure was passed by U.S. Congress to assist the Commonwealth government in the reestablishment of its public health activities and facilities.

Given priority in this emergency program were disease control (VD, TB, malaria, leprosy, malnutrition and immunization), general sanitation, maternal and child health, school health, health education, hospitals and public health laboratories.

Still reeling from the ravages of the war, the Philippines was given its independence in 1946 with Manuel A. Roxas as president. The tragic evidence of the war still stared at the country from the broken ruins of the city. Whereas, the Philippines were a land of comparative plenty, the brutal hand of war blotted its progress toward economic development (M. Roxas). The Republic was confronted with grave problems of rebuilding the nation's economy, restoring peace and order, coupled with facing the challenges and responsibilities of a newly attained independent nationhood.

Many agencies came to the rescue of the Department of Health such as the PCAU (Philippine Civil Affair Unit), an agency of the Army of Liberation, the U.S.P.H.S. under its Phil. Rehabilitation program and later on such other agencies as the U.S. Mutual Security Agency (MSA), UNICEF, WHO, etc.. The sad note was that in the Rehabilitation Act, the U.S. demanded parity rights, i.e. the same rights as the Filipinos enjoy in the exploitation of resources in the country.

On 17 July 1946, a joint cooperative agreement was signed by representatives of U.S.P.H.S and the Philippines Secretary of Health and Welfare which provided for programs related to communicable disease control (malaria, TB, VD, leprosy) general sanitation; MCH, Nutrition, Health Education, Public Health Training Centers, Rehabilitation of Public Health Laboratories and of the Philippines Quarantine Service.

Among the highlights of this period are:

- research on DDT saw dust as a larvacide and DDT residual spraying of horses in the control of malaria.
- construction of the National Chest Center, establishment of a control case registry for TB. Mass case finding surveys and mass BCG immunization for TB control.
- inauguration of an industrial hygiene laboratory
- the Bataan Enriched Rice Project which amply demonstrated the efficacy of enriched rice in the eradication of beri-beri and iron deficiency anemia.
- introduction of one - infection method for gonorrhoea with penicillin which established a routine procedure in all VD clinic.
- creation of a Central Health Laboratory of Philippine under the newly organized Department of Health.
- reorganization of government offices in 1947 resulting in the transfer from the Dept. of Health and Public Welfare of the Bureau of Public Welfare to assume the name of Social Welfare Administration, and the creation of the Bureau of Hospitals and Quarantine under the Dept. of Health.
- creation of the Institute of Nutrition - 1948 which was charged with the duty of unifying, centralizing and coordinating all nutrition research and activities. The Institute of Nutrition was transferred to the newly created Bureau of Research and Laboratories through Reorganization of 1958. In 1959, with the passage of Science Act of 1958, the Institute was transferred to the National Institute of Science and Technology and renamed Food and Nutrition Research Center - later named Food and Nutrition Research Institute.

In 1950, at the termination of the joint Department of Health and U.S. Public Health Program, through the request of the Phil. Government, a survey was carried out by the U.S. Economic Survey Mission known as the Bell Trade Mission, to look into the economic and financial situation of the country. One of the six-point recommendations of the mission was to restore the high degree of efficiency of the public health service to help in rural development. An offshoot, the Foster-Quirino Agreement, was signed in Nov. 14, 1950. A mutual assistance and cooperation was forged between the U.S. Economic Cooperation Agency (ECA) (forerunner of U.S. AID and the PHILCUSA (Phil. Council of U.S. AID) created by Pres. Quirino. The objective of the Phil.-American Public Health Program was to provide health services on a continuing basis to all areas of the country especially the rural areas.

On June 1951, Manila was selected as Headquarters for the Western Pacific Office. Other international organizations stepped in such as UNICEF, UNESCO, FAO, ILO. With the assistance of bilateral and international organizations, many projects sprouted.

Several milestones in public health marked this post-war period.

- Enactment on 19 June 1954 of Republic Act 1082 entitled: *An Act Strengthening Health and Dental Services in Rural Areas and providing funds thereof*, during the Magsaysay Administration. This concept of an integrated health service was first demonstrated in 81 pilot provinces. The staffing pattern included 1 MHO, 1 nurse, 1 midwife and 1 sanitary inspector for municipalities with 5000-10,000 population, with those with less than 5,000 could have no MD; those with over 10,000 will have a corresponding complement of health personnel.  
The RHU program was instrumental in-bringing to the people the basic health services and has improved the total health picture of the country.
- Reorganization of the DOH in 1958, a step towards decentralization. Eight regional offices were organized under which were the provincial and municipal health offices. The national office assumed only staff functions; line functions emanated from the regional offices.  
The same period saw the creation of several offices - The Dental Health Services (1963), the Malaria Education Services (1966), the Disease Intelligence Center (1961), the Division of Nutrition (1960), Food and Drug Administration (1963), National Schistosomiasis Control Commission, National Nutrition Program (1968).
- Enactment of R.A. No. 6111 otherwise known as the Philippine Medical Care Act of 1969, an answer to the long felt need of extending medical service to as many people as possible.
- Definitive programs were initiated with multilateral assistance such as: WHO/UNICEF assisted TB and BCG program, the piloting of an integrated TB control program into the basic services of rural health units, TB sputum case finding by microscopy; Serum and Vaccine production in Alabang; the Foreign assisted Expanded MCH Program, the Mental Health Program, WHO/UNICEF School Health Education Program; Training Program for midwives; strengthening of the graduate public health program at the Institute of Hygiene (now CPH); the WHO/MSA/PHILCUSA assisted health education project at the Dept. of Health, the setting up of demonstration training centers such as the WHO/UNICEF Rural Health Demonstration and Training Center in Quezon City and the U.P Comprehensive Community Health Program in Laguna which served as laboratories for extra mural activities of the students of the Institute of Hygiene and the College of Medicine and of other health allied colleges. In all these endeavors, coordination with other government sectors-education, social work, agriculture, welfare, community development, etc. and NGOs-Phil' Medical Association, PNHA, Phil. Hosp. Association, PRRM, PTS, PNRS, Nutrition Foundation of the Phil., CARE, Catholic Relief Services, Phil. Business for Progress, IIRR, etc. were promoted.

- The development of the Family Planning movement. This started as early as 1957 as a Family Relations Center of the Presbyterian and Congregational ministers. This gained momentum and in 1965, the Family Planning Association of the Philippines was organized while the Family Relation Center served as the nucleus for the Planned Parenthood Movement of the Phil. These two organizations eventually merged to form the FPOP (Family Planning Organization of the Phil.), an NGO partially funded by the International Planned Parenthood Federation. Meanwhile on 15 May 1970, the Commission on Population was created (Ex. Order 233) an intersectoral body, and on 16 Aug. 1971 R.A. No. 6365 approved the Population Act of the Philippines. Many foreign assistance came in - USAID, IPPF, Pop. Council of New Work, Pathfinder Fund, Ford foundation, WHO, UNDP, Rockefeller Foundation, Church World Service.
- Launching of health programs during Secretary Elpidio Valencia's term from the private sector on a volunteer basis to support on a volunteer basis to support the government's effort to provide services to the periphery. Project Helping Hand, Operation Tribal Minority, the Maria Way (Medical Assistance to Rural Indigent Areas) launched by PMA, AKAP, and supported by business firms and charitable institutions are some examples of efforts to attract doctors to doctorless areas.

Of note is the Philippine Rural Reconstruction Movement, (PRRM) another NGO, which was a potent force in the public health picture. From a handful of volunteers in 1952, the PRRM has demonstrated the importance of a wholistic and innovative approach to rural reconstruction, an integrated four-fold program including 1) livelihood (agriculture, cooperatives and cottage industries); 2) education; 3) health and; 4) self-government. The seat of the Project was San Leonardo, Nueva Ecija.

Three of the principal ways in which PRRM works with barrio people in health improvement are: 1) establishment of barrio health centers; 2) training and activities of young adults as auxiliary health workers and; 3) planning and carrying out urgently needed education for mothers. The PRRM is ahead of the DOH in establishing health centers at the barangay level which was institutionalized during the Primary Health Care Movement. PRRM has been utilized as a social laboratory for the training of public health workers.

Secretary Juan Flavio was honed into a public health man through his vast experience he accumulated while working as a physician at the PRRM which provided the materials for his book "Doctors to the Barrios" and his daily parables in the Philippine Star.

The PRRM was expanded into an international organization - The International Institute for Rural Reconstruction located in Silang, Cavite, catered to different countries in the world. Its field of operation covers 200 villages which serve as its social laboratory and training field.

- ***The Rizal Development Project*** an operation research of the DOH, the Institute of Hygiene U.P. and WHO led to the restructuring of the Dept. of Health Rural Health Care Delivery System. Additional responsibilities were given to nurses; treatment functions for common ailments were delegated to midwives. Each barrio was provided with a midwife with expanded role. This research became the centerpiece of the first national health plan.

This bright public health picture was marred when Cholera El Tor lurked its ugly head together with that of hemorrhagic fever and a new disease of man, intestinal capillariasis. For the first time, dengue virus was isolated from typical "H" fever cases in children. Philippine hemorrhagic fever, a new disease of children, was found to be one of the clinical manifestations of the local dengue virus strain (Hammon et al). The resurgence of cholera el tor paved the way to test the efficacy of three different cholera vaccines with Negros Occidental as the site of the study. These were found ineffective inasmuch as there were different strains of the bacilli. Proper waste disposal and safe water were found better measures in the prevention of the disease. Indiscriminate vaccination was stopped and restrictions especially to travellers were lifted as they as they were found to be unnecessary and ineffective. Five antibiotics were tested as it was clear that they shorten the duration of diarrhoea and the excretion of vibrio in the stool. Immunization against cholera was no longer obligatory.

By the early 70's improved biologicals were produced and widely used which proved as a breakthrough in public health in the prevention and control of communicable diseases.

### **The Martial Law Years (1972-1986)**

At the height of all these developments in public health came the declaration of the Martial law on Sept. 21, 1972. The nation was at a state of unrest. The nation was transformed from a Presidential to a Parliamentary form of government. Declared a new society, Presidential decrees were issued one after the other. The first decree created the National Economic Development Authority for economic planning. The DOH was changed to Ministry of Health with now 12 instead of 8 regional offices. In spite of the political change, the Ministry of Health continued to function in accordance with the public health structure previously laid. Acted as Ministers of Health during this period were Drs. Clemente Gatmaitan, Enrique Garcia and Azurin.

The following highlight the accomplishments of the period:

- Formulation of a National Health Plan drafted by NEDA and the MOH. An offshoot of this were the following events:
  - \* Implementation of the Restructured Health Care Delivery system where the three levels of care was evolved - the primary, the secondary and the tertiary. The problem of access was addressed by the establishment of Barangay Health Stations manned by

- midwives. Tertiary Hospitals were constructed - the Philippine Heart Center, the Lung Center, the Kidney Center, and the Children's Hospital (Lungsod ng Kabataan).
  - \* Implementation of Medical Care in 1972:
  - \* The Rural Health Practice Program of the Dept. of Health requiring graduates of Medicine and Nursing to render service in rural areas.
- **The adoption of PHC** as an approach to health development in the Philippines which was eventually launched nationwide on Sept. 11, 1981. After the Alma Ata Declaration in 1978, the Philippines, being a participant in the international gathering, introduced to the country the global goal "Health for all by the year 2000". The LOI 949 dated Oct. 19, 1979 provided the legal basis.
 

The Philippines has the distinction of being the first country in the world to implement Primary Health Care nationwide through the Ministry of Health then under Minister Azurin. In his words, The MOH "has been able to penetrate the world of lethargy and convert the people to the cult of self reliance". By early 1984, 39,000 barangays have been organized to carry out PHC with 9,000 midwives, 8,000 nurses and 4,500 physicians trained to implement the program. The national health program was given world recognition when it won the first Sasakawa Health Prize in Geneva.
- **Operation Timbang**, a nationwide nutrition program providing supplementary food for infants and school feeding programs.
- **The Integrated Provincial Health Office (IPHO)**. The brain child of Minister Azurin came into being which merged public health and hospital services at the provincial level and the various health units, personnel and financial resources of the different health programs were integrated. This is another move towards decentralization which was fully implemented in April 1988 by Dept. Administrative Order 144.
- **The Oral Rehydration Therapy** for the National Control of Diarrheal Disease became one of the major thrust of the DOH with the collaboration of the DECS, NMPC, Ministry of Information, NEDA, U.P, UNICEF, WHO, USAID, MLGCD, MWSS, LUWA and the PMA - Phil. Pediatric Society.
- **Community-Based Health Programs** - (CBHP) This was started in 1973 by three Catholic sisters and one physician nun of the Rural missionaries. The vision was to train lay people and utilize local and indigenous resources for preventive and curative health care instead of Western-oriented system. Many other groups have utilized the CBHP approach like AKAP.
- **Public Health Research** - Breakthroughs in public health are generally based and supported by research. The Martial law years can be credited with:

- \* Creation of the *Nutrition Council of the Philippines* in 1974 began to address the problem of malnutrition. This has significantly saved millions of lives of mothers and children from this dark scourge. Now the country is engaged in an aggressive campaign against micro-nutrients malnutrition. Through research linkages with the international institutions, the Center is able to contribute to the strengthening national policies and programs. Some of its outstanding output are the Vitamin A fortified star margarine, fortification of wheat flour, salt iodization program, assessing the health and nutrition status of 10 million school children and enlisting teachers in the detection and intervention of nutritional disorder in schools.
- \* The *Research Institute for Tropical Medicine (RITM)* was inaugurated on 23 April 1981 as the research arm of the Department of Health in infectious and tropical diseases. The vision is that advances in health technologies could have an impact of the survival and quality of life of millions of people. The research programs include acute respirator infections (ARI), diarrheal diseases schistosomiasis, human immunodeficiency virus (HIV-AIDS) infection, malaria, viral hepatitis, leprosy, TB, dengue, rabies, poliomyelitis and other infectious diseases. Recently, field studies on human immune response to recombinant *S. japonicus* antigens are being conducted in Leyte. This has promise of discovering immunization against schisto. The RITM has become the center of infectious diseases training for doctors and paramedical staff of both public and private hospitals, the national reference laboratory for polio eradication program, the coordinating center for anti-microbial resistance surveillance and reference center for problematic infectious and tropical diseases. Results of studies from Hepatitis Study Groups can be credited for the integration of Hepa B vaccine into the expanded Program of Immunization. It also showed the affinity of HBV for human liver and causes infection which may lead to liver cancer. The Rabies Research Group developed strategies for rabies control which have been implemented by local governments. The Virology Laboratory has established capabilities for virologic studies including virus isolation and identification, serologic diagnosis and preparation of dengue antigens for serologic methods.
- \* The *PCHRD (Philippine Council for Health Research and Development)* was created in 1982. Its mandate is to lead, direct and coordinate science and technology activities in health and nutrition. It sets the goals of health research in the country; establishes implementing mechanisms develops/strengthen research capabilities of institutions through manpower development pro-



grams or improvement of equipment and laboratories, monitors and evaluates research and promotes research utilization.

During the latter part of the so-called New Republic of the Marcos Administration, the nation was in a state of political unrest. This was aggravated by the assassination of Ninoy Aquino in 1983 which led to a chain of events culminating in the EDSA Revolution (People Power Revolution) on 24 February 1986. This spelled the collapse of the dictatorship and the end of the Parliamentary form of government. Hence, the Ministry of Health was again renamed as the Department of Health.

### **Post-Edsa Revolution - 1986**

A documentary by the Department of Health, titled *Panorama of Concerns of the Department of Health* featured the state of the nation's health condition, such as: increase in life expectancy have slowed down; morbidity and mortality rates from preventable causes stabilized at high rates; declines in infant and child mortality have decelerated malnutrition incidence has increased; practice of family planning has declined; health status of large pocket of disadvantaged sectors further deteriorated. In short, as the economic status of the nation worsened, advanced in health status slowed down.

## **State of Health Sector**

### *The Government Health Service*

- weakened by three years of budgetary strangulation demoralized by a politicalized reorganization in 1975-1985
- sustained a long tradition of service orientation and professionalism
- retained the basic technological capabilities for tackling infectious and communicable diseases
- retained a corps of experienced and technically prepared but poorly motivated middle managers
- stuck in ancient systems and procedures handed down from generation to generation
- blessed by clearly defined problems and priorities

### *The Private Health Sector*

- provided a parallel network of hospitals and practitioners
- sustained itself from its own efforts and revenues
- weakened by the post-assassination recession
- strengthened by market discipline
- lacked coherence and standardization

By virtue of Exec. Order 119 Jan. 1987, the DOH was reorganized. Five offices headed by undersecretaries and assistant secretaries were created. These were the Office of the Chief of Staff, Public Health, Hospitals and Facilities, Standard and Regulation and Management. At the lower level, the integrated scheme remained.

### **The Aquino Administration**

The new Constitution of 1987 contained more specific provision on health making available comprehensive health care and emphasized private sector and NGO participation. A new office to deal specifically with non-government organization was created called Community Health Service. Research was strengthened with the creation of the National Health Research Program.

During the term of Pres. Aquino and Secretary Bengzon foremost among the legislation and DOH activities influencing the health of the public were:

- The Milk Code Exec. Order 51, 1986 which required the marketing of breastmilk substitute to promote breast feeding.
- Proclamation No. 6, 1986 which committed the government to the goal of universal child and mother immunization by 1990.
- In 1987, the International Safe Motherhood Initiative was launched to reduce maternal deaths.
- Passing of RA 6725 in 1989 prohibiting discrimination against women with respect to terms and conditions of employment.
- Formation of Population and Development Foundation Inc. by the Phil. Legislator's Committee with its advocacy mission for formulating and reviewing population legislations.
- Start of the National Epidemic Surveillance System in 1988 under the Field Epidemiology Training Program in 8 sentinel sites in different region of the country to track down the occurrence of 14 diseases which have the potentials of causing outbreaks including HIV/AIDS surveillance. Established in 1986 with the support of USAID, the US Center of Disease Control, the FETP aims to strengthen the Department's epidemiological service.
- The National Drug Policy together with the Generic Act of 1988 to ensure the availability of safe, effective and affordable quality drug identified by their generic name and to give the patient the first decision in the choice of their drug.
- RA 7160, The Local Government Code of 1991, fully implemented in 1993 which devolved the process, resources and functions of the provincial and municipal governments from the national to the governors and mayors.
- RA 7170 - Organ Donation Act of 1991 - Legalizing donation of all or parts of the body after death for specified purposes.

- The Magna Carta of Public Health workers, RA 7305, dated March 1992 which seeks to promote and improve the social and economic well being of public health workers. Corollary to this was the passage of the Code of Conduct to Public Health Workers in 1992 to define the nature and scope of the duties of public health workers.
- RA 7277 in 1992, The Magna Carta for Disabled Persons providing the rehabilitation, self development and self-reliance of disabled persons and their integration into the mainstream of society and other purposes.

It was during the Bengzon years that the onslaught HIV/AIDS emerged. By August 1988, the Government of the Philippines and the World Health Organization approved the First Generation Medium Term Plan for the prevention and control of HIV/AIDS. By 1992 the DOH approved 12 policy statements developed by the National Program.

One legacy of the Bengzon administration is the face lifting of the DOH - the construction of four impressive building which greatly improved the image of the Department.

Significant external cooperation were negotiated with USAID (The Health Policy Development Project and the Health Finance Development Project) and with the German Agency for International Cooperation of the Federal Republic of Germany addressing the problem of health and management information system (HAMIS).

In 1986, the newly installed Aquino Administration launched the Community Employment Development Project (CEOP) as a major pump priming program of the government. This was an effort to institutionalize "people power" through active people participation in the process of development.

A survey in 1982 by the Philippine Business for Social Progress listed over 16,000 NGOs throughout the Philippines. Of these 284 are health-oriented NGOs. Others are welfare organizations, socio-civic and professional organizations, developmental issue-oriented organizations. The 284 health NGOs are scattered throughout the different regions. Some of these are religious based; some University based, some internationally funded, TV channel based. They cater to different clientele - children, mothers, occupational groups, welfare groups of general population or by urban and rural sector etc. Whatever programs they sponsor will have an impact on health, no matter how little. Some are very innovative and in general supplement and complement the DOH programs.

### **The Ramos Administration**

The 1992 election ushered in the Ramos administration with its rallying cry TOWARDS PHILIPPINE 2000! This was matched by secretary Flavier's slogan HEALTH IN THE HANDS OF THE PEOPLE and LET'S DOH IT! Being media friendly, the Flavier years continued to build on the foundations laid by this predecessors but added a great deal of adornment into it. His appeal was more affective rather than cognitive.

During the Flavier Administration, the DOH continued to adopt Primary Health Care as its strategy. Emphasis shifted from curative to preventive and promotive care; health services were gradually shifted from the Central Office to the local governments. The Flavier administration in his own words “sought to bring in a fresh wind of excitement to the existing health program” initiated by his predecessors. All the health programs carried a special touch best described by the acronym MEDIC which stands for Media-friendly, Exciting, Doable, Innovative and Current. Let’s DOH it became the national battlecry. The following are among the memorable initiatives conducted during his administration:

- ***Nationwide Immunization Day (NID)*** - While the expanded immunization program has already been launched by the Bengzon administration, there were still some who have not been reached because of peace and order conditions or were in inaccessible areas. A nationwide call for “Ceasefire for Children” was aired so people would devote one day for the immunization of their children against TB, Diphtheria, Tetanus, Pertusis, Polio and Measles. The response to the call was tremendous. Non-government and government sectors, religious organizations, civic groups, local officials, media, private corporations, international organizations and volunteers gave their support. This led to Reaffirming the Philippine Commitment to the U.N. Goal of Universal Child Immunization and Proclamation 147 declaring April 21 and May 19, 1993 and every third Wednesday of January and February thereafter for two years, as National Immunization Days. Later RA 746 required compulsory immunization against hepatitis for children below 8 years old.
- ***Mother and Baby-Friendly Hospital Initiative*** - In 1992 the Philippines gained the coveted distinction of being the only country that succeeded in meeting the UNICEF goal and was conferred an award by Director James Grant. This strategy ensures the survival and health of children through breastfeeding. This is supported by RA 7600 - “The Rooming in and Breastfeeding Act of 1992”
- ***Promotion of Philippine Traditional Medicine*** - While the DOST and DOH have been producing and packaging herbal medicine this was carried a step further towards developing other forms and innovative packaging. Traditional medicine has been institutionalized with the creation of the Traditional Medicine Unit at the DOH.
- ***Hospitals as Centers of Wellness*** - transforming 45 government hospitals from “disease palaces” to “centers of wellness”.

Other innovative projects were: Yosi Kadiri (anti-smoking campaign), Araw Sangkap Pinoy to prevent Vitamin A iron and iodine deficiencies, (Micro-Nutrient Campaign) voluntary blood donation, family planning (Kung sila’y mahal mo magplano) water for life, Oplan Sagipmata, Oplan Batang Buhay, Doctors to the Barrio, Disaster Management, Ur-

ban Health and Nutrition Program. Among other significant legislations passed during this period were:

- \* RA 7394 - "Consumer Act of the Philippines" An act providing penalties for manufacture, distribution, sale of adulterated food, drugs, cosmetics and other devices.
- \* Ra 7610- Special Protection of Children Against Child Abuse, Exploitation and Discrimination act.
- \* RA 7624 - Integrating drug prevention and control in the intermediate and secondary curricula.
- \* RA 7431 - Radiologic Technology Act of 1992 - regulating the practice of radiologic technology.
- \* E.O. 39 - Creating the Philippine National AIDS Council as a national policy and advisory body in the prevention and control of HIV/AIDS
- \* RA 7637-Mt. Pinatubo Assistance Resettlement and Development Fund-An act appropriating the sum of P10 B for the aid, relief, resettlement, rehabilitation and livelihood services as well as infrastructure support for the victims of Pinatubo.
- \* R.A. 7432 (1992) Senior Citizen's Act - to maximize the contributions of Senior citizens to national buildings, grant benefits and Special Privileges for other purposes.
- \* R.A. 7719 - National Blood Services Act of 1994 - to promote voluntary blood donation.
- \* R.A. 7876 - "Senior Citizens Center Act of the Philippines." An act establishing a senior citizens center in all cities and municipalities of the Philippines and appropriating funds therefore. (Feb. 14, 1995)
- \* R.A 8172 - "An Act for Salt Iodization Nationwide (ASIN)." An act providing for salt iodization nationwide. Approved 1996 or FIDEL, Fortified for Iodine Elimination.

These legislations lent sustainability to the above discussed initiatives but the intense and widespread response and enthusiasm of the people (different sectors, GOs and NGOs) were not sustained.

The fervor with which the programs were carried out antagonized some sectors, particularly the church and the Pro-Life as exemplified by the resistance to family planning and the rejection of the tetanus toxoid for pregnant women, allegedly an abortifacient and the practice of safe sex through condom use for STD/HIV-AIDS prevention.

One of the issues that marred the enchantment with the Flavier administration, in so far as the health personnel were concerned, was the implementation of the Local Government Code or the devolution of the DOH. In 1993, nearly all health personnel, 596 hospitals, and 12,580 health centers and barangay health stations were devolved

to local government units. The central DOH office was cut off from the peripheral level and the official link among the health services network became loose. The IPHOs and district health offices and hospitals are now administratively under the provincial governors, while the RHUs are under the mayors. What created furor was the fact that some offices are retained and enjoy the salaries and fringe benefits as dictated by the salary standardization and the Magna Carta for Public Health Workers whereas those devolved were deprived of these benefits. This demoralized the devolved personnel plus the fact that security of their position was at stake. The reporting of health events was disrupted and supervision of health personnel was greatly reduced. While, theoretically, the move to devolve was sound, it appeared that the field people were not prepared for its proper implementation resulting in many disgruntled personnel. The transitional problems cover organizational, fiscal and operational issues which may adversely impact on the coverage utilization and effectiveness of public health programs.

When the Flavier administration ceased, the aura he had created simmered down and the public image of the DOH became hazy. The short lived Ramiro administration somehow destroyed the credibility of the DOH in the eyes of DOH staff and the people it served. The solid foundation on which public health services had been built was shaken and almost collapsed and the Reodica administration had to wrestle with this problem upon her assumption to office.

The Reodica Administration's first task was to clean up the mess and resurrect the DOH from the graveyard of scandals. It has waged a plethora of information campaign focused on seven major health concerns and has adopted the health slogan "Five for Life in '95"; "Health for all Filipinos by 2000" and "health in the Hands of Filipinos by 2020" continued to be its guide posts.

Her seven strategy program focuses on.

- Expanded program on immunization (*Oplan Alis Disease*) to eradicate polio, eliminate measles and neonatal tetanus.
- Nutrition - Vitamin A, iron, and iodine utilization (*Araw ng Sangkap Pinoy*).
- Family Planning (Kung Sila'y Mahal mo, magplano).
- Tuberculosis prevention (Target, stop TB)
- Environmental Sanitation (Tubig, Kubeta, Oresol)
- STD-AIDS awareness and prevention
- Healthy Life style program.

### AFP'S CONTRIBUTION TO PUBLIC HEALTH

After the foregoing discussions on the public health developments, it would be folly to ignore the contributions of the Armed Forces of the Philippines to public health as part of the health system.

The broad functions of the AFP Medical Services include: care of the sick and injured; education of physicians, nurses, medical technicians and paramedical personnel; conduct basic and advanced courses for medical corps, nurses corps, medical administrative corps, veterinary corps and enlisted personnel; prevention of diseases and promotion of public health; advancement in medical research and maintenance of medical equipment.

In addition to the usual medical services being provided at the AFP Hospitals and Dispensaries, wherein the military personnel, their relatives and some civilians are provided such services, the AFP services are also felt in the community through the Civil Relations Services (CRS) and various out-reach undertakings. More specifically, the AFP has greatly contributed to public health through the following activities.

1. *Alay sa Barangay Project* – the concept of this project involves adopting a specific barangay, where local residents benefit from people-oriented activities like medical/dental services, lecture on cooperativism, activities which prevent drug abuse, conduct of values education, vaccination and peace rallies and the like.

2. *AFPOplan "Iwas Tigdas"* – this project was launched to check the spread of measles in the barangay. This is a yearly operation of the Task Force KANDILI a team composed of medical and dental staff. They provide medical services, medical supplies and vehicles for the team composed of staff from DOH, and NGOs in reaching for flung areas

3. *Bloodletting* with mostly civilians and soldiers as blood donors. This is regularly conducted in collaboration with GMA Channel 7, Phil. National Red Cross and DOH.

4. *CRS-REACT* – This Regional Emergency Assistance Team (REACT) assist and support the people in times of emergencies and calamities. This team is consistently sought after in times of various calamities that require prompt and adequate services that readily alleviate suffering.

The AFP also participates in environmental and livelihood endeavors like tree planing, forest conservation, and livelihood and skills training on cooperative establishment. They also provide Human Courses on Maintenance and Operation of Medical Equipment. The Human Resource Development primarily aims to support peace process and support the implementation of "Unlad Bayad" - where personnel are trained to train the community folks to be always prepared and be vigilant in times of calamities and to respond and to attend to peculiarities of their region with consideration of environmental factors. Secondary objective of the HRD program is to enhance skills on cardio-pulmonary resuscitation, disaster management and mitigation techniques.

In summary, after the Post War years, there was:

- A rapid decline in mortality and morbidity, but health improvements were stalled during the late 1970s to mid 80s due to severe economic contraction during the period. Infant mortality, an important health indicator,

declined but was stalled in the late 1970s and mid 80s but recovered modestly by late 1980s. However, it had the slowest decline in the Asian region.

- Steady progress has been made towards the control of infectious diseases, through the introduction of chemotherapy such as the multi-drug therapy (MDT) introduced in 1985 for leprosy, short course chemotherapy, for TB since 1986; praziquantil for schistosomiasis since 1978, the adoption of rehydration for management of diarrheal diseases, to name a few. However, important causes of illness and deaths still include pneumonia, TB, diarrhea, nutrition-related diseases, and measles, with deaths among infants constituting 15 percent of all reported diseases. Diseases of the heart and vascular system and malignant neoplasms also emerged as among the top causes of death. AIDS and HIV infection pose a potential serious health problem.
- The current status of service delivery infrastructure indicates that preventive and promotive health programs have not sufficiently covered the population. The consequences of the recent devolution are still unfolding and there are many recognized deficiencies in the health system. The proportion of medically attended deaths show that 60% do not have reliable access to medical care. There are still pockets of rural/urban areas unserved or underscored.
- On health programs from the 1980 primary health care strategy focused on the delivery of maternal and child care services (EPI, CDD, CARI, breast feeding promotion and maternal care), control of prevalent diseases (Schisto, malaria, and TB), nutrition and family planning. To these were added access to water, household sanitation, reduced risk to air and water pollution, and chronic degenerative diseases.

The Expanded Program for immunization is the most successful public health program. High level of coverage of 90% has been sustained. However, with devolution, this high coverage may not be sustained unless LGUs will have the political will to make the necessary investments for cold chain facilities, replacement and distribution of vaccines.

The pursuit of disease control facilities has had some degree of success - malaria, schistosomiasis and TB. Coordination of various facets of the program would have to be arranged between the DOH and LGUs.

As regards CDD and CARI, technical infrastructure for delivering case of effective management interventions have been established across the nation. Clinical protocols, trained health personnel, drug distribution and monitoring and supervisory systems have been installed. The challenge after devolution is to sustain all these activities.

Women's health and safe motherhood (family planning, breast feeding and maternal care) have been addressed through training, service delivery policies, innovative approaches.



The current health situation creates tremendous burden to households and the economy in terms of infant and maternal deaths from preventable and curable diseases, disabilities and ineffective health care services.

### **Future Challenges**

In the National Health Plan 1995-2020, the DOH vision is that "All Filipinos will be able to attain a level of health that will enable them to lead a progressively improved socially and economically productive life following the momentum of socio-economic development generated over the years."

Correspondingly, the Philippines will face many challenges that will have an impact on health.

- **Urbanization** – It is forecast that by the Year 2020, the urban population will comprise 65% to 75% of the total population. The chaotic growth of cities will result in a multitude of economic and social problems. The rise of slums, of criminality, of disease, of unemployment - all will pose a major health risk to the population. Overcrowding, inadequate housing facilities, poor environmental sanitation, a different life style and values all breed infections as well as non-communicable diseases.

The increase in transportation due to urbanization will result in air pollution, traffic accidents, traffic problems, stress and unhealthy life styles such as smoking, promiscuity, alcohol and drug dependence, sedentary habits, and poor eating habits.

- **Industrialization** would mean more women joining the working force. This may or may not have an adverse effect on the family. Care of children will be entrusted to caretakers. On the other hand, industrialization may result in family limitation. Occupational hazards becomes a major concern. Corollary to this are air, soil and water pollution and the management of toxic and hazardous waste. Industrialization and urbanization will affect agricultural production as more lands will be used for subdivisions, industrial and recreational centers and for malls or "trade palaces". More people will work in factories, reducing the number who work in the farms. Mechanized farming will display many farm hands.
- **Environmental Concern.** While the Philippine now boasts of having perked up its economy, this has its tradeoffs. Environmental degradation caused by deforestation, deterioration of seas and rivers due to industrial waste, dynamite fishing, siltation on-site toilets, domestic waste waters, indiscriminate garbage disposal and other destructive practices are problems to be reckoned with. Radical population growth, industrialization and urbanization all contribute to environmental degradation. No place is too remote or difficult/hazardous for intrepid adventurers, tourists or developers in search of exotic locales for golf courses, plush subdivisions or tourist resorts. All these lead to ecological imbalance

which leads to the emergence of new types of micro-organisms as the Ebola and the HIV virus.

- ***“The Revenge of the Germs”*** – The indiscriminate and over use of antibiotics has resulted in drug-resistant, bacteria, viruses and parasites, Staphylococcus and streptococcus have accomplished the feat of penicillin resistance. Super strains of staph that were resistant to huge numbers of potential drugs existed by 1990. Switching from inexpensive penicillins to other drugs increases drug treatment costs which is beyond the reach of the poor. The same patterns of resistance seen in staphylococcus and streptococcus were mimicked by other dangerous microbes; microbacterium leprae, gonococcus, shigella dysentery, salmonella, resistance of *P. falciparum* to chloroquine, etc.

### **What will be the future scenario?**

The Philippines, will emerge from its image as the ‘sick man’ of Asia into the newest economic tiger. It will be one of the significant economic players in the international arena. It will make a quantum leap forward, it will “poll vault”. There will be a rapid pace of technological advancement, especially in telecommunications and information technology. It will become a “wired world”, an “internet society”. This will be paralleled by advances in bio-medical technology. With more and better information in their hands, people will be empowered; will have more confidence in decision-making about their health. Health providers will have to keep pace with the new developments in the health field lest the people make demands with which they cannot cope. With advances in the bio-medical field (in molecular biology and genetic engineering) transplants, cloning, test tube babies will increase. New treatment technologies will be discovered.

Breakthroughs in the health and medical field will take place. Vaccines against communicable diseases like influenza, schistosomiasis, malaria etc. may be discovered. Correspondingly many ethical and moral issues will have to be resolved.

People will be more mobile due to faster and more efficient transport technologies. This will allow for more opportunities to gain greater access to health facilities. The DOH will have to be ready to meet increasing demands on its services. The new government health insurance scheme will be set in place; health demands would multiply. The private sector will take a greater role in meeting such demands. HMOs will further sprout; Privatization of the health sector will take place in consonance with the recent Asia Pacific Economic Conference’s recognition of the role of the private sector as the “engine of growth and the source of innovation”. The DOH then, in addition to its regulatory roles will have to act as a facilitator of private health initiatives. The shift in emphasis of HMOs for example, to promotive and preventive rather than curative roles will have to be encouraged and care will need to be taken to assure that HMOs do not dictate their terms to the medical profession.

There will be problems galore but knowledge will be the key to success of whatever health program is undertaken. As Toffler has said in his War and Anti-War, success in future wars will hinge on three factors - lethality, speed and range. Applied to public health, this means that public health programs must be effective (lethal), timely and comprehensive/farsighted. If young men have vision and knowledge, then the dreams of old people will come true!

## THE PHILIPPINE PUBLIC HEALTH IN THE LAST 100 YEARS

MANUEL M. DAYRIT  
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Dr. Tiglao's paper gives a wonderful perspective of how Philippine Public Health has been like these in last 100 years. I would like to frame my reactions to it within the framework of a story about a Chinese emperor who lived even much earlier in history. This way, we dig even deeper into the wisdom of the ages.

"This story is about Emperor Liu Bang who in the third century B.C. became the first ruler to consolidate China into a unified empire. To celebrate his victory, Liu Bang held a great banquet in the palace, inviting many important government officials, military leaders, poets and teachers, including Chen Cen, a master who had given him guidance during the campaign. Chen Cen's disciples, who accompanied him to the banquet, were impressed by the proceeding but were baffled by an enigma at the heart of the celebration.

"Seated at the central table with Liu Bang was his illustrious high command. First there was Xiao He, an eminent general whose knowledge of military logistics was second to none. Next to him was Han Xin, a legendary tactician who'd won every battle he'd ever fought. Last was Chang Yang, a shrewd diplomat who was gifted at convincing heads of state to form alliances and surrender without fighting. These men the disciples could understand. What puzzled them was how Liu Bang, who didn't have a noble birth or knowledge comparable to that of his chief advisers, fit into the picture. "Why is he the emperor?" they asked.

"Chen Cen smiled and asked them what determines the strength of a wheel. "Is it not the sturdiness of the spokes?" one responded. "Then why is it that two wheels made of identical spokes differ in strength?" one asked Chen Cen. After a moment, he continued, "See beyond what is seen. Never forget that a wheel is made not only of spokes but also of the space between the spokes. Sturdy spokes poorly placed make a weak wheel. Whether their full potential is realized depends on the harmony between."<sup>1</sup>

<sup>1</sup>From Phil Jackson, *Sacred Hoops. Spiritual Lessons of a Hardwood Warrior*. Hyperion New York 1995.

Where public health is concerned, what are the spokes, what are the spaces?

Dr. Tiglao has already provided some answers to these questions. Let me cite 3 main spokes (although I know there may be more).

First is the public health infrastructure - personnel, resources, organization, systems - which has been strengthened over the years. It is interesting to see how this has evolved to the decentralized public health system we have now.

Second is the scientific and technological advances which have been developed through research and development: drugs and vaccines, the bifurcated needle for the elimination of smallpox, technologies for vector control to cite a few.

Third is the mass-based approaches to mobilize populations and community participation to widen the coverage of tested interventions.

All these are spokes in the wheel of Public Health.

The other question however is what are the spaces? This is the trickier issue. The spaces represent the harmonizing elements which strengthen the spokes. Let me cite to you what I believe might represent the "unseen" - the spaces that are so important for strengthening the spokes.

First is the mindset for healthy living especially in present times when many of our illnesses are determined by lifestyle.

Second is the value placed on equity. This is anchored on a mode of socio-economic development which over the long term alleviates and eliminates poverty.

Third is the value placed on the preservation of the environment, prevent its degradation, and making life on the planet wholesome.

If these "spaces" do not harmonize with the spokes, I believe there is no way we can achieve long-term achievements in public health over the next 100 years.

The spaces are perhaps at the level of the spiritual - reverence for self (healthy living), reverence for others (equity), and reverence for the environment.

To conclude: I am persuaded that we are already seeing the epidemics of both the present and the future. Infectious disease problems are still commonplace but are said to be on the wane - perhaps. Over the next several decades, we shall be seeing chronic disease problems much of them related to what is probably the single largest risk factor of industrial times - tobacco use. Eventually I am told that we will be seeing a wave of depression as the stresses of living, the loss of meaning and meaningful relationships increase.

The ultimate management of the future will lie in managing "what is unseen" even as we manage with the traditional tools of public health known to us.

Thank you.

## **SOCIAL SCIENCE AND PUBLIC HEALTH IN THE PHILIPPINE CONTEXT**

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In reviewing the past, analyzing the present and scanning the future, Dr. Tiglao's paper emphasizes the role of social, cultural, political and economic factors on the status of public health in the Philippines. Further, the definition of public health cited at the beginning of her paper contextualizes her analysis. By pointing out that the nature of public health is ecological, multisectoral, collaborative and participative, the contribution of social science to public health strategies and policies is clearly indicated.

But in this short discussion, it must be pointed out that social science and medical science were not always on talking terms. Each ignored the other. If and when social science was acknowledged, it was relegated to the status of second class citizen. In the Philippines, it was only in the 70's and 80's when social science began to be more systematically integrated into attempts to improve health status, prevent disease and facilitate treatment. The work at the College of Social Science and Philosophy (CSSP), College of Public Health (CPH), Research Institute of Tropical Medicine (RITM) and health NGOs paved the way for the collaborations. In some of these early efforts, there was a certain degree of mutual distrust borne out of a lack of knowledge and understanding of each other's theoretical perspectives. But with each successive interaction between these fields grew the realization that the role of medicine did not diminish but was enhanced and supplemented with an application of the social sciences. The result was a more comprehensive understanding of disease and better health care. The input of scientists, researchers and consultants in the various control programs as well as the Essential National Health Research (ENHR) are now fairly well established. For its part, social science has followed the trend of other scientific disciplines which become more applied as they mature. The social sciences have the appropriate perspectives and methods for the interdisciplinary work in public health. The basic models and theoretical perspectives and methodologies of psychology, anthropology and sociology found ready application in the health fields.

The rest of this commentary will focus on the social and cultural context of public health and like Dr. Tiglao, I am making the assumption that "health and

illness can be understood only in a social context. It is not only that culture determines our notions of well-being and illness but the conditions of social life influence our biology as well as our psychology”.

What is this social context that affects the status of health and public health programs? The most pervasive and challenging is poverty. Dr. Tiglao points out for example that although progress has been made in slowing down infant mortality rate, we have the slowest decline in the Asian region. Castillo (1996) quotes a survey on low-income families done by Herrin and Racelis (1994) which provides further insight: . . . “Among the poor pre-natal and post-natal consultations are lower than the national population. Most of the deliveries are done at home attended to by traditional birth attendants.”

In trying to explain social class differentials in morbidity and mortality rates, it is important not only to identify socio-environmental factors that affect susceptibility to illness and have disease consequences, but also to identify the effects of social and psychological environment. Migration and population density have been studied as contributory factors in disease transmission and prevalence. Infectious diseases do not become endemic until enough people live in one place to provide a reservoir for a virus to recycle itself. It is not surprising that disease outbreaks are more probable in poor communities. Vaccines are of course available for most of these infectious diseases and these can significantly reduce new cases. Yet measles still causes death among infants in the disadvantaged families because of malnutrition and lack of knowledge concerning the disease among mothers. Social science has well defined principles regarding effective communication and these have been adopted to some extent in health campaigns. But the tyranny of poverty can be illustrated by some field observations in family planning and malaria control.

For example, one poster which aimed at encouraging participation in the collection of blood smears for malarial diagnosis showed a finger being pricked and dripping with blood. A nonliterate farmer interpreted the drawing to mean that malaria was caused by dirt under the fingernails. In another field research on family planning, a woman decided to take contraceptive pill. All she knew was that the pill would prevent her from getting pregnant. She was so poor that she decided to extend the use of the pills by dividing these into two and taking them only before having contact with her husband. Social science can assist the public health professional not only in terms of identifying errors and problems but more constructively in terms of planning and designing effective preventive control and intervention strategies, persuasion and communication, compliance and adherence to medical regimens.

Another example is adolescent sexuality. Social change associated with urbanization and industrialization has had a biological effect on the earlier onset of puberty. Added to this is precocious adolescent sexual activity resulting in unwanted pregnancies. The higher morbidity and mortality for mothers and infants associated with adolescent pregnancy can be reduced by better medical care but the social developmental consequences for the mother and the infant that she is

poorly prepared to nurture are well known. It is easy to declare a policy to provide sex education in schools but delicate cultural and ethical issues need to be tackled first, and here, social scientist can make a significant contribution.

Looking ahead and sharing Dr. Tiglao's note of optimism that economic good news is around the corner, and that there are significant improvements in the delivery of health care, a longer life span may be expected for most Filipinos (except that in an urbanizing context, there will be those who will become victims of violence, accidents and diseases related to lifestyle or environmental pollution). In this regard there is an urgency for social planning to anticipate the consequences of better health measures. It must be noted that the Reodica administration listed diseases related to lifestyles as one of DOH's primary concern. The advances made in behavior modification, attribution theory, persuasion and communication are directly relevant to these. Additionally advances in the medical field and treatment technologies enhance the need to tackle ethical issues. The culture of violence in urban areas and the clashes between the military and dissidents or rebels also require attention and systematic intervention for the child and family victims. On a limited scale, NGOs have directed their energies for the physical and psychological rehabilitation of these victims.

The most effective control programs have put emphasis on the dynamics of human interaction in the delivery of services and exposure to the disease. The detailed observational studies and training programs for barangay health workers done by an interdisciplinary team based at the RITM, on the work behavior patterns and sleeping arrangements of malari-prone community in Morong illustrate how social science work provide the basis for effective interventions in the delivery of services. Working at the control program level, it appears necessary to exercise "cultural wisdom" by exerting extra effort to study the personalities and politics within the organization, acculturating oneself so to speak, before initiating collaborative work with the control staff. This generally ensures that the disease control staff will better appreciate and eventually adopt one's findings.

The public health system directly services the needs of the disadvantaged sectors of our society. It is the public health professional who sees on a day-to-day basis the illness burden exacted by social inequity. Public health deserves the best social science can offer, for in the end, the abstractions of justice, equity, responsibility can be translated in creative collaboration with medical scientists.



## **PLENARY SESSION II**

### **ENVIRONMENTAL HEALTH: PAST, PRESENT AND FUTURE**

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#### **INTRODUCTION**

As the 20<sup>th</sup> century draws to a close, a consensus is emerging that we must make better decisions to protect the quality of our environment and public health. These decisions cut across our economic development directions, environmental policies and our perspectives of a quality life. Obviously there are interactions between development activities, consumption of natural resources and human health which are significantly influenced by demographic, social, cultural and political factors. In order that these decisions be made better, it's just fitting that we look back to what happened in the past and draw some lessons to enable us provide a sustainable development to the future generation.

This paper presents a treatise of development, environment, health in the country. Increasingly in recent years, it has been recognized that health depends largely on our ability to understand and manage the interaction between human activities and our physical environment. On the other hand, development means addressing people's needs at the same time attaining ecological sustainability so that natural resources are not depleted, damaged or degraded. This report covers the socio-political influences of the prewar era as well as the postwar trends in our economic development, environmental management and human health. The paper also tries to present the challenges and opportunities in integrating health and environment in our development efforts towards a newly industrializing nation by the turn of the century and in making better decisions for sustainable development in the next millennium.

### Historical Background

The Philippines is endowed with vast natural resources such as mountain ranges and forests, river systems, coastline and volcanoes. According to the book *History of the Filipino People*, the longest continuous mountain range in the country is the Sierra Madre while the largest river system is the Rio Grande de Mindanao. Philippine forests cover some 40,000 square miles while the coastline extends to about 11,000 statute miles.

There are at least ten active volcanoes, of which the Mayon volcano is the most active considering that it has erupted more than thirty times since 1615. Volcanic eruptions have been for centuries a natural cause of injuries, death and destruction of property. Because of the country's geographic location and topography it is also prone to natural hazards such as earthquakes, and floods. During the pre-colonial period, the economic life of the Filipinos was mainly dominated by agriculture and as such an abundance of rice, coconuts, sugar cane, cotton, hemp, bananas and oranges, and other crops existed. Kaingin system and tillage were the basic methods of land cultivation during those times. The rice terraces of the Mountain Province is a living proof of the irrigation technology used during the period. Other industries did exist such as poultry, fishing, mining, lumbering, shipbuilding and weaving. As chronicled, fishing was mostly done with the use of fish nets, bow and arrow, spear, wicker baskets, corals and fish poison. Mining, on the other hand was developed before the Spaniards came particularly in gold mining. This was evidenced by the goldchains, bracelets and earrings worn by the rich natives as reported by Legazpi in 1569. The reported gold mining technology used then as washing and placer mining. Shipbuilding technology then present was shown by the vintas and bancas used for travel and commerce.

Under the Spanish regime, during the nineteenth century, telecommunications, infrastructures and public utilities were developed. The railway line, 120 miles long extending from Manila to Dagupan was constructed by Filipinos before the Philippine revolution in 1896. Among the forms of street transportation were horse-drawn carriages, animal-pulled tramcar, tranvia de vapor, calesa and carretela. The Quezon bridge was the first suspension bridge in the Far East built during the period. The telephone system in Manila began working in 1890 servicing initially only 170 persons. Telegraph lines between Manila and Corregidor started in 1872 and the first interisland submarine cable linking Manila with the Visayas done in 1897. The lighting of Manila and suburbs using coconut oil started as early as mid 1800s and gradually changed to kerosene in the 1880s. The walled city was lighted by electricity in 1893.

Several economic development took place during the American occupation. The country developed with the establishment of industries such as textile, tobacco, sawmills coconut oil mills, cordage factories, alcohol distilleries sugar centrals, fishing and fish-canning industries to mention a few. All these industries were based on agricultural resources of the country. On the other hand, mining became the

backbone of the Philippine economy. The mining industry depended as well on the extraction of natural resources of the country. The country's then growing economic development, however, was affected by the world-wide economic depression of the 1930s.

Paralleling the economic development were progress in the transportation and communication sectors. Road and bridges were constructed, and the means of transportation also improved from the ancient bull carts and calesas to a railway network. Interisland shipping also developed which promoted domestic trade and industry by opening several ports to domestic shipping. In telecommunications, telephone lines, radio-telephone services and postal services were also opened.

Significant progress in education was achieved during the American occupation which enormously contributed to the increase of Filipino literacy. Enrollment of children became compulsory as well as the learning of the English language. Filipino students excelling in specific areas of learning were sent to the US as *pensionados*. The UP was established in 1908 to provide higher education. Other private educational institutions, like the University of Santo Tomas also formed part of the educational system of the country.

The progress in literacy had been attributed to the establishment of schools, colleges and universities between 1900 and 1935 as well as the adult education program introduced by American missionaries in non-Christian areas and the pioneering venture of the UP in launching literacy campaign in the rural areas.

Considered as an important contribution of the Americans to the country was the introduction of a scientific program of public health and welfare. Communicable diseases such as cholera, smallpox, tuberculosis and malaria ravaged the country before 1900. The Americans introduced new methods of preventing and treating these deadly diseases and educating the people on basic principles of hygiene and sanitation. The net results of the preventing programs were the decrease in mortality, improvement in standard of living and nutritional status of the Filipinos. The net results of the preventive programs were the decrease in mortality, improvement in standard of living and nutritional status of the Filipinos. However, the major limitation in the program was the ignorance and superstitious nature of the Filipinos.

The Japanese occupation of the country resulted to a massive destruction of the economy, as well as the loss of the social and moral balance of the Filipinos. Industry and trade suffered a major setback during the occupation since production virtually stopped. Filipinos then engaged on the "buy and sell" business which resulted to astronomical prices of basic commodities. The high inflation and the manufacture of "Mickey Mouse" money to encouraged buy and sell businesses resulted to high prices and scarcity of food. With the economic dislocation and food scarcity, many Filipinos died from malaria, malnutrition, tuberculosis and other communicable diseases.

### **Trends in Development, Environment and Health (1945 to 1997)**

The destruction brought about by the war on the national economy was incalculable. Poverty was prominent due to widespread destruction of property, the reduction in income and other means of livelihood and losses in industrial productivity. Consequently, social services, health and welfare suffered tremendously. However, the rebuilding of the country started with the reorganization of the government, the American financial aid for rehabilitation and reconstruction. Several changes occurred in our health development through the years, mainly influenced by demographic and social factors, economic development, trends in science and technology, management of our environmental resources and by the institutional and policy framework wherein our goals of total development are embodied.

#### **Demographic Trends**

The 1990 population of the Philippines, according to census, stood at 60, 703, 206. This population figure more than doubled from 1960 to 1990 considering the average annual growth rate of 3.5 percent between 1960 and 1970, 3.1 percent between 1970 and 1980, and 2.6 percent between 1980 and 1990. These rates are definitely higher than in the neighboring countries of Indonesia and Malaysia. The 60.7 million population in 1990 is occupying a land base of 300,000 square kilometers making the country one of the most densely populated countries in the world. Although the population growth declined to 2.4 percent by the end of 1995, such rapid growth is putting great pressure to the country's economy to generate employment. During the 1970s, migration patterns of population were towards the cities where employment opportunities abound. However, these patterns considerably changed when the country experienced economic crisis between 1980-1985 provoked by domestic economic policy and excessive bank lending. The reduction of job opportunities in Metro Manila and the collapse of the sugar, rice and corn industry, the declining family income for the population and the incentive for commercial logging contributed to upland migration that by 1985, some 30 percent of the population have migrated to the upland areas.

In 1990, the migration pattern was back towards urban and industrial centers of the country basically for employment. However, work opportunities were not always available thus the sprouting of urban slums where basic services such as water and sanitation and other social services were grossly inadequate. Poverty had stricken many regions of the country that in 1991 severely affected areas were the Bicol region, Central and Eastern Visayas as reported by the National Statistics Office (NSO).

Undernutrition of Filipino children, although decreasing in proportion in proportion was still high compared with our neighboring countries of almost similar development trends. Food expenditures were reported to share more than 50% of the

family income. A 1991 survey by the NSO indicated that food expenditures share more than 56% of the total income in the political regions of Eastern Visayas, Western and Northern Mindanao and the ARMM. This indicates that with the declining economic opportunities and consequently the family income, access to food had become more difficult and thus satisfying the nutritional requirement of the population was grossly neglected. Surveys of the FNRI in 1982 and 1990 indicated that 17% and 12% of 1-6 year old and 7-10 year old children respectively were underweight in 1982. These decreased to 14% and 5.5 percent in 1990. Stunting, an indication of chronic malnutrition was seen among 22% of 7-10 year old children in 1985 and reduced to 14.2% in 1990. This nutrition profile made our children vulnerable to several diseases and premature death. The prevailing demographic patterns required additional resources for basic health and social services and would strain our environmental resources which are rapidly deteriorating because of population pressures.

### **Urbanization**

Together with the significant population increase is the unprecedented rate of urbanization which poses multi-faceted problems including unemployment and underemployment, lack of infrastructure and basic social services as well as environmental degradation. In 1961 urban poor population was 28% of the total urban population and the proportion doubled thirty years later.

The level of urbanization in the country was 30% in 1960, 31.8% in 1970, 38% in 1980 and increased to 49% in 1990. The growth of urban population increased from 3.7% per year in 1960s, 4.4% in 1970s, and 5% in the 1980s. Unfortunately, the level of urbanization had not been matched by a correspondingly high per capita income and employment opportunities. The acceleration of urbanization in the 1980s occurred when the overall economic performance of the country was mediocre compared with our Southeast Asian neighbors.

Economic development policies in the 1960s were bias in favor of urban areas particularly in Metro Manila, the main urban center in the 1950s. This induced the concentration of manufacturing establishments, infrastructure and social services in the area. Gradually in the 1970s and 1980s this trend continued on to Central Luzon and the Southern Tagalog regions. Cebu in the Visayas and Cagayan de Oro and Davao in Mindanao emerged as the urban and industrial centers in those regions. With these developmental activities concentrated in urban centers, rural development was impeded due to lack of infrastructures and social services in the rural areas.

The macroeconomic environment in the country changed significantly in the 1980s and early 1990s towards a more open and market-oriented economy. However, the country's foreign debt and the ailing economy during this period severely limited the government's ability to provide the growing demand for infrastructure development and social services. Major problem spawned by urbanization was poverty as characterized by lack of employment opportunities, poor access to food, housing, health and education.

Given this socio-economic and political climate in the country during the mid-1980s to mid 1990s, efforts had been focused on development programs and projects designed to enhance food production, to improve delivery of social services and the establishment of infrastructure industries and utilities which aimed at generating greater productivity and promoting the social and economic growth of the country. Among the development projects implemented by the government agencies and private sector investments were infrastructure development covering transport, water resources, power and communications and social infrastructure including health, nutrition, population, education, manpower development, housing, community development and environmental management.

### **Trends in Science and Technology**

Postwar developments in science and technology were mainly focused on rehabilitating our economic and educational facilities and industrial productivity. This meant having new laboratory equipment and facilities, and training of Filipino scientists abroad in the fields of agriculture, industry and medicine. The US government supported most of these rehabilitation activities through aids and grants. In agriculture, research and capacity-building were in the fields of horticulture, agronomy, pest control, farm mechanization, the Masagana system of rice culture, animal husbandry, livestock and fish culture – all aimed at increasing the yield of our natural resources. Research projects also concentrated on industrialization of local products including coconut by-products, leather tanning, plywood, pulp and paper, ceramics and medicinal plants among others.

Development of science in the country after the war was attributed to several factors such as 1) the increasing number of scientists in the fields of agriculture, medicine, engineering and other specialist in allied technological fields sent abroad for training, 2) the educational trend wherein colleges and universities offered science and technology courses geared towards meeting the needs of our economic development and 3) the revitalization of activities and membership in professional scientific organizations. In 1968, a special science fund sourced from tax imposed on motor private vehicles and science stamps on legal travel documents sustained the science and technological research program of the country which aimed at developing our science resources and the application of such resources in accelerating our social and economic development. However, the science and technology sector suffered a setback in human resource development in the so-called “brain-drain” phenomenon in the 1970s. This problem was countered by the government with the Balik-Scientist program during the martial law period and the “development diplomacy” on transfer of technology invoked in 1986.

Henceforth, several government efforts were directed towards the formulation of a comprehensive plan for science and technology, the establishment of S and T council, science communities and the scientific career system, and the integration of regional activities to enhance the promotion of science and technology in the

countryside. The Science and Technology Master Plan focuses on directing efforts towards making the nation a newly industrializing country through the development of 15 "leading edges" in priority sectors aimed at yielding substantial contributions to our GDP. The 15 production and services sectors are: agriculture, aquaculture, forestry, metals, textile industry, mining, process industry, food and feed industry, energy, transportation, construction, information technology, electronics, instrumentation and control, pharmaceuticals and emerging technologies. As such, strategies formulated to realize development goals include the development of high quality S & T manpower in growth areas, expansion of S&T education and training, development of S&T institutions, expansion of private sector participation, and development of an S&T culture.

### **Economic Development Trends**

After the war, agricultural production was the focus of the government to increase people's productivity and improve standard of living. From 1946 to 1956, crop production almost doubled to some 7 million metric tons. This increased production resulted from expansion of land area for cultivation and the application of improved methods of production to increase yield per hectare such as the use of irrigation systems, better types of seeds, chemical fertilizers and pesticides to destroy plant pests and diseases. Philippine exports also tremendously increased from 300,000 metric tons in 1946 to 2.77 million metric tons in 1956. Copra and sugar were then the leading exports of the country. Recovery in the fisheries and livestock production was partial and thus resulting to reduced importation from 1946 to 1956. Increased activity in the lumber and timber industries was inevitable as demanded by the post war reconstruction. The timber and lumber production soared to 2.5 billion board feet in 1964 as an outcome of domestic and foreign demands.

Even with the rehabilitation efforts, the national economy remained weak, so that in 1950 a US economic mission headed by Daniel Bell initiated an assessment of the economic situation of the country as well as to identify measures to improve production and living standards in the country. Among the major findings were inefficient production and low income, and excessive volume of imports. The recommendations listed in the mission report to address these difficulties included efforts to improve agricultural production through modern and efficient methods, expand the services of agricultural extension stations, and the establishment of rural banks; to diversify the economy by encouraging new industries, developing power and transportation facilities, coordinating activities of government corporations and abolishing ineffective ones, and giving financial assistance to productive enterprises; and to improve social and welfare services through an adequate program of public health and education, to provide better facilities for urban housing, to pass laws for the protection of workers and to establish a minimum wage for urban and agricultural workers; and the provision of foreign financial assistance through grants and loans for carrying out a program of economic development and technical assistance.

It was in 1955, during the term of President Magsaysay when the country started to avail a US \$10 million loan from the International Monetary Fund for economic stabilization and increased to US \$ 18 million in 1957. President Garcia's economic program anchored on austerity and Filipino-first policy to complete Philippine economic independence. Other aims of his program was to achieve a balanced economy by providing equal impetus to agriculture and industry and to minimize or possibly eliminate graft and corruption. In spite of his nationalistic policies, the economy of the country continued to deteriorate and graft and corruption continued to perpetuate.

During the term of President Macapagal in 1961, the stabilization loan of the country amounted to US \$330 million and the Philippine peso devalued from 50 US cents to 25 US cents. Among the major goals of his presidency were to solve the problem of unemployment, self-sufficiency in staple food and the establishment of practices that will strengthen the moral fiber and reintroduce those values which invigorates our democracy. To radically change the economic and social conditions of the farmers, the Macapagal administration saw the need to prepare the agricultural land reform code. This code was passed by Congress and signed by Macapagal into law in 1963. The code intended to establish the economic family-size farm as the basis of Philippine agriculture and divert landlord capital in agriculture to industrial development. Consequently, a viable social and economic structure in agriculture will be achieved.

During the inauguration of President Marcos in 1966, he promised that he will implement the land reform code. Among the objectives of the Marcos administration then were self-sufficiency in rice production and diversification of crops, implementation of the land reform program, and intensification of community development program in order to strengthen further "our social, economic and political base". In the early years of his administration the country's foreign loan amounted to US \$500 million and reached to some US \$ 19.5 to 215 billion in 1983.

In 1972 when martial law was declared, a reform program to restructure the Philippine society through rationalization of development planning and decision-making was deemed necessary by the administration. The creation of NEDA in 1973 provided the necessary institutional framework for development planning and policy-making in the country. NEDA estimated that the GNP of the country increased from P40 billion in 1974 to P43 billion in 1975. Agricultural growth was documented and attributed to the liberal credit policy of the government and to good climatic conditions in 1974 to 1975.

### **Industrialization**

Serious efforts at industrialization started in the mid 50s as a result of attempts to rehabilitate and recover from the ravages of war. The pace accelerated in the 1970s and moved to high gear in the early 1980s. The Philippine industrialization strategy then was based on accelerated implementation of 11 major industrial projects namely:



aluminum smelter, copper smelter, integrated steel mills, phosphate fertilizer, diesel engine manufacture, heavy engineering industries, cement industry, coconut industry, petrochemicals, pulp and paper and alcogas.

To realize industrialization goals and accelerate the country's development, export processing zones (EPZs) were established in the early 1970s. Three export processing zones were established between 1972 and 1979: 1) the Bataan export processing zone established in 1972 with a total proclaimed area of 1,600 hectares, 2) the Baguio City export processing zone established in 1979 covering a proclaimed area of some 114,000 hectares, and 3) the Mactan export processing zone in Cebu made operational in 1979 with a total land area of about 120,000 hectares. In 1980, the Cavite export processing zone established but became operational only in 1986.

Nine special export processing zones were also developed to cater the major industrial projects of the country. Among these special EPZs are the Leyte industrial estate for copper smelting and fertilizer manufacture, the Tabangao special EPZ in Batangas for LPG production, industrial estates in Cavite, and Tarlac, industrial and business parks in Cavite and Laguna. In 1986 these regular and special EPZs housed only 51 companies. This figure doubled in 1989 and increased to 178 in 1991. The export earnings of the three regular EPZs in 1980 amounted only to US \$ 71 million which increased to US \$ 236 million in 1981. The exports in 1986 only valued some US \$ 277 million. Five years later, exports increased by 250%. With industrial development, employment improved as demonstrated by the increase of employment of some 24,000 in 1986 to 43,000 in 1991. The net trade balance of these zones in 1986 was US \$ 129.6 million which slumped to million US \$M 6.3 in 1987 due to the then weak political stability of the government. However, this was able to recover in 1988 to about US \$ 100 million and in 1991 soared to US \$ 245 million.

## **Agriculture**

As the agricultural technology in the 70s called for intensive use of chemical fertilizers and pesticides to increase crop yield, 1974 census data indicated that 30% (10,117,000 hectares) of the total land area of the country was devoted to food and commercial crop production. The Masagana 99 rice production program was introduced in 1973 with the aim of increasing and attaining self-sufficiency in rice.

To realize this end, dissemination of high-yielding variety, expansion of irrigated areas planted to rice and the scientific application of fertilizer and pesticides were called for. In 1973 a total consumption of some 677,000 metric tons which peaked in 1974 to 740,000 metric tons of fertilizers were recorded. However, in 1976, this value decreased to 650,000 metric tons. The local production of fertilizers could only supply 50% of the requirement. By 1977 the Masagana 99 areas planted total some 64,000 hectares and the total fertilizer allocation for the vegetable, rice, corn programs was estimated at 268,000 metric tons. Pesticides were also extensively used to maximize crop production. Over 80 kinds of pesticides were used then including DDT and

endrin which were banned for use in vegetable production. In 1976, 32 firms engaged in pesticide industry and the annual importation for the year.

### **Mining**

Mining of mineral continued to contribute to economic development of the country. Mineral production included gold, silver, iron, copper, chrome, manganese, coal and other minerals and non-metals. The application of modern methods for exploration and mining coupled with increasing world demand for metals and industrial minerals led to the unusual mining industry in the 1970s. This was demonstrated by the country being the biggest producer of copper in Asia and its consistent production of significant quantities of gold, silver, nickel and cement.

In 1977, total mineral production was 1.15 million metric tons consisting of nickel, refractory chromite and copper concentrates. A major portion of production went to mineral export and valued at 2.322 billion pesos while mineral product imports totalled P108.5 million. In 1987, the total ore reserves were 6 billion metric tons (BMT) for metallic ores and 25 BMT for nonmetallic ore. Due to increasing exploration activities, known ore reserves were estimated to have increased. In 1994, the estimated ore reserve was 6.8 BMT for metallic and 51.6 BMT for nonmetallic ores. The metallic ore was dominated by copper while limestone dominated the non-metallic reserve. The economic dependence of the country on the mining industry led to increased volume of mineral production resulting in increased exploitation of mineral resources which are nonrenewable and the consequent depletion of mineral reserves.

### **Energy**

Parallel to the industrial development is the increasing demand for energy to fuel agro-industrial and other economic activities. The Philippines has always been dependent on foreign supplies of commercial energy. Ninety four percent of the country's energy needs in the 1970s were supplied by foreign countries through oil imports with the rest supplied by hydropower and coal. The global fuel crisis in 1973 which severely affected the country's fuel supply forced the country's decision-makers to develop and utilize indigenous sources of energy and reduce dependence on costly imported petroleum.

The mid 1970s witnessed the acceleration of energy resource exploration and development activities in the Philippines. The first oil discovery in Palawan recorded in 1976 prompted the government to broaden its energy perspectives by launching a more comprehensive energy resource development program which will explore other indigenous resources such as coal, geothermal and nuclear fuels and strengthen research and development activities on other less conventional forms of recoverable energy such as solar, wind and biomass.

In 1977, coal production recorded at about 250,000 metric tons, the highest production figure so far in the country. The coal regions were identified in 1977 as the

Cagayan basin, Southern Mindoro, Semirara, Cebu, Davao, Malangas, Eastern Negros Catanduanes, Surigao, Polilio, Bataan and Lanao-Misamis.

The Philippines remains the second largest producer, next to the US, of geothermal power in the world. Potential reserves are estimated at more than 5000 megawatts (MW). In 1977-1978, a total of 28 development wells were drilled for geothermal exploration and development. Of these, three became operational in the late 1970s namely the geothermal plants in Tiwi, Albay, in Makiling-Banahaw in Laguna, and in Tongonan, Leyte. In the 1980s an additional geothermal plant in Palinpinon in Southern Negros was added to the list. In 1995, there were five geothermal fields with the addition of Bacon-Manito in Albay, generating a total geothermal power of 1,153 MW representing only 20% of geothermal reserves.

The energy program was able to reduce the country's dependence on imported oil down to 65% in 1983. Domestic oil and coal production contributed only about 5% and 3% of the country's energy needs respectively. A couple of years later, there was a decreasing trend in the use of imported coal and oil. However, the trend reversed when imported energy fuel use increased from 55.6% in 1986 to 65.7% in 1990, and 71% in 1994. Records have shown that the total energy consumption of the country from 1980 to 1995 was at its lowest during the period 1984 to 1986 and started to show an upward trend in 1987 to 1995. This profile maybe attributed to the level of economic activities, population growth and the political climate of the country. The crippling power crisis in the early 1990s led to the redefinition of the institutional framework of the energy sector.

Historically, energy affairs were in the hands of the Petroleum Board in 1972. This was changed to consider other energy sources with the creation of the Energy Development Board in 1976. The Ministry of Energy was created in 1977 and with the change in the political leadership in 1986, the BOE was placed under the Office of the President. The power crisis in early 1990s led to the creation of the Department of Energy to rationalize the organization and functions of government agencies related to energy. The energy sector has spurred the economic development of the country through the years and have generated beneficial and detrimental environmental consequences which may have affected as well the quality of life of the general population.

### **Environmental Resources**

With the increasing pace of industrialization and urbanization occurring in the 1960s, the Government and Congress were indicatively concerned with the deterioration of our environmental resources and thus has created the National Pollution Control Commission in June 1964 under RA 3931. The Commission was mainly tasked to prevent, abate and control environmental pollution for the more effective use of the country's natural resources. Reports of environmental quality monitoring and surveillance were then available, however, organized information as to how the physical environment has been affected by the economic development activities was

virtually insufficient. The reports on overall status of the quality of our environment started in 1977 with the creation of the National Environmental Protection Council composed of 13 member agencies. President Marcos then chaired the Council. It was tasked to undertake a comprehensive and nationwide study of the overall status of the Philippine environment and thus had to coordinate with other government agencies and non-governmental organizations involved in the country's development. It continued to produce environmental status report until 1985 when the task was charged to the Environmental Management Bureau with the structural reorganization of environmental management affairs. The EMB henceforth produces situationers which chronical developments in the country's ecosystems and identify importants issues and concerns in the environmental management efforts of the country.

### **Land Resources**

The country's land resources have undergone marked changes since the 1960s. From 1960 to 1990, there was a steady increase of alienable and disposal lands from 39.6% to 47% in 1990. Of these lands, about 64% were allotted for agricultural use in 1960 and increased rapidly to 92.7% in 1990. However, a 1991 nationwide study indicated that the conversion of agricultural lands to human settlements and industrial purposes have been going on rapidly at a rate of 2,267 hectares per annum. From 1987 to 1991, a total of 11,337 hectares had been converted which meant that some 3 to 5 times that area of rainfed and rolling uplands have to be cultivated in order to replace the loss in food production due to conversion. From 1982-88, conversion of agricultural lands to residential areas was evident in large cities of Metro Manila, Cebu, Iloilo and Davao, 42% of this conversion occurred in Region 4. A 1993 study identified 15 provinces covered by the study as critically affected by this conversion namely the CALABARZON provinces; Pampanga, Nueva Ecija and Bulacan in Region 3; Cebu, Aklan and Negros Occidental in the Visayas and South Cotabato and Lanao del Norte in Mindanao.

Other problems confronting our land resources which indirectly affect health development are erosion, siltation and misuse and overuse of chemical fertilizers and pesticides. A study undertaken in 1993 estimated that approximately 5.2 million hectares of land are severely eroded, 46% of which are in Mindanao. Overuse of agricultural chemicals and siltation also resulted to soil acidification, nutrient loss and deterioration of drainage conditions. The implementation of Masagana 99 and the green revolution program of the government also introduced high yielding varieties of rice and other crops which required extensive use of chemicals and the consequent manifestation of pest resistance. Misuse of pesticides also resulted to residues in the soil, groundwater and food exceeding the standard limits as reported in the monitoring in 1986-87 by the National Crop Protection Center and succeeding monitoring by concerned agencies.

## **Air Quality**

Air quality monitoring has been limited to Metro Manila where motor vehicles and industries have been concentrated. In the 1970s, vehicular emissions contributed approximately 75% of the total daily air pollution load. It remained as the dominant source of air pollutants through the 1980s and 1990s. In 1994, the total number of vehicles in Metro Manila exceeded 959,000. In four years (1990-1994) consumption of gasoline increased by more than 30% and diesel by more than 60%. Information available provides a basis for the estimation of total motor vehicle emissions for Metro Manila in 1990: about 497,100 metric tons of carbon monoxide, 80,900 metric tons of hydrocarbons, 65,200 metric tons of oxides of nitrogen, 1,750 metric tons of sulfur dioxide, 650 tons of lead, and 11,100 tons of suspended particulate. These emission levels increased by approximately 50% over the following four years.

Although industries are second chief source of air pollutants, they contributed more sulfur dioxide (99% in 1970s, 88% in 1990s) and nitrogen dioxides than motor vehicles. More than half of the country's manufacturing and industrial firms are in Metro Manila. Power plants remain responsible for a high proportion of the pollution load from stationary sources. The emissions from power plants in Metro Manila are low compared to motor vehicle emissions. The emissions of carbon dioxide from power plants are only 0.1% of motor vehicles; oxides of nitrogen are 13% of motor vehicles; and total suspended particulate is 40% of motor vehicles. On the other hand, power plants are the largest sources of sulfur dioxide, generating 35 times more than motor vehicles.

## **Water Resources**

The Philippine inland waters comprise primarily of 421 rivers, 58 natural lakes and more than 100,000 hectares of freshwater swamps. With an annual average rainfall of about 2,200 mm and annual run-off of some 257,000 million cubic meters (MCM), theoretically, the country has enough water resources to supply the water demand for domestic, industrial and agricultural needs. However, the rapid population growth and increasing industrialization and economic activities, have threatened the availability of these resources and their optimum utilization.

By 1978 only a quarter of the 421 rivers have been classified as to their best usage and monitored as to its quality. As of end of 1994, the number almost doubled to 207. Records show that in 1977 the rivers in Metro Manila were severely affected by pollution from domestic discharges (55% of the organic load) and from 313 industrial firms along the Pasig-San Juan-Marikina river systems while 140,000 tons of tailings daily from 24 active mining firms in the country were discharged into the rivers and streams outside Metro Manila. About nine rivers in Luzon, three in the Visayas, one each in Palawan and Marinduque have been polluted by mine tailings and consequently have damaged 128,000 hectares of irrigable lands. Other than mining operations, sugar mills and alcohol distilleries polluted 19 river systems in Bulacan, Capiz, Negros Occidental and Oriental.

Pollution of inland waters by domestic and industrial discharges continued to progress through the years. In 1989, it was reported that all the four major river systems in Metro Manila have become biologically dead. The rivers were contaminated by organic matter from sewage and solid wastes, heavy metals from industries, pesticides, and oil spills from gasoline stations, oil depot and barges and tanks. From 1990 to 1995, Pasig river quality has slightly improved but still did not meet the quality criteria for its classification of optimum usage (Class C) as for fishery and recreational purposes. The Marikina river which was classified as source of public water supply that required complete treatment (Class A) in 1975 has deteriorated through the 1990s; it is now found to meet only Class C water quality requirements. The other two river systems, the Paranaque-Zapote and the Navotas-Malabon-Tullahan-Tinajeros river systems which were classified as Class C rivers still do not meet the quality criteria and could satisfy only class D quality, that is, for agricultural irrigation and livestock watering.

Similar pollution trends were noted outside Metro Manila where the river systems have been continuously damaged by mine tailings, organic discharges from sugar mills and distilleries. In the 1970s through the 1980s, among the heavy metals monitored were copper, lead, cadmium in Luzon and mercury in Mindanao, concentration levels of which exceeded the quality standards set by the government. In 1990-1995, only 75 out of the 207 classified rivers were monitored. Reports indicated that only about 55% have met their classification criteria and 13% are considered dead. The rest are in their different stages of pollution. Of the dead rivers, two are in La Union, three in Pangasinan, five in Bulacan, one in Quezon.

Laguna Lake, Southeast Asia's biggest lake has been polluted by excessive organic discharges from domestic, agricultural and industrial sources that fish kills due to algal blooms in the early 1970s have occurred. A survey done in 1976 indicated that an estimated 25 million kilos of fertilizers and 100 tons of pesticides were used in irrigated and rainfed agricultural lands around Laguna lake. In 1978 the lake was reported to be in semi-eutrophic condition and siltation has resulted to a shallower lake from a depth of 7 meters to 2.8 meters. In the 1980s, the lake was considered hyper-eutrophic wherein algal populations abound. With the deteriorating lake water quality, occurrences and increases of fish diseases as *kitong* or *korikong* in the late 1980s have been attributed to the polluted condition of the lake.

Development pressures have continuously affected the quality of Laguna de Bay through the years. From 1978 to 1994, it was reported that nitrogen and phosphorus loads have increased by 10.8% and 24% respectively making the lake eutrophic. In the 1990s although almost 47% of the 1,500 industries around the lake have wastewater treatment facilities, they still significantly contribute to the total waste load of the lake together with domestic (about 4.8 M people or 60% of the population in the region discharge liquid and solid waste directly into the lake) and agricultural sources. Among the pollution problems threatening the lake quality are thermal pollution due to discharges of industrial cooling waters, toxic and hazardous wastes, and oil spills from barging and oil transport operations.

The importance of the coastal zone in the economic development of the country could be gleaned from the fact that 82% of the provinces are located in the zone. In 1981, fish production totalled 1.77 million metric tons, and increased up to 2.05 million metric tons in 1985 and to 2.4 million metric tons in 1989. Through the 1990s, fish production from all sectors almost doubled at 2.5 million metric tons in 1990 and gradually increased to 2.7 million metric tons in 1994. However, a serious problem experienced by the sector is overfishing. Commercial fishing vessels have intruded waters reserved for municipal fishing. Fishery experts signified that the limits of sustainability of marine fisheries have already been reached or exceeded since the estimated actual marine catch of 1.3 million tons per year is nearing the potential catch which is 1.45 to 1.85 million tons per year.

Mangroves serve as nursery grounds for aquaculture, and thus vital to our coastal fisheries. The mangrove resources of the country as recorded in 1918 were about 500,000 hectares. In 1970 or 52 years later, the mangrove forests reduced to 288,000 hectares and again to 242,000 hectares in 1980. A decade later, mangrove area further decreased to 132,000 hectares which gradually decreased at an annual average rate of 2.4% to 120,500 hectares by 1994. The reduction of mangrove cover has been attributed to conversion to fishponds, harvesting of mangroves for fuel wood production and expansion of coastal human settlements.

As early as 1977, the country's coral reefs have been observed as progressively degenerating. The first comprehensive assessment of the coral reef areas of the country was conducted in 1976-1981. The study results established that 32% of the Philippine reefs were already considered in poor condition and less than 6% were considered excellent. The destruction has been attributed to siltation, natural calamities, destructive fishing practices, harvesting of corals for handicraft, ornamentals and construction purposes and the pollution of the marine environment.

The coastal waters of the country have rapidly deteriorated over the past decades primarily due to domestic wastewater and industrial effluents from urban areas, mine tailings from mining operations, agricultural run-off and oil from shipping operations. Monitoring of Manila Bay indicated that many beaches in its eastern part arc already unfit for bathing while nearshore waters are also unfit for fisheries and shellfish growing.

The red tide phenomenon in our coastal waters has also consequently affected the fishery sector and has resulted to paralytic shellfish poisoning (PSP) of persons consuming the red-tide contaminated shellfish. The first episode of PSP was reported in 1983 when red tide phenomenon occurred in Samar, Capiz, Masbate, Sorsogon, Camarines Norte, Angeles City and Mati in Davao. A total of 279 PSP cases and 23 deaths were reported then. The next episode was reported in 1987 this time affecting Zambales and Samar again. In 1988, 307 cases of PSP were reported in red tide occurrences in Manila Bay, Samar, Negros Occidental and Carigara Bay. Henceforth, yearly occurrences of red tide and PSP cases were reported and each time more areas are affected. Since 1983 a total of 1871 PSP cases with 113 deaths have been reported up to mid 1997.

### Health Trends

The factors that determine the health status of populations can be classified as: the socioeconomic/psychosocial environment, environmental pollution, individual practices and behavior, health care services, and genetic endowment. The socioeconomic/psychosocial environment includes factors related to income, education, occupation, and impact of childhood experiences, social support and influence of political system in buffering stress. Environmental pollution includes all aspects of the physical environment which affect health. Individual practices and behavior includes smoking, drinking, personal hygiene, food choices, and other health risk factors which are thought to be based on individual's choice. Health care services include all aspects of human institutions designed to protect and promote health and cure diseases.

There is an overlap of the above factors as determinants of health and thus boundary lines between these factors are vague. For example, in the relationship between the socioeconomic environment and individual health practices, the ability to eat healthy food depends upon its availability and affordability. In a country where healthy food is readily available and affordable to the population, dietary patterns become an "individual's choice". In the Philippines, the availability and affordability factors will be most important, and individual health practices and behaviors are best construed as responses to socioeconomic conditions. In other words, choosing health food is as much as socioeconomic factor as it is an individual health practice.

The quality, availability and affordability of health care services will also be largely determined by socioeconomic conditions. In 1990, the country spent approximately 2% of GDP on health care which places it in the lower end of the 2-7 % by countries in its income bracket as reported in a 1993 World Bank report. In the same report, it was stated that the public share of total expenditure is a low 30% whereas the mean for its income bracket is approximately 45%. Spending on environmental and public health services is affected by these patterns and may be inadequate to protect the public from the effects of environmental pollution.

The country's gain in life expectancy has been modest considering that from 1960 to 1990, the life expectancy of the Filipinos rose only by 5 years (from 59 to 64 years) compared with an average of 17 years in the demographically developing world and 12 years in the world as a whole. Definitely, there is a positive correlation between GDP and life expectancy. However, the average level of income per person in a country is only one among many social, economic, cultural and political factors that influence people's health. In general, the Philippines is "average" as far as health status among developing countries is concerned. According to the 1991 UNDP *Human Development Report*, of the 127 developing countries in the world, we ranked number 61 in life expectancy at birth at 64.2 years in 1990, number 51 in real GDP per capita for 1985-88 and ranked number 51 in terms of human development index (HDI) in 1991. The HDI is a composite indicator incorporating life expectancy, educational attainment (adult literacy and mean years of schooling) and real GDP per capita.



Definitely, we then lagged behind our Asian neighbors like Malaysia and Thailand which ranked number 20 and 33 in HDI, respectively.

The country has outstandingly achieved a very low illiteracy rate among adult females – among the lowest in its income bracket. Several of our health studies indicate the significance of adult female literacy in providing better and more effective care of children from the stand point of nutrition, sanitation and stimulation. However, the proportion of low birth weight of newborns and the rate of malnutrition among our young children are still unacceptably high. This makes interpretation of environmental health data more complicated. Malnutrition may be both a risk factor for environmental disease and a result of it. As reported in many health studies, malnourished children often have poor immune systems and compromised intestinal function which increase the severity of pollution-related infections.

The health profile of the Filipinos has been improving in terms of control of communicable diseases mainly because of public health interventions such as immunization, improved water and sanitation services, and health education programs. Although there is a downward trend among communicable diseases which are related to the quality of our physical environment, they still are the leading causes of morbidity such as acute respiratory infections, cholera, typhoid, diarrhea, hepatitis A, malaria, schistosomiasis and filariasis. Philippine statistics from mid-1940s to 1991 indicate that a changing disease pattern is emerging, that is, the increasing trend in non-communicable diseases particularly malignant neoplasm and diseases of the heart which ranked number 5 and 1, respectively as the leading causes of mortality in 1991.

The health profile of the working population is also a major concern since it drives the economy of the country. Studies have shown that occupational health problems relate mainly to agriculture, mining, manufacturing, transportation and construction. Contractual workers are identified as high risk population in terms of health considering that they do not enjoy the same working conditions and social benefits that regular employees do. The emergence of the informal sector particularly in the urban areas is also another health concern. The informal sectors in major urban areas made up of street vendors, jeepney and tricycle drivers, professionals sex workers, and people engaged in diverse cottage industries. These workers exist close to poverty in some of the poorest living conditions in the country.

The employment of child labor is very alarming as reported by a survey done by the NSO in 1995. Results showed that of the 13.7 million children aged 10-17 years in the Philippines in 1995, 15% or 2.1 million have worked in 1995, two-thirds of whom were working in the rural areas predominantly in agriculture including fishery and forestry. Other child labor consumers are the services (20%) and manufacturing (7%) sectors. Definitely, child labor has specific detrimental impact to the physical, mental and social development of children. Lack of educational opportunities, hazardous working conditions, separation from family members as in the case of domestic helpers are just few of the problems which threaten the development of a child as a total human being.

Specific occupational health hazards identified among our working population are work-related injuries and illnesses, pesticide and chemical exposure and poisonings, communicable diseases including malaria, schistosomiasis, dengue and tuberculosis. Reports from establishments employing 10 or more workers in 1993 indicate that the agriculture sector had the highest in number and severity of injuries (20 per 500 full time workers and some 5,000 lost work days per 500 full-time workers) during the year, followed by the manufacturing, transportation and construction sector. There are very limited available epidemiological data as to occurrences of occupational diseases, however, the Government has released a list of few occupational diseases which are compensable under the compensation law.

### **Air Pollution and Health**

The average annual measurements of concentrations of total suspended particulate and sulfur dioxide from the routine air quality monitoring of DENR in 1988-1992 showed that particulate dust is found at levels which could affect human health. Epidemiological studies done by the UP College of Public Health in 1990 and 1992 defined exposures to dust by specific populations. These populations were jeepney drivers, air-conditioned bus drivers, commuters, school children and street child vendors. One major finding of these studies is that being a jeepney driver in Metro Manila increases the risk of chronic obstructive pulmonary disease by more than doubled.

Blood lead levels of children in Metro Manila were also determined. Data from these studies indicate the tendency to higher blood lead levels among children in the inner city areas of Metro Manila, where traffic flows are greatest. With the exception of Muntinlupa, all areas have mean blood levels among the school children that are or above those of lead smelter towns in most developed societies (Hertzman 1991). Among child vendors, the distribution of their blood lead levels are higher, one-third of them have levels above 20 micrograms per deciliter. The range of lead exposures found among children in Metro Manila is public health significance, because lead's toxic effects on brain development affect behavior and learning in ways which can interfere with success in school, and have potential for lifelong impact.

### **Water Pollution and Health**

The health issues surrounding water supply begin with the quality of surface waters used by the population. The widespread pollution of surface water used for recreation and bathing with coliforms as reported by the DENR and the water quality issues facing Laguna de Bay, as a drinking water source are some environmental problems which may be related to health. By 1990, about 42% of the population were supplied with Level III, 31% by Level II and 25% by Level I systems as reported by the NSO. Sanitary toilet coverage was very limited at 67% with the rural communities suffering most considering that only half of the population has access to sanitary facilities. What could be the impact of these to health? An evaluation of the Water

Decade in 1992 by the DOH showed that using a public or neighbors water supply increased the risk of diarrheal disease by 25% compared with using a household source. Similarly, living in a household without a toilet increases risk by 42% compared to a household with a toilet. The use of inaccessible water supply increased the risk of skin disease by 74% compared with a household source while the lack of a toilet increased risk by 28%. For both diarrhea and skin disease, the worst combination is a household without either a toilet or with an accessible water supply since the risk is increased by 76% compared with households that have both.

### **Pesticide Use and Health**

A major opportunity for pesticides to adversely affect human health when farmers come in contact with them. Another opportunity comes as a result of pollution of the environment where they are used. A study conducted in 1993 compared the prevalence of various health impairments for 57 exposed farmers and 39 controls. Results showed that pesticide users had higher prevalence of eye, skin, respiratory, heart, digestive, and blood related conditions than controls. The modal number of conditions among the users is four, whereas for the controls is one. Moreover, the risk of developing pesticide-related symptoms increases with frequency of pesticide use; doing two to three applications per season increases the risk for conditions in each of five body-organ systems. Since more than 8.5 million Filipinos are employed in agriculture, this would suggest that there are actually millions of pesticide poisoning episodes per year in the Philippines. In 1993, only 100 pesticide poisonings were reported to the National Poison Control Center, indicating that most pesticide poisonings are not recorded.

As reported in 1992 and 1994, surveys conducted in Nueva Ecija and Laguna showed that well water in irrigated areas not only yielded the pesticides that were in current use, but also pesticides which were not reported as being used as well. These observations raise a number of questions about the persistence of pesticides and the accuracy of reporting of pesticides currently in use and also about the knowledge of users of pesticides used.

### **Pollution and Food**

It has been reported that fish has been concentrating heavy metals point and non-point sources, bacteria multiply under market conditions to reach unacceptable levels, and a wide range of pesticide residues persist. The public health effects of daily exposure to food contaminated with metals, microbes and pesticides are uncertain and has not been systematically studied in the Philippines. Several outbreaks of foodborne diseases have been reported by the DOH in 1989-1994, all of which are related to microbiological contamination of food.

### Solid Waste and Health

Waste generation of Metro Manila in 1992 is estimated to range by 2,600-3,300 tons per day, 7% of which is burned, goes into waterways and seers or is left lying around. Close 71% is handled by the municipal collection system, and is subject to landfilling and scavenging. In the past, most of the public health attention was focused on the Smokey Mountain because of the presence of squatter communities and scavengers on other dumpsites in the country. The health profile of child scavengers in Smokey Mountain was worse than those of schoolchildren and child vendors as reported in 1991. One out of three child scavengers had bronchitis or ashtma. More than half of the children have pulmonary function below normal, and mean blood lead level was three time higher than the schoolchildren and 73% of child scavengers have blood lead levels above the 20 $\mu$ g per liter deciliter compared with 10% for school children and 33% for child vendors.

How much of the diseases prevailing in our country are attributable to environmental pollution which is major impact of development activities for the past 50 years or so? Epidemiological data are so limited and the multi-dimensional nature of health makes the quantification of health impact almost impossible. Due to these constraints, recent studies on the assessment of environment health status of the country utilized the burden of disease morbidity and mortality in terms of days of life lost due to premature death, due to conditions that cause death, losses due to chronic disability that do not cause death, and losses due to acute phases of disease.

The estimates are in two major groups: those for which there is direct evidence regarding the impact of pollution, and those for which only informed estimates are available. These estimates are expressed as "days of healthy life lost" (DHLL). The days lost relate to current losses due mostly to morbidity episodes and losses in future years due mainly to premature death arising from such morbidity. From this assessment study, conservative estimates have been made of the share of the burden of morbidity and premature mortality in 1990 attributable to pollution namely:

- 294 million DHLL for bronchitis due to dust
- 47 DHLL for heart disease due to dust
- 54 million DHLL for diarrhea due to poor water supply and no excreta disposal facility
- average 5 points of IQ loss among all children due to lead exposure
- 184,000 years of life lost due to premature mortality from bronchitis, diarrhea and heart disease.

The study also estimated that the present value of the loss of discounted future earnings due to premature death in 1990, of people participating in the work force, amounted to 2 to 4% of Gross Domestic Product.

### **Institutional and Policy Framework on Health, Environment and Development**

The Rio declaration of 1992 proclaimed the first principle of Agenda 21: *"Human beings are at the centre of concerns for sustainable development. They are entitled to a healthy and productive life in harmony of nature"*.

The Philippine Medium-term Development Plan for 1993-1998 (MTPDP) explicitly focuses on sustainable development and people empowerment towards achieving the vision of Philippines 2000. This is evidenced by the incorporation of strategies on environmental protection and public health promotion in development plans and by clearly defining the duties of local communities. A UNDP/WHO study in 1993 on the integration of health and environment issues in development plans has identified certain institutional and policy deficiencies in operationalizing the plans.

At present, there are two institutional bodies legally created to oversee the environment and health concerns of development: the Philippine Council for Sustainable Development (PCSD) and the Interagency Committee on Environmental Health (IACEH). The PCSD oversees the fulfillment of the Philippine commitments to sustainable development principles made at the 1992 United Nations Conference on Environment and Development (UNCED), particularly those related to conservation and management of resources for development. The Council is chaired by NEDA and co-chaired by DENR. There are 14 other government member agencies and seven representatives from the non-governmental organizations.

The PCSD has facilitated the integration of sustainable development principles in the MTPDP through its subcommittees, by adopting a resolution that endorses local NGOs and people's organizations as lead entities in the implementation of development programmes, and by the inclusion of sustainable development activities in the 1994 Philippine Government budget. However, a major finding of the UNDP/WHO study identified in the execution of its mandate is that the PCSD will only function as effectively as the extent of its commitment and adherence of its members to sustainable development principles. The report further stated that the PCSD subcommittees are also faced with a certain dilemma that an agency is a member in three or more subcommittees resulting in poor representation in subcommittee meetings and in limited participatory consultations.

In 1995, President Ramos directed the PCSD to formulate the Philippine Agenda 21. The document was drafted which embodied the consensus of sustainable development by the different sectors of the Philippine society. Regional cluster consultations and by NGOs, business, youth, health, labor, urban poor and other sectors were conducted to generate the needed information for the document. The document was finalized with the formulation of the Principles of Unity which represent the common ground among the key actors in the sustainable development process - government, business and civil society. The Philippine Agenda 21 "envision[s] a better quality of life for all Filipinos through the development of a just, moral, creative, spiritual, economically vibrant, caring, diverse yet cohesive society charac-

terized by appropriate productivity, participatory and democratic processes, and living in harmony and within the limits of the carrying capacity of nature and the integrity of creation.”

The implementation of the Philippine Agenda 21 is seen to be done by participatory action and shared responsibilities. Strategies of implementation identified are: 1) strengthening the role of major stakeholders, 2) information, education and communication plan for sustainable development, 3) financing means and strategies such as market-based instruments and command-and-control measures by the LGUs, the National Government, and the Economic Sectors, and 4) through the formulation of a local action agenda for sustainable development.

The Interagency Committee on Environmental Health (IACEH) was formed prior to the PCSD with 11 major government member agencies. The committee is chaired by the Department of Health and vice-chaired by the Department of Environment and Natural Resources. The IACEH focuses on environmental health issues and support the Department of Health mechanisms for streamlining national health policy implementation in response to the devolution of power to local government units. As reported in the UNDP/WHO study in 1993, the IACEH has never been involved in the development of the national MTPDP and its role had been limited to reactive responses to health issues of development projects. Similarly with the PCSD, it was faced then with administrative problems and an uncertain commitment of its member agencies. In the more recent years, the IACEH has taken a proactive stance through the development of action plans and research projects that address health issues related to environmental pollution. The IACEH also supported information drives of local government units in preventing communicable diseases such as dengue, typhoid, cholera and diarrhea.

The PCSD, IACEH and regional and local development councils complement the official mandate of government departments such as DENR and the DOH to integrate health and environment issues into the development and implementation of plans for sustainable development. The regional and local development councils are channels by which development projects maybe made acceptable to local populations through a process of consultation.

The policy instrument that gives legal force to the integration of environment and health concerns in development projects is the Environmental Impact Statement (EIS) system, which has been in operation for more than a decade. It remains a useful tool for pursuing environmental and development policies and enhancing sustainable use of natural resources. It also promotes consultations, dialogues and public hearings for evaluating the technical feasibility and social acceptability of projects, while facilitating understanding and formal agreement among project participants. In 1996, the DOH through the IACEH endorsed the inclusion of Health Impact Assessment in the existing EIS system implemented by the DENR.

## **Challenges of the Future**

The challenges facing us today are undoubtedly daunting but in making reasonable choices about safeguarding environmental health, the choices must blend and balance scientific facts, societal values and economic realities which are the building blocks of environmental decision. However, much of the information and understanding needed to resolve our environmental health problems is still incomplete. At the same time varied social, economic, political, scientific, technological and legal issues color our decision making processes must build strong bridges between scientists and decision makers; improved mechanisms for using science to devise, implement, and evaluate environmental health policies and strengthen the scientific underpinnings that support environmental decisions.

### **Development and Environmental Policies**

Development policies designed to improve the economic conditions and living standards of communities often have unintended effects on health. While these effects can be positive, many policies created additional health risks for vulnerable groups, thereby compromising the welfare objectives of development policies. These policies can also create or exacerbate the diseases of poverty as well as health problems of industrialization. Our country is now confronted with this double health burden and places additional pressure on the limited national resources available for health services provision and public health intervention. A review of our macro-economic policies as well as the agricultural, energy, industrial, and housing policies must be objectively done since these may positively or adversely impact on health.

Our environmental policies and programs need to be reviewed as well and assess if these have taken a holistic approach to preventing and reducing pollution, based on the best scientific methods with appropriate characterization and communication of the associated scientific uncertainties, that these have been made with the participation of stakeholders, and that these recognize shared responsibility among Government, businesses, and communities. The implementation of the Philippine Agenda 21 deserves objective monitoring and evaluation and to derive specific learnings in reforming existing environmental regulations.

### **Research in Science and Technology**

The dearth of scientific information as to the health risks associated with industrialization and development activities and the health burden brought about by environmental pollution must be timely addressed to enable stakeholders at all levels make better decisions and for regulatory purposes. Epidemiological studies as to the health effects of certain hazards are urgently called for. Risk assessment studies which incorporate community values must be conducted to provide better solutions for risk management. Research in scientific methods and product technology that

appropriately addresses prevention, reduction and control of environmental pollution must be conducted.

### **Public Participation and Education**

This should strengthen communication and involve more interest groups, professionals, politicians at the national and local levels, to heighten environmental and health awareness and create demand for helpful change. Mass and targeted communication with the assistance of NGOs are some helpful change. Mass and targeted communication with the assistance of NGOs are some helpful mechanisms in this process. The formal education system could be used further to involve students and academics and encourage self-help and behavioral change.

### **Human Resource Development and Capacity Building**

Strengthening the capabilities of implementing agencies in terms of staff training and adequate facilities to implement and monitor their programs are urgently needed. Health professionals need to be trained to enhance the identification of environmental and occupational hazards as causes of disease and to provide feedback to relevant agencies. A network of trained health workers at local level can form a basis for an improved system of problem identification and monitoring. To this end, it will also be necessary to create analytical laboratory capability and capacity.

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## PLENARY SESSION III

### AN OVERVIEW ON CLINICAL PEDIATRICS IN THE PHILIPPINES

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The talk this morning will be an overview of clinical pediatric practice in the Philippines - past, present and future. I graduated in medicine a few months before the start of World War II and my young colleagues in the profession consider that as "ANCIENT". Having been placed in that category, I presume that I am qualified to talk about the past, and as I am still in the midst of the present with high hopes for the future, my comments on the three phases will be a typical description of the eras.

#### **Pre-Spanish Era**

In the pre-Spanish era, the health care of children as well as of the adults was in the hands of herb doctors, seers and (?) witches which, sad to say, are still so in some parts of the islands. Western medicine was introduced when Spain took possession. Some doctors were in the expedition and the friars dispensed their own type of healing. Much later, Filipinos were trained to be doctors and then augmented in number with the coming of the Americans who established a government-supervised medical school. Pediatrics was not well delineated at the time although it was already taught as a separate subject. It was an accepted fact that children are not merely miniature adults, neither in size nor in their diseases. Some of the doctors were known to be proficient in treating children while still treating adults, they became the prototype of the present day pediatricians. The doctors of old were usually kindly middle-aged men (no women as yet) who exacted respect and trust. The doctor was one with acuity of perception, a high degree of an educated intuition, and without the help of technology, would arrive at a rational diagnosis and treatment. There was an empathy between physician and patient which contributed much to the well being of both patients and their parents. This human touch is slowly disappearing today.

House visits and home medications were administered. Specific treatments were not available - for febrile reactions and mild illnesses simple procedures were usually followed. The child was wrapped to produce sweating and the ubiquitous castor oil would always be in the regimen. The evening enema will end the ritual. Windows were closed during defervescence and diet was restricted. The rationale behind the procedure was to cleanse the system and starve the cause, to produce cure.

More serious illnesses needed hospitalization. The more common conditions that would require confinement would be severe diarrheas and respiratory infections. Rehydration technique was poor as this was effected by the slowly absorbed "hypodermoclysis". There were no facilities for intravenous infusions - there were no tubings as are used at present. The only ones available would be rubber tubings as used for enemas. The forceful infusion in the subcutaneous tissues was very painful and traumatic as it bloated the thighs until bursting, so as the interscapular areas of smaller babies. If this happens today, the doctor will surely be accused of physical abuse. Because of this lack of proper hydration, cholera was almost always fatal, as the main treatment is fluid replacement.

One common disease during the prewar era was a condition caused by vitamin B1 deficiency popularly known as beri-beri. It was so rampant that sanitary inspectors, or even layman, in the rural areas diagnose the condition. Young doctors have only a hazy idea about the condition but it caused a high morbidity and mortality among our children and even adults. Treatment with a dose of 10 mg. of vit. B1 would be enough to result in almost immediate improvement or cure. Rice bran became the panacea for the cure and the byword among Filipinos "tikitiki Zamora" was born. A foreigner in the concentration camp was saved by the "darak" cookies that were smuggled to them during the war. With the spate vitamin preparations these days scarcely would one hear of the condition - vitamins have been used for so many purposes even in cosmetics and cancer prevention. I would be remiss if I do not mention intestinal parasitism. Tuberculosis has always been with us - was and still at present ranks high as a leading cause of morbidity and mortality. Antitubercular drugs did not reach the islands until after the cessation of the war. It did not spare anyone - even high officials. If a child contracted the meningitic form it was tantamount to a death sentence. Within six weeks all told, without treatment, will end up in death.

### **Post Era**

I will term the immediate post war era as the recent past as not so long ago, it was part of the present. Advances in medicine in the other parts of the world did not reach the Philippines until the end of the war. Then, there was an exodus of doctors in all levels. The faculty went abroad for reorientation - to refresh and absorb new trends in Pediatrics. The young graduates went for training with a vision for specialization and because the Americans needed doctors, they gobbled up our manpower. This situation reminds me of Abraham Jacobi, the father of American Pediatrics who in the

1860's felt that there was already a trend in organ specialization, of which he was not in favor, had this to say "If they honestly believe a human organ can be treated separately like the wheels of a watch, they do not have the brains enough to be a physician. And if they do it for mercenary reasons, they haven't the right to be." I wonder what he would have to say today when there is a possibility of specialization of the left or right eye or leg!

Antibiotics had come into the islands, and their impact on the virgin organisms in the country was almost magical. Penicillin for the bacterial infections and streptomycin for the long suffering tubercular patients. With all the enthusiasm for the new management, the Filipinos were among the first to use both intrathecally. Other antibiotics and antituberculous drugs were to follow with good results.

Well-baby clinics were introduced, prevention became a new thrust in the care of infants and children. Immunization against infectious disease with growth monitoring spelled a better life for the young population. There were only a few vaccines available before the war but it was a proof that prevention is the best way to curb diseases. Small pox vaccination is the best example in this category. Since 1970, smallpox vaccination has been discontinued as it was declared globally that it was no longer a menace. DPT, polio, measles, mumps, rubella were the first to receive backstopping by an Expanded Program on Immunization (EPI), together with BCG. The target of the DOH for polio eradication is the year 2000. We hope each of these preventive measures will go the same way of the small pox experience.

Since then so many types of vaccines have come out. There are Hepatitis A & B, Hemophilus Influenza B, a new type of typhoid vaccine, and the latest are the varicella vaccine, and influenza virus, pneumococcus, and many more under trials - such as for H. fever and malaria.

One phase of pediatrics that has progressed by leaps and bounds is the care of the newborn. Whereas before, the care of the newborn infant was in the hands of the obstetricians; the special care needed by high risk infants demanded a full time attention. It was obvious that if the mother had some difficulties in delivery resulting in high risk babies, the obstetricians' primary concern would be the mother. As a result, neonatology as a subspecialty was born. Neonatology means knowledge of the newborn and the term was coined by Alexander Schaeffer in 1960 in his book "Diseases of the Newborn". The neonatologist, then came into being. Smaller babies were saved with the aid of high technology and a better understanding of the pathophysiology of such types of babies. Neonatal disorders being the result of perinatal difficulties, antepartum assessment of the fetus became a must so that difficulties may be anticipated and therefore remedial measures can be done. High risk babies including those with very low birth weight, survived with fairly good outcome postnatally as the quality of life is considered. This brings in another subspecialty- perinatology. In some institutions the pediatrician has a corresponding level in the faculty of the Department of Obstetrics and Gynecology Intrauterine intervention; surgical and non-surgical have been going on since two to three years ago. In one of our talks in the latter part of the 80s, we predicted this to happen in the

year 2000. It happened in less than five years. Such is the rapid advances in medicine. The latest addition is the cure of small babies who had trouble in maintaining respiration after the first breath with the use of artificial surfactant. This is a very costly procedure. About P20,000 to P30,000 is needed per application which is administered twice.

Pediatric practice is likened to a pendulum; it sways forward but somehow backtracks and sometimes old ideas and procedures resurface.

Breastfeeding practices cannot be more than over-emphasized. It benefits both mother and child, and of course, is less expensive.

The Philippine hemorrhagic fever has been in our textbooks, but apparently did not merit a second look until the mid fifties when there was an epidemic of the disease. The virus was here; dengue virus and the vector has been in our midst -the *Aedes aegypti*. Poor sanitation brought about by over population (uncollected garbage, stagnant canals) have caused the high incidence during this last few years.

### **The Present**

One of the most talked topics is child abuse - physical, sexual, substance. This is critical and must be addressed as soon as possible. Centers where the aggrieved can be taken cared for, have to be established. This is again a very expensive proposition. A multidisciplinary team of experts is needed and should be available 24 hours a day to be effective.

Cancer in children is not as rare as some people want to believe. Among adults, more and more cases are diagnosed due to better awareness. Advances in the management of cancer in children have resulted in longer survival and sometimes total cure.

Children do contact AIDS most often from their mothers at birth. Other routes are less, except in cases of sexual abuse.

The high cost of technology which of course has been beneficial to mankind, the support of researches, and the continuing search for newer drugs have led to spiraling cost of health care. Ways and means to minimize this situation were devised and one of these was the establishment of the Health Maintenance Organization (HMO). HMO is composed of business interest groups or organizations in health care delivery which provide a specific set of comprehensive services to a defined population of patients for a prepaid, fixed sum. And like any corporation, the priority is for profit while the health care seem to be of less importance. This type of health care providers had been going on in the US for some time now. It is true that HMOs have succeeded in controlling cost of health care, but this seems to be at the expense of the doctors and the patients' coverage. Doctors are given minimum reimbursement for services rendered while the patients are shortchanged because of limitations in their health coverage. Due to this flaw, new models in health care delivery have emerged in the USA. One of these is the Preferred Provider Organization (PPO) which is like a group practice without walls, where the health care is managed, controlled

and owned by doctors or physician groups. This type of organization has taken ground here in the Philippines.

Genetics is very much in the forefront since the 1950's scientists have proven that the so called "thread of life" - deoxyribonucleic acid (DNA) is the basic stuff of heredity. A detailed map of the human gene has been drawn roughly showing the known locations of mutations uncovered by scientists. With this knowledge, they have search for mutant imperfect genes that cause the appearance of the disease in the person. The genetic map shows the known locations of mutations already uncovered. Once they are discovered, they have to be fixed. Several approaches have been tried as transplantaion of a healthy copy of the gene for the affected cells. An immunodeficiency disease has been one of the first gene therapy tried with success. This what we would like to see in the future for our Filipino children. Scientists are now very concerned in Genetic Engineering. Once this is mastered, genetic therapy will certainly follow. How far can man meddle in creation? Frankly, I am ambivalent with regard this situation. There are already talks about genetic screening which in itself is a good procedure if used for good purposes. But if the secrets of the individual hidden in his gene will be used against him - this again will be a major constitutional issue of the next generation.

### **Toward the Future**

What really do we expect in the future? Concisely, we would like the following to happen:

Preventable diseases suppressed and wipe out.

Genetically preventable diseases will be corrected.

With the help of the public health sector there will be a clean community with ample potable water good sewage and sewerage system.

The environmentalist will give our children fresh air, water, and land free of pollution.

All these will create a Philippines where children will have the best chance to grow and develop their full potential, so they can really be the future backbone of the nation.

# 100 YEARS OF INTERNAL MEDICINE IN THE PHILIPPINES

CECIL Z. TADY

*Regent*

*Philippine College of Physicians*

This paper presents the outstanding achievements and contributions in the field of Clinical Medicine in the Philippines during the last 100 years

## **Early Achievements**

It was in the latter part of the 19<sup>th</sup> century when the Philippines began to produce its own doctors. The first batch of graduates in 1875 were from the University of Santo Tomas. Subsequently, other universities (University of the Philippines, University of the East, Far Eastern University, Manila Central University, Cebu Institute of Medicine, etc.) graduated their doctors.

The initial graduates were first and foremost internists, but likewise, surgeons.

During the early years, the achievement in Internal Medicine focused in the molding of exemplar Internists - Internists who form the backbone of present day Internal Medicine.

Those years saw the likes of: Dr. Joaquin Pardo de Tavera, Dr. Luis E. Guerrero, who had the distinction of simultaneously holding two chairmanship positions (UP Department of Medicine and UST Department of Pediatrics); Dr. Fernando Calderon, Dr. Ariston Bautista-Lim, Dr. Antonio G. Sison, Dr. Mariano Alimurung, Dr. Francisco Tangco, Dr. Agustin Liboro, Dr. Francisco Roman, Dr. Gonzalo Austria, and others.

Their qualities and ideals were the unmatched achievements and contributions in Internal Medicine.

## **Present Day Achievements**

- \* The syndrome of conjugated hyperbilirubinemia without either intrahepatic or extrahepatic obstruction was first described by Drs. Arturo Rotor, Angel Florentin and Lourdes Manahan of the UP College of Medicine. This became known internationally as the Rotor-Manahan-Florentin Syndrome (presently Rotor Syndrome). It is mentioned in any standard textbook in Internal Medicine. The importance of the syndrome like in the insight into the basic mecha-



nism of bilirubin mechanism and transport. Subsequently, this knowledge found practical application in the treatment of a number of liver diseases.

- \* The one and only pathophysiologic description of hepatosplenic *Schistosomiasis japonica* by Drs. Q.M. Sulit, E.O. Domingo, D. de Peralta and E. Imperial. This contribution provided the basis for locating the block that gives rise to the portal hypertension in this disease and also suggested the hemodynamic consequences. This knowledge is a major contribution to the pathophysiology of the disorder. It also suggested the surgical intervention appropriate for the condition.
- \* Basic immunology of the *Schistosoma granuloma* by Drs. Ernesto Domingo and Edito Garcia which provided the mechanism of the hepatic pathology in hepatosplenic *Schistosomiasis japonica*. Their works contributed significantly in accelerating research on the immune mechanisms in delayed hypersensitivity reaction as exemplified by the *Schistosoma granuloma* and set the groundwork and the search for an appropriate vaccine against the parasite.
- \* The investigations on viral hepatitis and *Hepatocellular carcinoma* by the UP Liver Study Group which has produced the most comprehensive epidemiologic data on viral hepatitis. The group also published the most authoritative studies on the etiology, clinical manifestation and treatment of *Hepatocellular carcinoma*. Its data have been used extensively by the Department of Health in policy making in relation to the control of hepatitis B.
- \* The clinical studies on hepatobiliary tuberculosis by Dr. Sol Alvarez and co-workers at the University of Santo Tomas provided clinical information that are among the most comprehensive in the world.
- \* Rheumatic Heart Disease/Rheumatic Fever in the Philippines.

The magnitude of the problem was initially pointed out by Dr. Mariano Alimurung in 1955 where he found that the leading etiology of heart disease among hospital admissions in four hospitals in Metro Manila was RF/RHD. This observation was confirmed by Dr. Imperial et. al. on 6,000 autopsies at PGH(1953-60) showing that RF/RHD was the major cause of death (50.1%). Children age 5-15 are at high risk of developing RF/RHD. School children studied in 1978 by Yason showed that the prevalence rate of RF/RHD was 1/1000. Comparative prevalence rate in other developing countries range from 1-20/1000 while in developed countries (USA, Japan and Australia) the rate is 0.1/1000.

- \* RF/RHD.

Limson (1978-1979) studied the epidemiology of streptococcal throat infection and observed seasonal variation; about 20% streptococcal throat infection during the rainy season and only 7% during the dry season.

Same authors observed higher ASO titer (>200-300 Todd units) among school children coming from the low income group compared to children from middle income group (<200 Todd units).

Mabilangan and Reyes studied the clinical manifestations of acute RF and showed that the most frequent findings are arthritis and carditis; rarely erythema marginatum and chorea.

De la Paz reviewed 511 cases of RF/RHD and found the following clinical manifestations:

Carditis	72.6%
Migratory arthritis	36.5%
Subcutaneous nodules	0.98%
<i>Erythema marginatum</i>	0.78%
Chorea	0.39%

Juvenile Mitral Stenosis was studied by Dr. Lopez and found that signs and symptoms appeared from 5 months to 4 years after the onset of acute RF. In the western countries, the symptoms of MS usually appeared 10-20 years after the onset of acute RF.

Dr. Calleja et al studied 309 cases of RF/RHD and established criteria for those who will undergo surgery and those who benefit more from medical management. The basis for assessment of the cases were by 2D-Echo and cardiac catheterization. Criteria were set for those who need surgical management.

Dr. Ortiz studied 28 patients with ARF by 2D-Echo doppler. An important observation was the findings of cardiac enlargement, prolapsed of aortic or mitral valve and or increased in echodensity of the valve leaflets in patients with no evidence of clinical carditis. These observations are important because they indicate that the heart can be involved early in the process of the disease even in the absence of clinical carditis.

Dr. Ongtengco reported on the use of balloon catheter in performing valvotomy in rheumatic valvular disease. This is a percutaneous procedure which reduces mortality and is cost effective in properly selected patients.

Dr. Viscayno et al reported the results of utilizing primary health care workers in the recognition and secondary prophylaxis of patients with RHD. Midwives and nurses were able to recognize heart murmurs. These were referred for confirmation to designated consultants. Once cases were verified to have RHD, secondary prophylaxis were administered by these primary health workers in the rural health unit.

RF/RHD Hospital Registries for secondary prevention. Registries were established in the Philippine Heart Center (PHC) and UP-PGH in 1976-78. The penicillin given to registry patients were subsidized from grant-in aid from NSDB. Patient compliance on secondary prophylaxis was 80-85% when penicillin was subsidized and dropped to 30% when penicillin subsidy was discontinued.

- **Respiratory Tract Infection**

The research works of Drs. Thelma Tupasi and M.E. Velmonte on incidence and risk factors of respiratory tract infections in a community and in hospital provided information on how government sectors, health workers, and non-government organizations could help reduce morbidity and mortality.

Research works of doctors from the Philippine College of Chest Physicians, Lung Study Group (UP PGH) help formulate guidelines on the recognition and initial antibiotic treatment for community acquired pneumonias, thereby implementing a cost-effective approach to treatment.

- **Pulmonary Tuberculosis**

The National Consensus on Pulmonary Tuberculosis (Part I -1989; Part II - 1992; Part III - 1996) established a common consensus on the diagnosis and management of Pulmonary Tuberculosis in the country. This was organized by the Philippine College of Chest Physicians. Its aim was to address controversies in Tuberculosis therapy, thereby have a cost effective approach to patient with PTB.

- In the field of Clinical Allergology, foremost achievements are the works of Drs. F. Cua-Lim, Payawal, Laserna and the works of Agbayani, Bulalacao and Ferreria. Their initial description of aeroallergens in the Philippines include housedust mites (*D. farinae* and *D. pterimosinus*), mold spores (*Penicillium*, *Aspergillus*, *Hormodendrium*, *Curvularia*) and pollen (mainly *Cynodor dactylor* and *Sorghum halepense*). Tree pollens and weed pollens are not as significant as grass pollens. Other common allergens include cockroach, kapok, feathers, tobacco, wool, perythruom and horse danders.

- Their findings are clinically significant in the management and treatment of patients with Bronchial Asthma, Allergic Rhinitis and other allergic conditions.

- The investigative work on the 'Pre-Diabetic' kidney (Annals of Internal Medicine April 1961) by Dr. Alberto Daysog contributed significantly to the understanding of Diabetes Mellitus. The study consisted of doing needle biopsies of the kidney of pre-diabetic subjects without clinical renal disease. These were selected on the basis of a family history of diabetes and other stigmata of it. The author and his associates were the first to show the presence of glomerular basement membrane changes similar to that seen in overly diabetic subjects. This finding contributed to a better understanding of the pathophysiology of renal disease in Diabetes Mellitus. The same author has done extensive work on the Nephrotic Syndrome, Leptospirosis and Renal Failure.

- **Hypertension**

Clinical Practice Guidelines on the Detection and Management of Hypertension headed by Drs. Ramon Abarquez, Antonio Dans and Bernadette Tumanan. The objectives were: (1) the identification of priority issues or concerns in hypertension control that should be addressed in the Philippines; (2)

draft the guidelines thru multisectoral task forces for addressing these priorities.

\* Cancer in the Philippines

\* Studies on survival of Filipino cancer populace from a population-based data source (1987-1993) were a major achievement in the cancer control program in the Philippines. This was conducted by doctors from the Department of Health and the Philippine Society of Medical Oncology. Since treatment and stage data were poor and unavailable, only overall observed survival rates were estimated (regardless of stage/treatment) except for survival by stage of breast and cervix cancer patients. Survival from breast cancer was the most favorable while liver cancer had the poorest survival rate. Survival from stage II Breast Cancer was 73.2% compared to 24.4% for stage III and 6.67% for stage IV. Survival from stage I Cervix cancer was 46.7% compared to 23.6% for stage II, 12.17% for stage I and 9.5% for stage IV. The difference between stages was significant, depicting the need for implementation of early detection of breast cancer and primary prevention/early detection of cervix cancer. This study reflects the survival of cases with the top ten sites in an era where primary, secondary, and tertiary prevention are wanting. Perhaps the survival picture will change for patients diagnosed in the year 2000.

\* Algorithms for the management of common medical oncology problems and guidelines on chemotherapeutic agents were done by doctors from the Philippine Society of Medical Oncology (1994-1995). This project was necessary to continuously review and/or fortify the medical oncologist's practice of his/her skill.

## SURGERY TODAY TOWARDS TOMORROW

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Surgery started with the first successful operation under anesthesia on October 16, 1846. Since then, there has been an extraordinary explosion of scientific information leading to the advancement in the field of Surgery.

Today, surgery can best be described as both aggressive and minimally invasive. We are witnessing an awesome development in surgical technology and technique. Present day surgeons have also realized that they need to work together to be able to correct and help patients suffering from serious injuries and illnesses.

With a better understanding of the nature of diseases, surgeons are developing and perfecting newer and bolder techniques in almost all field of surgical specialization. Twenty years ago, patients with advanced malignancies would constitute a contra-indication to definitive surgery. Today, many surgeons are working together as a team; they offer procedures that would address the problem and give a chance to adjuvant therapy to work, thereby prolonging survival.

The most common cancer among women is still breast cancer. Two decades ago, once a stage III breast cancer is diagnosed, surgery was no longer advised. Presently, the same patients can be given induction chemotherapy thereby downgrading the stage of the disease and allowing surgeons to perform local control surgically. This is followed up by completion of chemotherapy and hormonal therapy giving the patient a better chance of survival.

Hilar-malignancy of the biliary tree was considered in the past, as a death certificate. Today we are able to perform respective procedure because of a better understanding of the physiology and anatomy of the affected organs. Again, giving the patient a chance to survive.

General surgeons are working very closely with Plastic & Reconstructive Surgeons, allowing them to perform radical resection of tumors of the skin and subcutaneous tissues, including the face, with minimal disfigurement. The advent of microvascular surgery has tremendously contributed to a more acceptable outcome. These and many more attest to new aggressive efforts that pervade surgery today.

We are also swept by a storm called surgical endoscopy. Today endoscopy is no longer limited to diagnosis. It is now being used more and more for therapy. Newer fiberoptic scope that are smaller, more manageable and with better vision are being developed to answer the need of surgeons with regards minimal access surgery.

In general surgery, the current state of the art is laparoscopic surgery. It started with laparoscopic cholecystectomy but today a surgeon gains experience and expertise in the use of the instrument; its application is being extended to other procedures such as herniorrhaphy, colectomy, fundoplication, and others. Laparoscopic surgery definitely has an edge over open conventional surgery. For one, it has very little post-operative pain thereby reducing the number of hospital stay and allowing early return to work. It used to be said that surgeons loved to see and touch what they are doing. Today, surgeons are contented with watching their own operation on the monitor which is actually a magnified field. Currently, this technology could be used for just about all procedures in general surgery.

There are newer instruments being developed in general surgery. One of them is the harmonic scalpel. This device uses the earlier knowledge of ultrasonic wave. It allows surgeons to cut through tissues with minimal bleeding. It can also coagulate vessels. This device heralds a new era in which procedures can be done expeditiously with less bleeding.

Thoracic and cardiovascular surgeons are now using the technology of laparoscopic surgery. They are now performing Video-Assisted Thoracic Surgery (VATS). The procedure can be used to diagnose and manage different chest diseases with less post-operative pain and pulmonary dysfunction.

In cardiac surgery, the current favor is the MIDCAB or the minimally invasive coronary artery bypass grafting wherein up to 2 vessels can be done through right mini-thoracotomy on a beating heart. This obviates a long median sternotomy and the use of the heart-lung machine.

The advent of the operating microscope has given plastic & reconstructive surgeons more room to practice their specialty. It is now said that what general surgeons destroy through their surgical aggressiveness can now be repaired and reconstructed by the plastic and reconstructive surgeons. They can now literally transfer tissue (from skin to muscle) freely from one area of the body to another with a very high rate of success. They are no longer inhibited by the length of the vessel. Their greatest success is in limb salvage. Fingers and toes following trauma need no longer be disarticulated or amputated. They can now be reconnected resulting to functional organs. Plastic surgeons are also using endoscopy for procedure like face-lifting and augmentation mammoplasty.

Pediatric surgeons are now performing fetal surgery. Fetal surgery was first conceived to treat unborn babies with anomalies gastroschisis. Today, new developments have been made to correct congenital anomalies before a child see the light. Babies are now undergoing surgical correction while still inside their mothers' wombs.

Laser surgery is now occupying a very important role in the field of urology. Ureterolithiasis no longer requires open surgical removal. They can now be crushed with the combined use of endoscopy and laser. This same procedure is used to operate on prostate making the procedure almost blood-less and obviating the use of continuous bladder irrigation.

Stereotactic neurosurgery is presently occupying the limelight in neurosurgery. Together with the Gamma knife and linear accelerator, neurosurgeons are able to accurately remove brain tumors without performing a craniotomy. Neuro-endoscopy is also being utilized especially for placement of ventricular catheters and resection of intraventricular tumors.

Finally, we don't see any new procedures in orthopedic surgery. What we have are new implants or prothesis. A good example is the rod used for patients with scoliosis, which before was very rigid but the newer ones allow more degree of flexibility.

### **What is in store for surgery in the next millennium?**

The next millennium will see further development in minimal access surgery. The scope will be finer, more flexible and more user-friendly. We will also see more and more genes being tagged and identified as the culprit in many diseases which will eventually lead to gene therapy wiping them out even before they can manifest themselves.

We are also looking into tissue engineering especially in the field of organ transplant. A diseased organ can be replaced by a healthy organ from the patient himself after undergoing tissue engineering thereby obviating the need for donors and tissue reactions.

The next millennium will definitely be an exciting period for medicine in general and surgery in particular.





## PLENARY SESSION IV

### MEDICAL EDUCATION IN THE PHILIPPINES

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Medical Education in the Philippines dates back with the establishment of the College of Medicine of the University of Santos Tomas in 1871. It has continuously been in operation and has produced a long list of illustrious medical graduates and some very prominent medical practitioners including our national hero, Dr. Jose P. Rizal. Among the illustrious graduates of the University of Santo Tomas College of Medicine are: Joaquin Pardo de Tavera, Joaquin Gonzales, Santiago Bartolome who was the physician of Gen. Emilio Aguinaldo during the revolution, The Guerreros: Alfredo and Luis, and many others.

In 1899, the Malolos Congress established the second medical school of the Philippines, the "Universidad Cientific-Literaria de Filipinas." At the beginning, it was referred to as College of Medicine and Surgery. This school was short-lived; but it is interesting to know that a number of the professors of this short-lived medical college later on became members of the faculty of the medical college of the new University of the Philippines which was established in 1907.

Among the illustrious members of the faculty connected with both and formed linkages between the two were the following: Dr. Fernando Caldero; Don Luis Guerrero who later became Chairman of the Department of Medicine in U.P.; Dr. Ariston Bautista-Lim who later on took post-graduate studies in Europe, also became the first Chairman of the Department of Medicine in U.P.; and Dr. Jose Albert who became Chairman of the University of the Philippines, Department of Pediatrics for many years.

The initial requirements of the university was a high school diploma before entry to the College of Medicine.

In 1959, the Medical Act was passed into law that required a baccalaureate degree in premedicine for admission to the College of Medicine; and a Board of

Medical Education was created to oversee and insure high standards of education in the country.

### Curriculum

The curriculum was traditional in approach: subject-oriented, teacher-oriented, crisis hospital-oriented, and largely patterned from the western model of medical education from the very beginning. There was a drive to adopt the technological advances and to train specialists perceived to be the highest level of professionalism, both economically and academically. Our graduates became adept with health conditions of the west and yet woefully unprepared to meet the challenge of serving the health needs of the Filipino people. Results were well known: large scale migration of physicians, high cost of health care with greater inequity in health care delivery, dissatisfaction with the medical profession which placed undue emphasis on the curative aspect of medicine at the expense of preventive and promotive care.

As early as the 60's, these problems had already been identified. The University of the Philippines-College of Medicine (UPCM) in its Seventh Teaching Institute on May 28-30, 1962. I had the privilege to Chair this institute, as Chair of the Department of Medicine. We reviewed the Objectives, Curriculum and Methods in the medical school.

The objective of undergraduate medical education is to provide an adequate preparation for the medical student so that he can go into general practice or proceed further for graduate training in a specialized practice, research and/or teaching of medicine.

Our observations in that institute are still as relevant at this time:

1. The a curriculum stressed general principles, scientific approach, correlation between basic science and clinics.
2. The importance of research in medical education was particularly emphasized at this early stage. The Department of Medicine through the initiative of its Chairman, set up the first Medical Research Laboratory in the College of Medicine funded by grants from the Rockefeller Foundation, China Medical Board and the National Science Development Board. The laboratory succeeded to attract many young members of the faculty who later on became the outstanding leaders in their various disciplines.

Dr. Campos who was sent by the College to attend the Oakridge Training Course in Nuclear Medicine initiated the establishment of the first Radioisotope Laboratory in the country. Under his dynamic leadership of the Department of Medicine, this department went on to establish specialty clinics both in the Out-Patient- Department and in the In-patients so that a time came to pass when half of the faculty of the College were in the Department of Medicine.

3. Students were immature, irresponsible, with poor communication skills, and prefer spoon feeding.
4. Concern about quality of teachers was felt: desirable traits of dedication, capability of imparting knowledge and concern for the students learning and even of what they teach, intellectual honesty.
5. Modification of the curriculum started allowing students exposure to various health problems in the community.
6. Teaching and learning methods were reviewed (lecture method, size of the class, site of learning activity). Already the importance of ambulatory medicine which closely approximates what a graduate would have to meet in actual practice of the profession entered curricular planning and methodology.
7. The institute came to the conclusion that graduate medical education is a must; so our doctors be conversant not only for increased scientific knowledge as required in specialty practice, in teaching and research but also for their continuing education.

In 1967, the Association of Philippine Medical Colleges was established to facilitate and coordinate the affiliation of the various medical schools, undergraduate course requirements with the government hospitals and facilities and working for necessary amendments to the Medical Act.

The herein proponent in a Symposium on Science and Technology on December 3-4, 1970 emphasized that brain drain is a reflection of failure in our part. Successful medical education is intended to produce doctors for rural areas, should be oriented towards work in these areas and new curricular need to be designed for such purposes. Questions like the following need to be answered: What type of doctors are needed in the country? How much of these types of doctors should be produced? and Where-what parts of the country are these doctors needed?

### **Addressing the Problems**

The necessity for familiarity of health workers, especially doctors, with the problems of the community was clearly obvious. To address this need:

1. University partnership with the community was explored in Los Baños in 1961. As Chairman of the Department of Medicine, we started with the Department of Medicine fielding its interns for one (1) month every year in Los Baños as a part of the agricultural extension of the College of Agriculture.

In 1963, under the initiative of the Department of Medicine, a formal Memorandum of Agreement (MOA) between U.P. and the Department of Health (DOH) resulted in the organization of the Comprehensive Community Health Program (CCHP) at Bay, Laguna with Dr. Paulo C. Campos, acting as Chairman of the Department of Medicine and concurrently its Director. With the University of the Philippines CCHP meeting this health needs of Bay, Calauan, Los Banos, Pila, CCHP served as the

community laboratory of U.P. established as an autonomous health district, a furtherance of devolution of the Department of Health.

Eventually, CCHP involved almost all health sciences schools of the university including Arts and Sciences, specially the Social Sciences. No less than Alfredo Landa Jucano wrote his well-known book on medical anthropology while he was working with the CCHP.

Various government policies were initially tried here. The inclusion of *hilots* as a part of the community health team resulted from studies done in CCHP. The initial government policy of barring them from the health team was reversed and instead replaced it with a new policy officially including them so long as they will gain some initial instructions; provided with necessary tools and supervised by the official community health worker.

Initial studies in devolution of the Department of Health were made here when an initial attempt to develop an autonomous health district such as the Bay project, including close working relationships between public and private health care groups utilizing common facilities.

The initial studies on the use of iodized oil for the prevention and eradication of goiter were made in Bay using Dr. Campos' initial experiences in his goiter work. By the time Dr. Campos left the directorship of Bay, Laguna, he has succeeded in placing community medicine officially in the curriculum of the College of Medicine starting from the first year up to Internship. He demonstrated the need not only for community orientation but more importantly for community based learning in Medicine so that it can be more relevant to the needs of the country and to instill in medical students the sense and dedication to the service of their countrymen. Attempts were made to discredit this but it has remained solidly and indelibly a part of the curriculum not only of the U.P. College of Medicine but all other medical and health science schools in this country.

It is my firm belief that the Tacloban experiment needs a candid review. It was originally conceived to be the answer to the increasing medical services to the remote areas of the Philippines. It is now over 20 years. It is increasingly evident that the problem is more of socio-economic one which is slowly being resolved with improved socio-economic conditions. In this more remote area that modifying and/or inventing new curricula on new methodologies does not seem to contribute much to its solution. It is unusual for medical schools to run first medical curricula simultaneously. The UP seems to be an experimentation running not only two but actually three curricula simultaneously over the last almost 20 years. They are possibly justifiable as experimental curricula; but surely after 20 years this university possibly must come to a decision which to continue and expand so that it can channel its resources, its energies, and its talents to the more desired functions of great universities - research and development.

So much has been said about Intarmed and after a generation of specialization with Intarmed it is about time we decide whether we will have to take Intarmed and drop the conventional curriculum or vice versa. I have some advantage since I have

been around in the medical academe for a much longer period than most of our faculty and medical administrators. I feel that shortening the curriculum may have advantages but can create disadvantages. We started with a four year curriculum in 1909. One or two years later we added a shorter pre-medical work. By 1918 we required an associate in arts (two years of pre-medical work). By the 1950's we were convinced that we need a longer preparation period for our medical students; So the Medical Act of 1959 required a baccalaureate degree for admission to medicine. The pendulum is definitely unstable and tends to swing back. Fortunately the UP preferred to experiment initially but the other medical schools did not seem to waste their time experimenting and wanted a shortened medical course. The reasons are not always arguistic. To shorten this medical course we have to refill the Medical Act of 1959. It allows only experimental courses as an exception and only in credible medical institutions. Shortening it will require a refill of the Medical Act of 1959. It may not be too bad for UP but it can be disastrous for medical education in the country. Even for UP, after 17 years of experimenting, we definitely cannot say it is better for many reasons: (a) It is a highly selected course curriculum and access is limited to a very few fortunate souls who have the money and/or the connections to get into science high schools and to qualified institutions like UP, Ateneo, an a few other institutions: (b) Even UP has to spend extra money to provide the faculty and the resources for such an intensive curricular offering and the products of this 17 years have not shown any distinct advantage of the graduates of Intarmed to the graduates of the conventional four year curriculum. As a matter of fact, casualties are higher in the Intarmed program. The committee that studied the products seemed to feel that the Intarmed projects are less mature than conventional graduates, and (c) Lastly, accessibility to such a curriculum really has become very limited so that there is not one to replace the conventional program.

2. In 1975, the National Teachers Training Unit was established to handle the training of medical school faculty in pedagogy.
3. A step-ladder type curriculum was adopted in 1976 at the UP Institute of Health Sciences in Palo-Leyte wherein the students were nominated by the community and passed through steps starting with barangay health worker - community health worker-midwife-general nurse-bachelor of science in community health - public health nurse-medicine proper with internship.
4. Alma Ata Declaration of Health for All in the Year 2000 promoted the concept of primary health care, an approach to the provision of health services emphasizing promotive and preventive care, partnership between health and other professionals and the community and insuring that basic health services are "available, accessible, affordable and acceptable."
5. Intarmed curriculum was adopted. It is a seven year Integrated Medical Curriculum in UPCM with students accepted directly from high school, providing for integration of premedicine subjects into the basic medical sciences.

6. The World summits/conferences in Medical Education in 1988 and 1993 established directions for medical education through reforms such as curriculum based on national health needs, commitment to primary healthcare, competency based learning, identifying new challenges in medical education such as increasing population, economic recession, shrinking resources, emerging new diseases as well as resurgence of previously controlled ones as tuberculosis, medicine becoming a business.
7. World Health Organization (WHO) in 1994: Changing Medical Education Agenda for action, identified the expanded roles of a five-star doctor.

The 1993 call for changes was basically an endorsement of the conclusions and suggestions of the 7<sup>th</sup> teaching Institute (1962) of the UP-College of Medicine: (a) changing of institutional missions towards improving health rather than simply providing care; (b) revision of outdated curricula, balance of demand for generalists and specialists; (c) newer methods of teaching and learning; (d) population-based setting for learning-stress ambulatory care; (e) ethics in medical education - basic principles of medical ethics together with newer issues raised by new biomedical technologies and changing social perspectives.

In the Philippine, there have been several responses to the call for change:

1. DOH Partnership for Alternative Health Science Education (PAHSE) - similar to the step-ladder type curriculum
  - Bicol Christian College of Medicine in Legaspi City
  - University of Northern Philippines in Ilocos Sur
  - Western Mindanao State University in Zamboanga City
2. Community-based curriculum,
  - Mindanao State University in Iligan City
3. Problem-based community oriented education
  - Zamboanga Medical School Foundation
4. Establishment of Clinical Epidemiology Units
  - Centro Escolar University
  - University of the Philippines in 1982
5. Association of Philippine Medical Colleges (APMC) - promotion of reforms in medical education thru seminars, workshops in training faculty

### **Challenges of the Future**

1. Faculty and administrative response
  - resistance to change, coming from traditional medical education
  - scarce resources for faculty incentives: part-time faculty is more the rule rather than the exception

- basic sciences faculty recruitment: clinical sciences more economically rewarding

Solution: Need to emphasize that the faculty members' first goal is to foster students' lifelong learning by helping them to develop their learning skills, as mentors guiding learning rather than providing information that students are expected to memorize for examinations.

Faculty development: Educator track for both basic science and clinical faculty; need to reward excellence in teaching, providing support, create meaningful cross-disciplinary teaching opportunities to encourage faculty members to assume educational responsibilities beyond their specialized area of practice or research.

Facilities are needed to train faculty in the use of computer for medical education.

2. Student selection - continuing search for a screening tool that will bring out desirable social attitudes and personal values of the student in addition to academic qualifications
  - need to provide equitable access to medical education thru scholarships
3. Evaluation - change from the traditional multiple choice objective questions to problem -oriented, objective examinations that will assess solving and patient evaluation skills: Objective Structured Clinical Examination (OSCE)
  - Licensure examinations intended to maintain standards but there is no flexibility to adapt to changing learning activities of integration, etc.
4. Extensive technological advances and rapid accumulation of knowledge requires accompanying support in library system, methods of supporting learning and communication and data management system with the use of computers.
5. Need to foster and maintain humane and caring attributes of the physicians in view of the over-developed technology as well as the trend of medicine and health care delivery system being seen as a form of "business".
  - support team work among health professionals
  - introduction of social sciences in medical education
  - environmental concerns in the form of threats to ecology and growing awareness of interaction between health and environment should be properly emphasized.
  - ethics in medicine - newer issues as organ transplantation, genetic engineering, etc.
6. Need to provide adequate positions for postgraduate training with training with the closing of American hospitals to foreign medical graduates (this should be seen as an opportunity rather than as a threat).

### **Curriculum Change**

The seven year integrated liberal arts - medicine programme was designed to shorten the medical curriculum to seven years and to implement the concept of medical education as a continuum that would result in early exposure of the student to the philosophy of the medical profession while allowing for ethical, humanitarian and social development of the student. The incorporation of internship in the undergraduate curriculum assures that progress and forward step along the line of continuing medical education will be achieved and facilitate acquisition of graduated and increasing levels of professional responsibilities.

There were other reasons for the curriculum change. One was the undesirable B.S. pre-med students' attitude to aspire for higher grades rather than learning and education. In the third year proper, there were too many subjects which led to frequent examinations. Students could not cope with the daily topics nor prepare adequately for the tests. There was also no observable difference between the performance of the B.S. graduates and those of the previous two year pre-medicine era.

### **The Intarmed Program**

The new curriculum was geared to achieve significant purposes. One was to provide a general education that would develop nationalism in the students while achieving the goals and objectives of the College of Medicine. A second aim was to apply the philosophy of "relevance in education". It was also geared to achieve savings in time and educational expenses.

The main goal of the seven year INTARMED PROGRAM is to graduate physicians who will have, not only the scientific competence to practice medicine, but also the social conscience which will motivate them to continuously improve medical services in the Philippine society. The program is guided by the aim of the UP College of Medicine to produce graduates who will be primary physicians with potentials to be teachers, researchers and specialists.

The program aims to develop in the student a clear perception of the medical profession by early clinical exposure. A liberal education is emphasized which includes critical thinking, logical decision making, appreciation of community role and the development of attitudes of compassion, respect for the rights and privacy of others. Other objectives include the acquisition of competence as a primary physician as well as the capability to develop in many directions in medicine. Comprehensive health care and the role in a health team are emphasized. The program assists the students to assume personal responsibility for his own education.

### **Curriculum Strategies, Content and Evaluation Plan**

The relevant strategies include the teaching of the humanities throughout the program horizontal and vertical synchronization of subjects and simple to complex progression in course content. Whenever possible the problem-oriented and integrated approaches are used. Basic sciences are taught with a clinical orientation and



clinical subjects are studied with a basic science orientation. The other significant methodologies are a more equitable distribution of subject load, rural internship and the provision of time for electives and self-study. In addition there is an early and greater exposure to clinical situations with increasing but graduated responsibilities in patient care.

The following are the fields of study in the INTARMED program: humanities, social sciences, mathematics and physics, chemistry, biology, human biology, human illness, neuroscience, family and community health, pre-internship clinical experience, research, pedagogy and management.

With the assistance of the late Professor Corazon Paulino-Gonzales, an evaluation plan was also outline which was envisioned to run parallel with the program implementation. The intention was to identify program weaknesses, determine degree of attainment of objectives and assess quality of the products.

### **Early Revisions: Integrated Clinical Clerkship (ICC)**

In 1986, Dean Alberto G. Romualdez Jr. introduced the Integrated Clinical Clerkship (ICC) as a manner of implementing the clinical years of the INTARMED Program. In effect, it was a curricular revision which was approved by the University Council of Manila on May 26, 1987 and confirmed by the Board of Regents during its 1000<sup>th</sup> meeting on June 25, 1987.

In the original INTARMED Program, clinical clerkship was described only at Year Level VI as a half-day morning schedule. The afternoons were intended for didactic lectures. The ICC revisions however, instituted a 2-year clinical clerkship (year levels V and VI) which integrated the didactic and ward rotation of all clinical courses. The "block system" was also introduced wherein the class was divided into small sections with each section taking only one subject at a time.

On April 17, 1986 the Up system adapted a general education program (GE). On November 20, 1987, the University Council of Manila approved the incorporation of the GE program requirements into the first two years of the INTARMED Program.

### **Initial Evaluation**

The initial formative evaluation of the program in terms of its implementation and effects was completed in 1989 during the term of Dr. Marita V.T. Reyes. Professors Maria Lourdes Rebullida and Laurie Ramiro of the UP Manila College of Arts and Sciences conducted the study at Year Levels 1, 2, and 7. The evaluation focused on determining the effects of the program on the self esteem, social adjustment, adaptability and social distance of the direct high school entrance. (Non-BS students). The implementation aspects of the evaluation included the following: system of classroom teaching; teacher factors; relevance, description and scope of subjects; and facilities.

The important conclusions of the initial evaluation are the following:

1. The non-BS students are able to cope with the academic demands of the program without much difference from the BS students.
2. The emotional maturity of non-BS student are not fully well developed. More time should be provided for developing personal social relations, and enjoying extra-curricular activities and experiences.
3. On the objectives of social awareness, concern, commitment and responsibility, there is an expression of willingness to serve in rural communities and in the country instead of practicing medicine abroad.
4. INTARMED students at Year Levels I and II should be mixed with students of other courses.

On the part of the Curriculum Committee, an Internal Evaluation Report was submitted by Dr. Genara Limson. The report concluded that the academic performance of the Non-BS students is about the same as the BS students. The report emphasized that at this point of the program, it is not fair nor reliable to assess the leadership qualities and social consciousness of both groups of students. The report further recommended that the admission of Non-BS students be expanded.

The Integrated Clinical Clerkship was also evaluated. The result showed an over-all satisfaction from the students because of the implementation of several learning activities other than the lecture method. The implementation of electives added to students satisfaction. However, there were isolated complaints on unclear objectives with regards to patient care and responsibility. Some faculty members continue to be unhappy over the faculty intensive schedule of the ICC.

### **Latest Revisions**

In January of 1992 during the term of Dean Alfredo T. Ramirez, a next set of revisions to further enrich the INTARMED Curriculum were approved by the University. The implementation started in Academic Year 1996. Pediatrics, Surgery, Obstetrics-Gynecology were restored in Year Level 6. These revisions will remove the learning gap in these subjects and improve the entering knowledge and skills at Year Level 7 (internship).

Dean Ramirez encouraged the use of innovative and modern curricular approaches. The faculty of all academic departments held workshops and seminars on competency-based learning and problem oriented method of instruction. The faculty was encouraged to use more interactive learning strategies like group discussions where the student is an active participant in the learning process. Seminars were held concerning teaching methods that are process-centered rather than content centered and student directed rather than teacher directed. These approaches will gradually replace the traditional, less efficient subject matter/discipline based instructional method.

A Medical Education Unit was formed composed of medical faculty members with education degrees or units. This unit conducts seminars and workshops on teaching methods and evaluation of students. Attendance in these pedagogy workshops is now a requirement for faculty permanency.

### **Community-Oriented Medical Education (COME)**

A major area of concern is how to translate the UPCM Vision of community-orientation in the INTARMED curriculum. Dean Ramirez in 1992 launched in the 5-Point Strategy to Implement Community Orientation: 1) enrichment of Family and Community Health Courses; 2) revival of the Multidisciplinary Community Health Program; 3) extending curricular credits to participation in socially-oriented student/campus organizations; 4) designing community-oriented departmental courses, projects and extension/consultancy services; 5) enrichment of the 6-week community preceptorship program for interns.

### **The 1994 Evaluation**

An external evaluation was completed in 1994 by an Ad Hoc Chancellor's Committee headed by Dean Cristina Mencias of the UP National Teacher Training Center for the Health Professions. The report helped the College to re-focus on the original design of the INTARMED Program and to assess the extent of the achievement of the objectives. Weaknesses and unintended effects were also identified. One of the objectives of the INTARMED Program is to improve the health service in the Philippines. Available data on the whereabouts of graduates indicate that these objectives has not been attained. An almost equal percentage (48.5% for direct and 48.7% of lateral entrants) are abroad, working or taking up residency.

The 1994 evaluation report also gave specific suggestions on the following areas of concern: Community orientation, schedules, teaching methods, instructional materials, faculty matters, selection and evaluation of students.

### **Current Curriculum Enrichment**

The Management Action Plan (MAP) of current Dean Amelia R. Fernandez gives priority to continuing INTARMED curriculum development strategies. A conceptual framework was developed by the present Curriculum Committee, Chaired by Dr. Lourdes L. Ignacio. There are three areas of current concern: 1) introduction of a gradual, early, and sustained community-orientation, correlation and field experience starting at Year Level 3, 2) strengthening the development of values, humaneness and compassion; 3) integration of bioethics in the curriculum.

Dean Fernandez and the curriculum committee agree on the implementation of the INTARMED Program as originally designed consistent with the College vision-mission statement. New models for horizontal and vertical integration of courses are being developed (e.g. reproductive and cardiovascular systems) using the Integrated

Neuroscience Program experience as reference. More concrete curricular guidelines are being outlined by defining specific Year Level competencies (Year Levels 3 to 7). The following strategies are being enhanced: competency-based learning, self-directed learning and problem-solving skills.

### **The Ideal UPCM Graduate: A Multipotential Primary Physician**

The INTARMED Program desires to develop the ideal UPCM graduate. A physician who is able to develop in many directions in medicine (Multipotential Graduate) but who will possess the basic knowledge, skills and attitudes necessary for being a PRIMARY PHYSICIAN capable of participating in a comprehensive continuing care and working as a member of the health team. He is a committed, professionally competent, socially conscious Filipino physician and community leader who is able to develop as a basic scientist, medical practitioner, specialist, researcher, teacher, and health administrator.

## AN OVERVIEW ON MEDICAL EDUCATION

RAMON L. SIN

*Faculty Dean of Medicine and Surgery  
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We would like to congratulate Dr. Paulo C. Campos for his extensive dissertation on "Medical Education, Past, Present and Future". We also congratulate UP College of Medicine for adapting its Medical Foundation program in such manner as to be an effective instrument in providing health care to our people. Its programs and innovations can serve as models for other Medical Institutions.

I have here with me a videotape on Medical Education in the UST Faculty of Medicine and Surgery, past and present. I wonder if time will allow us to view it.

But what about our projections for the future?

In April 3-7, 1997, all Department Chairs of the UST Faculty of Medicine and Surgery gathered together for five-day seminar workshop in Calaruega, Batulao, Batangas to contemplate on the destiny of our Medical School in the year 2000 and beyond; to make a forecast on what the vision of our Medical School ought to be for the next millennium, to define its mission and to plot strategies on how to attain them.

Making forecasts with numerous unknowns could be a challenging and provocative experience. Nonetheless, allow me to share with you some of the highlights, which I believe are relevant to our discussions today, which the Caleruega experience brought about:

- I. The principal goal of the Medical School continues to be the maintenance and development of outstanding programs in health sciences in order to advance knowledge judged to be important for the future.
- II. To educate medical students in the most modern aspects of science and practice of Medicine anticipating the indivisibility and interdependence of research and education.
- III. To achieve these goals, there is need to give highest priority to recruiting and retaining the very best and most enthusiastic young minds (both faculty and students) for all active medical specialized areas and other areas we wish to develop for continued evolution and growth.

- IV. The faculty must be convinced that their primary goal and mission is not to provide information but to develop skills, attitudes and study habits to guide students in the learning process.
- V. Student selection must be based not just on academic qualification but on desirable social attitudes, motivation and personal values.
- VI. Passive didactic teaching must be progressively replaced by active participatory learning based on self-initiated study and problem solving.
- VII. In response to the powerful revolution in biology that precipitated the advent of recombinant DNA technology and genetic engineering, oncology, virology and immunology, basic departments will increasingly have to disregard traditional department boundaries in favor of new and interdisciplinary alliances and functional groupings whose versatility and flexibility allow them to focus on novel research projects.
- VIII. Vertical and horizontal integration of basic and clinical departments, of medical and surgical specialization including support services to form new, discrete functional units.
- IX. Traditional departmental boundaries of clinical departments will have to give way to more centralized interdisciplinary units, for both teaching and research, where physician-scientists, regardless of their departmental affiliation, can join forces in using similar approaches to study human biology and sharing with each other recent body of knowledge, research techniques and equipment available. Thus, all of the facilities we have set up – the Molecular Biology Unit, Electron Microscopy Unit, Learning Resources Unit, Clinical Epidermology Unit, the Medical Research Laboratory, the Neuropathology Laboratory, the Mycology Laboratory, should be available to all units and departments.
- X. Since the consensus now is that basic scientists have most of the answers while the clinicians have most of the questions – it is best to develop a program to train physicians conversant with both the new biology and clinical medicine to form physician-scientists.
- XI. Physician-investigators participating in research would retain membership in their parent clinical departments but they would also have the opportunity of being appointed to a basic science department. Recruiting would be a joint responsibility.
- XII. Strengthening of primary-care medicine with the horizontal integration of Family and Community Medicine to such specialties as pediatrics, General

Medicine and General Surgery. Such a primary-care unit or department would be assigned the responsibility for Geriatrics, Environmental Medicine or Ecology, Clinical Nutrition and Epidemiology.

XIII. Revision of the curriculum in a gradual, step-wise manner rather than a sudden and comprehensive revision, at least in the foreseeable future. A sudden, massive revision would generate more heat and polarize the faculty.

XIV. Gradual inclusion in the curriculum of:

A. Oriental or Traditional Medicine:

- Herbal Medicine to be part of Pharmacology -- emphasizing on herbs indigenous in the country
- Practices like acupuncture -- both the Oriental Methodology and the MRA system practiced in the USA, can be explored as to scientific basis.

B. Social Sciences

C. Humanities

XV. Strengthening of:

A. Bioethics as part of the curriculum. (Value education shall be the responsibility of Bioethics.)

B. Health Sciences Research Management Group and the various Research Laboratories now existing

- Molecular Biology Laboratory
- Learning Resources Unit
- Electron Microscopy Unit
- Clinical Epidemiology Unit
- Neuropathology Laboratory
- Infectious-Tropical Diseases Laboratory, as part of the
- Medicine Research Laboratory
- Mycology Laboratory

C. Medical Library

D. Faculty Development Program

E. Continued Medical Education Program

F. Computer system and computer-assisted teaching

G. Internet – the present volume of knowledge and data gathering can be facilitated by these modern technologies advances.

XVI. Setting up of facilities for:

- A. Distance Education through Tele-Medicine for an on-the-spot exchange of knowledge and expertise. This facility would allow the faculty and students to interact and discuss with Medical Centers abroad the latest developments in Medical Science, to gather more information for research, and to observe the latest techniques in medical practice through video screens.
- B. More outreach stations like the one we have in Sapang Palay -- the San Martin de Porres Community Center.

XVII. The University Hospital remains an essential tool and laboratory for Medical Education. It must be strengthened to make it a more appropriate place for the student learning process. This, however, shall be supplemented by more exposure to community service and rural medicine.

XVIII. Logistics or funding of the Medical School remains a big problem particularly since private institutions do not receive subsidy from the government. The only source is the tuition fee, which is hardly able to meet the demands of the increasing cost of medical education. This problem, however, has to be addressed by Administration, whether through Foundation, Alumni, Benefactors, Supporters, etc.

XIX. A review of the system of rewards or incentives for faculty.

XX. Incentives for good and deserving but financially depressed medical students through more scholarships.

XXI. Our vision for Medical Education in the next millennium:

A deep and fruitful integration of basic and clinical medicine; a gradual adaptation of the curriculum to reflect the realignment of scientific and clinical specialties; and a value system mandating that growth and expansion be invariably linked to maximal intellectual and educational returns.

Difficult as these proposals may seem, they are stepping-stones to the future. We anticipate that by year 2000 and beyond, economic and societal pressures, if not expediency, will have brought about many of these changes.



## AN OVERVIEW ON MEDICAL EDUCATION

FELICIDAD G. CUA-LIM, M.D., FPCP  
*Immediate Past President*  
*Philippine College of Physicians*

Dr. Dayrit suggested that I direct my remarks as a Reactor to the subject of integrating a subspecialty into the mainstream of the medical curriculum, and I would like to do so in the time allowed me. I would also like to share, in relation to that issue, my thoughts on the larger pursuit of medical education.

In my view, medical education possesses three crucial components: (1) the administration, (2) the mentors or teachers, and (3) the students.

To the administrators fall the task of selecting qualified mentors and providing the best facilities possible. The possibility of creating an optimal teaching environment rests in their hands.

Mentors for their part, must endeavor to keep up to date with current advances and concepts in the discipline. Yet knowledge alone, however current, is not sufficient. The mentor must also be dedicated to teaching as well as be adept at imparting complex ideas in an accessible manner.

Now I would like to comment on the third player in the medical education, the students. Future physicians must possess not only aptitude, motivation, and ethical maturity but also scholarly humility. In my many years as a doctor, I have tried to consider myself a student at all times. I recall that, when I first returned from my postgraduate specialty training in 1963, a distinguished "dermatologist-allergist" alleged that my postgraduate subspecialty of allergy and immunology was analogous to quackery. I was then, and remain to this day, very grateful to Dr. A. B. Rotor who came promptly and effectively to the defense of a "debutante speaker" under attack. But that experience solidified my resolution to treat criticism from my detractors as an impetus for self improvement, rather than an occasion to defend my pride.

Scholarly humility must also be present in every physician's commitment to continuing education, so that, while the physician does not forget his or her general training, he or she also remains open to innovation and advancement in the field. Molecular biology and genetic engineering are here for better understanding of disease(s) and disorders.

Coming at last to the question of integrating subspecialties into the curriculum, I would advocate that this be done as early as the first year of medical school. For

example, medical instruction can emphasize the links between specific cell(s) and products.

How do cells communicate with each other? How are they recruited to inflammatory site(s), thus affecting the target tissues / organs? Colleagues of noted that "cytokines", "adhesion molecules", and other concepts have become buzzwords at the conferences they attend. Indeed, today's mentors are faced with the challenge of increasing the range of their competence by familiarizing themselves and their students with current interests and debates in our discipline.

My remarks today obviously underscore the necessity of hard work on our part. Yet however difficult it may be to establish and uphold rigorous standards of medical education, the result, I think, speaks for itself - the training of physicians worthy of their patients' trust for after all, our patients are the greatest teachers. Our commitment to learning is, fundamentally a commitment to this idea.

Thank you and good day.

## SCIENTIFIC PAPERS

### *AGRICULTURAL SCIENCES*

#### **IMPROVING QUALITY AND SHELF LIFE OF VEGETABLES AND FRUITS BY EVAPORATIVE COOLING STORAGE**

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#### **ABSTRACT**

*Fresh fruits and vegetables deteriorate in quality very rapidly after harvest due to high rates of water loss and senescent changes. These processes rendered rambutan fruits and pechay crowns unmarketable in 2-3 days of storage and banana, mango, tomato and sweet pepper, in 7-13 days at ambient (27-32°C; 64-88% RH). Using a simple box-type evaporative cooler (EC), a cool (23-28°C) and humid (>90% RH) storage condition was established and inhibited fruit shrivelling and leaf wilting, reduced weight loss, retarded fruit ripening and rambutan pericarp browning, improved banana peel color quality, and as a result, prolonged shelf life considerably. However, the increased humidity favored disease infection in the later period of EC storage. Sodium hypochlorite washing minimized disease incidence in tomatoes, sweet peppers, pechay, and mango. Ethanol application was ineffective but retarded ripening of fruits, except in bananas. In rambutan, ascorbic acid did not retard pericarp browning. EC storage and the various treatments employed had no adverse effects on the chemical and sensory quality attributes of stored produce.*

**Keywords:** *Vegetables, fruits, postharvest quality, storage life, evaporative cooling, high humidity, hypochlorite, ethanol*

## INTRODUCTION

Vegetables and fruits play a decisive role in the country's economy and in human nutrition. Economic contributions as of 1993 of the vegetable and fruit industries are estimated at 6.5 and 10.5 billion pesos, respectively (Bautista and Acedo, 1975). These commodities are also the richest source of vitamins, minerals, and plant proteins. A major constraint in the production of these crops is their high perishability which, together with lack of storage systems, greatly account for the serious losses of harvested produce (Bautista and Acedo, 1995; Acedo, 1996). Refrigerated storage is seldom available but evaporative cooling storage could minimize the problem. In this storage system, a cool and humid environment can be established since the heat from the storage chamber is dissipated with the evaporation of water. The decrease in temperature is usually small but the increase in humidity is substantial so that product quality deterioration due to moisture loss can be inhibited. A number of evaporative coolers (EC) has been found effective in improving the keeping quality of vegetables and fruits (Garcia and Bautista, 1984; Labios and Bautista, 1984; Olea, 1984; Redulla et al., 1984; Rama et al., 1990; Thiagu et al., 1991). These ECs were typically simple and inexpensive since target users are the small, resource-poor farmers and entrepreneurs who dominate the industry. However, several gaps in the use of EC have not been addressed. These include disease development during storage, unretarded fruit ripening, inapplicability of some EC systems to certain produce, and unknown responses of many commodities to EC conditions. This paper describes the effectiveness of simple box-type ECs in maintaining quality and prolonging shelf life of important vegetables and fruits and the commodity treatments that could be integrated with EC storage.

## MATERIALS AND METHODS

### Vegetable and Fruit Sampling

Leading commercial cultivars of three vegetables and three fruits were used; tomato ('Improved Pope'), sweet pepper ('Grossum'), and rambutan ('Maharlika'). Freshly harvested mature-green tomatoes, peppers, mangoes (about 14 days from flower induction), and bananas (full three-quarters), red-ripe rambutans, and 25-day old crowns of pechay were obtained from local growers and sorted, retaining defect-free samples of comparable size. Ten to 20 fruits or 5-10 pechay crowns were used for each treatment per replicate.

### EC Structures and Storage Condition

The EC consisted of a wooden main frame 60 x 50 x 100 cm in length, width, and height, respectively. The storage compartment was raised 40 cm above the ground and had three drawer-type commodity holders made of screen wire. Two types of wall structure were used. In the first, the storage compartment including

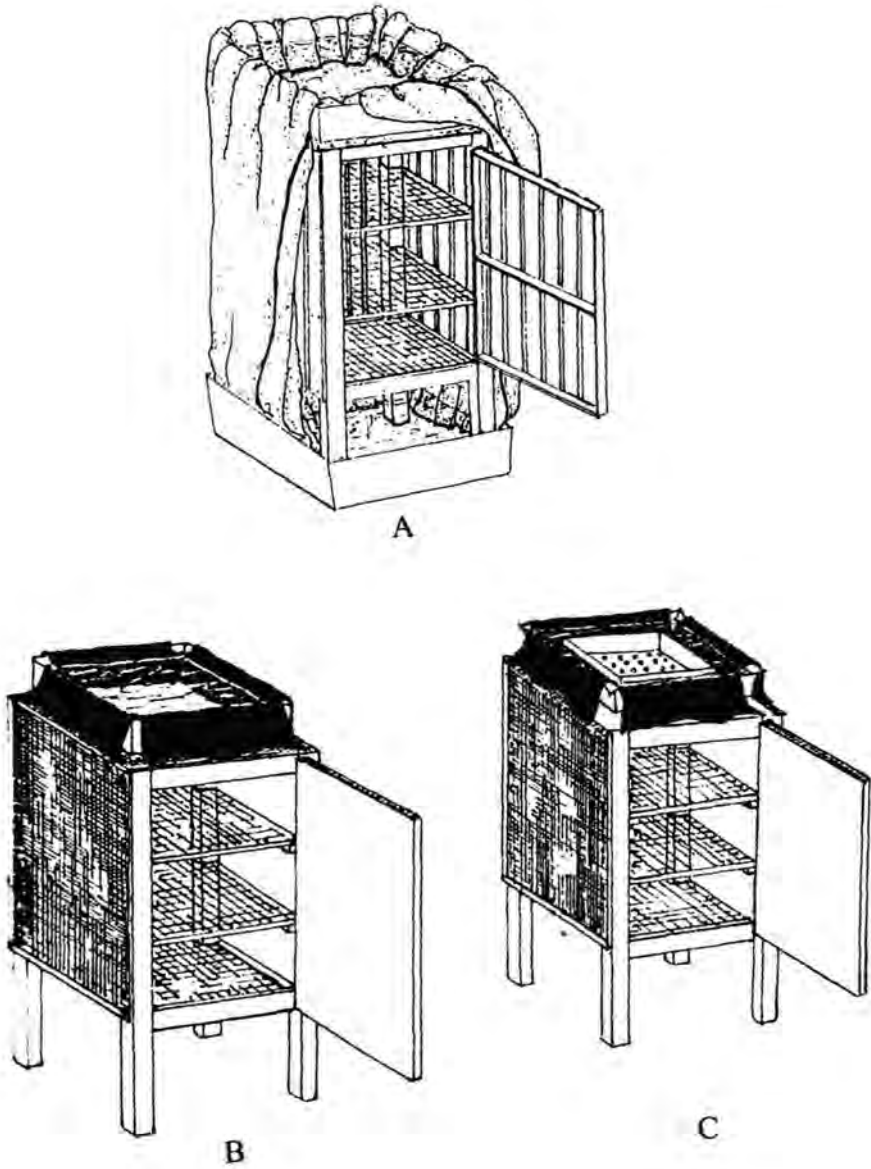


Figure 1. The evaporator coolers (EC) with jute sack walling (A) and with rice hull wall insert without vents (B) and with vents (C).

the door assembly were walled with wooden slabs spaced at 2 cm. The whole structure was covered with just sacking kept wet by dipping its top and bottom ends in water contained in a pan made of plain galvanized iron (GI) sheet. The jute sacking was sewn on except across the front to facilitate opening of the door (Figure 1A). In the other type, the inner side of the five walls and that of the door assembly were made of GI sheet with fine holes spaced at 5 x 5 cm while the outer side was made of 0.32 cm fine-meshed wire. The 1.5-cm space between the inner and outer walls was filled with rice hulls. A GI pan of water was placed on top, with cloth to draw water to the rice hull insert. This type of EC was either unvented (Figure 1B) or vented with 32 2-mm diameter holes in the top (Figure 1C). Storage in these ECs was compared with that at ambient in the same laboratory. The temperature and relative humidity (RH) were measured using a *Tri-Sense* digital meter. The vapor pressure deficit (VPD) in millibar (mb), a measure of the capacity of an atmosphere to dry out commodities, was traced from a psychrometric chart. The data presented are means of daily readings taken at three times, about 6 am, 12 noon and 6 pm.

### Commodity Treatment

Sodium hypochlorite (NaOCl) was tried for disease control in tomatoes, peppers, pechay and mangoes, using commercial bleach diluted to 0.5 and 1.0% NaOCl and washing for about 1.5 minutes. Tap water was included for comparison. For ripening retardation, ethanol application was tried, as vapor or as dip. In the former, a 3.5-liter glass jar capped with rubber stopper was used and inside it was a filter paper-wick. Absolute ethanol at 1-3 ml/kg fruit was put onto the wick using a plastic syringe: it evaporated within 15 minutes, and the fruits were left for 6-12 hours. In the dip treatment, fruits were dipped in 20-40% ethanol for 2-4 minutes.

### Measurement of Storage Characteristics

In tomato, pepper, mango and banana, the changes in peel color were visually determined using a color index (CI) of 1-6, with 1-green, 2-breaker, first trace of yellow or red, 3-more green than yellow or red, 4-more yellow or red than green, 5-yellow or red with trace of green, and 6-full yellow or red. The number of days to reach CI 4-5 (banana) or CI 6 (tomato, pepper, and mango) was taken as the ripening period. At the ripe stage, total soluble solids (TSS), titratable acidity (TA), and overall flavor were determined. TSS was measured using an *Atago* hand refractometer while TA, by titration against NaOH and phenolphthalein; results are given on the wet weight basis. The overall flavor was evaluated by 10-15 trained panelists using a hedonic scale of 9 (liked extremely) to 1 (disliked extremely). In rambutans, TSS, TA and overall flavor were measured when the fruits reached the limit of marketability.

In pechay, yellowing of the leaf surface was rated on a scale of 1 - green, 2 - up to 10% of leaf surface yellow, 3 - li - 30% yellow, 4-31-60% yellow, 5-61-90%

yellow, and 6-more than 90% yellow. Chlorophyll content was also analyzed by the acetone method (Yoshida et al., 1976) using the second leaf from the base of the crown.

In rambutan, pericarp browning was scored using an index of 1-6 (Lam et al., 1987), with 1- none, 2 - up to ¼ of spintern brown, 3 - ¼ to ½ brown, 4 - ½ to ¾ brown, 5 - more than ¾ brown, and 6 - entire spintern brown.

The percent loss in weight of each vegetable and fruit was measured. The degree of fruit shrivelling or leaf wilting was scored 1-4 for none, slight, moderate and severe.

Disease-infected tomatoes and peppers were counted and expressed as percent of the total. In pechay and mango, severity of disease infection was rated on a scale of 0 for none to 5 for severe or more than 30% of the fruit or crown affected.

Shelf life was the number of days for each vegetable or fruit to reach the limit of marketability, due to shrivelling or wilting, overripening or leaf yellowing, and/or decay.

All experiments were in a completely randomized design using 3-4 replicates. Each experiment was repeated at least once. The results obtained were similar and the data presented here are pooled means of 2-3 trials.

## RESULTS AND DISCUSSION

The ECs had lower temperatures and higher RH than that at ambient (Table 1). VPD consequently decreased from about 8.8 mb at ambient to 1-2 mb in the ECs. This indicates that the ambient atmosphere was 4-5 times drier than the EC atmosphere. Storage conditions in the three types of EC did not differ much. Similarly, storage characteristics of the vegetables and fruit did not vary with EC design. In the succeeding discussion, commodity responses to storage in the vented EC (Figure 1C) were compared to responses to ambient storage.

### Fruit Ripening Characteristics

EC storage retarded ripening of tomato and mango by 1-2 days and that of sweet pepper, by 9-10 days later than the ripening periods of fruits at ambient (Table 2). It effected also normal peel reddening of peppers whereas at ambient, less than 50% of the fruits turned red-ripe. Ethanol vapor and dip treatments further

Table 1. Average temperature, RH and VPD during storage of vegetables and fruits.

Storage of Treatment	Temperature, °C	RH, %	VPD, mb
EC-jute sacking	26.7 ± 1.1	96.1 ± 1.8	1.8 ± 1.0
EC-rice hull	26.1 ± 0.9	96.2 ± 1.6	1.2 ± 0.6
EC-rice hull, vented	25.6 ± 0.8	93.8 ± 2.5	2.0 ± 1.2
Ambient	29.1 ± 1.4	78.1 ± 5.3	8.8 ± 2.5

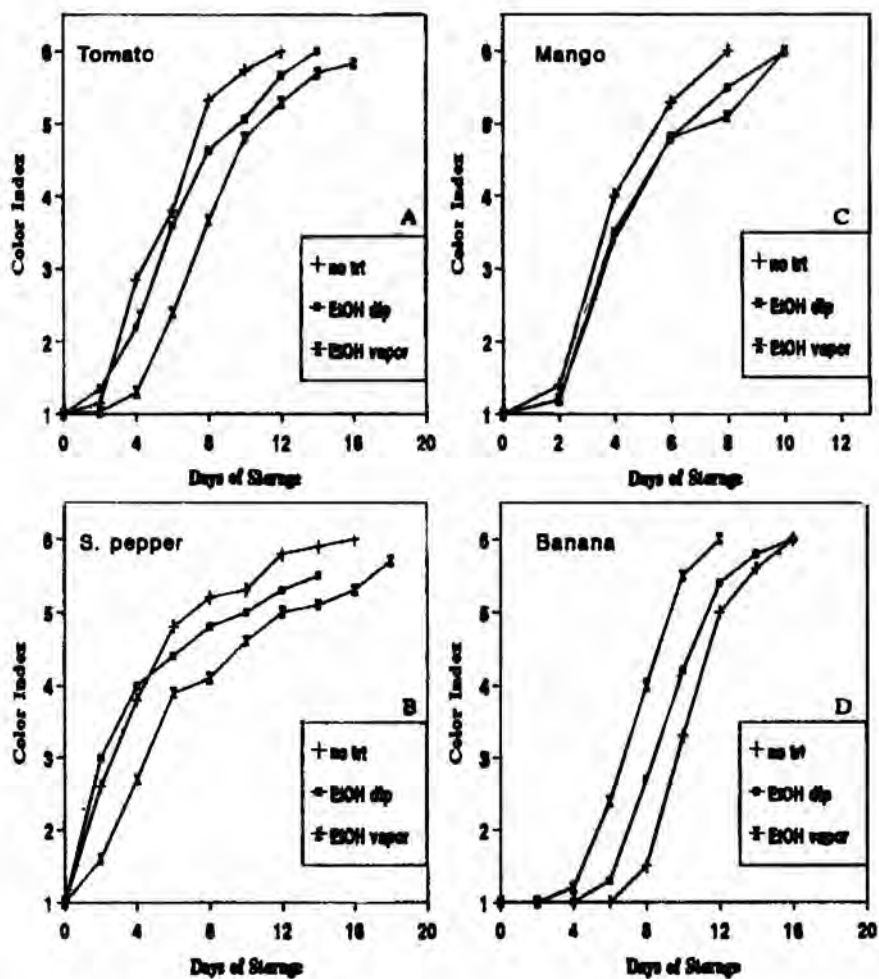


Figure 2. Peel color development of EC-stored vegetables and fruits treated with ethanol vapor or dip.



slowed down ripening manifested as reduced rate of peel color development, except in banana (Figure 2). Vapor treatment was more effective than the dip treatment, particularly in tomato and pepper. The optimum treatment was 1 ml ethanol per kg fruit for 6 hours (vapor) and 40% ethanol for 4 minutes (dip). Use of lower or higher rates of application had no added benefit. In bananas, the ripening period of EC-stored fruits was comparable to that of ambient-stored fruits. Ethanol treatment also promoted ripening (Figure 2D). However, the EC fruits developed better ripe peel color than the ambient fruits (Table 2).

Ripening retardation by EC can be attributed to the cool and humid condition during storage (Table 1). It is well known that metabolic processes proceed at a slower rate at lower temperatures. High humidity is also retardant for ripening (Grierson and Wardowshi, 1978). In bananas, it is surprising that the humid EC condition had no marked inhibitory effect on ripening, contradicting previous reports (Tung et al., 1987; Shukor et al., 1990; Xue et al., 1995). Factors such as ethylene accumulation in the storage chamber may have negated the ripening-inhibitory effect of low temperature and high RH in the EC. Among the fruits and fruit-vegetables used here, banana is the most sensitive to ethylene. On the other hand, the inhibition of tomato, pepper and mango ripening by ethanol concurs with results of earlier studies (Saltveit, 19889; Saltveit and Sharaf, 1992). But again, the response of banana contradicted that obtained in 'Senorita' and 'Cavendish' fruits whose ripening was retarded by ethanol (Esguerra et al., 1993). It can be speculated that the rates of ethanol application used were already excessive for 'Saba' and probably inductive to stress ethylene production and ripening (Hyodo, 1991).

As the rice stage, the soluble solid and acid contents were generally lower in EC-stored fruits than in ambient-stored ones, including that in rambutan (Table 2). This could not possibly be due to respiratory breakdown of TSS and TA components since this process was apparently retarded by the low temperature and high RH condition in the EC. If one examines Table 3, it can be noted that the degree of weight loss and shrivelling of EC-stored fruits was much reduced compared to that of ambient fruits. These data suggest that the EC fruits contained more water than the ambient ones and that the TSS and TA decrease in EC fruits was not due to chemical breakdown but rather to dilution of the chemical constituents. Supportive to this possibility is the results on the overall flavor which did not change with the decrease in TSS and TA contents (Table 2). The EC and ambient tomatoes, mangoes and bananas had comparable flavor. In rambutan and sweet pepper, EC storage even improved flavor due to increased juiciness.

### **Shelf Life**

The EC increase the shelf life of tomato, sweet pepper, pechay and rambutan by about twice as long as that at ambient or longer (Table 3). Mangoes also lasted in the EC for longer periods of about 2 days more than that at ambient. Shelf life of EC- and ambient-stored bananas did not differ significantly.

Table 2. Ripening characteristics of EC- and ambient- stored vegetables and fruits.

Commodity Parameters	EC	Embient
<i>Tomato</i>		
Ripening period, days	10.8a	8.2b
TSS, °B	5.0B	5.8a
TA, % citrate	0.22b	0.36a
Overall flavor	7.4	7.1
<i>Sweet pepper</i>		
Ripening period, days	16.1a	6.8b
Red-ripe fruits, % of total	97a	45b
TSS, °B	4.6b	6.1a
TA, % citrate	0.43b	0.59a
Overall flavor	6.8	4.8
<i>Mango</i>		
Ripening period, days	10.5a	8.9b
TSS, °B	19.7b	23.7a
TA, % citrate	0.17	0.18
Overall flavor	8.4	8.1
<i>Banana</i>		
Ripening period, days	9.0	8.6
Peel color quality	bright yellow	pale yellow
TSS, °B	11.7	11.9
TA, % citrate	0.40	0.35
Overall flavor	8.2	8.4
<i>Rambutan</i>		
TSS, °B	8.4	8.6
TA, % citrate	0.48	0.54
Overall flavor	6.9a	4.1b

Mean separation within rows by LSD, 5%.

Quality maintenance and shelf life extension by EC storage were due largely to the marked reductions in weight loss and degree of fruit shrivelling or leaf wilting (Table 3). These are expected responses to EC conditions (Table 1). However, disease infection increased at the later part of the storage period and accounted mainly for the quality deterioration of EC-stored tomato, pepper, pechay and mango (Table 3). Prestorage NaOCl wash significantly minimized disease incidence by delaying symptom manifestation, reducing the number of disease-affected produce, and/or decreasing the severity of infection (Figure 3). A concentration of 0.5% NaOCl was sufficient for tomatoes and peppers while for pechay and mangoes, 1% NaOCl, but this treatment did not extend shelf life further since the other causes of quality deterioration were not affected.

Table 2. Storage characteristics of EC- and ambient-stored vegetables and fruits.

Commodity Parameters	EC	Embient
<i>Tomato</i>		
Shelf life, days	26.1a	13. b
Weight loss, %	15.8b	27.2a
Degree of shrivelling	1.5b	
3.4a		
Diseased fruits, % of total	62.7a	14.3b
Overripe fruits, % of total	37.3	36.7
<i>Sweet pepper</i>		
Shelf life, days	22.1a	8.1b
Weight loss, %	12.0b	38.9a
Degree of shrivelling	1.2b	3.7a
Diseased fruits, % of total	69.7a	4.0b
Overripe fruits, % of total	31.3a	0.0b
<i>Pechay</i>		
Shelf life, days	5.1a	2.3b
Weight loss, %	14.0b	37.5a
Degree of wilting	1.0b	3.9a
Degree of yellowing	4.6a	1.6b
Chlorophyll loss, %	63.0a	5.3b
Degree of decay	3.3a	1.0b
<i>Mango</i>		
Shelf life, days	11.5	10.3
Weight loss, %	13.7b	20.5a
Degree of shrivelling	1.6b	3.0a
Diseased fruits, % of total	60.4	53.3
Disease severity	2.9a	1.8b
<i>Banana</i>		
Shelf life, days	11.5	10.3
Weight loss, %	9.5	12.0
Degree of shrivelling	1.5b	2.7a
Overripe fruits, % of total	100	100
<i>Rambutan</i>		
Shelf life, days	5.8a	3.2b
Weight loss, %	9.7b	19.0a
Degree of browning	5.0	5.7

Mean separation within rows by LSD, 5%.

In rambutan, browning of spinterns was the primary cause of quality deterioration which was inhibited by EC (Table 3 and Figure 4). In another experiment, ascorbic acid as an antioxidant was tried but did not influence browning symptom

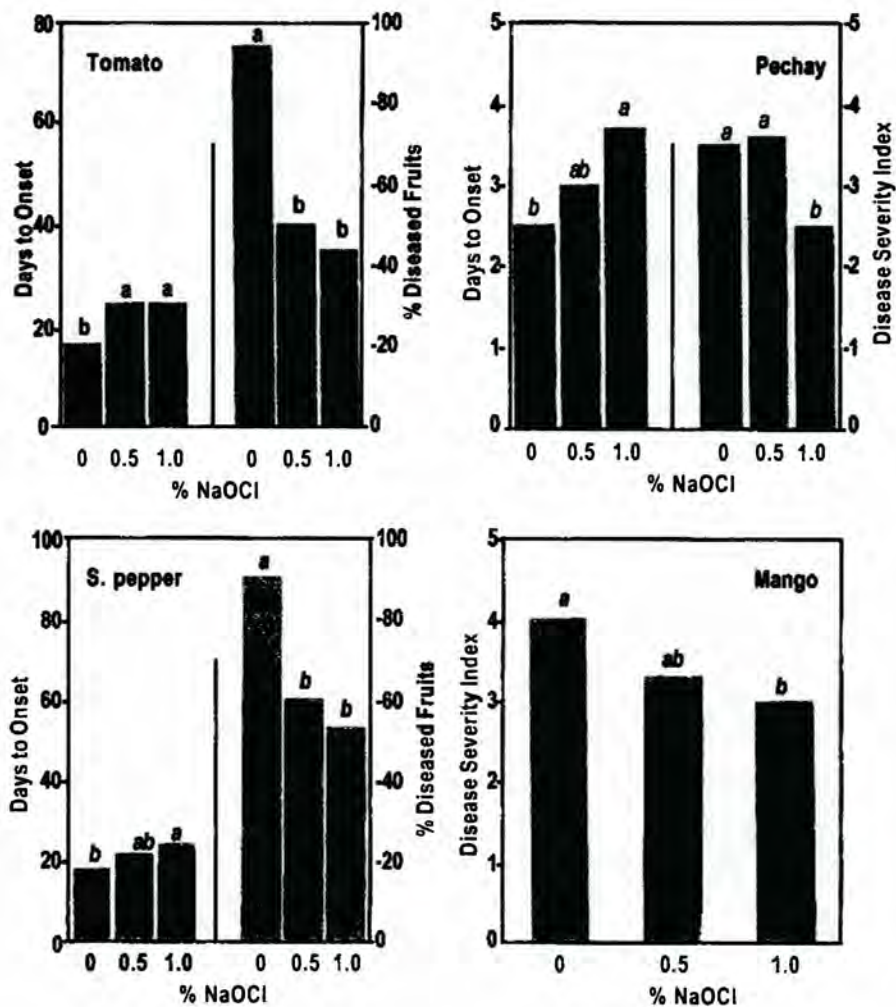


Figure 3. Disease incidence during EC storage of NaOCl-treated and untreated vegetables and fruits.

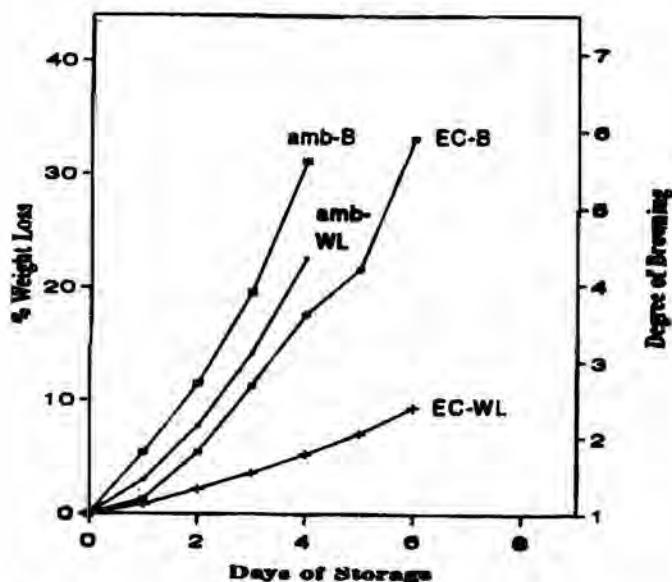


Figure 4. Weight loss and degree of browning of rambutan fruits during EC and ambient storage.

development (results not shown). From this result and that presented in Figure 4, it can be deduced that pericarp browning was primarily due to water loss. In bananas, overripening caused the fruits to deteriorate. As presented earlier in Table 2, banana ripening proceeds at comparable rates at ambient and EC condition and as a result, the fruits turned overripe and reached the end of their shelf life at comparable periods. Beneficial effects of EC storage included only reduced weight loss and shrivelling in addition to improved peel color of ripe fruits.

### CONCLUSION

Storage in the simple box-type EC was very effective in prolonging the postharvest life of tomatoes, sweet peppers, pechay, mangoes and rambutans. Two major limitations were disease development and unretarded ripening. Disease incidence can be minimized by NaOCl washing while fruit ripening can be slowed down by treatment with ethanol. In bananas, the EC and ethanol treatment did not retard ripening and as a consequence, shelf life was not prolonged. Treatments that can inhibit banana ripening need to be developed to extend fruit shelf life during EC storage. The combined application of disease and ripening control measures should (also be looked into as a strategy to further extend shelf life of EC-stored produce. More importantly, larger scale trials need to be done to establish the economics of the process and the commercial viability of the technique.

## ACKNOWLEDGMENT

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# COMPARISON BETWEEN ACUPUNCTURE ANALGESIA AND XYLAZINE-KETAMINE ANESTHESIA FOR EXPLORATORY LAPAROTOMY IN SHEEP

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## ABSTRACT

*The effectivity of conventional acupuncture analgesia using needles inserted at Acupoints 26 and 31 was compared to anesthesia induced by xylazine-ketamine for right flank exploratory laparotomy in ten sheep. Acupuncture stimulation produced surgical analgesia comparable to that induced by xylazine-ketamine combination in five out of six sheep. Respiratory and heart rates were stable, and white blood cell and differential white blood counts were higher in sheep under acupuncture analgesia, compared to the xylazine-ketamine group. The results show that acupuncture analgesia can be used as an alternative to xylazine-ketamine anesthesia in the performance of abdominal surgeries.*

*Keywords: acupuncture, analgesia, ketamine, laparotomy, pain, sheep, surgery, white blood cells, xylazine.*

## INTRODUCTION

The use of conventional anesthetics and analgesics such as ketamine and xylazine has been widely accepted in the performance of various surgical procedures in sheep. However, considering the cost, side effects and complications of using anesthetics and analgesics, acupuncture analgesia can provide an alternative due to its distinct advantages such as no side effects, superior post-operative recovery and minimal cost (Chuan, 1995).

Acupuncture analgesia has proven its effectivity for many surgical procedures in domestic animals such as dogs, pigs, cattle and horses (Klide and Kung, 1977; Lin, 1984; Yung *et al.*, 1984). However, limited studies have been conducted on the use of acupuncture analgesia in sheep (Bossut *et al.* (1986; Orilla (1980).

This study was conducted to compare the effects of acupuncture analgesia and xylazine-ketamine induced anesthesia on pain responses, physiological responses (heart rate and respiratory rate) and blood cellular responses (total white blood cell

{WBC} count, absolute and relative differential WBC counts) before, during and after right flank exploratory laparotomy in sheep.

## MATERIALS AND METHODS

### Materials

Ten one to eight-year old female Philippine sheep were randomly selected from 38 sheep of University of the Philippines College of Veterinary Medicine Experimental Animal Farm were used in the study. The sheep were subjected to standard management procedures. The sheep was fasted 18 hours before the scheduled surgery.

Xylazine 20% (Rompun) was used as a sedative and preanesthetic drug. Ketamine 100 mg/ml (Ketaset) was used to induce and maintain anesthesia. Straight round sharp filiform needles, 0.65 cm. in diameter and 7 cm long were used for acupuncture. The acupuncture needles were sterilized before being used.

### Methods

Two acupuncture points were used in the study, Acupoints 26 and 31. The acupuncture points were selected based on their degree of analgesic effect as demonstrated in a preliminary study. Acupoint 26 (Tian-Ping) is located on the dorsal midline between the spinous processes of the thirteenth thoracic vertebrae and the first lumbar vertebrae (Figure). Acupoint 31 (Bai-Hui) is located on the dorsal midline between the spinous processes of the sixth lumbar vertebrae and the first sacral vertebrae.

The ten sheep were divided randomly into two groups. Four sheep composed the xylazine-ketamine (control) group and six sheep the acupuncture (treatment) group. All sheep were subjected to right flank exploratory laparotomy.

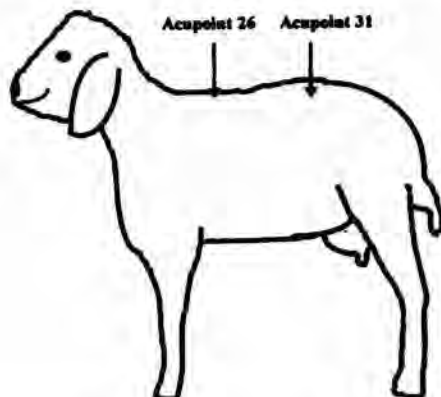


Figure 1. Acupoints used for a acupuncture analgesia in sheep.



For the control group, anaesthesia was induced via administration of xylazine (0.15 mg/kg body weight) intramuscularly followed by the administration of ketamine (11 mg/kg body weight) intramuscularly five minutes after xylazine administration.

For the treatment group, acupuncture analgesia was induced via insertion of acupuncture needles on Acupoints 26 and 31. The acupoint areas were disinfected using 1% iodine solution and 70% isopropyl alcohol prior to acupuncture needle insertion. The needles were inserted perpendicularly for about two to three cm deep depending on the size of the animal. The needles were stimulated via continuous rotation and thrusting for a duration of one minute and repeated every five minutes. Stimulation was continued for the whole duration of the surgery.

The surgical area in the right flank was clipped prior to induction of acupuncture analgesia or xylazine-ketamine anesthesia. After induction of anesthesia for the control group, each sheep was placed in left lateral recumbency and prepared for final asepsis and draping. For the treatment group, each sheep was restrained at left lateral recumbency on the surgical table. Acupuncture needle insertion then followed after which final aseptic preparation of the surgical area and draping was made.

After reaching the desired level of anesthesia, an 8-10 cm vertical skin incision was made in the paralumbar fossa midway between the last rib and the tubercosae and about 2-3 cm ventral to the transverse processes of the lumbar vertebrae. The skin incision was continued deeply, cutting through the fibers of the external abdominal oblique muscles and internal abdominal oblique muscles and exposing the transverse abdominal muscles. An incision through the transverse abdominal muscle and peritoneum was made and extended with scissors allowing entry into the abdominal cavity.

After opening the abdominal cavity, the cecum and a substantial length of the intestines were exposed and exteriorized. The rumen, abomasum, right kidney and pelvic organs were palpated. Exploratory laparotomy lasted for approximately 15 min before the incision was closed.

Closure of the laparotomy was achieved by three layers of suture. First, the peritoneum and the transverse abdominal muscles were closed together with a 3-0 chromic cat-gut using simple continuous suture pattern. The internal and external abdominal oblique muscles were closed with a second continuous layer using a 3-0 chromic cat-gut. The skin incision was closed with a simple interrupted suture pattern using a 3-0 silk and disinfected. Prophylactic antibiotic (250,000 units of procaine penicillin and 250 mg Dihydrostreptomycin per 10 kg body weight), was administered intramuscularly to each sheep once a day for three days.

Pain response to the surgery was graded as follows: 1) Good, if there was no pain response to surgery; 2) Fair, if responses were noticed but the operation can be accomplished smoothly; and 3) Poor, if intensive pain responses and struggling was present and the operation can no be carried out; in such case surgery was discontinued.

For both groups, blood samples were collected ten minutes prior to induction of analgesia or anesthesia and ten minutes after surgery. Total WBC and relative and absolute differential WBC counts were determined using standard procedures. Heart

and respiratory rates were monitored every ten minutes prior to xylazine administration or acupuncture stimulation. Monitoring lasted until last ten minutes after recovery from anesthesia and until ten minutes after surgery, for control and treatment groups, respectively.

The pain response to surgery of the control and treatment group was compared and the differences in the mean values of the blood cellular and physiological responses for both groups were analyzed statistically.

## RESULTS

All sheep responded to pain stimuli induced by pin prick and pinch probe applied on the surgical site prior to xylazine-ketamine administration or acupuncture stimulation and after the sheep were restrained with ropes in the treatment group. The sheep would kick towards the offending stimuli, grind their teeth, raise and shake their head violently and struggle hard.

All sheep subjected to right flank exploratory laparotomy in the control group were graded as good on their pain responses (Table 1). The animals stayed asleep during the course of surgery up to 10-25 minutes after surgery. For the treatment group, five out of six sheep operated under acupuncture analgesia were graded as good and one was graded as fair. For those animals grade as good, the analgesia produced can be described as comparable to xylazine-ketamine anesthesia. There were no pain responses observed, no struggle, no unnecessary movement that interfered with the performance of the surgical procedure. The five sheep stayed calm and did not respond to noise stimuli.

For one sheep graded as fair, there was minimal movement such as raising of head, movement of hindlimbs, and raising of body when there is excessive tissue manipulation such as during stretching of the flank muscles and clamping of the viscera. This was observed during the early part of the surgery. The needle inserted as Acupoint 3 I was then withdrawn and reinserted allowing surgery to be accomplished smoothly with no observable indication of pain during suturing of the flank muscles, subcutaneous tissue and skin.

Table 1. Pain responses of sheep (no. of animals) subjected to right flank exploratory laparotomy under either xylazine-ketamine anesthesia or acupuncture analgesia.

Group*	Pain Response		
	Good	Fair	Poor
Control	4	0	0
Treatment	5	1	0

Control: Xylazine-ketamine group; Treatment: Acupuncture group.

All sheep in the treatment group stayed awake throughout the course of surgery. After the surgery, the animals readily rose up and were allowed to walk by themselves towards their pen.

Table 2 shows the mean WBC counts of the sheep in the two groups ten minutes before anesthesia or analgesia and ten minutes after recovery. In the control group, there was a significant decrease in the total WBC count after recovery ( $p < 0.05$ ). In the treatment group, the WBC count increased significantly after recovery ( $p < 0.05$ ).

Table 3 shows the mean relative differential WBC counts of the sheep in the two groups. In the control group, the eosinophil count was lower while the lymphocyte and neutrophil counts were higher after recovery than before surgery but the differences were not significant ( $p > 0.05$ ). In the treatment group, the monocytes, eosinophils and neutrophils increased significantly after recovery ( $p < 0.05$ ). Table 4 shows the mean absolute differential counts of the sheep. All components of WBC in the control group decreased after recovery, but the differences were not significant ( $p > 0.05$ ). In the treatment group, segmented neutrophils increased significantly after recovery ( $p < 0.05$ ) (Table 5). There was a decrease in the heart rate during surgery in the control group as compared to that before surgery ( $p < 0.05$ ) (Table 6). In the treatment group, there were no changes in the respiratory and heart rates before, during and after surgery.

### DISCUSSION

In small ruminants such as sheep and goats, provision of suitable restraint procedures can include physical restraint, chemical restraint or acupuncture analgesia.

Xylazine-ketamine combination is one of the most common drug combinations used in inducing anesthesia both in small and large animal species. The dose range used in the study has been reported to produce the best sedation, analgesia, and muscle relaxation without side effects (Kurdele et al., 1988). It also provided sufficient muscle relaxation and post-operatively no adverse effects was observed. Sheep are the most sensitive domestic animals to the effect of xylazine, producing deep sedation and

Table 2. Total WBC counts ( $/\alpha$ ) of sheep before and after right flank exploratory laparotomy under either xylazine-ketamine anesthesia or acupuncture analgesia.

Group*	Time of Collection			
	Before mean	SD	After mean	SD
Control	11846.9 <sup>a</sup>	2379.4	10118.8 <sup>b</sup>	3739.3
Treatment	11893.8 <sup>a</sup>	1742.5	15364.6 <sup>b</sup>	2999.6

Control: Xylazine-ketamine group, Treatment: Acupuncture group.

† Values with different superscripts in the same row are different ( $p < 0.05$ ).

Table 3. Relative differential WBC counts (%) of sheep before and after surgery under either xylazine-ketamine anesthesia or acupuncture analgesia.

Group*	Relative Differential WBC Count†			
	Before		After	
	mean	SD	mean	SD
<b>CONTROL</b>				
Lymphocyte	45.2 <sup>a</sup>	3.6	51.2 <sup>a</sup>	6.8
Eosinophil	11.5 <sup>a</sup>	0.6	1.0 <sup>a</sup>	0.8
Band Neutrophil	0.8 <sup>a</sup>	0.5	1.0 <sup>a</sup>	0.8
Segmented neutrophil	52.5 <sup>a</sup>	4.0	46.8 <sup>a</sup>	7.3
<b>TREATMENT</b>				
Lymphocyte	58.8 <sup>a</sup>	10.1	52.0 <sup>a</sup>	5.8
Eosinophil	0.7 <sup>a</sup>	0.8	0.8 <sup>b</sup>	0.8
Band Neutrophil	1.7 <sup>a</sup>	0.5	1.5 <sup>a</sup>	0.8
Segmented neutrophil	38.3 <sup>a</sup>	10.6	45.3 <sup>a</sup>	4.5

Control: Xylazine-ketamine group, Treatment: Acupuncture group.

†Values with different superscripts in the same row are different ( $p < 0.05$ ).

Table 4. Absolute differential WBC counts (/ $\mu$ l) of sheep before and after surgery under either xylazine-ketamine anesthesia or acupuncture analgesia.

Group*	Absolute Differential WBC Count†			
	Before		After	
	mean	SD	mean	SD
<b>CONTROL</b>				
Lymphocyte	5408.6 <sup>a</sup>	1494.2	5090.6 <sup>a</sup>	1793.3
Eosinophil	81.6 <sup>a</sup>	59.8	187.4 <sup>a</sup>	103.9
Band Neutrophil	85.7 <sup>a</sup>	69.1	86.9 <sup>a</sup>	62.7
Segmented neutrophil	4823.4 <sup>a</sup>	2364.0	6163.9 <sup>a</sup>	952.8
<b>TREATMENT</b>				
Lymphocyte	6985.8 <sup>a</sup>	1168.4	8035.7 <sup>a</sup>	1869.7
Eosinophil	76.4 <sup>a</sup>	92.5	120.7 <sup>a</sup>	109.1
Band Neutrophil	195.5 <sup>a</sup>	53.4	217.8 <sup>a</sup>	126.0
Segmented neutrophil	463.18 <sup>a</sup>	1306.9	693.72 <sup>b</sup>	1263.7

Control: Xylazine-ketamine group, Treatment: Acupuncture group.

†Values with different superscripts in the same row are different ( $p < 0.05$ ).

Table 4. Relative differential WBC counts (%) of sheep before and after surgery under either xylazine-ketamine anesthesia or acupuncture analgesia.

Group*	Absolute Differential WBC Count <sup>†</sup>			
	Before		After	
	mean	SD	mean	SD
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Band Neutrophil	85.7 <sup>a</sup>	69.1	86.9 <sup>a</sup>	62.7
Segmented neutrophil	4823.4 <sup>a</sup>	2364.0	6163.9 <sup>a</sup>	952.8
<b>TREATMENT</b>				
Lymphocyte	58.8 <sup>a</sup>	10.1	52.0 <sup>a</sup>	5.8
Eosinophil	0.7 <sup>a</sup>	0.8	0.8 <sup>b</sup>	0.8
Band Neutrophil	1.7 <sup>a</sup>	0.5	1.5 <sup>a</sup>	0.8
Segmented neutrophil	38.3 <sup>a</sup>	10.6	45.3 <sup>a</sup>	4.5

Control: Xylazine-ketamine group; Treatment: Acupuncture group.

†Values with different superscripts in the same row are different (p<0.05).

Table 5. Respiratory rates (breaths/min) of sheep before, during and after surgery under either xylazine-ketamine anesthesia or acupuncture analgesia.

Group*	Respiratory Rates <sup>†</sup>					
	Before		During		After	
	mean	SD	mean	SD	mean	SD
Control	72.7 <sup>a</sup>	44.4	57.3 <sup>a</sup>	33.7	58.8 <sup>a</sup>	29.8
Treatment	45.0 <sup>a</sup>	9.6	57.0 <sup>a</sup>	10.6	50.2 <sup>a</sup>	12.6

Control: Xylazine-ketamine group; Treatment: Acupuncture group.

†Values with different superscripts in the same row are different (p<0.05).

Table 6. Heart rates (beats/min) of sheep before, during and after surgery under either xylazine-ketamine anesthesia or acupuncture analgesia.

Group*	Heart Rates <sup>†</sup>					
	Before		During		After	
	mean	SD	mean	SD	mean	SD
Control	105.0 <sup>a</sup>	10.0	582.0 <sup>b</sup>	13.0	78.5 <sup>b</sup>	1.9
Treatment	99.5 <sup>a</sup>	11.2	100.5 <sup>a</sup>	22.6	102.8 <sup>a</sup>	23.8

Control: Xylazine-ketamine group; Treatment: Acupuncture group.

†Values with different superscripts in the same row are different (p<0.05).

analgesia (Booth and McDonald, 1982). Xylazine-ketamine combination provides good degree of muscle relaxation, analgesia and anesthesia in ruminants (Gray and Wayne, 1986).

Several studies have demonstrated the analgesia-inducing effect of acupuncture in many surgical procedures in other domestic animals. The present study proves that acupuncture analgesia using two points, Tian-ping (Acupoint 26) and Bai-hui (Acupoint 31), can produce sufficient analgesia in the performance of right flank exploratory laparotomy in sheep. Acupoints 26 and 31 are two of the many sets of acupoints which can be used for acupuncture analgesia in the performance of abdominal surgery in ruminants (Chuan, 1995).

In the present study, acupuncture analgesia was comparable with xylazine-ketamine anesthesia in several aspects. Firstly, the sheep appear to be drowsy that rough movement in the surgery area, presence of other sheep and production of noise did not elicit any distractive response from the sheep. Such finding is in agreement with Whie *et al.* (1985) on the use of electroacupuncture analgesia for laparotomies in two dairy cows. Cockrill (1975) observed that rumenotomy in a four year old bull under acupuncture analgesia alone allowed partial evacuation of rumen contents, regurgitation, chewing and reswallowing.

Secondly, acupuncture provides enough analgesia to the flank skin muscles as depicted by no painful response during incision, tissue clamping, stretching and suturing. In cows, the acupuncture analgesia effect was described to be similar in many respects to paralumbar nerve block (White *et al.*, 1985). O'Boyle and Vajda (1975), observed excellent analgesia in several cases of dogs subjected to various abdominal surgeries under acupuncture analgesia alone.

Thirdly, acupuncture provides sufficient analgesia in the abdominal viscera. Tissue clamping, invasive palpation of the abdominal organs, exposure of the intestines with subsequent stretching and replacement did not elicit any painful response.

In comparison with xylazine-ketamine anesthesia, acupuncture analgesia provides additional advantage because it allows surgery in an awake sheep; thus, producing superior post-operative recovery. The sheep could rise up and walk right after the surgery (Chuan, 1995). Such aspects is of considerable advantage because it decreases the length of time the animal stays recumbent with concurrent hypoxemia (Gray and Wayne, 1986). In addition, animals operated under acupuncture analgesia are assured of no adverse and toxic effects of general and local anesthetics.

A decrease in the respiratory rates and heart rates of sheep under xylazine-ketamine anesthesia was observed in the present study. However, these values were within normal ranges. Denghani *et al.* (1991) and Coulson *et al.* (1989) also reported decrease in heart rates and respiration rates with this combination. Because of this action, xylazine-ketamine combination is contradicted in animals with compromised heart function.

Compared to xylazine-ketamine anesthesia, animals operated under acupuncture analgesia had stable respiratory rates and heart rates. This is in agreement with several studies using acupuncture in different animals (Dill *et al.*, 1988; Janssens *et al.*, 1979;

Lakshmi pathi and Ramakrishna, 1988; O'Boyle and Vajda, 1975; Wright and McGrath, 1981). The absence of changes in the respiratory and heart rates of sheep during and after acupuncture stimulation makes acupuncture analgesia an excellent alternative to anesthetic risk patients (Celo and Luzuriaga, 1980; O'Boyle and Vajda, 1975).

Although there was reductions in the total WBC and differential WBC counts in sheep under xylazine-ketamine anesthesia, in agreement with previous studies (Komar, 1988; Moore *et al.*, 1988), the values obtained were within normal ranges. Induced stress to the sheep might play an important role in the decrease in the WBC of sheep after xylazine-ketamine anesthesia and exploratory laparotomy. It has been reported that stress is one of the culprit of decrease in WBC count (Coles, 1980). There is, however, no explanation on how xylazine-ketamine anesthesia exerts a direct affect on these changes in the WBC pictures of sheep and even in other animals.

The WBC, lymphocyte and neutrophil counts observed in sheep under acupuncture analgesia were above normal levels. In humans, other studies reported an increase (Kita *et al.*, 1979; Lin *et al.*, 1980), decrease (Cracium *et al.*, 1973) and a decrease followed by an increase (Omura, 1979) in the leucocyte count after acupuncture stimulation. Leucocytosis indicates introduction of foreign substances to the body or disease invasion. It requires proper stimulation and presence of stimulating agents such as antigen and chemotactic factors which generally indicates bacterial or viral disease and tissue damage (Tizard, 1992; Schalm, 1982). Whether acupuncture has a direct effect on leucocytosis can not be explained in this study. The relationship between acupuncture analgesia and its role as a possible immunostimulant has to be investigated further.

The above results show that acupuncture analgesia can be used as an alternative method in the performance of abdominal surgeries in sheep, in place of xylazine-ketamine anesthesia, with less side effects, minimal cost and superior post-operative recovery.

#### ACKNOWLEDGMI

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# PHILIPPINE FRESHWATER RESOURCES: STRATEGIES FOR SUSTAINABLE DEVELOPMENT

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## ABSTRACT

*Water is essential for life and sustainable human development. Despite its vast freshwater resources consisting of lakes, swamps, rivers, reservoirs and groundwater, the Philippines has an impending "water crisis" because of rapid depletion and deterioration of such resources brought about by increasing demand for human, agricultural and industrial.*

*Policies and laws for national conservation and management of the country's freshwater resources seem adequate. There is an urgent need, however, to restructure and strengthen the national coordinating and regulating agency for water, promote public and private participation for rehabilitation of watersheds and the efficient delivery of water services as well as the exercise of political will for water policy/law enforcement to ensure sustainable development of such vital resources.*

## INTRODUCTION

Water is essential for life and sustainable human development. Freshwater constitutes only 2.7% of the earth's water resources. Two-thirds of the water from rainfall goes back to the atmosphere through evaporation. Of the remaining water, more than one-half flows out into the sea. Only 8% of the world's freshwater is used for human and sanitation needs, while 60-70% is used for agriculture and 20% for industry (Singh, 1996).

The Philippines has a rainfall of 2500 mm and freshwater resources consisting of surface inland waters (lakes, rivers, reservoirs and swamps) and groundwater. There are about 70 lakes in the country with an aggregate area of 200,000 hectares and 106,328 hectares of freshwater swamps. There are also 424 principal rivers and 30,000 hectares of dams and reservoirs in the country. The groundwater in the aquifers of the Philippines has been estimated to be under a 100,000 square kilometer area (Guerrero, 1995).

There is an impending freshwater crisis in the world because of the increasing demand for human, agricultural and industrial needs, and the depletion as well as contamination of freshwater resources. The water availability per person in Asia that was 10,000 m<sup>3</sup> in 1950 is expected to be only 3,000 m<sup>3</sup> per person by the year 2000 (Singh, 1996). In the Philippines, only 76% of the population had access to potable water in 1992.

This paper will assess the current status of freshwater resources in the Philippines, review water conservation and management policies and discuss strategies for the sustainable development of such resources.

### STATUS OF FRESHWATER

In 1950, per capita water availability in the Philippines was 9,600 m<sup>3</sup>. It is now down to about 3,300 cubic meters. Destruction of forest watersheds and drainage of wetlands have reduced the capacity of such resources to hold and release water. The country's present forest cover is much lower than the optimal 54% (Liew, 1996). There has been a drop of 30-50% in the country's water resources in the past 20 years (Alejandrino, 1996).

As of 1994, there were 1.532 million hectares of agricultural land in the country which were provided with irrigation facilities. The effectively irrigated area only represents 49% of the total irrigable area of 3.126 million hectares. With lahar flows in Central Luzon and decreasing water supply due to watershed denudation, irrigation efficiencies in the country have decreased (NIA, 1991). Water use efficiency for rice irrigation in the Philippines is only 30-40% in the wet season and 40-60% in the dry season (Spurgeon, 1995).

Major lakes and reservoirs in the country are adversely affected by sedimentation due to watershed denudation and soil erosion. The fisheries productivity of such waters has declined because of water quality deterioration and habitat destruction. The hydroelectric power generating capacity of heavily silted dams has been much reduced.

The future of Laguna de Bay, the country's largest lake, is threatened with man-induced stresses as sedimentation, over-exploitation of its fisheries and pollution. Forest cover of the lake's watershed has been reduced from 93,000 hectares in 1963 to less than 18,000 hectares in 1988 because of human activities. Soil erosion from deforested areas is the main contributor of sediment to the lake. The sediment loading has been estimated at 1.5 million cubic meter per year (Guerrero, 1996).

Of the 74 designed water quality monitoring stations surveyed in various parts of the country, 65% showed water quality already deteriorated beyond the beneficial use of the stations. Forty-seven percent of the river stations surveyed had water quality lower than the worst classification. Some 40 rivers which are major sources of water for industry and major population centers were found to be polluted to a degree of critical concern (NWRB, 1995).

Groundwater nitrate levels in some agricultural areas in Ilocos Norte have been found to be almost double the safe limits. Endosulfan, a commonly used pesticide, was found in 79% of well water samples in rice growing areas of the country. The levels of toxic metals such as mercury, lead, cadmium and zinc have been found to be increasing in Laguna de Bay (Tuong, 1955).

Saltwater intrusion of aquifers in heavily urbanized areas as Metro Manila as a result of overpumping of groundwater has been reported (Haman, 1996).

### **WATER CONSERVATION AND MANAGEMENT POLICIES**

There appears to be adequate laws and regulations for the protection, conservation and management of the country's freshwater resources. Among others, such laws include the creation of a National Water Resources Council, a Water Code, the establishment of the Presidential Committee on Water Conservation and Demand Management and the National Water Crisis Act.

Presidential Decree No. 424 (1974) created the National Water Resources Council (NWRC) to coordinate and integrate water resources development in the country for social and economic progress and to meet present and future needs for water. The NWRC which was later restructured into the national Water Resources Board is tasked to "coordinate and integrate on a sound and logical basis the national plans and policies for the appropriation and utilization of surface and groundwater; undertake river basin surveys and establish, operate and maintain observation networks and a centralized water resources data center for the scientific surface and appraisal of surface and groundwater potentials of the country; and to conduct or promote special studies and researches with other government or private agencies on related aspects of water resources development."

Presidential Decree No. 1067 (1976) instituted a Water Code which consolidated laws governing the ownership, appropriation, utilization, exploitation, development, conservation and protection of the country's water resources subject to the control and regulation of the government through the National Water Resources Council.

The Philippine Constitution of 1987 provides that the exploration, development and utilization of natural resources (including water) to be under the full control and supervision of the State (Article XII).

Executive Order No. 222 (1995) established the Presidential Committee on Water Conservation and Demand Management to prepare a nationwide Water Conservation Plan to cover conservation measures focusing on both the quality and quantity of water and undertake a nationwide information campaign. The Committee came about because of the "water crisis" that impelled the need for a national water program to be implemented through coordinative, consultative and mutually supportive mechanisms where the private sector plays an active role."

The National Water Crisis Act of 1995 (Republic Act No. 8041) addresses the country's water problem through an integrated water management program and the development of new water resources and the conservation of identified watershed, among other provisions.

### **STRATEGIES FOR SUSTAINABLE DEVELOPMENT OF FRESHWATER RESOURCES**

Sustainable development implies the utilization of the earth's natural resources (including water) "in a regenerative manner so as to preserve them not only for the present generation but for all generations to come" (La Vina, 1991).

Despite the existing constitutional and legal framework governing the utilization of the country's freshwater resources on a supposedly rational and sustainable basis, the depletion and contamination of such resources due to the factors earlier mentioned go on unabated at an alarming rate. Why is this so?

According to B. Gujja of the World Wildlife Fund International's Freshwater Programme, ensuring the availability of freshwater on a sustainable basis requires an ecosystems and cross-sectoral approach. There is need to manage water resources considering "social equity and ecological sustainability" (Singh, 1996).

Various strategies and action programs have been proposed for the sustainable development of freshwater resources in the Philippines.

The National Irrigation Administration (1991) proposed the following measures for maximizing use of available water:

1. Construction of reservoir-type projects
2. Redesign of irrigation facilities to reuse return flows
3. Engineering measures to minimize water conveyance losses
4. Involvement of farmers for better planning of cropping calendars and irrigation delivery schedules.

Cruz (1995) endorsed the holistic, integrated and systematic approach for effective planning and management of the country's watersheds as a multi-resource with social equity and multi-sectoral participation.

Liew (1996) recommended the following action plans for addressing the country's "water crisis":

- i. Formulation of policies that stress integrated water resource management;
2. Mapping out of a water resource management system which should include the inventory of available land and water resources, projection of development potential of water resources, demand-management action plans projection of community water demand and environmental impacts assessment for future development projects.

3. Decentralizing water projects such as irrigation, sewerage and flood control to local government units (LGUs);
4. Rehabilitation of deforested watersheds for minimizing floods and soil erosion; and
5. Promoting reforms in the water service sector such as mobilizing private sector participation and strengthening the ability of water supply institutions to serve their clientele.

There is also an urgent need for legislative action to restructure and structure and strengthen to national coordinating and regulating agency for water resources, the National Water Resources Board. While the NWRB has the legal mandate to carry out the herculean tasks of conserving and managing the country's freshwater resources, it does not have the control and financial capability to carry them out.

Water conservation and management should be every citizen's concern. Thus, there is need for public information dissemination to instill awareness and discipline for water saving practices and consciousness.

Lastly, the strategy for exercising political will at all levels of society for the implementation of water development based on ecological sustainability and the unrelenting enforcement of water laws and policies is strongly urged.

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# MYCORRHIZAE FROM SELECTED TREE SPECIES IN MT. PANGASUGAN, LEYTE, PHILIPPINES AND THEIR EFFECT ON TREE GROWTH

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## ABSTRACT

*A study was conducted to determine and identify indigenous mycorrhizae associated with selected tree species growing in Mt. Pangasugan, evaluate grasses as trap crop for their culture and mass production, test their efficacy for tree growth improvement and evaluate the most efficient delivery system for the mycorrhizae. There were 14 tree species sampled and based on root sample assay seven (7) were found positive for mycorrhizal association. These were Kaliandra (*Calliandra calothyrsus*), Antoso-an (*Cassia javanica* L.), Paguringon (*Craoxylum celebicum* Blume), Fire tree (*Delonix regia* (Bojer Hook) Raf.), Yamane (*Gmelina arborea* Roxb.), Ipil-ipil (*Leucaena leucocephala* (Lamarck) de Wit) and Narra (*Pterocarpuz indicus* Willd). However, all the soil samples contained vesicular-arbuscular mycorrhizal (VAM) spores with Paguringon having the highest spore count of 359 while Dao had the lowest count of 65 spores. Four major genera of VAM fungi were identified associated with the tree species namely: *Glomus*, *Gigaspora*, *Acaulospora* and *Sclerocystis*. All the three grasses evaluated as trap crops supported the build up of mycorrhizal fungi with Napier grass producing the heaviest roots (21.57 g) and containing the highest mycorrhizal spores (476). In terms of inoculum produced in the soil there was an increase of 61.6%, 48.1% and 39.8% for Paragrass, Napier grass and Guinea grass, respectively after 3 months. Mycorrhizal roots and soil containing mycorrhizae as inoculum increased both the height (cm) and stem diameter of tree seedlings tested in the screenhouse and field. The general observation was that the soil with mycorrhizae had greater effect on seedling growth.*

**Keywords:** *Mycorrhizae, Vesicular-arbuscular-mycorrhizae, Glomus, gigaspora, Acaulospora, Sclerocystis, Seedling growth, Mycorrhizal spores, Tree species Trap crop*



## INTRODUCTION

Mycorrhiza is a symbiotic relationship between a fungus and the roots of plants. This association is widespread in the plant kingdom. The fungus derives all its food requirements from the plants. In return, the fungus provides benefit to the plants such as: increased absorption of nutrients and water, increased drought resistance of plants, control of root pathogen infection, production of growth promoting substances, promotion of the activity of other beneficial organisms and improvement of soil structure and soil aggregation (Kothari et al., 1990 and Dela Cruz, 1993).

Mycorrhizal fungi are classified into ectomycorrhiza and endomycorrhiza. Endomycorrhiza is found in many trees such as narra, ipil-ipil, kakawate, yemane, falcata, mangium, rain tree and others. The ectomycorrhizal fungi form a loose network of hyphae on the root surface. Endomycorrhiza invade the cortical cells where they develop spherical to ovate structures (vesicles) and multibranched hyphae (arbuscules), thus called vesicular-arbuscular mycorrhiza (VAM). The VAM fungi belong to the family Endogonaceae a lower form of fungi resembling molds.

There has been considerable interest in vesicular-arbuscular (VA) mycorrhizal fungi in the past two decades because of the overwhelming published evidence indicating their growth promoting aspects. In a study conducted by Bagyaraj et al. (1989) they screened seven VA mycorrhizal fungi for symbiotic response with Hawaiian giant cultivars of *Leucaena leucocephala*. They found three promising ones from a preliminary trial conducted with fungi obtained from different parts of the world and the other four were local isolates obtained from the rhizosphere of *Leucaena*. A local isolate of *Glomus mosseae* was found to be the best mycorrhizal fungi for inoculation of *Leucaena* in order to obtain healthy, vigorously growing seedlings.

According to Kothari et al. (1990) root infection with vesicular-arbuscular (VA) mycorrhizal fungi can increase efficiency of nutrient absorption and enhance growth and development of mycorrhizal plant particularly in soil with low phosphorus. They noted that VA mycorrhizal association may alter plant-water relations since more rapid recovery from water stress and higher soil moisture extraction at low water potential have been observed in mycorrhizal plants. On the other hand, Dela Cruz (1993) considered mycorrhiza as a biofertilizer because it contains beneficial fungi that can increase the absorption of nutrients that are already in the soil. He said that the fungus that can increase the absorption of nutrients that are already in the soil. He said that the fungus has the ability to increase efficiency and recover of bound nutrients from the soil. The addition of chemical fertilizers had helped improve the survival and growth rates of tree seedlings in reforestation sites. However, the cost of chemical fertilizers is very expensive. It is also detrimental to the soil microfauna and other soil-inhabiting beneficial microorganisms when continuously used. Other alternative technologies to replace these chemical fertilizers is important.

Mycorrhiza is one of the alternative technologies known to substitute for chemical fertilizers. Once the fungus invades plant roots, it can proliferate within the roots as the latter grows. There is no need to reinoculate the plants especially in the field. It is convenient to inoculate reforestation seedlings during the nursery phase. In addition, mycorrhiza is safe to use since it is part of the natural reforestation. This study was conducted with the following objectives: (1) to determine and identify indigenous mycorrhizae associated with selected tree species growing in Mt. Pangasugan, (2) assess trap crop for the culture and mass production of potential mycorrhizae, (3) test their efficacy for tree growth improvement and (4) evaluate the most efficient delivery system for these mycorrhizae.

### MATERIALS AND METHODS

Different tree species at the closed canopy area in Mt. Pangasugan were tagged. Soil and root samples were collected from them. The root samples were then processed following the standard procedures by Kormanik and McGraw (1983). Likewise, the soil samples were processed for mycorrhiza following the sucrose-flotation method of Allen et al. (1979).

Soil collected from different tree species was thoroughly mixed to 1 part sand and was placed in 20 cm pot. The potted sand-soil mixture was planted with Guinea grass (*Panicum maximum* Jacq.); Napier grass (*Pennisetum purpureum* Schumach.) and Paragrass (*Brachiaria mutica* (Forssk.) Stapf.) as hosts for mycorrhizal culture and mass production. The initial mycorrhizal spore count in the soil prior to planting the grasses was determined. Build up of mycorrhizal fungi in these three grass species was assessed three months after planting. Percent increase of mycorrhizal fungi population in the soil was computed.

#### Screenhouse Experiment

Tree seedlings namely: Dao (*Dracontomelon dao* (Branco) Merrill et Rolfe, Paguringon (*Cratoxylum celebicum*) Blume, Hindang, Bagalunga (*Melia dubia* Caranilles), and Kalumpit (*Terminalia microcarpa* Decaisne) acquired from the GTZ nursery area were evaluated for mycorrhizal infectivity using mycorrhizal roots and mycorrhizal soil as inocula. Data on plant height and stem diameter were taken before VAM inoculation and at monthly interval until five months after VAM inoculation. Roots and soil samples were collected and processed following the procedures stated earlier. Infection of VAM in roots was determined by randomly picking 30- 1 cm root segments, placed on a slide and observed under the microscope and the percent infection was computed. VAM spores extracted from soil samples were counted under the microscope with the aid of a multiple hand tally counter. The following treatments were used: T1-uninoculated; T2-inoculated with 100 g soil with mycorrhizae and T3-inoculated with 25 g mycorrhizal roots. Each treatment was replicated three times.

## Field Experiment

Evaluation of the effective mycorrhizal fungi for tree growth and development under field condition was conducted. The experiment was set up at Punta, Baybay, Leyte using Narra and Mahogany seedlings. Soil samples were randomly collected in the area. These were processed in the laboratory to assess the population of native VAM fungi present in the area. One month after transplanting, the seedlings were inoculated with VAM fungi. Data on plant height and stem diameter were taken before and after inoculation until 12 months. The following treatments were used; T1-uninoculated control; T2-inoculated with 25 g mycorrhizal roots; T3-inoculated with 25 g mycorrhizal roots + 50 g MYCOVAM 1 and T4-inoculated with 50 g MYCOVAM 1. Each treatment was replicated five times.

## RESULTS AND DISCUSSION

### Determination and Identification of Mycorrhizae

There were fourteen (14) tree species sampled namely: Ayangili (*Acacia confusa* Merr.), Kariskis (*Albizia lebbekoides* (D.C.) Berth.), Kaliandra (*Calliandra calothyrsus*), Antoso-an (*Cassia javanica* L.), Kapok (*Ceiba pentandra* (L.) Gaertn.), Paguringon (*Cratogeomys celebicum* Blume), Fire tree (*Delonix regia* (Bojer Hook) Raf.), Dao (*Dracontomelon dao* (Branco) Merrill et Rolfe), Ani-I (*Erythrina fusca* (Lour.)), Dapdap (*Erythrina orientalis* L. (Murr.)), Yemane (*Gmelina arborea* Roxb.), Ipil-ipil (*Leucaena leucocephala* (Lamarck) de Wit), Narra (*Pterocarpus indicus* Willd) and Anabiong (*Trema orientalis* (L.) Blume). Of these tree species, seven (7) were identified to have mycorrhizal association through root sample assay. These included: Kaliandra, Antoso-an, Paguringon, Fire tree, Yemane, Ipil-ipil and Narra. However, all the soil samples (150 g) collected from these tree species contained various VAM spores with the soil sample from Paguringon having the highest spore count of 359 spores while the soil sample from Dao had the least count of 65 spores (Table 1). Mean spores count in soil samples collected from these tree species was 180 spores.

Four major genera of VAM fungi were identified associated with trees in Mt. Pangasungan namely: *Glomus*, *Gigaspora*, *Acaulospora* and *Sclerocystis*. Description of each genera is presented in Table 2.

### Mass Production/Culture of Mycorrhizae

All the three grasses tested as trap crops supported the build up of mycorrhizal fungi as revealed by VAM spore count in both the soil and root samples. Napier grass produced the heaviest roots (21.57 g) and contained the highest mycorrhizal spores in the roots (476 spores). This was significantly different to Guinea and Para grasses (Table 3). In terms of inoculum production in the soil, spore count after 3 months was 467, 374 and 299 spores or an increase of 61.62%, 48.12% and 39.80% for Paragrass, Napier grass and Guinea grass, respectively.

Table 1. Number of mycorrhizal spores from the soil and roots collected from selected tree species at the closed canopy area in Mt. Pangasugan, Leyte, Philippines.

Scientific Name	Common Name	Mycorrhizal Spores	
		Soil <sup>1</sup>	Roots <sup>1</sup>
<i>Acacia confusa</i> Merr.	Ayangili	325	-
<i>Albizia lebbekoides</i> (D.C.) Berth	Kariskis	187	-
<i>Calliandra calothyrsus</i> .	Kaliandra	331	+
<i>Cassia javanica</i> (L.)	Antoso-on	213	+
<i>Ceiba pentandra</i> (L.) Gaertn.	Kapok	80	-
<i>Cratogeomys celebicum</i> Blume	Paguringon	359	+
<i>Delonix regia</i> (Bojer Hook) Raf.	Fire tree	141	+
<i>Draxontomelon dao</i> (Branco) Merrill et Rolfe	Dao	65	-
<i>Erythrina fusca</i> Lour.	Ani-i	151	-
<i>E. orientalis</i> (Murr) L.	Dapdap	84	-
<i>Gmelina arborea</i> Roxb.	Yemane	97	+
<i>Leucaena leucocephala</i> (Lamarck) de Wit	Ipil-ipil	178	+
<i>Pterocarpus indicus</i> Willd	Narra	167	+
<i>Trema orientalis</i> (L.) Blume	Anabiong	144	-

<sup>1</sup>Based from 150 g soil sample<sup>2</sup>+and-indicates presence or absence of mycorrhiza

Table 2. Description of genera of vesicular arbuscular mycorrhiza (VAM) fungi associated with selected tree species in Mt. Pangasugan, Leyte, Philippines

GENERA	DESCRIPTION
<i>Acaulospora</i>	Azygospore produced singly in soil, large generally globose or subglobose, with oily content, borne laterally on the stalk of a large, terminal, thin-walled vesicles. Vesicles about the same size as the spore. Spore walls continuous except for a small occluded spore. Germ tubes produced directly through walls near spore base. Forming endomycorrhizae with lobed vesicles and arbuscules.
<i>Gigaspora</i>	Azygospore produced singly in soil, large, generally globose or subglobose, with oily contents, borne terminally on a bulbous suspensor-like cell to the spore. Spore wall continuous except for a small occluded spore. Germ tubes produced directly through wall near spore base, Thin-walled vesicles borne in soil on coiled hyphae, forming singly or in clusters. Forming endomycorrhizae with arbuscules.
<i>Glomus</i>	Chlamyospores borne terminally on single (rarely two) undifferentiated, nongametangial hyphae in sporocarps or individually in soil. Spore contents at maturity separated from attached hyphae by a septum or occluded by spore wall thickening.
<i>Sclerocystis</i>	Chlamyospores arranged side by side in a single layer, elongate, radiating out from a central plexus of hyphae.

Table 3. Fresh root weight (g) and mycorrhizal spores in three grass species three months after planting to soil collected in Mt. Pangasugan closed canopy area.<sup>1</sup>

Grass Host	Fresh Root Weight	Mycorrhizal Spores	
		Soil <sup>2</sup>	Roots <sup>3</sup>
Guinea Grass ( <i>Panicum maximum</i> Jacq.)	14.6 b	299.0 c	394.0 b
Napier Grass ( <i>Pennisetum purpureum</i> Schumach.)	21.6 a	347.0 b	476.0 a
Para Grass ( <i>Brachiaria mutica</i> (Forssk.) Stapf.	11.6 b	469.0 a	341.0 b

<sup>1</sup>Average of 10 replications. Means within a column followed by the same letter are not significantly different at 5% level (DMRT)

<sup>2</sup>Based from 150 g soil sample.

<sup>3</sup>Based from ten 1 cm root sample.

### Screenhouse Experiment

The different tree seedlings showed varied growth response to VAM inoculation. Inoculation of VAM regardless of type of delivery system increased both plant height and stem diameter for all tree species tested except for the plant height of Bagalunga seedling. It was observed that Dao had the greatest growth response in terms of plant height increase (12.34 to 31.09) compared to the other tree seedlings. However, in terms of stem diameter increase, Hindang had the highest stem diameter growth (13.05 to 31.05) (Table 4). The use of soil with mycorrhizae (T2) as inoculum had a greater effect on seedling growth compared to the use of mycorrhizal roots (T3). Although results are not statistically different from that of the uninoculated treatment (T1) a variable trend was observed as to the effect of VAM inoculation on both growth parameters studied. This may be due to the fact that the inoculum used contained different VAM fungi species since it was field collected. According to Daniels and Menge (1981) mycorrhizal species differ significantly in their ability to stimulate growth to host plant because of differing rate of infection, greater infectivity related to spore size, more external hyphae or more rapid translocation of essential nutrient elements.

As presented in Table 4, the highest percent root infection was observed in Hindang followed by Paguringon and Dao seedlings inoculated with soil with mycorrhizae (T2). The treatments inoculated with mycorrhizal roots (T3) gave the second highest root infection to the same tree species. It was noted that root infection was higher in Hindang, Paguringon and Dao seedlings and minimal root infection was observed that there was an infection even on the uninoculated treatment (T1) for Paguringon, Hindang, Dao and Bagalunga except for Kalumpit.

Table 4. Plant height (cm) and stem diameter (mm) growth of five tree species, percent root infection and spore count in soil as affected by VAM fungi inoculation five months after under greenhouse condition.<sup>1</sup>

Treatment <sup>3</sup>	Growth <sup>2</sup>		% Infection <sup>4</sup> (mm)	Spore in Soil <sup>5</sup>
	Plant Height	Stem Diameter (cm)		
<b>PAGURINGON</b>				
Uninoculated	32.4	2.0	5.6 c	116.0 b
100 g soil with mycorrhiza	36.3 (10.8)	2.7 (25.1)	60.0 a	1,186.7 a
25 g mycorrhizal roots	33.7 (3.8)	2.8 (29.3)	40.0 b	1,075.3 a
<b>HINDANG</b>				
Uninoculated	9.2	3.3	6.7 b	355.0 b
100 g soil with mycorrhiza	12.2 (24.8)	4.8 (31.1)	75.6 a	1,431.7a
25 g mycorrhizal roots	9.8 (6.4)	3.8 (13.1)	67.8 a	1,361.3 a
<b>DAO</b>				
Uninoculated	16.3	3.8	5.6 b	49.0 b
100 g soil with mycorrhiza	23.7 (31.1)	5.3 (28.1)	38.9 a	195.0 a
25 g mycorrhizal roots	18.6 (12.3)	4.5 (14.9)	27.8 a	167.0 a
<b>KALUMPIT</b>				
Uninoculated	12.8	2.7	0.0 b	18.7 b
100 g soil with mycorrhiza	17.2 (25.5)	2.8 (5.7)	7.0 ab	32.0 ab
25 g mycorrhizal roots	16.7 (23.3)	3.0 (11.0)	12.2 a	39.7 a
<b>BAGALUNGA</b>				
Uninoculated	13.7	1.3	2.2 b	16.0 b
100 g soil with mycorrhiza	14.2 (3.7)	1.7 (20.4)	8.9 ab	34.7 a
25 g mycorrhizal roots	13.2 (-3.6)	1.7 (20.4)	12.1 a	37.0 a

<sup>1</sup>Average of three replications. Means within a column followed by the same letter are no significantly different at 5% level (DMRT)

<sup>2</sup>Figures in parenthesis denote the percent increased based from the uninoculated control.

<sup>3</sup>Inoculum level of 370 spores per 150 g soil.

<sup>4</sup>Based from 30 l cm root segment.

<sup>5</sup>Based from 300 g soil sample.

The reason for this may be because the seedlings acquired were already potted in small plastic bags and the soil used for potting was not sterilized and may have contained native VAM fungi spores.

In terms of VAM spore recovery from the soil, Hindang and Paguringon gave the highest spore recovery even in uninoculated treatment compared to the other tree seedlings. There was inoculation but very low spore recovery from Kalumpit and Bagalunga (lower than that of the inoculum level) was noted. In other words, the said tree seedlings may be poor host of the VAM fungi used. Abbott et al. (1988) mentioned that in many soils, spore number is not a good predictor of VAM formation. Also, the total number of infective propagules in a soil has little predictive value for subsequent mycorrhizal formation, particularly where there are different species of VAM fungi and where there are also different types of propagules of individual VAM fungi and where there are also different types of propagules of individual VAM fungi species.

**Field Experiment**

Table 5 shows the result of the field experiment conducted at Punta, Baybay, Leyte, Philippines. Inoculation of VAM fungi increased both plant height and stem diameter of Narra and Mahogany. Plant height and stem diameter increase (%) due

Table 5. Plant height (cm) and stem diameter (mm) growth of Narra and Mahogany as affected by VAM fungi inoculation 12 months after under field condition.<sup>1</sup>

Treatment	Growth <sup>2</sup>	
	Plant Height (cm)	Stem Diameter (mm)
<b>NARRA</b>		
Uninoculated	88.4 b	8.0 b
25 g mycorrhizal roots	95.2 b (7.2)	10.4 b (23.1)
25 g mycorrhizal roots + 50 g Mycovam I	193.4 a (54.3)	21.4 a (62.6)
50 g Mycomvam I	142.9 ab (38.1)	18.8 b (32.2)
<b>MAHOGANY</b>		
Uninoculated	70.2	12.2
25 g mycorrhizal roots	91.9 (23.6)	14.2 (14.1)
25 g mycorrhizal roots + 50 g Mycovam I	117.8 (40.4)	17.0 (28.2)
50 g Mycovam I	103.9 (332.4)	4.7 (17.0)

<sup>1</sup>Average of five replications. Means within a column followed by the same letter are not significantly different at 1% level (DMRT)

<sup>2</sup>Figures in parenthesis denote the percent increased based from the uninoculated control.

to VAM fungi application ranged from 7.18-54.29 and stem diameter increase ranged from 23.07-62.64 for Narra. For Mahogany, the increase ranged from 23.55-40.39 and 14.08-28.23 for plant height and stem diameter, respectively.

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## DEVELOPMENT OF PLANT REGENERATION SYSTEMS IN AVOCADO *Persea americana* Mill.

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### ABSTRACT

*The first success in developing regeneration systems via somatic embryogenesis and shoot organogenesis in avocado (*Persea americana* Mill.) which are suitable for *Phytophthora cinamomi* resistance selection in vitro are reported.*

*Direct shoot regeneration was achieved from shoot tips or meristems excised from axillary buds cultured initially on MS medium supplemented with 0.5mg/L BAP and 0.1 mg/L NAA and subcultured on the same medium or with BAP in combination with 0.5 mg/L thidiazuron (TDZ). Increasing sucrose concentration of 70 g/L reduced the incidence of browning and improved shoot growth favoring the formation of multiple shoots.*

*Embryogenic calli were obtained from immature seeds of avocado cv RCF Purple cultured on modified Murashige and Skoog (MS) medium supplemented with 5.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 1.0 mg/L 6-benzylaminopurine (BAP). Formation of somatic embryos was achieved among calli subcultured at 3-4 week interval for at least 4 cycles into either the same medium or with only 100 mg/L coconut water as additive. Various stages of somatic embryo development from the globular, heart-shaped to torpedo stages were observed.*

*Somatic embryos were subjected to maturation using MS-based medium containing 5 to 10 mg/L abscissic acid (ABA). Consequently, complete and efficient plant regeneration was obtained from mature embryos recultured in MS medium with 0.1 mg/L TDZ and 0.5 mg/L gibberellic acid (GA3).*

**Keywords:** *avocado, Persea, somatic embryogenesis, organogenesis, tissue culture, plant regeneration.*

### INTRODUCTION

Avocado (*Persea americana* Mill.) being the most nutritious of all fruits, has promising potential both in the local and export industry. Production in the

Philippines however, is limited to backyard planting and in addition, is beset by a fatal disease which also affects viable avocado industry in the United States of America and the South American countries. *Phytophthora cinnamomi* Rands causes root rot and attacks trees of all ages, including young nursery trees on tolerant rootstocks. The most obvious symptoms are gradual decline or dieback of foliage which are often wilted in appearance. Progressive defoliation occurs and entire branches dieback. Fruit production drops and eventually the entire tree dies (Coffey, 1991).

The use of resistant cultivars as rootstocks is so far the most practical and durable disease control measure. However, most avocado cultivars are susceptible with the exception of 'Martin Grande' (Dolan, 1986). Other related species known to be resistant are either graft- or sexually-incompatible with avocado. Recent advances in cell and tissue culture enable the selection of resistance at the cellular level. This may potentially be applied in the selection of *Phytophthora*- avocado cell lines that subsequently grow into complete plants. For this strategy to work, two basic requirements must be met: a) an efficient plant regeneration system from cultured avocado cells or calli and b) the identification of an *in vitro* selection strategy.

This study was conducted to obtain basic information needed for the genetic improvement of avocados using biotechnology tools. Specifically, the objective was to develop a cell culture regeneration system of avocado.

## MATERIALS AND METHODS

### Direct Plantlet Formation

**Plant Material.** Healthy apical shoots were collected from greenhouse- and field-grown avocado cv RCF Purple during the dry and rainy season. With the leaves trimmed off and petioles intact, the shoots were washed with soap and water 5 times, followed by immersion in 70% (v/v) ethyl alcohol for 2 min and rinsed with sterile distilled water. This and all subsequent procedures were done inside the laminar flow hood under sterile conditions. Explants were soaked in varying concentrations of sodium hypochlorite (Zonrox, a.i. 5.25%) for 5-10 min followed by a rinse with sterile distilled water. Explants were cut into single nodes and again soaked in Zonrox and rinsed thoroughly in 5-8 changes of sterile distilled water. Meritems and axillary buds (0.5 mm) were excised from these materials and cultured on to shoot initiation medium.

**Culture Media.** Explants were cultured on three basal nutrients media, namely: Murashige and Skoog's (MS) (1962) with half-strength macro element components, standards MS medium and Schenk and Hidebrandt (SH) (1972) medium. All media were supplemented with 30g/L sucrose, 0.1 mg/L 1-Naphthalene Acetic Acid (NAA) and 0.5 mg/L BAP. Shoots formed *in vitro* were subcultured onto half strength MS medium with all possible combinations of TDZ (0.1 mg/L), GA<sub>3</sub> (0.5 mg/L), BAP (0.2 mg/L), sucrose (30 g/L) and agar (5 g/L) for shoot multiplication and rooting.

**Culture Condition.** The meristems/axillary buds were incubated in airconditioned room under diffused light approximately  $10 \mu\text{E m}^{-2} \text{sec}^{-1}$  and photoperiod of 16 hr.

## **Callus Initiation, Somatic Embryogenesis and Plant Regeneration**

### *Establishment of Embryogenic Cultures*

Immature, 30 days old fruits of avocado cv RCF Purple were collected from the germplasm collection of the National Plant Germplasm Resources Laboratory, University of the Philippines Los Banos then rinsed in sterile distilled water. Under aseptic conditions, the immature seed was removed from the fruit and was dissected along its longitudinal and horizontal axes, thus producing eight explants. The seedpieces were placed onto sterile callus induction medium in test tubes so that the cut portion was in contact with the medium. The cultures were kept in complete darkness and subcultured onto fresh medium at 3 wk interval. The callus induction medium consisted of MS medium with half-strength macro components supplemented with 5.0 mg/L 2,4-D and 1.0 mg/L BAP.

### *Somatic Embryogenesis and Plant Regeneration*

Somatic embryos on its globular stage formed in the same medium were transferred to similar medium with only 100 ml/L young coconut water as additive. Subsequently, somatic embryos on its cotyledonary stage were transferred to similar medium added with ABA at 5-10 mg/L. After 3 wk in culture, mature embryos were cultured onto MS medium with all possible combinations of TDZ (0.1 mg/L), BAP (0.2 mg/L), GA<sub>3</sub> (0.5 mg/L), sucrose (30g/L) and agar (5g/L) for germination.

## **RESULTS**

### **Direct Plantlet Formation**

The tissue culture requirements for initiating aseptic shoot tip/meristem and callus cultures were established in both field and greenhouse-grown avocado cv RCF Purple trees. Surface sterilization was achieved in most explants by soaking in 20% (v/v) Zonrox for 10 min and final washing in 10% (v/v) Zonrox for 5-8 min. Among field-collected materials, 60 and 100% asepsis was obtained during dry season (January to May) from axillary and meristem explants, respectively. During rainy season (June to November), per cent asepsis was lower and possible only through the use of smaller shoot tip/meristem explants. These results attested the failure of the technique which may be attributed to the change in weather conditions resulting to presence of some common contaminating organisms in field-grown trees, namely: *Aspergillus flavus*, *A. niger*, and *Collectotrichum* sp. However, these variations were not pronounced among leaf explants obtained from the greenhouse.

Shoot initiation was observed from intact axillary buds and exercised shoot tip/meristem cultured in modified MS medium supplemented with 0.5 mg/L BAP and 0.1 mg/L NAA (Fig. 1). However, the ability of these cultures to develop directly onto shoots was greatly influenced by the source of explant. The excised shoot tip/meristem responded more efficiently (i.e., 100%) to shoot formation and did not exhibit callus formation as compared to axillary buds (Fig. 2). Subsequent transfers to media with varying concentrations of GA<sub>3</sub>, and/or reduced concentrations of BAP and/or NAA resulted in further shoot growth. When these shoots were transferred to medium containing 0.5 mg/L GA<sub>3</sub> and 0.1 mg/L TDZ, rapid shoot elongation and multiple shoot formation occurred 2 wk after transferring the shoots to medium. Subsequent transfer to MS basal medium induced root to form.

#### **Callus Initiation, Somatic Embryogenesis and Plant Regeneration**

Compact yellowish calli initiated from the lower half of the sectioned immature seeds of approximately 1 mo old avocado cv RCF Purple fruits cultured in MS medium with 2,4-D and BAP have regeneration potential (Fig. 3). The upper half of the sectioned immature seed germinated directly into shoot, and later ungerminated portion formed callus. Proliferation and maintenance was best achieved in MS medium with 5.0 mg/L 2,4-D and 0.5 mg/L BAP. Starting from the 5th monthly subculture, embryo-like structures formed in high frequency (Fig. 4). ABA at 5 and 10 mg/L added onto MS medium promoted maturation of somatic embryos while



Figure 1. Stages in shoot regeneration from avocado meristems.

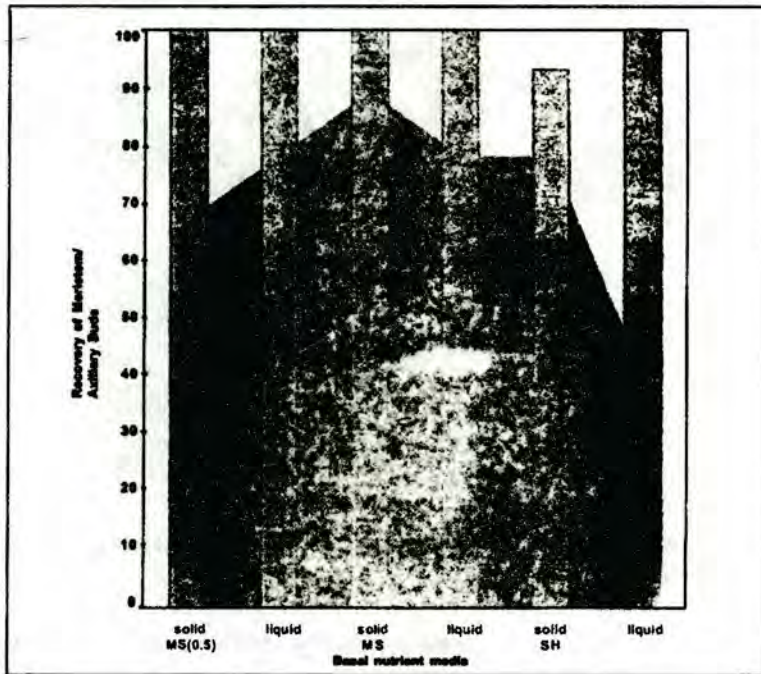


Figure 2. Effects of basal nutrients media on the recovery of responsive meristem/axillary buds of field-grown avocado



Figure 3. Compact seed-derived calli of avocado with high regeneration potential.



Figure 4. Pro-embryoids and mature somatic embryos from seed-derived calli of avocado

mamitol at 15 and 30 g/L resulted to browning (Fig. 5). Results from 3 separate trials indicated that complete plant regeneration from seed-derived calli may be achieved following subculture of mature somatic embryos to MS medium with 0.1 mg/L TDZ and 0.5 mg/L GA<sub>3</sub>, (Fig. 6).

## DISCUSSION

The results demonstrate that by manipulating media formulation, high frequency shoot regeneration can be achieved. From a single meristem/axillary bud, as many as 25 shoot buds was obtained in MS medium with TDZ and GA<sub>3</sub>. Initiated meristem/axillary bud failed to elongate when subcultured to initiation medium but elongation and faster multiple shoot formation was achieved upon subculture to medium with 0.1 mg/L TDZ and 0.5 mg/L GA<sub>3</sub>. The promotive effect of GA<sub>3</sub>, on shoot bud elongation of avocado was evident, as was reported earlier in *Nicotiana* (Engelke, et al. 1973) and *Morus australis* (Pattnaik, et al. 1996). Root formation and elongation followed upon transfer to hormone-free MS medium with half-strength macro elements after 3 wk, however, the phenomenon of profuse growth of slender and thin roots was not observed.

Immature seed-derived calli having somatic embryogenesis potential may be induced to form proembryoids in medium containing auxin. While Picloram was cited as a critical source of auxin for somatic embryogenesis of avocado (Hammerschlag and Litz, 1992), findings show that 2,4-D at 5.0 mg/L in combination with BAP at 0.5 mg/L may be a good substitute for direct somatic embryogenesis. Likewise, secondary somatic embryogenesis from mature somatic embryos occurred

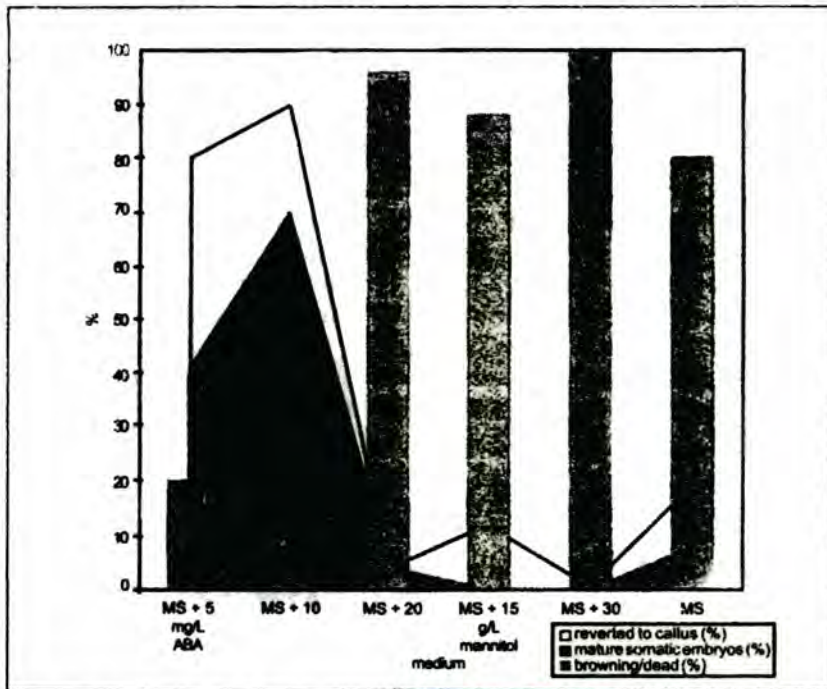


Figure 5. Effect of ABA and mannitol on maturation of seed-derived somatic embryos.



Figure 6. Complete plantlet regenerated from avocado meristem (A) and seed-derived somatic embryos (B)

very efficiently in avocado, as in the case of *Vitis* (Krul and Worley, 1977; Gray, 1987) in medium with or without auxin. Somatic embryos subjected to maturation medium formed white cotyledonary embryos while browning of other portions not embryogenic was common. The browning may be the effect of the ABA used in maturation of somatic embryos which was effective in suppression of formation of accessory or secondary embryos from globular or early heart-stage somatic embryos (Hammerschlag and Litz, 1992). The effect of GA<sub>3</sub> on germination of matured embryos of avocado was in congruence with its effect on *Citrus sinensis* (Kochba, 1974). The control in maturation and germination by ABA inhibition of precocious germination and GA<sub>3</sub> promotion of germination (Hammerschlag and Litz, 1992) was true for avocado seed-derived somatic embryos.

The regeneration systems for avocado are highly efficient and therefore suitable for in vitro selection strategy to select Phytophthora-resistance at the cellular level.

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## **BIOLOGICAL SCIENCES**

### **LIGHT INHIBITION OF *Photobacterium leiognathi* AS BIOINDICATOR OF HEAVY METAL POLLUTION**

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#### **ABSTRACT**

*Heavy metal pollution in the biosphere has become a serious health and ecological concern. Its occurrence in nature is usually monitored by chemical analyzes with the use of high-cost instruments. An alternative method is presented for determination of water pollution with heavy metals and detergents using biological system. It is a test tube assay involving the naturally luminescent *Photobacterium leiognathi* as bioindicator. Three-milliliter bacterial suspension ( $10^6$  cells/ml) is mixed with 0.5 ml of the test solution to find the minimum anti-luminescent concentration (MAC) of the pollutant. Light inhibition is determined simply by gross visual observation inside the dark room. Tests showed MAC at 714.29 ppm for  $PbNO_3$ , 142.86 ppm for  $CuSO_4$ , 14.29 ppm for  $ZnSO_4$  and 1.43 ppm for  $HgCl_2$ . A local commercial detergent inhibited bioluminescence of the bioindicator bacterium at 14 ppm MAC. Inhibition of light emission in the bioassay literally means "turning off" the light of the bioindicator bacterium. Light is "turned on" again, i. e., light emission is restored, when the bacterium is transferred to a fresh medium. Since the assay simply involves "on" and "off" of luminescence, this can serve as a preliminary test for toxicity of heavy metals in polluted waters.*

**Keywords:** *Bioluminescence, Photobacterium leiognathi, Bioindicator, Heavy Metals, Bioassay, Minimum Anti-luminescent Concentration (MAC), Light Inhibition*

## INTRODUCTION

Metals mostly exist as mineral deposits in nature. They may be derived from the lithosphere by mining and leaching or from the more familiar industrial, agricultural, and domestic wastes. With the advances in technology, the accumulation of, and pollution with, heavy metals in the biosphere especially in rivers, lakes, and estuaries in urban areas has become a serious health and ecological concern.

Cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn) are important trace metals for growth and development in most living systems. However, other metals like lead (Pb), mercury (Hg), and cadmium (Cd) do not seem to have any importance and, in large quantities, may become hazardous or toxic to various life forms in the environment (Haslam 1990). Because of the need to determine the occurrence and monitor the concentrations of these elements in nature, chemical analyzes are done with the use of high-cost instruments. On the other hand, recent studies explore the possibility of using alternate biological systems such as bacterial bioluminescence and fish bioassay in determining the concentrations of these metals (Bulich 1979).

Bioluminescence is a natural lighting phenomenon associated with fireflies (e.g. *Photinus pyralis*) and bacteria, for example, *Photobacterium* and *Vibrio* (Saylor *et al.*, 1990). It refers to the ability of the organism to give off light. Luminescence here is a result of endogenous enzymatic process that transforms the chemical energy into light energy. The enzyme involved is luciferase which catalyzes the bioluminescent mixed-function oxidation of reduced flavin mononucleotide (FMNH<sub>2</sub>) and a long chain aldehyde (RCHO) in the presence of molecular oxygen. The balanced over-all equation for the reaction (Brock *et al.*, 1994) is:



Bioluminescent microorganisms exist symbiotically with nocturnal and benthic marine life forms although some are free-living. They can be found within sac-like organs in fishes. The emitted light functions in communication and mating (Atlas and Bartha 1993).

The bioluminescent *Photobacterium* and *Vibrio* species are gram-negative, straight or curved rods measuring 1.8-2.4 x 0.8-1.3 μm. They are motile with 1-3 unsheathed polar flagella and capable of respiratory and fermentative metabolisms. They accumulate poly-B-hydroxybutyrate but do not metabolize exogenous B-hydroxybutyrate monomers. They do not form endospore or microcyst. These bacteria inhabit the marine environment and thus, have an absolute requirement for Na<sup>+</sup> (Baumann and Baumann 1977).

The objectives of this research work are to: isolate, characterize, and identify bioluminescent bacterial from local marine fishes; devise a bioassay method of determining the pollution levels of heavy metals using inhibition of bioluminescence in the bioindicator bacterium; and, determine the applicability of the

bioassay to other pollutants such as detergents. However, this work does not aim to establish the mechanism involved in the inhibition of luminescence.

## MATERIALS AND METHODS

### Isolation of Bioluminescent Bacteria

Specimens of four marine fishes (asungos, bangus, loro, lapu-lapu) were obtained for the isolation of the organism. The eyes, scales and intestine of the samples were removed and teased apart with scalpels, and mixed thoroughly. The mixture was suspended in distilled water containing rock salt. A 10-ml aliquot was transferred to 125-ml Erlenmeyer flask containing 100 ml 2% NaCl solution. The flask was then subjected to shaking for few minutes; thereafter, 5 ml was added to a 500-ml Erlenmeyer flask containing 100 ml modified nutrient broth for enrichment for 12 hr in a shaker. Final isolation and subsequent purification were done by streaking on modified nutrient agar plates which were then incubated at 18-20°C. Bioluminescent bacterial colonies were identified in the dark room.

### Characterization and Identification of Bacterial isolates

Cultural characterization of the two bioluminescent isolates was done based on colony growth on Egorova-Yarmolink agar (EYA). Gram-staining and morphology of the bacterial isolates were also done.

For the biochemical characterization, the isolates were tested for: *gas production* from glucose using glucose-yeast extract broth; *xylose* and *maltose utilization* using basal medium agar, *acetate utilization* using acetate agar; and, *gelatinase production* using nutrient gelatin. The temperature requirement for optimal growth was also determined. NaCl (3%) was added to all media, and the pH in all cases was adjusted to 7.0.

### Bioassay of Heavy Metals

For the assay of heavy metals, a 100-ml EY broth culture grown for 9 hr at room temperature was centrifuged for 5 min at 3000 rpm. The harvested cells were then washed and suspended in 2% NaCl solution. Cell density of the inoculum was quantified and adjusted to  $10^6$  cells/ml using Breed's method.

Four heavy metals in salt form ( $ZnSO_4$ ,  $HgCl_2$ ,  $PbNO_3$ ,  $CuSO_4$ ) were tested. One gram of each heavy metals salt was dissolved in 100 ml of water for an initial standard of 10,000 ppm. From this standard, concentrations of 7000, 5000, 3000, 2000, 1000, 100, 10, 1 and 0.1 ppm were prepared. Aliquots (0.5 ml) of these preparations were added to 3 ml bacterial suspension in test tubes to come up with final concentrations of 1000, 714429, 286, 143, 14.3, 0.14 and 0.014 ppm. The tubes were shaken for 5 min to allow aeration and then were observed in the dark room. The minimum anti-luminescent concentration (MAC) was recorded.

### **Bioassay of Complex Effluents**

A commercial detergent (Tide powder) (0.01 g) was diluted with 10 ml distilled water to come up with a standard concentration of 1000 ppm. From this standard solution, serial dilutions were done down to a concentration of 1 ppm. The same procedure was done as in the assay of heavy metals. The final concentrations of the diluted detergent would then be 143, 14, 1.4, 0.14 and 0.014 ppm. The MAC was recorded.

### **Bioassay of Polluted Water**

A 10-ml aliquot of a polluted water sample from San Juan River was serially diluted in 9 ml 2% NaCl solution down to a concentration of 1 ppm. Aliquots (0.5 ml) of each solution were transferred to tubes containing 3 ml of the bacterial suspension. These were shaken and observed inside the dark room. The MAC was recorded.

## **RESULTS**

### **Identification of Bacterial Isolates**

Two bacterial isolates were obtained from fish specimens. Both were identified as *Photobacterium* sp. belonging to the family Vibrionaceae. The cells were gram-negative rods, grew at room temperature, did not produce gas from glucose, and utilized acetate but not maltose and xylose as carbon source. Results for gelatinase production were negative.

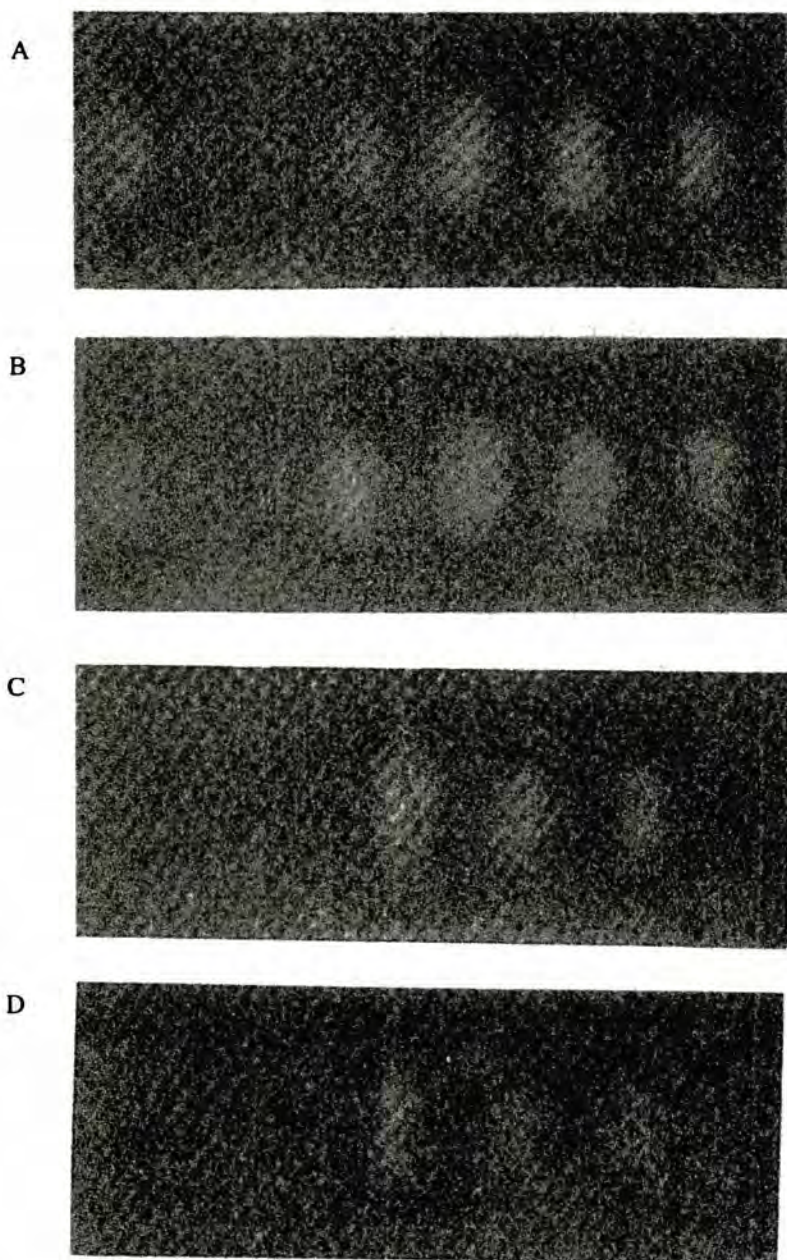
Of the three known species of *Photobacterium*, two were able to emit light: *P. phosphoreum* and *P. leiognathi*. The biochemical characteristics of these two species and those of our two isolates (from asungos) are summarized in Table 1. Our isolates clearly are allied to *P. leiognathi* and thus, were identified to be this species.

### **Light Inhibition Assay for Heavy Metals**

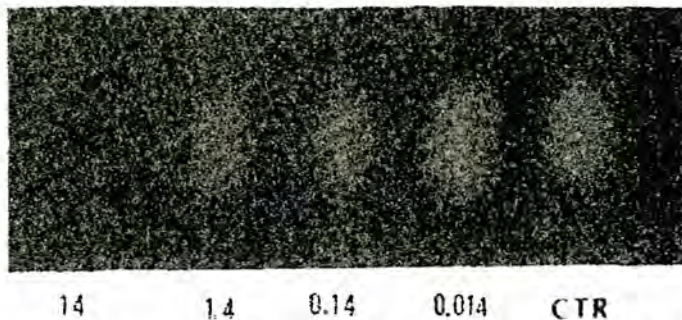
With the test tube assay done at room temperature, the minimum anti-luminescent concentration (MAC) (Fig. 1) for the four heavy metals tested was lowest at 1.43 ppm for mercury and highest at 714 ppm for lead. MAC for zinc was 14.3 ppm, and for copper, 143 ppm (Table 2; Fig. 1).

### **Light Inhibition Assay For Detergent and Polluted Water**

The detergent concentration of 14 ppm was the minimum anti-luminescent concentration (Table 3) (Fig. 2). The water sample from San Juan River was diluted down to  $10^{-4}$ . All dilutions in three trials as well as the control (saline solution) and undiluted water sample failed to inhibit bioluminescence of the bacterium.



**Figure 1.** Minimum anti-luminescent concentration (MAC) of Pb (A), Cu (B), Zn (C), and Hg (D).



**Figure 2. Minimum anti-luminescent concentration (MAC) of detergent.**

**Table 1. Biochemical characteristics of bioluminescent bacteria from fishes**

Biochemical Test	Isolate A	Isolate B	<i>P. phosphoreum</i>	<i>P. leiognathi</i>
Gelatinase Production	-	-	-	-
Utilization of				
Xylose	-	-	-	-
Maltose	-	-	+	-
Acetate	+	+	-	+
Fermentation and Gas Production	-	-	+	-
Optimum Growth (°C)	33.35	33.35	4	35

(+) positive; (-) negative test

**Table 2. Minimum anti-luminescent concentration (MAC) of heavy metals**

Concentration (ppm)	Hg	Zn	Cu	Pb
1000	--	--	--	--
714	--	--	--	--
429	--	--	--	+
286	--	--	--	+
143	--	--	--	+
14.3	--	--	--	+
1.43	--	+	+	+
0.14	+	+	+	+
0.014	+	+	+	+

(+) light on; (-) light off

Table 3. Minimum anti-luminescent concentration of detergent (Tide powder)

Concentration (ppm)	Detergent
143	--
14	--
1.4	+
0.14	+
0.014	+

(+) light on; (-) light off

## DISCUSSION

The bioluminescent *Photobacterium leiognathi* could be isolated from the intestines and guts of non-luminous Philippine marine fishes (asungos). Growth at 35°C or at room temperature (Hasting 1977) makes easy identification of this species. The morphological, cultural and biochemical characteristics (Table 1) confirmed that our two isolates belong to this species of *Photobacterium*.

Bacterial luminosity has been employed in determining toxicity in aquatic environments (Bulich 1979). Its sensitivity and reliability compared to the fish test has already been established. A good correlation exists between bacterial luminescence assay and the conventional fish test (Bulich *et al.* 1981).

In this experiment, the luminescence of *P. leiognathi* was used to determine the minimum anti-luminescent concentration (MAC) of four heavy metal samples. These metals were chosen based on their solubility in water and their occurrence in polluted waters. The MAC for mercury was lowest at 1.43 ppm. Mac values for copper and zinc were 143 and 14.3 ppm, respectively. Lead had the highest MAC at 714 ppm (Table 2). These concentrations inhibited light production of our bioindicator bacterium (isolate B). Light inhibition could well be due to the inhibition of the enzyme luciferase by heavy metals which are known to targets the cell's functional enzymes. However, light inhibition is not related to the death of the cells. This was proven when light-inhibited bacterial cells used in the assay were restreaked in fresh culture media. After 9 hr of incubation at room temperature, the cells grew and again exhibited luminescence.

The assay procedure was further applied to detergent, a common water pollutant, and to a polluted water sample. A local commercial detergent (Tide powder) gave a MAC of 14 ppm (Table 3). This concentration level is the same MAC for Hg (Table 2). Light inhibition here again did not mean cell death but simply turning-off the light.

Rebioluminescence or the resumption of light emission upon transfer of the bioindicator bacterium to a fresh medium from the assay tube needs some explanation. Microorganisms have specialized transport systems for different solutes and toxic inorganic ions such as heavy metals. Thus, the influx of sodium ions in

the cells acts as a transport system for inorganic ions transforming toxic heavy metals to less toxic and sometimes volatile forms (Selifonova and Barkay 1994; Mitchell, 1991). Perhaps the same mechanisms holds true in the bioluminescence of *P. leiognathi*.

The other unexpected result was the failure of the indicator bacterium to turn off its light in the presence of the polluted water sample from a river. It is not likely that heavy metals were not present in that river. According to the National Pollution Control Center, heavy metal levels in San Juan River in 1980-1985 decreased from 2.8 to 0.4 ppm. Based on our results with Cu, Zn, Pb, Hg (Table 2), this level would not be sufficient to inhibit bioluminescent of our test bacterium.

### CONCLUSION

Our experiment proved that inhibition of bacterial luminescence can be used to indicate heavy metal pollution in aquatic environment. The applicability of the bioassay using *Photobacterium leiognathi* worked for both metals and detergents. Its sensitivity to low concentrations of pollutants makes it an effective, alternative biological method for testing pollution.

### ACKNOWLEDGMENT

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# MOLECULAR METHODS FOR EARLY IDENTIFICATION OF BANANA (*Musa* spp. AAA) DWARF SOMACLONAL VARIANTS

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## ABSTRACT

*Micropropagation offers a rapid method of producing disease-free planting materials in vegetatively reproduced species such as banana. However, widespread use of this technique is limited by the high incidence of somaclonal variation, with dwarfism as the major micropropagation induced variant (hereafter referred to as off-types) in the Cavendish subgroup (*Musa* spp. AAA). Dwarf off-types are rarely detected in vitro and commonly not apparent after until field planting has taken place. Identification of dwarf off-types at an early stage during in vitro culture is important. A random amplified polymorphic DNA (RAPD) marker specific to dwarf off-type was identified. Primer OPJ-04 (5'CCGAACACGG-3') consistently amplified a fragment of approximately 1,500 bp which was present in normal (true-to-type) but absent in the dwarf off-type plants of two Cavendish cultivars including New Guinea Cavendish and Williams. This RAPD fragment was cloned and a 1,625 bp nucleotide sequence was obtained. Primers homologous to this sequence were designed and used for specific polymerase chain reaction (PCR). The specific PCR offers a rapid and reliable method for identification of dwarf off-types.*

## INTRODUCTION

Micropropagation offers a rapid method of producing large quantities of disease-free bananas (*Musa* spp.) for commercial production. However, widespread use of such materials is hindered by the high percentages of tissue culture-induced variants (off-types), especially with Cavendish (*Musa* spp. AAA) bananas (Smith 1988; Israeli *et al.* 1991). Off-types have been noted for plant stature (mainly dwarf), leaf variegation and fruit bunch characters (small bunches, hairy fruit). Dwarfism is by far the most common off-type, accounting for more than 75% of the observed micropropagation-induced variation in the Cavendish subgroup (Israeli and Reuveni 1995). Fruit bunches produced by the dwarf off-types are of less commercial value, causing serious economic losses to growers.

Early detection and elimination of off-types from micropropagated materials is highly desirable but not easy to undertake. Dwarf off-types are not visibly detected *in vitro* and can be observed in the later stages of nursery production (i.e. two to three months after deflasking) when plants are taller than 20 cm and growing vigorously (Smith and Hamill 1993). Application of GA<sub>3</sub> at the deflasking stage increased the efficiency of detection, however, the GA<sub>3</sub> response at the *in vitro* stage did not give a clear discrimination between normal and dwarf off-type plants (Damasco *et al.* 1996). The use of polymerase chain reaction (PCR)-based marker to detect dwarf off-types following micropropagation offers several advantages over these and other detection technique such as RFLP and isozymes. The technique is rapid, results can be obtained in two days in contrast to three months for nursery evaluation or seven months for field evaluation. Only small amount of DNA is needed, such as would be available from small *in vitro* leaf. There is no requirement for greenhouse or field space since analysis of the plants can be done while they are still *in vitro*. Being DNA-based, the technique is more reliable as it is not influenced by the developmental stage of the plant or the environmental conditions under which plants are grown.

Random amplified polymorphic DNA (RAPD) analysis a PCR-based technique uses random primers to generate DNA fragments which can be used as genetic markers (Williams *et al.* 1990). Polymorphisms generated by RAPD analysis can be used for fingerprinting many plant species, genetic analysis and tagging specific traits (Michelmore *et al.* 1991; Tingey and del Tufo 1993; Howel *et al.* 1994). The conversion of RAPD marker into a sequence characterised amplified region (SCAR; Paran and Michelmore 1993) allows for a fast and robust DNA assay.

This paper describes the development of a robust molecular assay based on PCR for the identification of dwarf off-types generated from banana micropropagation.

## MATERIALS AND METHODS

### Plant materials

All plant materials were obtained from the banana collection of the Queensland Department of Plant Industries, Maroochy Horticultural Research Station, Nambour, Queensland, Australia. Normal and dwarf off-type plants of Cavendish (*Musa* spp. AAA) cultivars New Guinea Cavendish and Williams were obtained originally from several different micropropagation events (Table 1). 'Dwarf Parfitt', a naturally occurring extra-dwarf Cavendish cultivar, was also included in the study to determine if the dwarf mutation generated from micropropagation is the same as that occurring in somatic mutants. Suckers harvested from these plants were cultured *in vitro* following the procedure of Drew and Smith (1990). *In vitro*- and glasshouse-grown (New Guinea Cavendish accession C420, Williams accessions C76 and C117, and Dwarf Parfitt) and field-grown (New Guinea Cavendish accessions C75 and C529 and Williams accessions C251, C271 and C117) normal and dwarf off-type plants were analyzed using RAPD. The accession number (e.g. C117) represents the original clonal plant from which the original tissue cultures were initiated. In total, 116 (57 normals, 59 dwarfs) plants were used for RAPD analysis.

Table 1. Normal (N) and dwarf off-type (D) plants of Cavendish cultivars New Guinea Cavendish (NGC), Williams and Dwarf Parfitt (DP) used in RAPD analysis. All plant materials were obtained from QDPI Maroochy Horticultural Research Station, Nambour, Australia. Each accession represents at least one independent tissue culture event. Plants from sucker were regenerated through axillary and/or adventitious budding while plants from floral apex tissue were regenerated from callus through adventitious budding. The different accessions were collected from the South Johnstone Research Station, South Johnstone (SJRS), Redland Bay (RB), Cleveland (C), Mena Creek (MC), Kennedy (K), and Wamuran (W), Queensland, Australia.

Cultivar	Plant type	Accession number	Number of plants tested			Initial explant	Place of collection
			<i>In vitro</i>	Glasshouse	Field		
NGC	N	C420	14	7	–	sucker	SJRS
	D	C420	14	5	–	sucker	SJRS
NGC	N	C75	–	–	4	floral apex	RB
	D	C75	–	–	5	floral apex	RB
Williams	N	C76	5	5	–	floral apex	SJRS
	D	C76	5	5	–	floral apex	SJRS
Williams	N	C251	–	–	7	sucker	MC
	D	C251	–	–	2	sucker	MC
Williams	N	C117	8	3	4	sucker	K
	D	C117	9	3	3	sucker	K
DP	D	M503	2	2	1	sucker	W
<b>Total</b>			57	30	29	116	

### DNA extraction

Total genomic DNA was extracted from fresh leaves of *in vitro*-, glasshouse- and field-grown plants using a modified CTAB (hexadecyltrimethylammonium bromide) method (Graham *et al.*, 1994). Leaf tissues (1 to 2 g) were frozen in liquid nitrogen and ground in a mortar and pestle. For some *in vitro* plants with small leaves (<0.2 g), leaf tissues were frozen and ground in a microfuge tube (1.5 mL) using a micropestle. The DNA pellet was resuspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and yield quantified using a Beckman Du-64 Spectrophotometer equipped with a soft pac module for nucleic acid determination (Beckman Instruments Inc., Fullerton, CA, USA).

### DNA amplification and electrophoresis

RAPD reactions were performed in volumes of 25  $\mu$ L containing 1 x reaction buffer (10mM Tris-HCl pH 8.3, 1.5 mM  $MgCl_2$ , 50 mM KCl), 2.5 to 4.0 mM  $MgCl_2$  (depending on primer), 400  $\mu$ M each of dATP, dCTP and dGTP and dTTP (Promega, Madison, WI, USA), 0.25  $\mu$ M of arbitrary decamer primer (Operon Technologies, Alameda, CA, USA), 50 ng of banana genomic DNA, 1.0 unit of *Taq* DNA polymerase (Boehringer Mannheim, Mannheim, Germany) and overlaid with a drop of paraffin oil. Amplification reactions were performed in a 480 Perkin Elmer DNA thermocycler (Perkin Elmer Corp., Norwalk, CT, USA). An initial denaturation temperature of 94°C for 5 min was followed by 45 cycles each at 94°C for 1 min, 37°C for 1 min and 72°C for 2 min. For specific PCR, DNA amplification reactions were performed in volumes of 20  $\mu$ L containing 1 x reaction buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM  $MgCl_2$ , 0.1 mg  $mL^{-1}$  gelatin), 200  $\mu$ M each of dATP, dCTP, dGTP and dTTP, 3.0 mM  $MgCl_2$  (including that in the reaction buffer), 0.1  $\mu$ M primer, 1 unit of *Taq* DNA polymerase and 50 ng of banana genomic DNA. Amplification reactions were performed in a PT-100-96 Thermal Controller (Bresatec Pty. Ltd., Thebarton, SA, Australia). An initial denaturation at 94°C for 3 min was followed by 35 cycles each at 94°C for 10 s, 50 to 65°C (depending on primers) for 10 s and 72°C for 1 min. The amplification products were analyzed on a 1.2% agarose (Promega, Madison, WI, USA) gel in 1 x TBE buffer containing ethidium bromide (0.25  $\mu$ g  $mL^{-1}$ ). The amplification products were visualized under UV light (302 nm) and photographed using 667 polaroid film (Polaroid (UK) Ltd., Hertfordshire, England).

### Identification of polymorphisms between normal and dwarf off-type plants

Initially, the usefulness of RAPD analysis in detecting polymorphisms between normal and dwarf off-type bananas was determined using *in vitro*-grown New Guinea Cavendish accession C420. Sixty-six arbitrary decamer primers from kits A (OPA-01, 04, 06, 07, 09, 10, 12, 13, 18-20), H (OPH-01-20), J (OPJ-01-15), S (OPS-01-05) and U (OPU-01-15) were used. Five *in vitro* normal and 5 *in vitro* dwarf off-type plants were tested for each primer. The primers which initially

revealed polymorphisms between normal and dwarf off-type plants were further evaluated for markers specific to the dwarf off-type plants using 81 micropropagated plants from New Guinea Cavendish (20 normals, 21 dwarf off-types) and Williams (22 normals, 18 dwarf off-types). The RAPD marker identified as being specific to the dwarf off-types was further tested on Williams accession C76 (10 normals, 10 dwarf off-types) and Dwarf Parfitt (5 plants).

### **Cloning and sequencing of the RAPD marker band**

The polymorphic RAPD marker band was excised from an ethidium bromide stained low melting temperature agarose gel (2.0%) under UV illumination. The DNA was extracted from the agarose gel slice using phenol following the procedure of Sambrook *et al.* (1989). The purified RAPD marker band was cloned using the TA cloning system (Invitrogen Corp., San Diego, CA, USA) following the manufacturer's instructions.

DNA sequencing was performed using the Prism™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing (Applied Biosystems Inc., Foster City, CA, USA) according to the "Protocol for Cycle Sequencing" supplied by the manufacturer. M13 forward and reverse primers were used to sequence the insert from both ends and the remaining sequences were obtained by progressively moving the priming site along the DNA sequence (Sambrook *et al.* 1989). Oligonucleotide primers were synthesized by the Oligonucleotide Synthesis Facility, Centre for Molecular Biology, The University of Queensland, Brisbane, Australia. The sequences generated were analysed by the computer program SeqEd™ (Version 1.0.3; Applied Bio Systems Inc.).

### **SCAR analysis**

Three pairs of primers were designed and synthesized to be used as dwarf off-type SCAR primers (Table 2). The first pair contained the RAPD primer sequences plus the next 10 internal bases from both ends of the RAPD marker sequence. The other two pairs were the primers used in the sequencing of the RAPD marker band. The 3 pairs of SCAR primers were tested on 12 normal and 12 dwarf off-type plants previously analyzed by RAPD (Table 1).

### **Specific PCR for detecting dwarf off-types**

The effectiveness of the specific PCR technique to detect dwarf off-type plants while still at the *in vitro* stage was tested in collaboration with a commercial tissue culture laboratory. A random sample of 158 *in vitro* plantlets of Williams (accession C724) was taken at subculture cycle 4 of the multiplication stage and DNA extracted. Amplification reactions were performed using a standard reaction mixture, 2 µL of unquantified DNA, SCAR primer pair B<sub>1</sub>B<sub>2</sub> and an annealing temperature of 50°C. Plants were assayed for the presence (normal) or absence (dwarf) of the SCAR<sub>1343</sub> band.

Table 2. Sequence of the 20-mer specific oligonucleotide primers derived from the RAPD marker and the expected size of SCAR band. The underlined sequences in primers A<sub>1</sub> and A<sub>2</sub> represent the sequence of the OPJ-04 RAPD primer. Primers B<sub>1</sub>, B<sub>2</sub> and C<sub>1</sub> were used in sequencing the RAPD marker band.

Primer	Primer Sequence (5' to 3')	Expected size of SCAR band (bp)
A <sub>1</sub>	CCGAACACGGGACTTATACA	1,625
A <sub>2</sub>	CCGAACACGGGCTAACCTAG	
B <sub>1</sub>	CTGTGGTTGCATTCTCATAC	1,343
B <sub>2</sub>	GTGAATCATACTCGGAACC	
C <sub>1</sub>	TTAAGCCTTGGATTGACTGC	994
B <sub>2</sub>	GTGAATCATACTCGGAACC	

## RESULTS

### Polymorphisms in New Guinea Cavendish and identification of dwarf off-type specific marker

The initial 66-primer screen amplified a total of 234 products with between 1 and 10 products generated per primer. Nineteen of the 66 primers (28.8%) revealed polymorphisms between normal and dwarf off-type plants, with 34 polymorphic products generated. Three primers (OPJ-02, OPJ-08, OPU-04) did not amplify any products. The DNA and MgCl<sub>2</sub> concentrations in the reaction mixture were important for successful DNA amplifications in banana. Using 50 ng of banana genomic DNA as template and 2.5 to 4.0 mM MgCl<sub>2</sub> (depending on primer), field-, glasshouse- or *in vitro*-grown plants of a particular cultivar and/or accession produced the same scorable banding pattern.

The profiles of amplified products from New Guinea Cavendish (accessions C420, C75 and C529), and Williams (accessions C271, C251 and C117) and their associated micropropagation-produced dwarf off-types were compared for identification of markers specific to dwarf off-types. Of the 19 primers which revealed polymorphisms in the initial primer screen using normal New Guinea Cavendish accession C420 and its respective dwarf off-type, only 10 primers revealed polymorphisms for New Guinea Cavendish accessions C75 and C529 and its respective dwarf off-type, 7 primers for Williams accessions C251 and C271 and its respective dwarf off-type, and 4 primers for Williams accession C117 and its respective dwarf off-type (Table 3). The majority of the polymorphisms generated were not associated with dwarfism. Six primers (OPA-06, OPH-13, OPJ-04, OPJ-10, OPJ-13, OPU-06) were able to differentiate between normal and dwarf New Guinea

Cavendish (3 accessions), and 2 primers (OPJ-04, OPJ-13) differentiated between normal and dwarf Williams (2 accessions; Table 4). Of the DNA bands which differentiated normal and dwarf off-type plants of different accessions, only the 1,500 bp band from primer OPJ-04 (referred to as OPJ-04<sub>1500</sub>) was found consistently present in all normal but absent in all dwarf off-type plants of New Guinea Cavendish (3 accessions) and Williams (2 accessions; Table 4, Figure 1). This primer was further tested on normal and dwarf Williams accession C76. The OPJ-04<sub>1500</sub> band was consistently present in all normal but absent in all dwarf off-type plants. The naturally occurring extra dwarf cultivar, Dwarf Parfitt, gave an OPJ-04 RAPD profile similar to that of normal plants of Williams and New Guinea Cavendish (Figure 1), and the OPJ-04<sub>1500</sub> band was present in all Dwarf Parfitt plants tested.

### DNA Sequencing, primer construction and SCAR amplification

Sequencing of the dwarf off-type RAPD marker band generated 1,625 bp sequence. The terminal 10 bases exactly matched the OPJ-04 primer sequences. Three pairs of 20-mer SCAR primers were synthesized and tested using genomic DNA from normal and dwarf New Guinea Cavendish and Williams. In all normal plants, a 1,625 bp band was amplified using SCAR primer pair A<sub>1</sub>A<sub>2</sub> (Figure 2A), a band 1,343 bp was amplified using SCAR primer pairs B<sub>1</sub>B<sub>2</sub> (Figure 2B), and a band of 944 bp was amplified by primer pair C<sub>1</sub>B<sub>2</sub> (Figure 2C). The SCAR bands were not amplified from the dwarf off-type DNA. The RAPD polymorphism was retained as the presence or absence of the band with the corresponding SCAR primers. All the three pairs of SCAR primers tested showed SCAR amplification only in normal plants and not in the dwarf off-types and therefore either of the three primer pairs could be used for detecting dwarf off-type plants. Thus, in all *in vitro* detection experiments, only the SCAR pair B<sub>1</sub>B<sub>2</sub> was used in assaying for normal and dwarf off-type plants.

Apart from the major band, sometimes less intense bands were also amplified in both normal and dwarf off-type plants using the SCAR primers. Elevating the annealing temperature from 50°C to 65°C prevented the amplification of minor bands but did not affect the amplification of the SCAR band.

### *In vitro* detection of dwarf off-types using specific PCR

Of the 158 plantlets analysed from the commercial micropropagation system (Figure 3), 10 plantlets showed no amplification of the SCAR 1343 band, indicating that those plantlets were likely to be dwarfs. Those 10 plants showing the absence of amplification were then assessed by RAPD analysis and confined to be lacking in the OPJ-04<sub>1625</sub> band.

Table 3. Number of amplification and polymorphic products from New Guinea Cavendish (NGC, 3 accessions) and Williams (3 accessions) obtained with 19 arbitrary primers. Each accession represents at least one independent tissue culture event.

Cultivar and accession number									
Primer	Sequence 5' to 3'	NGC (C420)		NGC (C75, C529)		Williams (C27), C251)		Williams (C117)	
		No. of amplifi- cation products	Polymor- phic products	No. of amplifi- cation products	Polymor- phic products	No. of amplifi- cation products	Polymor- phic products	No. of amplifi- cation products	Polymor- phic products
OPA-06	GGTCCCT	6	2	6	1	6	0	7	1
OPA-12	TCGGCG	5	1	5	0	5	0	6	1
OPA-13	CAGCAC	10	1	10	1	10	0	10	0
OPA-19	CAAACG	6	1	6	0	6	0	6	0
OPH-05	AGTCGT	5	3	5	0	5	0	5	0
OPH-06	ACGCAT	3	1	3	0	3	0	3	0
OPH-09	TGTAGCT	7	1	7	1	7	0	7	0
OPH-13	GACGCC	6	3	6	1	5	1	5	0
OPH-17	CACTCTC	7	1	7	1	7	0	7	0
OPH-19	CTGACC	6	1	8	0	8	0	8	0
OPJ-04	CCGAAC	7	2	7	1	7	1	7	1
OPJ-10	AAGCCC	6	2	6	1	6	1	6	0
OPJ-13	CCACACT	5	1	5	0	6	1	6	1
OPS-03	CAGAGG	5	3	5	0	5	2	5	0
OPS-04	CACCCCC	6	4	6	0	6	0	6	0



Table 3. (continued)

Primer	Sequence 5' to 3'	Cultivar and accession number							
		NGC (C420)		NGC (C75, C529)		Williams (C271, C251)		Williams (C117)	
		No. of amplifi- cation products	Polymor- phic products	No. of amplifi- cation products	Polymor- phic products	No. of amplifi- cation products	Polymor- phic products	No. of amplifi- cation products	Polymor- phic products
OPU-06	ACCTTTG	7	1	7	1	7	1	6	0
OPU-07	CCTGCTC	7	3	7	1	7	1	7	0
OPU-08	GGCGAA	7	1	7	1	7	0	7	0
OPU-15	ACGGGC	5	2	5	0	5	0	5	0

Table 4. Primers that revealed polymorphisms between normal and dwarf off-type plants of New Guinea Cavendish (NGC, 3 accessions) and Williams (2 accessions). The (+) indicates presence and (-) absence of amplification. Each accession represents at least one independent tissue culture event.

Primer	Approx fragment size (bp)	Cultivar, accession number and stature							
		NGC (C420)		NGC (C529,C75)		Williams (C251)		Williams (C117)	
		Normal	Dwarf	Normal	Dwarf	Normal	Dwarf	Normal	Dwarf
OPA-06	600	-	+	-	+	+	+	+	+
	1,300	-	+	-	-	-	-	-	+
OPH-13	1,400	+	-	+	-	-	-	-	-
	1,300	-	+	+	+	-	+	+	+
OPJ-04	1,500	+	-	+	-	+	-	+	-
OPJ-10	1,300	-	+	-	+	+	+	+	+
	1,400	+	+	+	+	+	-	+	+
OPJ-13	1,300	-	+	-	+	+	+	+	+
	700	+	+	+	+	+	-	+	-
OPU-06	700	-	+	-	+	+	-	-	-
OPU-08	800	-	+	+	+	+	-	+	+

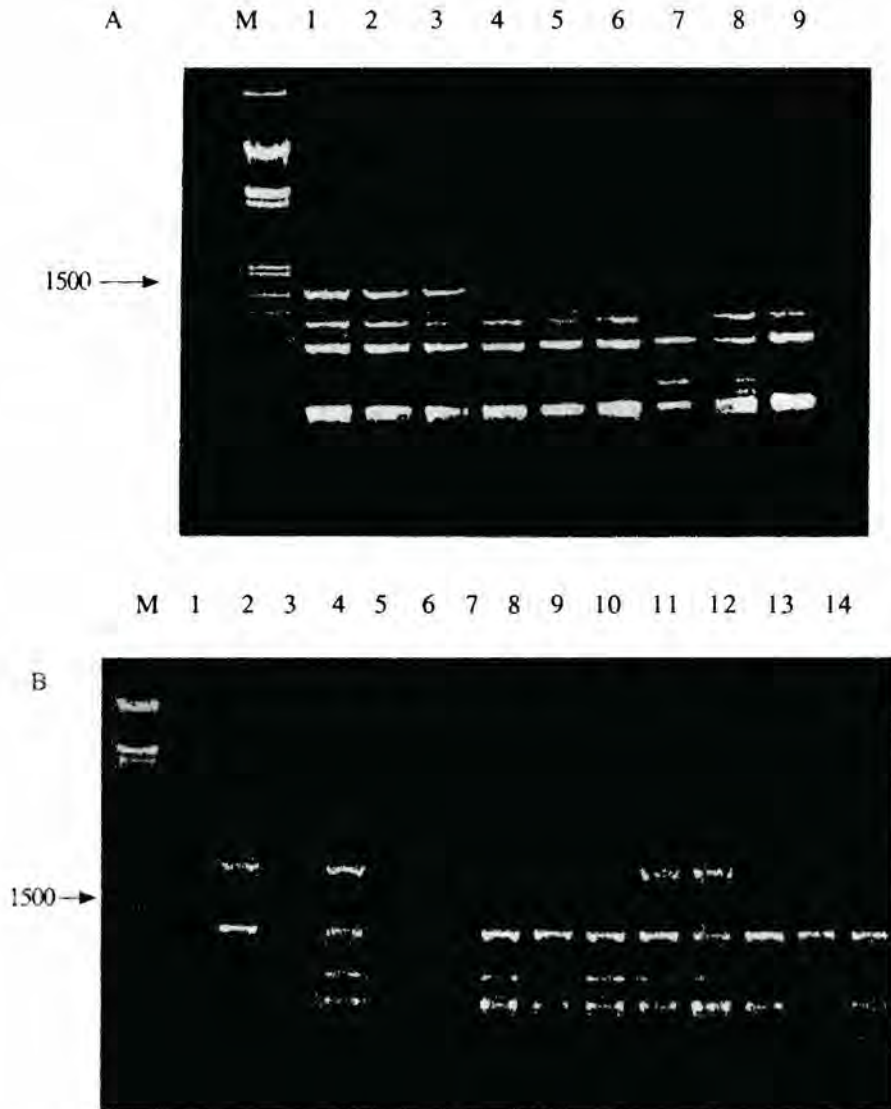


Figure 1. RAPD profiles of Cavendish cultivars obtained with primer (OPJ-04: (A) lanes 1-3 New Guinea Cavendish accession C420 normal plants, 4-8 Dwarf Parfitt, (b) lanes 1-6 Williams accession C251 normal plants, 7-9 dwarf off-types, 10-11 Williams accession C117 normals, 12-14 dwarf off-types, M-molecular weight marker, lambda DNA cut with EcoRI and HindIII (Progen Industries Ltd., Qld, Australia). Arrow indicates the OPJ-04<sub>1500</sub> band.

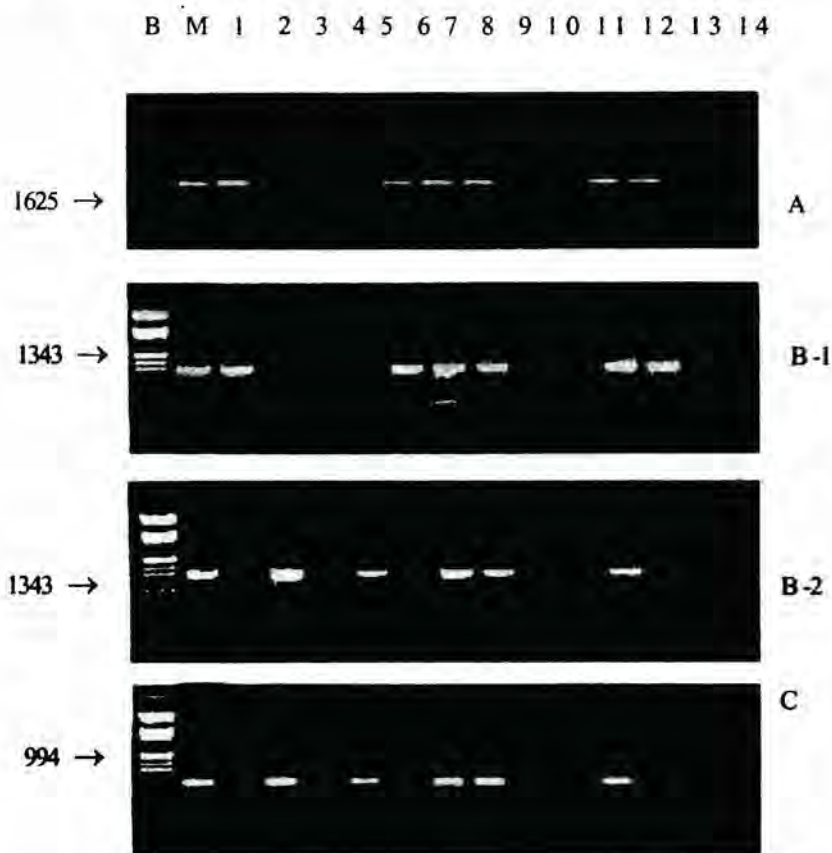


Figure 2. Amplification of SCAR in New Guinea Cavendish (NGC) and Williams. A amplification of SCAR<sub>1625</sub> using primer pair A<sub>1</sub>A<sub>2</sub> and annealing temperature of 65°C. Lanes 1-2 normal, 3-5 dwarf (NGC accession C75, field grown plants), 6-8 normal, 9-10 dwarf (Williams accession C251, field grown plants), 11-12 normal, 13-14 normal, 13-14 dwarf (Williams accession C117, field grown plants). B amplification of SCAR<sub>1343</sub> using primer pair B<sub>1</sub>B<sub>2</sub> and annealing temperature of 50°C. B-1 lanes 1-14 the same as in A, B-2 lanes 1 normal, 2 dwarf (NGC accession C420, in vitro plants), 3 normal, 4 dwarf (Williams accession C117, in vitro), 5 normal, 6 dwarf (Williams accession C76, in vitro), 7-8 normal, 9-10 dwarf (NGC accession C420, glasshouse), 11 normal, 12-14 dwarf (Williams accession C117, glasshouse). C amplification of SCAR<sub>994</sub> using primer pair C<sub>1</sub>B<sub>2</sub> and annealing temperature of 50°C. Lanes 1-14 the same as in B-2. M-molecular weight marker, lambda DNA cut with EcoRI and HindIII. Arrow indicates the SCAR 1625, 1343 and 994 band.

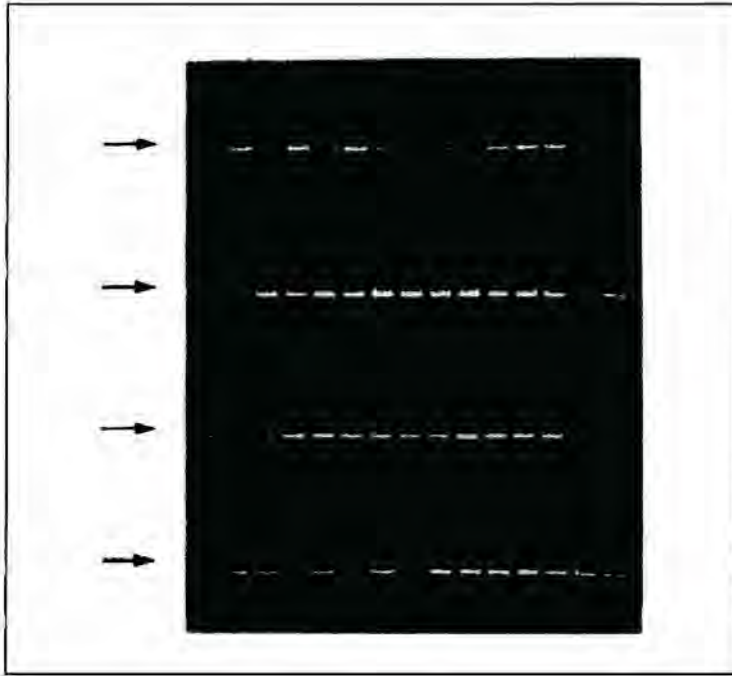


Figure 3. Assay for the presence (+) and absence (-) of SCAR<sub>1343</sub> band in micropropagated plants. The presence of band indicates normal plant and absence dwarf off-type. Arrow indicates the SCAR<sub>1343</sub> band. Amplification reactions were performed using primers B<sub>1</sub>B<sub>2</sub>, 2 mL of unquantified template DNA obtained from a PCR mini prep extraction and 50°C annealing temperature. The amplification products were analysed by electrophoresis on 1.2% agarose gel run for 1 h at 100 V. The first lane on top is the molecular weight marker (lambda DNA cut with EcoRI and HindIII). The assay was performed on *in vitro* plants of Williams (accession C724) samples at subculture cycle 4 of the multiplication stage.

## DISCUSSION

A RAPD marker specific to the dwarf off-type plants from micropropagation was identified after an analysis of 57 normal and 59 dwarf off-type plants generated from several different micropropagation events. The RAPD band, OPJ-04<sub>1500</sub>, was consistently present in all normal and absent in all dwarf off-types for all accessions of New Guinea Cavendish and Williams banana cultivars. Furthermore, the band was absent in all dwarf off-types tested regardless of initial explant source (sucker or floral apex) or mode of shoot regeneration (axillary or adventitious budding). The results therefore suggest that only one type of dwarf mutation exists in all of these micropropagated plants.

Other DNA polymorphisms observed in micropropagated bananas were not associated with the dwarf trait and could be due to other somaclonal changes during micropropagation. The polymorphism observed with the Dwarf Parfitt (a naturally occurring extra-dwarf) and micropropagation-induced dwarf off-types from New Guinea Cavendish and Williams suggests that different dwarf mutations must have occurred and that the dwarf trait marker seems to be associated with dwarfism in micropropagated Cavendish bananas only.

The conversion of the dwarf off-type specific RAPD marker into a dominant SCAR marker allowed for the development of a more rapid and robust PCR-based detection technique for dwarf off-types. All pairs of specific oligonucleotide primers resulted in the amplification of a single major band of expected size based on the sequence of the RAPD marker. The amplification of the specific band occurred only in normal plants and not in any of the dwarf off-type plants of New Guinea and Williams. For the first time, this PCR marker presents the opportunity to reliably identify all dwarf off-type plants *in vitro*, a situation not achievable based on morphology (Smith and Hamill 1993), even with the aid of GA<sub>3</sub> application (Damasco *et al.* 1996).

In other crops, the conversion of RAPD into PCR markers gave a more reproducible assay than could be obtained with RAPD analysis (Paran and Micheltore 1993; Francis *et al.* 1995; Yang and Korban 1996). This would facilitate the use of molecular markers for other applications such as in marker-aided selection, fingerprinting and in molecular taxonomy (Paran and Micheltore 1993; Yang and Korban 1996). In banana, the use of the dwarf off-type specific SCAR marker of the *in vitro* stage affords a reliable means for early detection of dwarf off-types allowing for their elimination before planting of micropropagated plants in the field. In addition, this SCAR marker could be particularly useful for testing the genetic integrity of banana *in vitro* stock materials for micropropagation. Those shoots with a normal plant banding pattern could be multiplied further while those showing the dwarf off-type banding pattern could be discarded. The use of proliferated shoots with a normal banding pattern for further multiplication would reduce the cost of micropropagating bananas as compared to initiating multiplication from new dwarf off-types. Further, micropropagation of these normal

shoots, however, could also result in production of new dwarf off-types. This dwarf off-type SCAR marker will be a useful tool in characterising the nature of the somaclonal variation mechanism that has produced the dwarf. Of particular interest is pinpointing the locus of this genomic alteration. Moreover, this marker can be used in determining whether the polymorphism is pre-existing and enriched by the micropropagation technique, or if the genomic alteration is actually induced in culture.

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# SUBMERGED BATCH FERMENTATION OF CITRIC ACID PRODUCTION USING *Aspergillus niger*: OPTIMIZATION AND KINETIC MODELLING

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## ABSTRACT

*The demand of citric acid requirement in the country is met by importation. It is used in food, pharmaceutical, feed and medical industries. This study was carried out to optimize the effect of initial ammonium (1.0, 2.0 and 3.0 g/l) and glucose (50, 100 and 150 g/l) concentrations and pH (3.0, 4.0 and 5.0) using the multiple regression equation, and to model the kinetics of submerged batch fermentation of citric acid production by *Aspergillus niger*.*

*Initial ammonium concentration effects on glucose, biomass and citric acid concentrations are shown by ANOVA ( $P > F = 0.0001$ ). The highest citric acid concentration, 84.11 g/l was obtained from 1.0 g/l initial ammonium concentration at 30°C, 3.5 pH, 0.8 vvm aeration and 400 rpm agitation rate.*

*The fermentation kinetics related to growth model, product formation, substrate uptake were used to determine the kinetic parameters ( $m$ ,  $q_m$ ,  $k$ ,  $C$ ,  $D$ ) using Leudeking-Piret equations. At the optimum fermentation conditions, the specific growth rate,  $m$  for 1 g/l initial ammonium concentration was  $0.071 \text{ h}^{-1}$ . The non-growth related parameter,  $q_m$  ( $0.0095 \text{ g.product/g.biomass.h}$ ) was obtained at 1.0 g/l initial ammonium concentration indicating that product formation is growth related. Substrate consumption was also growth related since higher value of growth related parameter  $C$  ( $1.6558 \text{ g. substrate/g.biomass}$ ) was obtained compared to the non-growth related parameter  $D$  ( $0.0458 \text{ g.substrate/g.biomass.h}$ ).*

*Computed parameters were used to predict biomass, glucose and citric acid concentration using the model. The model satisfactorily represented the glucose concentration at 1 g/l initial ammonium concentration.*

## INTRODUCTION

Citric acid is the most widely used organic acid of biological origin in the food, feed, pharmaceutical, medicinal and chemical industries. A greater demand for citric acid production could be the result of a large increase in citric acid



consumption among industrialized countries (Sassi et al. 1991). Worldwide sales of citric acid are divided among the principal fields of use. Approximately 75% of citric in the market is used for food and feed, the remaining 10% for pharmaceutical and medicinal purposes and 15% for industrial applications (Milson et al. 1983).

The most important source of citric acid is the fungal fermentation of carbohydrates which is attained either in surface or in submerged process. Submerged process is advantageous because less manpower is required, higher product formation rate is attained, and low level of usable concentration of sugar is needed (Tuompo 1985). Although citric acid can be obtained by chemical synthesis, the cost is much higher than fermentation (Prescott and Dunn 1982). There are many microorganisms, including fungi, yeasts and bacteria that can produce the acid by fermentation. Among them, *Aspergillus niger* is the most well-known and media-based on either purified glucose syrups or beet or cane molasses were utilized as substrate (Milson et al. 1983)

Many researchers have been conducted on the fermentation kinetics of the bioprocess specifically in submerged culture. These resulted in larger variations in the conditions recommended for a successful fermentation and a large number of models developed in describing and understanding the process. Variations include the type of microorganism used, incubation, temperature, pH, aeration, medium composition, etc. (Kristiansen and Sinclair 1978). Citric acid fermentation is characterized by a lot of nutrient limitations such as trace metals, phosphate and ammonium known to change the kinetics of the process through growth of the microorganisms. This study was conducted to optimize the fermentation conditions of glucose and ammonium concentrations and pH of medium on the kinetics of citric acid production, and to establish the fermentation model as well as compare with the experimental data.

### Fermentation Kinetics

Many researches have dealt with fermentation kinetics and the knowledge of kinetics of such process is essential in understanding of the bioconversion. Different kinetic models which describe growth, product formation and substrate consumption can be used in process control to obtain the maximum yield of fermentation (Roehr et al. 1981).

### Biosynthesis

Three main metabolic processes are involved in citric acid accumulation by *Aspergillus niger* namely: 1) breakdown of carbohydrates by the glycolytic pathway to produce pyruvate; 2) anaplerotic formation of oxaloacetate from pyruvate, and 3) accumulation of citric acid within the tricarboxylic acid cycle (Fig. 1) (Milson 1983). The net reaction is as follows:



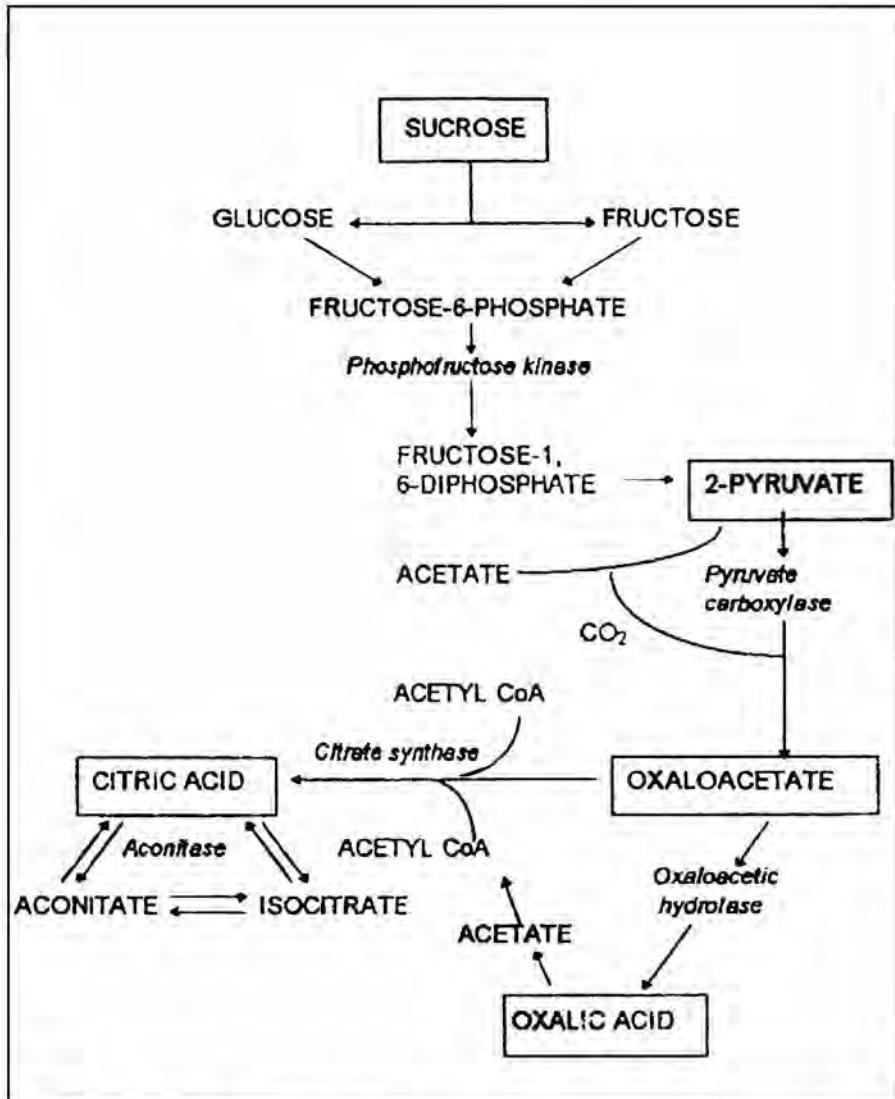


Figure 1. Metabolic pathway of citric acid production from carbohydrates.  
 (Source: (Milson and Meers, 1985))

### Growth Model

The most basic of all growth models is:

$$\mu = \mu_{\max} (1 - X/X_{\max}) \quad (1)$$

Where:

- $\mu$  = specific growth rate, ( $h^{-1}$ )
- $X$  = cell concentration, (g/l)
- $\mu_{\max}$  = maximum specific growth rate, ( $h^{-1}$ )
- $X_{\max}$  = maximum cell concentration, (g/l)

From equation (1), the specific growth rate is predicted to be zero once the maximum cell concentration has been reached. It also estimates the decline in growth rate as the cell concentration approaches its maximum (Klasson et al. 1991).

### Product Formation

Product formation can be related to substrate utilization and growth. The Leudeking-Piret equation for product formation was as follows:

$$1/X \cdot dP/dt = q_m + k\mu \quad (2)$$

Where:

- $q_m$  = non-growth related parameter, (g.product/g.biomass.h)
- $P$  = product formation, (g/l)
- $k$  = growth related parameter, (g.product/g.biomass)

From equation (2), Klasson et al., (3) identified four types of production formation as:

1. For production that is entirely growth related,  $q_m$  is zero or very small and  $(dP/dX) = k = Y_{P/X}$ , the yield of product per cell.
2. For production that is non-growth associated,  $k$  is zero or very small and  $(1/X) dP/dt = q_m$
3. For production that is directly associated with energy production, both  $k$  and  $q_m$  have positive values and  $(1/X) (dP/dt) = q_m + k\mu$
4. For production that is initiated at an intermediate point during the bioprocess,  $k$  is negative and its value determines at which critical specific growth rate production occurs and  $(1/X) (dP/dt) = q_m + k\mu$  ( $k < 0, P > 0$ ).

### Substrate Uptake

The last relationship needed to describe the bioprocess is the rate of carbon sources uptake (Weiss and Ollis 1980) which may be written as:

$$-dS/dt = 1/Y_{X/S} (dX/dt) = 1/Y_{P/S} (dP/dt) + mX \quad (3)$$

Where: S = concentration of substrate, (g/l)  
 $Y_{X/S}$  = yields of cells from substrate, (-)  
 $Y_{P/S}$  = yield of product from substrate, (-)  
 m = maintenance coefficient, (-)

Substitution of equation (2) to equation (3) yields:

$$-dS/dt = (1/Y_{X/S} + k/Y_{P/S})(dX/dt) + (q_m/Y_{P/S} + m)X \quad (4)$$

Equation (4) can be rewritten as:

$$-dS/dt = C (dX/dt) + DX \quad (5)$$

Where: C = growth related parameter for substrate consumption, (g.substrate/g.biomass)  
 D = non-growth related parameter for substrate consumption, (g.substrate/g.biomass.h)

Equation (3), (4) and (5) are valid only for  $S > 0$  and  $P > 0$ .

## MATERIALS AND METHODS

### Microorganism

Microbial strain of *Aspergillus niger* BIOTECH 3104 was obtained from the BIOTECH culture collection in UP Los Baños. The strain was subcultured on potato-dextrose agar slants that has the following composition (g/l): 25, potato; 10, dextrose; and 15, agar. The subcultured slants were incubated at 30°C for 3 days. The cultures were maintained at 4°C until used.

### Inoculum

The seed medium had the following composition (g/l): 30.0, dextrose; 0.5,  $\text{NH}_4\text{NO}_3$ ; 0.5,  $\text{KH}_2\text{PO}_4$ ; 0.1,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ;  $0.1 \times 10^{-3}$ ,  $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ ;  $0.1 \times 10^{-3}$ ,  $\text{ZnSO}_4 \cdot 5\text{H}_2\text{O}$ ;  $0.06 \times 10^{-3}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 2.0, peptone; 1.0, yeast extract; and 0.2, casamino acid. The seed medium was sterilized at 15 psi for 15 min. and cooled to room temperature. Inoculation was done by transferring 4 loopfuls of culture on a slant into 100 ml seed medium in 250 ml Erlenmeyer flask. The flasks were shaken for 24 h at room temperature.

### Experimental Design

Three parameters of glucose (50, 100, and 150 g/l), pH (3.0, 4.0 and 5.0) and ammonium ion (1.0, 2.0 and 3 g/l  $(\text{NH}_4)_2\text{SO}_4$ ) were conducted to optimize the fermentation conditions in the stirred tank fermentor. The experimental set up is shown in Figure 2. Other fermentation parameters were set constant such as 30°C, 0.8vvm aeration and 400 rpm agitation rate.

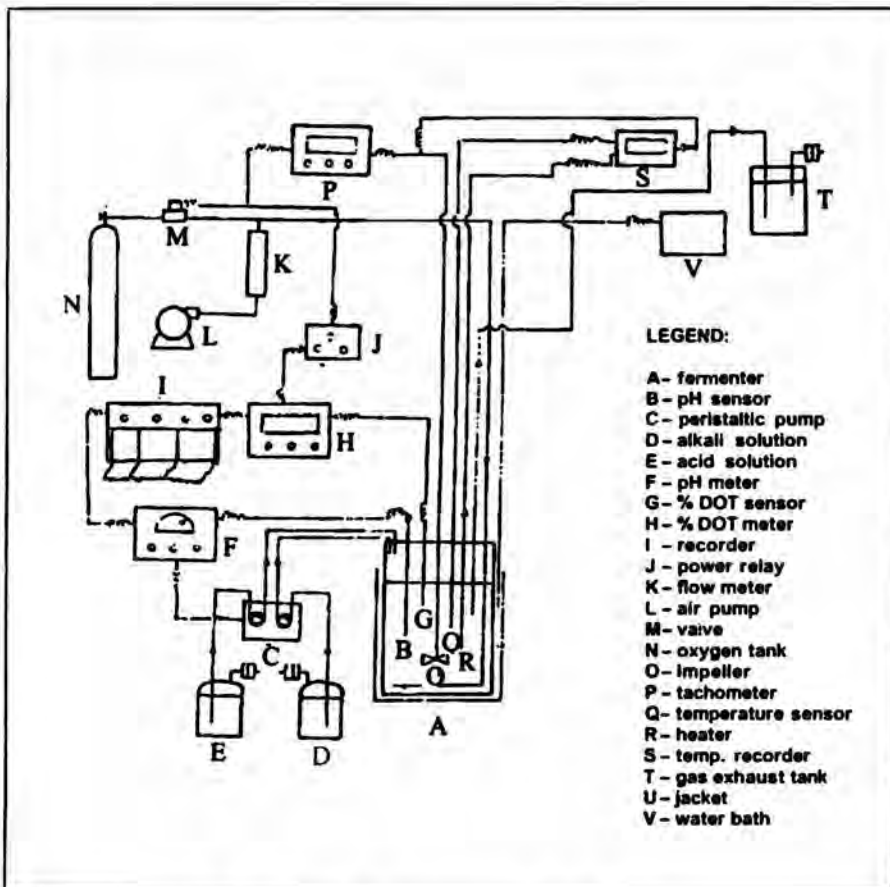


Figure 2. Schematic diagram of the fermentation set-up.

### Fermentation Process

The fermentation medium had the composition as follows (g/l: 50-150, glucose; 0.5,  $\text{NH}_4\text{NO}_3$  or 1.0 -3.0,  $(\text{NH}_4)_2\text{SO}_4$ ; 1.0,  $\text{KH}_2\text{PO}_4$ ; 1.0,  $\text{K}_2\text{HPO}_4$ ; 0.5,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 0.06,  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ; 0.01,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 3.0 peptone; 2.0, yeast extract and 1.0, NaCl. The pH of medium was adjusted to 5 using 2N HCl. The medium was sterilized at 15 psi for 15 minutes and cooled to room temperature. The inoculation rate was 10% (volume of seed/volume of fermentation medium).

### Analytical Methods

*Biomass determination.* The cells concentration was determined by dry weight in the oven at 100°C overnight.

*Glucose determination.* Sugar concentration was analyzed by the colorimetric method of Somogyi (1952).

*Citric acid determination.* The concentration of citric acid was assayed using the improved pyridine-acetic anhydride method (Marier and Boulet 1958).

*Determination of ammonium ion.* The concentration of ammonium ion was analyzed using the colorimetric method (AOAC 1985).

### Statistical Analysis

Data of the effect of ammonium ion concentration on citric acid production were subjected to statistical analysis. The analysis of variance (ANOVA) was carried out to test the treatment difference. Duncan's Multiple Range Test (DMRT) was used to determine the degree of differences among means.

## RESULTS AND DISCUSSION

### Optimization of Fermentation Conditions

*Effect of pH medium.* The effect of pH medium on citric acid production during time course of fermentation at 30°C, 350 rpm and 0.7 vvm aeration rate is shown in Figures 3 to 5. pH was kept constant at set values by automatically adding of 2 N HCl or 2 N NaOH. Dissolved oxygen (DO) was also always kept at 30% saturation by the constant supply of pure oxygen. At pH 3.0 (Fig. 3), the maximum citric acid obtained was 17.6 g/l for 96 h of fermentation. This is 35.1% yield to initial sugar concentration. At pH 4.0 (Fig. 4), the maximum citric acid concentration was 38.2 g/l and 76.5% yield to initial sugar used. The increase of pH medium from 3 to 4, resulted in the increase of citric acid production. At pH 5 (Fig. 5) the maximum citric acid concentration was 25.3 g/l which is 50.7% yield to initial sugar used. The citric acid produced decreased as pH medium increased from 4 to 5. These findings were confirmed by Tsay and To (1987) that the pH ranges from 3.8 to 4.5 provided the highest citric acid.

The negative relationship between glucose consumption and citric acid production was also obtained. The highest sugar which remained was 19.2 g/l at pH 3.0 (Fig. 3), while at pH 4.0 (Fig. 4), the glucose which remained was 1.6 g/l.

**Effect of sugar concentration.** Figure 6 shows data obtained from varying of the initial sugar concentrations in the fermentation medium at pH 4.0. The citric acid concentration was 38.2, 38.6 and 31.3 g/l with the initial substrate concentration of 50, 100 and 150 g/l, respectively, after 96 h of fermentation. It is worthy to note that the fermentation time was short, so that citric acid concentration did not reach the maximum values at stationary phase. These facts are supported by the findings of Choe and Yoo (1991). In their experiments, the fermentation process was carried out for 144 to 168 h in order to obtain the maximum citric acid production. This suggestion is also supported by the remaining glucose concentration after 96 h of fermentation (Fig. 7) which will ably maintain the growth and metabolism of *A. niger* to produce higher citric acid.

**Effect of ammonium concentration.** Figures 8 to 10 show the effect of ammonium concentration on citric acid and biomass during time course of fermentation. The drastic increase of citric acid was obtained after 96 h of fermentation, while glucose as substrate was depleted. The increase of ammonium concentration from 1 g/l to 3 g/l in the fermentation medium resulted in decreasing of citric acid concentration from 84.1 g/l to 34.6 g/l, while biomass concentration was increased from 16.5 g/l to 37.0 g/l for 120 h of fermentation.

The highest yield of 70.1% (Fig. 8) was achieved in 1 g/l ammonium concentration, followed by 40.6% (Fig. 9) in 2 g/l and 29.5% (Fig. 10) in 3 g/l initial ammonium concentration. These results agreed with the findings by Kristiansen and Sinclair (1978). They stated that the effect of ammonium nitrate concentration on production of citric acid may be described by considering streaming of cytoplasm. The streaming may lead to rearrangement of the cell material and the transformation into a storage or citric acid producing cell. Accordingly if streaming is too pronounced due to osmotic pressure by high concentration of ammonium, mitochondria will move toward the nonproducing tip of the hyphae and result in reduced citric acid production. The drastic increase of biomass cells was obtained as the ammonium ion was increased. It could probably be the environmental conditions that affected the cells preferably towards the synthesis of new cells rather than production of citric acid.

The ammonium concentration was constant decreased and depleted after 120 h of fermentation (Figs. 8 and 10).

ANOVA was carried out to determine the significance of treatments (Table 1). Results showed that the initial ammonium concentration significantly affects biomass, glucose and citric acid concentrations  $P > F = 0.0001$ . DMRT was also used to determine the degree of difference among means at 5% level of significance. For biomass, glucose, citric acid and ammonium concentrations, significant differences among means were observed in 1.0 g/l and 3.0 g/l initial ammonium concentrations.

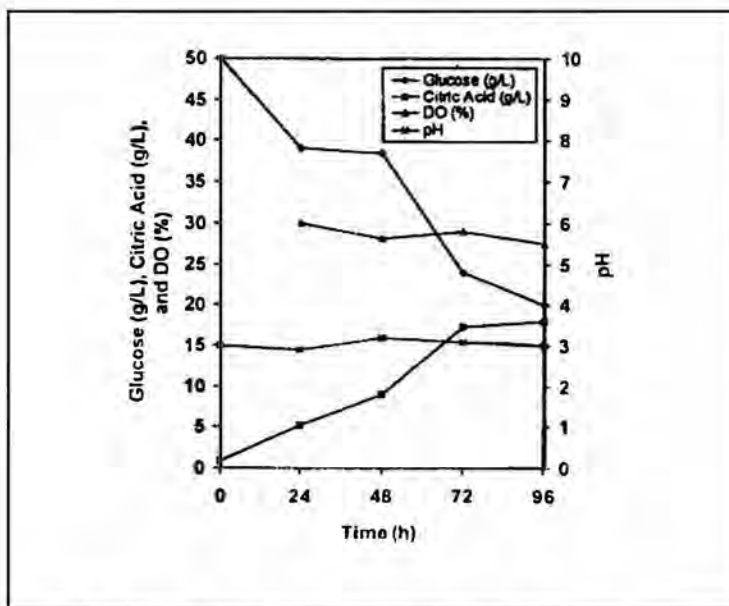


Figure 3. Effect of pH 3 on citric acid production at 50 g/l glucose and 0.5 g/l  $\text{NH}_4\text{NO}_3$ .

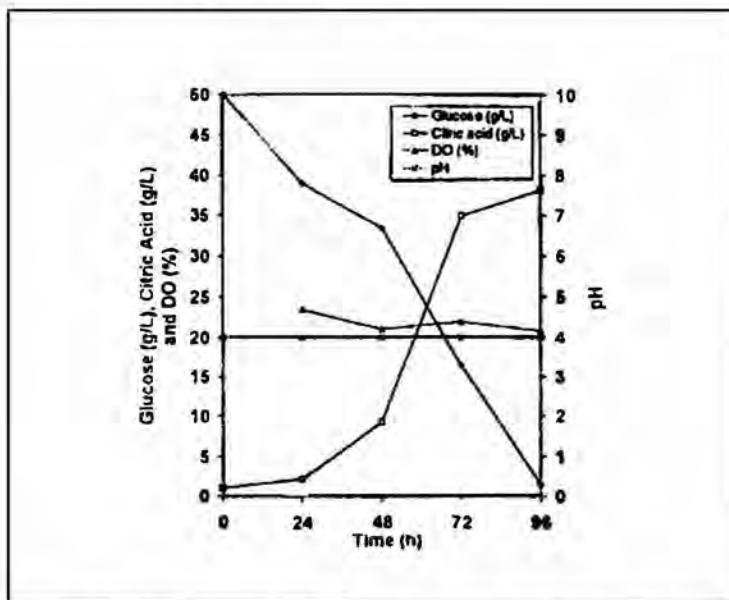


Figure 4. Effect of pH 4 on citric acid production at 50 g/l glucose and 0.5 g/l  $\text{NH}_4\text{NO}_3$ .



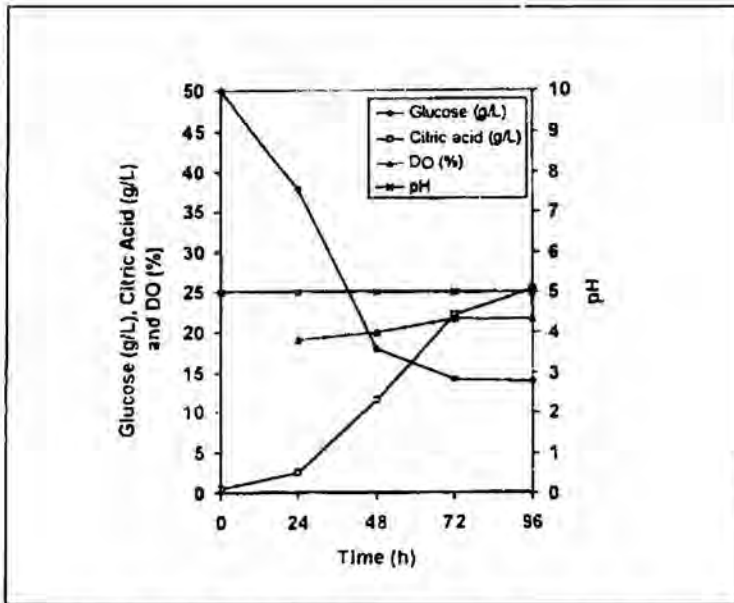


Figure 5. Effect of pH 3 on citric acid production at 50 g/l glucose and 0.5 g/l  $\text{NH}_4\text{NO}_3$ .

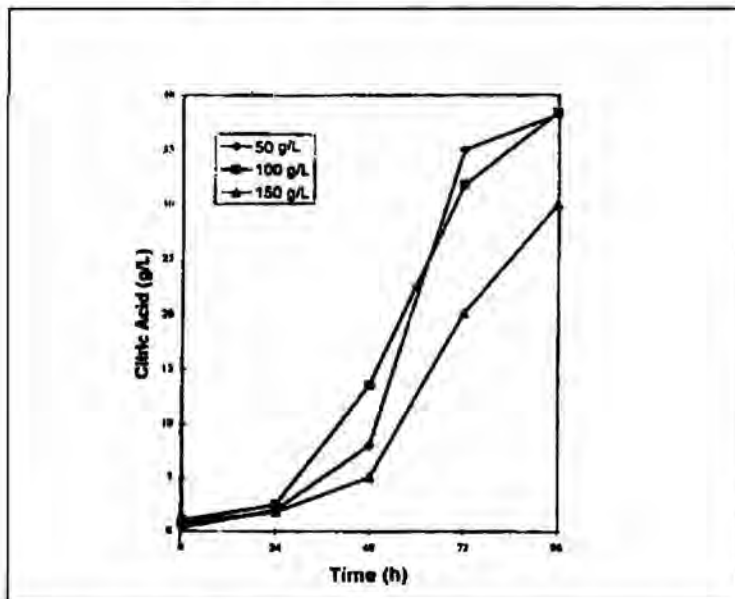


Figure 6. Comparison of citric acid production using initial sugar concentration of 50, 100 and 150 g/l.

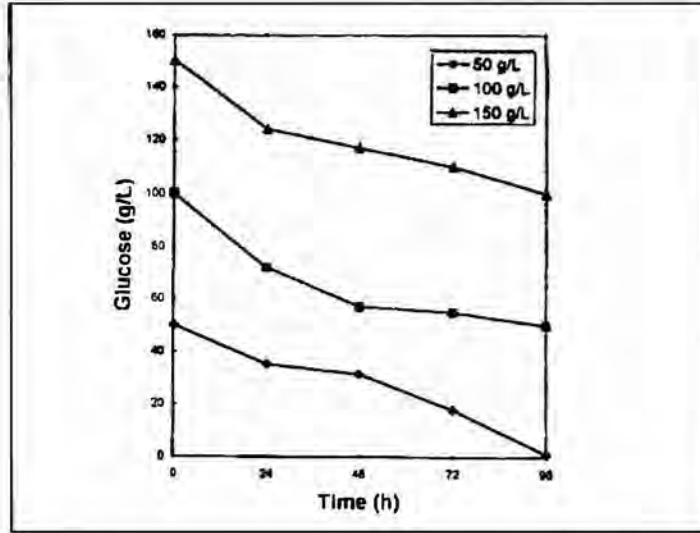


Figure 7. Chang of glucose concentration during the batch fermentation of producing citric acid using *Aspergillus niger* at 0.5 g/l  $\text{NH}_4\text{NO}_3$  and pH4.

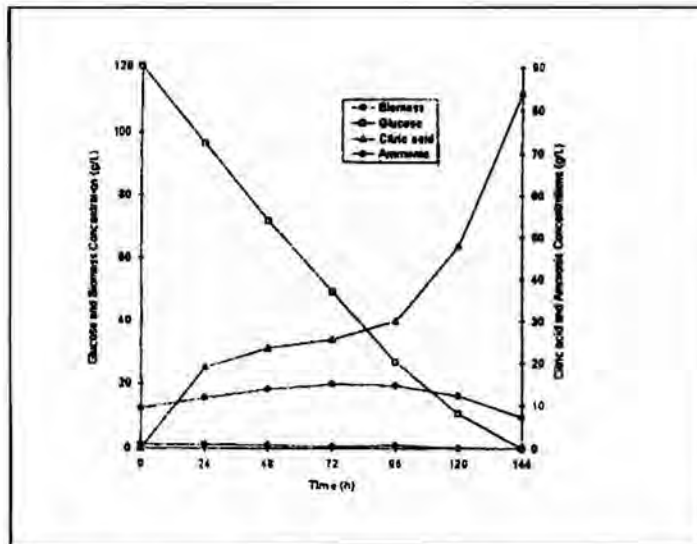


Figure 8. Biomass, glucose, citric acid and ammonia profiles at 1.0 g/l initial ammonia concentration, at 120 g/l glucose and pH 4.0.

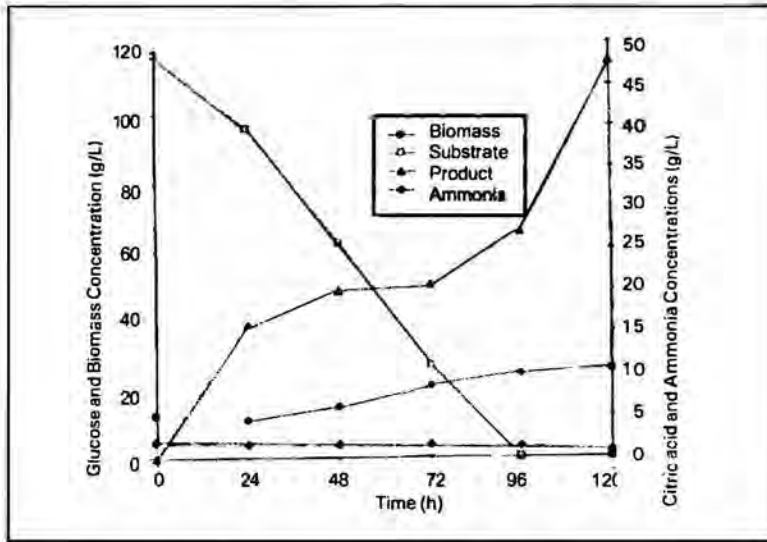


Figure 9. Biomass, glucose, citric acid and ammonia profiles at 2.0 g/l initial ammonia concentration, at 120 g/l glucose and pH 4.0.

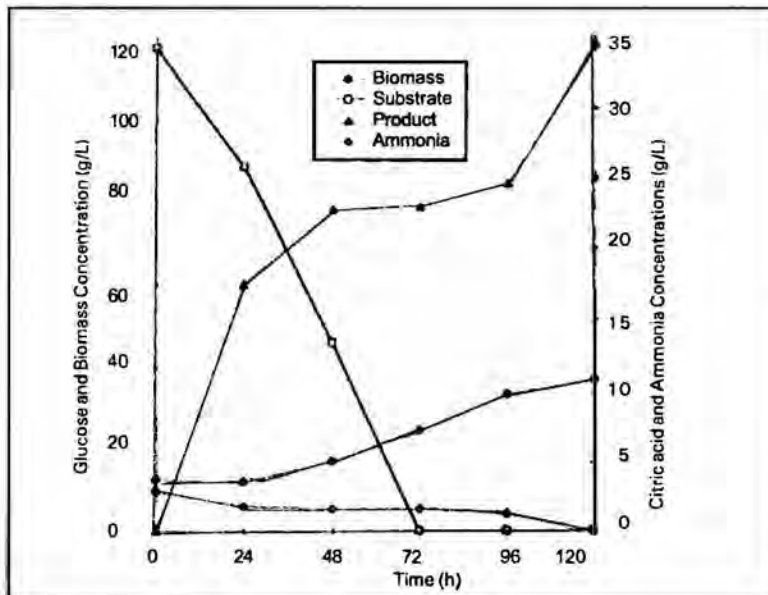


Figure 10. Biomass, glucose, citric acid and ammonia profiles at 3.0 g/l initial ammonia concentration, at 120 g/l glucose and pH 4.0.

For citric acid concentration, the test showed that from 0 to 24 h of fermentation time, significant differences among means were observed. However, from 48 to 96 h, no significant difference was observed. From 120 to 144 h, significant differences among means were observed indicating that there is an increase in citric acid concentration with fermentation time. It is suggested that the prolonged time of fermentation would provide better yield of citric acid.

Table 1. ANOVA of ammonium concentration on citric acid production.

Source	df	Sum of Squares	Mean Squares	F value	P > F
Model	8	13,321.76	1665.220	26.33	0.0001
Error	33	2,086.99	63.242		

$$r^2 = 0.865$$

### Kinetic Parameters Determination

The kinetic parameters of batch fermentation data at the optimized process conditions were determined and summarized in Table 2. These parameters were obtained using the graphical methods (13).

Table 2. Kinetic parameters determined using batch fermentation-data at the optimized process conditions.

Parameter	1 g/l Initial Ammonium Concentration
$\mu$ ( $\text{h}^{-1}$ )	0.0701
$q_m$ (g. product/g.biomass.h)	0.0095
$k$ (g. product /g.biomass)	3.4725
+ D (g.substrate/g.biomass.h)	0.458
+ C (g.substrate/g.biomass)	1.1558

Specific growth rate,  $m$  at 1 g/l initial ammonium concentration was determined using the equation:

$$\mu t = \ln(X_{\max}/X_0 - 1.0) + \ln(X/(1-X)) \quad (6)$$

A plot of  $\ln(X/(1-X))$  versus time gives  $\mu$ . Data from exponential phase was only considered, and  $\mu$  equals 0.0701 for 1 g/l initial ammonium concentration.

Product formation parameters,  $q_m$  and  $k$  were obtained using the Leudeking-Piret equation. At stationary phase ( $dX/dt = 0$ ), non-growth related parameter  $q_m$  was calculated using the following equation (13).

$$q_m = (dP/dt)_{\text{stat}}/X_{\text{max}} \quad (7)$$

Calculated value of  $q_m$  for 1 g/l initial ammonium was 0.0095 g.product/g.biomass.h.

Substituted equation (1) into equation (2) and integration gives:

$$P(t) = P_o + kX_o (e^{\mu t} / (1 - X_o / X_{\text{max}} (1 - e^{\mu t}))) - 1 + q_m (X_{\text{max}} / \mu) \ln(1 - X_o / X_{\text{max}} (1 - e^{\mu t})) \quad (8)$$

A plot of  $X_o (e^{\mu t} / (1 - X_o / X_{\text{max}} (1 - e^{\mu t}))) - 1$  versus  $P - P_o - q_m (X_{\text{max}} / \mu) \ln(1 - X_o / X_{\text{max}} (1 - e^{\mu t}))$  gives the growth related parameter  $k$  as slope. The value of  $k$  determined for 1.0 g/l initial ammonium concentration was 3.4725 g.product/g.biomass.

At stationary growth phase  $(dX/dt) = 0$ , the equation (5) becomes:

$$D = (dS / dt)_{\text{stat}} / X_{\text{max}} \quad (9)$$

Value of the non-growth related parameter  $D$  computed from equation (9) was 0.0458 for 1.0 g/l initial ammonium concentration.

Integration of equation (5) gives:

$$S_o - S(t) = C(X - X_o) + (DX_{\text{max}} / \mu) \ln(1 - X_o / X_{\text{max}} (1 - e^{\mu t})) \quad (10)$$

A plot of  $(X - X_o)$  versus  $S_o - S - (DX_{\text{max}} / \mu) \ln(1 - (X_o / X_{\text{max}}) (1 - e^{\mu t}))$  gives slope  $C$  which is the growth related parameter for substrate consumption.

Calculated value of  $C$  was 1.6558 (g.substrate / g.biomass) at 1.0 g/l initial ammonium concentration. From Table 2, computed value of  $q_m$ , 0.0095 (g.product / g.biomass.h) was smaller compared to a 3.4725 (g.product / g.biomass) value for  $k$  indicating that the production of citric acid is growth related. For substrate consumption, it was confirmed that it is more growth related as can be seen in higher value of the growth related parameter,  $C$  (1.6558) compared to the non-growth related parameter  $D$  (0.04558).

Theoretical values of biomass, glucose, citric acid were computed after the parameters obtained. For biomass (Weiss and Ollis 1980) the following equation was applied:

$$X(t) = X_o e^{\mu t} / (1 - X_o / X_{\text{max}}) (1 - e^{\mu t}) \quad (11)$$

For glucose and citric acid, equations (10) and (8) were used, respectively.

The experimental and theoretical values of biomass, glucose and citric acid concentrations at 1.0 g/l initial ammonium in the fermentation medium were plotted in Figure 11. For biomass, the graph coincides at the stationary phase (72 to 96 h) and remains constant up to 144 h, while the experimental plot declined after the

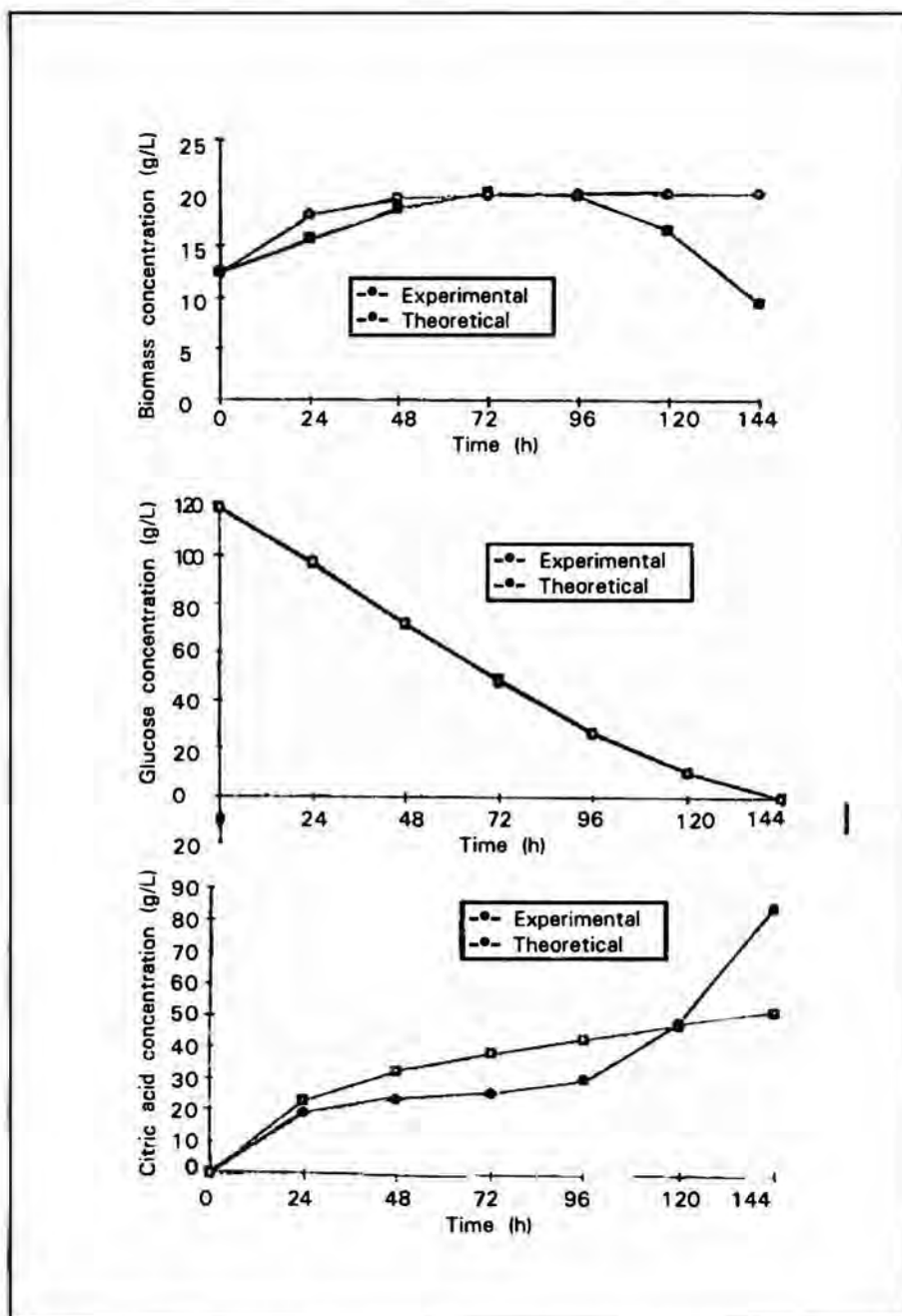


Figure 11. Experimental and theoretical biomass, glucose and citric acid concentrations at 1 g/L initial ammonia concentration.

stationary phase. This could be due to tendency growth up of mycelia pellets when the fermentation was prolonged. This resulted the error in getting of the samples for biomass determination. For substrate consumption, the experimental data fitted very well the theoretical plot. It shows that the parameters, C and D satisfactorily represented substrate consumption at 1.0 g/l initial ammonium concentration. There was the deviation for citric acid plot from 24 to 120 h. It could be possible that the secreted citric acid was entrapped in mycelia pellets and subsequently attributed to low citric acid concentration in the fermentation broth. However, after 120 h of fermentation, large deviation of experimental values for citric acid was obtained. This increase could be attributed to another factor such as streaming of cytoplasm (Kristiansen Sinclair 1978) which would be further studied in the next experiment.

### CONCLUSION

Bioconversion of glucose into citric acid by *Aspergillus niger* in a batch fermentation process was greatly affected by pH medium, substrate and ammonium concentration. The optimal growth and production of citric acid was at a pH range of 4.0 to 4.5. At high substrate concentration (150 g/l glucose), *A. niger* strain exhibited slight inhibition at beginning of fermentation. This inhibition was overcome after 24 h and provided high concentration of citric acid in fermentation broth if the fermentation time was prolonged up to 7 or 8 days. The ammonium concentration was also responsible in the growth and production of citric acid. At the optimized ammonium sulfate concentration (1 g/l), the highest yield (70.1% is obtained).

Kinetic parameters ( $m$ ,  $q_m$ ,  $k$ , C and D) of fermentation were determined. The values of C and D indicated that the production of citric acid is growth related. The model was also established to use in the process control for large scale production.

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## TOXICOLOGICAL STUDIES ON PHILIPPINE NATURAL GRADE CARRAGEENAN

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### ABSTRACT

PNG carrageenan, a new class of food additive derived from *Euchema cottonii* and *E. spinosum* seaweeds, has been globally marketed since 1980. To prove that it is non-toxic and to earn the recognition of the Food Codex Alimentarius and the FAO/WHO Joint Expert Committee on Food Additive (JECFA) as a food additive and, thereby, eliminate future international trade barriers, several toxicological studies were carried out using the conventionally processed (CP) carrageenan as the positive control. A three-month subchronic toxicity test was carried out in rats to examine the adverse effects over a period of time and the cumulative toxicity on target organs and on physiologic and metabolic tolerance from repeated exposure. The following parameters were observed; general observation, body weight, feed efficiency, intake of test materials, clinical examinations (hematology, serum biochemistry, blood coagulation analysis, urinalysis, and fecalysis), and gross, light microscopic and TEM examinations of tissues and organs. A battery of genotoxicology tests, namely Rec assay to determine direct DNA damaging potential, Ames test to assess the mutagenic potential before metabolic activation, Host-mediated assay to determine the mutagenicity after metabolic activation, Micronucleus test to investigate the chromosome breaking effect as well as antigenotoxic activities were done. The subchronic toxicity test in rats revealed the following findings: 1. There were no toxic manifestations or adverse effects observed with PNG and CP carrageenan in rats at a dose that is 80 times more than the Acceptable Daily Intake (ADI) set by the FAO/WHO JECFA for CP carrageenan 2. There was no evidence that PNG and CP carrageenan absorbed from the GIT.



The genotoxicity tests showed that PNG and CP carrageenan 1. do not possess any direct DNA damaging potential, 2. are not mutagenic before and after metabolic activation, 3. have no chromosome breaking effects, and 4. reduced the chromosome breaking effects of the three known genotoxins. From all the studies that were done, it can be concluded that PNG carrageenan has the same toxicological attributes as CP carrageenan and does not present any food safety concern. An ADI for PNG carrageenan, similar to CP carrageenan, can be assigned by the FAO/WHO JECFA. So far in the past years, the Philippine Government has made several significant success in its campaign for PNG carrageenan's international recognition as a food additive and they are as follows: 1. In 1995, the Food Codex Alimentarius, Codex Committee on Food Additives and Contaminants, granted PNG carrageenan a Code No. E 407a under the International Numbering System, 2. In 1996, the European Union lifted the ban on PNG carrageenan as a food additive and allowed its entry in the European Single Market. 3. In 1994, the FAO/WHO JECFA required additional data on PNG carrageenan and calendared it for another round of review in their 1998 meeting to assign a final ADI. Consequently, the new status of PNG carrageenan triggered significant growth in the carrageenan industry in terms of strengthening the economy of the Philippines, expansion of the carrageenan market, improvement of technology and employment security to the marginalized fisherfolks of the Southern and Central Philippines. The seaweed is now one of the nation's 14 top export industries. The industry's total export in 1996 reached US\$120 million compared to only about US\$16 million in 1990, 750% increase.

**Keywords:** E407a, PNG, Carrageenan, Rats, Genotoxicity, Antigenotoxicity, Subchronic Toxicity, Food Additive, Seaweeds, Eucheuma.

## INTRODUCTION

Carrageenan is a mixture of highly sulfated polygalactosides extracted from seaweeds (Stoloff 1959). Carrageenan is further classified as degraded (polygeenan) and native (undegraded) carrageenan. Degraded carrageenan is prepared by mild hydrolysis of iota carrageenan resulting to low molecular substance of 20,000 to 30,000. It has no food value since it lacks gelling and thickening properties, but has medical value (Bonfils 1970; Di Rosa 1972) and toxicological importance (Marcus and Watt 1969). The native carrageenan is widely used as food additive for its gelling and thickening properties and has been evaluated for acceptable daily intake at 50 mg/kg body weight/day in man by the joint FAO/WHO Expert Committee on Food Additives in 1969 and 1974. As of 1984, however, the estimate of Acceptable Daily intake for man has not been specified by the same committee. The detailed structure varies slightly depending on the source, but there are three main types: kappa, which produces a stronger and more brittle gel; iota, which produces a weaker and elastic gel; and lambda, which has no gelling property but has value as thickener (Technical Dossier 1991).

Approximately 70 to 80% of carrageenan worldwide is being produced today from two main species of seaweeds, *Eucheuma cottonii* and *E. spinosum*, mainly because these two species have been successfully cultivated on large scale farms,

*Eucheuma cottonii* is mainly composed of kappa-carrageenan, while *E. spinosum* is mainly composed of iota carrageenan (Technical Dossier 1991).

Food grade carrageenan has been produced mainly in Europe and the United States for more than 30 years by subjecting certain red seaweeds to conventional refining process. In the mid-1970's, a new technology which requires low energy of inputs for refining eucheuma seaweeds was developed in Japan and later on adopted in the Philippines, where eucheuma seaweeds abound, as an alternative refining process. This process retains the carrageenan together with the cellulose after removing the impurities as opposed to the conventional process where the cellulose is removed with the impurities leaving only the carrageenan behind. Thus, PNG carrageenan's main difference from the CP carrageenan is the presence of higher percentage of cellulose, which mostly makes up the acid-insoluble matter, at 12% against less than 1%, and, correspondingly, lower inorganic salt contents at 10 to 15% against 25%. The molecular weight of pure carrageenans from both carrageenans is more than 100,000 and range as high as 800,000 (Technical Dossier 1991).

In the Philippines, carrageenan from eucheuma seaweeds, produced by the alternative refining process, is known as Philippine Natural Grade (PNG) carrageenan. It has also been known by other names. The toxic effects of orally administered carrageenan, mostly of degraded type, have been shown to cause ulcer, metaplastic changes and tumor formations in the large intestine of several species of animals (Watt and Marcus 1969-1970; Grasso 1973; Fabian 1973; Benitz 1973; Wakabayashi 1978; Oohashi 1981). However, orally administered native (undegraded) carrageenan for 13 to 39 weeks from different botanical sources in rats revealed no evidence of carrageenan storage in the body and subchronic and chronic toxicity (Abraham 1985; Nilson and Wagner 1959) administered native carrageenan in rats and mice up to 25% level in their diet for 2 years and failed to demonstrate any adverse biological effects. However, it was able to produce ulcerations in the large intestine of the guinea pig and rabbit, but failed to produce the same lesion in rat, hamster, squirrel, monkey and ferret (Grasso et. al, 1973).

In 1980, PNG carrageenan was marketed in other countries as a new class of food additive carrageenan. The Philippines emerged as heavy exporter of refined carrageenan that competed with the giant manufacturers in the US and Europe.

In 1982, the US Food and Drug Administration (USFDA) imposed a ban on PNG carrageenan. It declared it unsafe to the 250 million American consumers. The new US carrageenan specification restricted AIM to 2%. The new definition denied PNG carrageenan the classification and label "carrageenan." Moreover, the USFDA reported that 12% AIM, although made up of cellulose and minerals, is toxic to man.

The Seaweed Industry Association of the Philippines or SIAP submitted physicochemical and microbiological data to the USFDA through the Department of Trade and Industry (DTI) and the Department of Foreign Affairs (DFA). In 1990, the eight-year import ban was finally lifted by the US government.

The victory of the seaweed industry was negated, in that same year. The European Union (EU) imposed a ban on PNG carrageenan since the FCA-CCFAC does not list PNG carrageenan as food additive.

In 1992, a Memorandum of Agreement was made and entered into by the Central Visayas Regional Projects Office (CVRPO), the Regional Office of the Department of Agriculture (Reg. VII), the SIAP represented by Mr. Benson U. Dakay, and the Philippine Council for Health Research and Development Foundation (PCHRD), Department of Science and Technology.

The CVRPO provided the funds for the toxicological studies, while the PCHRD was responsible for contracting laboratory facilities and services of the best qualified Filipino scientists. The PCHRD tapped Dr. Quintin L. Kintanar, as the Project Leader of the PNG Carrageenan Toxicological Studies. He collaborated with Professor Clara Y. Lim-Sylianco to undertake the genotoxicity and antigenotoxicity studies. The research staff of the BFAD Experimental Animal House Section, Laboratory Services Division conducted the subchronic toxicity test. Dr. Helen A. Molina and Dr. Kiyoshi Imai conducted the pathological examinations.

The research project aims to prove that PNG carrageenan is non-toxic and present no food safety concern is non-toxic. The result will be used to earn recognition from the Food Codex Alimentarius and the FAO/WHO Joint Expert Committee on Food Additive (JECFA) as a food additive and, thereby, eliminate future international trade barriers.

After the completion of the studies, the Philippine government formed a mission composed of representatives from the DTI, DFA, BFAD-DOH and the SIAP. It presented the findings before the food additive experts at the FAC-CCFAC meeting in Brussels and the 44th Meeting of JECFA in Rome.

## MATERIALS AND METHODS

### Three-Month Toxicity Test of PNG Carrageenan in Dietary Administration to Rats

**A. Test and Control Materials.** Philippine Natural Grade carrageenan (PNG Carrageenan, Bengel 350, lot no. NS 960A), refined using the alternative process by Shemberg Marketing Corporation in Cebu City, Philippine, was used as the test material. Conventionally processed carrageenan (Carrageenan) composed of approximately 90% kappa and 10% lambda carrageenan (Gelcarin GIP 812; Product Specification No. 303), manufactured last January 1992 by Marine Colloids Division, FMC Corporation, United States of America was used as the positive control. The standard laboratory powdered diet for rats manufactured by the Bureau of Food and Drugs was used as the vehicle control.

**B. Animals.** One hundred twenty 4-week old specific pathogen-free outbred Sprague-Dawley (SD) rats of both sexes, 60 each, were transferred from the barrier-system production area to the testing area of the experimental animal house. Test male and female rats, 50 each, were selected after the acclimatization period. At the onset of the treatment period, the male and female rats were 6 weeks old.

The rats were selected at random and distributed to each experimental group, such that all experimental groups have nearly the same average body weight. Each experimental group consisted of 10 males or 10 females.

The animals were individually housed in hanging-type cages with mesh wire floorings provided with trays. The animals were allowed free access to their diet.

The room temperature, relative humidity, dark/light hour were set at 22 to 26°C, except on last quarter of feeding when temperature reached 29°C for 5 days due to air-condition malfunction; 40 to 90% relative humidity and 12 hours dark/12 hours light (7:00 am lights noon/7:00 pm off), respectively.

**C. Preparation and Administration.** PNG and CP carrageenan were admixed with powdered laboratory diet in the feed mixer. The mixing level of each test material is shown as follows:

Dose Group	Weight of Vehicle (kg)	Material and Weight (name, kg)	Total kg
0.5% PNG carrageenan	99.5	PNG carrageenan, 0.5	100
1.5% PNG carrageenan	98.5	PNG carrageenan, 1.5	100
5.0% PNG carrageenan	95.5	PNG carrageenan, 5.0	100
5.0% CP carrageenan	95.0	CP carrageenan, 5.0	100

The diet was orally administered using an aluminum feed containers with perforated covers for three months. The groupings and dose level of materials were as follows:

Sex/No.	Group No.	Animal No.	Test Compound	Level
<b>Male</b>				
10	1	11-20	None	Not applicable
10	2	21-30	PNG carrageenan	0.5%
10	3	31-40	PNG carrageenan	1.5%
10	4	41-50	PNG carrageenan	5.0%
10	5	51-60	CP carrageenan	5.0%
<b>Female</b>				
10	1	111-120	None	Not applicable
10	2	121-130	PNG carrageenan	0.5%
10	3	131-140	PNG carrageenan	1.5%
10	4	141-150	PNG carrageenan	5.0%
10	5	151-160	CP carrageenan	5.0%

#### D. Observation and Examination

1. **General Observations.** All animals were observed daily for any clinical signs and behavioral changes, including the gross appearance and consistency of the stools.

2. **Body Weight.** The weight of each animal was measured at pretreatment period, at the start of the treatment, and once a week thereafter until the termination of feeding.

3. **Feed Consumption.** Feed consumed by each rat were measured twice a week.

4. **Efficiency of Feed Utilization.** To weekly feed efficiency (%) the following formula was used was:

$$\frac{\text{Body Weight Gain (g)}}{\text{Feed Consumed (g)}} \times 100$$

5. **Intake of Test Materials.** Individual intake of test materials (mg/kg/day) was computed based on the following formula:

$$[(\text{Feed Consumed (g)} \times 1000) \times (\text{Dose Level (\%)/100})]$$

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$$\text{Body Weight (g)} / 1000$$

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$$7 \text{ days}$$

## 6. Clinical Examination

a. **Hematological Examination.** The following tests were conducted: white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, and differential count.

b. **Blood Coagulation System Test.** The following tests were conducted: prothrombin and activated partial thromboplastin time.

c. **Blood Serum Biochemical Examinations.** The following tests were conducted using automated chemistry analyzer: sodium, potassium and chloride; inorganic phosphorus, albumin, A/G ration, SGOT, SGPT, cholinesterase, alkaline phosphatase, phospholipids, total cholesterol, triglycerides, blood urea nitrogen, total bilirubin, glucose.

d. **Urinalysis.** The following tests were conducted: 1. fresh urine-pH, glucose, ketone bodies, bilirubin, blood, urobilinogen, and 2. 24-hour urine-volume, turbidity, color and specific gravity.

The presence of kappa carrageenan with metachromatic test modified from the method of Beattie et al [5] with a detection limit of 10 ppm.

e. **Fecalysis.** Fecal samples were collected on the 6th and 13th week of feeding and tested for the presence of occult blood using the Guaiaac method.

## E. Pathology

1. **Necropsy.** All animals were sacrificed at the end of the administration period by exsanguination under light ether anesthesia. Full necropsy, which included examination of the external surface of the body, all orifices and the cranial, thoracic, abdominal and pelvic cavities and their contents, were done.

2. **Organ Weight.** The following organs were weighed:

Brain	Spleen
Pituitary Gland	Kidneys (left and right)
Submandibular Glands	Adrenal Glands (left and right)
Thymus	Testes (left and right)
Lungs	Epididymis (left and right)
Thyroid	Prostate Gland
Heart	Ovaries (left and right)
Liver	Uterus
Cecum (with and without contents)	

3. **Microscopic Examination.** Fixed tissues and organs were trimmed, processed (ETP-12V; Sakura), embedded in paraffin (TECS 4589, Sakura Miles) and cut into slices of 3 to 4 micron in thickness using sledge microtome (IVS-400; Sakura) and stained with hematoxylin and eosin.

The liver, spleen, mesenteric lymph node, and gastrointestinal tract of the group which were fed with 5.0% PNG and CP carrageenan, were stained with 0.05% toluidine blue and examined for the presence of metachromatic materials.

4. **Transmission Electron Microscopy.** Electron microscopic observation was performed on the liver and kidney from two males and two females in the vehicle control group and the 5.0% PNG carrageenan groups.

**F. Statistics.** The YUKMS Statistical Library I, Version 4.1; Division Statistical Analysis, Yukms Corp., Tokyo, Japan; Copyright 1987, 1989, 1991, a computer program, was used to calculate statistics.

#### **Mutagenicity, Clastogenicity and Antimutagenicity Potential of Carrageenan**

The following battery of tests were conducted on PNG carrageenan as the test substance and CP carrageenan as the positive control:

**Rec Assay** was used to determine direct DNA damaging potential (Kada, T.K. et al.; 1980).

**Ames Test**, without -9 mix, was employed to assess the mutagenic potential before metabolic activation (Ames, B.N.; 1971).

**Host-mediated Assay** was used to study mutagenicity after metabolic activation (Moriya, M.; 1980).

**Micronucleus Test** was used to investigate the chromosome breaking effect as well as antigenotoxic activity (Schmid, W.; 1978)

Genotoxins dimethylhydrazine, dimethylnitrosamine and benzo(a)pyrene were used to induce mutagenicity.

The Swiss Webster mice were used in the Micronucleus test. ANOVA was used in statistical evaluations.

## RESULTS

### Three-Month Toxicity Test of PNG Carrageenan in Dietary Administration to Rats

#### A. General Observations

There were no mortalities in all groups of either sex.

There were no clinical signs and behavioral changes related to the administration of PNG carrageenan and CP carrageenan, except for changes in the consistency and appearance of feces (Table 1; 1.A and 1.B). A few rats fed with PNG carrageenan, particularly at 5.0% dietary level developed soft formed stool sporadically starting on the middle third of administration. Big sized and fragmented stools were also occasionally observed. Beaded appearance of stools, which were not observed with CP carrageenan, were also noted.

All animals fed with CP carrageenan passed soft formed stools, which had an unusual pungent odor, frequently from the initiation up to the termination of feeding. Big sized and fragmented stools were also observed frequently from the middle period up to the end of feeding.

The findings on fecal consistency and appearance with PNG carrageenan-fed rats were definitely observed at a lower frequency and involved fewer rats than CP carrageenan-fed rats in either sex.

**Body Weight.** There were no significant differences in body weight among all groups in either sex.

**Feed Consumption.** There were no significant differences in feed consumption among all groups in either sex.

**Efficiency of Feed Utilization.** There were no significant differences in the efficiency of feed utilization among all groups in either sex.

**Intake of Test Materials.** The average daily intake of PNG carrageenan at different mixing levels were 382, 1140 and 3887 mg/kg/day in male rats and 410, 1292 and 4170 mg/kg/day in female rats. On the other hand, the average daily intake of CP carrageenan in male and female rats were 3917 and 4249 mg/kg/day, respectively (Table 2).

Average daily intake of PNG carrageenan at 0.5, 1.5 and 5.0% level of the diet were approximately 8, 25 and 80 times, respectively, more than the JECFA acceptable daily intake of 50mg/kg/day (1974) for an adult man.



Table 1. General and Fecal Observations  
Table I.A. In Male Rats

DIETARY GROUP	OBSERVATIONS	No. of Weeks After Initiation of Feeding														Total
		Frequency of Observation (No. of Rats Affected or Observed)														
		1	2	3	4	5	6	7	8	9	10	11	12	13		
Vehicle Control	STOOL															
	Soft						4(2)								2(1)	6
	Big-Size														2(1)	2
	Fragmented															
	Wound on gums												2(1)	2(1)		2
PNG (0.5%) Carrageenan	STOOL															
	Soft		8(4)	1(1)							1(1)				1(1)	11
	Big-size			1(1)			1(1)									2
	Fragmented										4(2)	2(2)		7(3)	13	
PNG (1.5%) Carrageenan	STOOL															
	Soft								1(1)						2(1)	3
	Big-size		2(1)			1(1)	1(1)	5(2)							8(4)	17
	Fragmented									3(2)	2(1)	2(1)			6(3)	13
	Chromodacryorrhea													6(1)	6	
PNG (5.0%) Carrageenan	STOOL															
	Soft		10(6)			12(6)	3(1)	3(1)	10(4)	6(2)	4(2)	12(4)	9(5)	21(7)	90	
	Big-size					8(4)	18(5)	13(2)	5(3)	13(5)	14(5)	2(2)	6(5)	17(6)	96	
	Fragmented					4(2)	11(4)	12(4)	12(6)	14(5)	20(7)	19(5)	7(4)	13(4)	112	
	Bead-Shape						7(4)	3(3)			2(1)				12	
CP (5.0%) Carrageenan	STOOL															
	Soft	70(10)	102(10)	78(10)	85(10)	75(10)	72(10)	52(10)	39(10)	98(10)	78(10)	35(10)	68(10)	836		
	Big-size					4(3)	10(5)	34(7)	25(9)	26(10)	22(10)	10(6)	13(7)	26(8)	170	
	Fragmented					2(1)		25(10)	62(10)	16(10)	17(9)	40(10)	16(8)	178		

Table I.B. In Female Rats

DIETARY GROUP	OBSERVATIONS	No. of Weeks After Initiation of Feeding													Total
		Frequency of Observation (No. of Rats Affected or Observed)													
		1	2	3	4	5	6	7	8	9	10	11	12	13	
Vehicle Control	STOOL														
	Soft		1(1)												1
	Big-Size							1(1)							1
	Fragmented													2(2)	2
	Wound on gums													1(1)	1
PNG (0.5%) Carrageenan	STOOL														
	Soft														0
	Big-size					1(1)									1
	Fragmented							3(3)							3
PNG (1.5%) Carrageenan	STOOL														
	Soft		1(1)	1(1)	1(1)	1(1)									4
	Big-size		12(6)					7(2)			2(2)				21
	Fragmented									6(1)		1(1)	2(1)	9	
PNG (5.0%) Carrageenan	STOOL														
	Soft					2(1)	2(1)	2(1)	3(3)		2(1)	7(2)	4(3)	3(2)	25
	Big-size		5(2)			4(2)	6(3)			1(1)			4(2)		20
	Fragmented							1(1)	7(4)	6(3)	2(1)	8(4)	10(2)	2(1)	36
	Bead-Shape							8(5)		1(1)	1(1)			10	
CP (5.0%) Carrageenan	STOOL														
	Soft	5(5)	26(10)	94(10)	62(10)	68(10)	80(10)	67(10)	48(10)	40(10)	63(10)	52(1)	30(1)	49(1)	684
	Big-size					1(1)	3(3)	17(8)	10(4)	10(4)	5(3)	11(7)	6(4)	7(3)	78
	Fragmented						3(3)	14(7)	19(8)	43(8)	21(9)	15(8)	43(9)	13(8)	171
	Swollen Gums					2(1)								2	

Table 2. Daily Intake of Test Materials

Sex	Dietary Groups	No. of Animals	Average	Weeks After Initiation of Administration													Daily Intake
				1	2	3	4	5	6	7	8	9	10	11	12	13	
Male	Vehicle Control	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
	PNG (0.5%)	10	744	604	518	433	394	359	332	313	296	268	271	225	209	302	
	Carrageenan		18 <sup>b</sup> (9)	31	33	20	40(8)	15	18(9)	15	16	24	8	10	12	151	
	PNG (1.5%)	10	2250	1785	1517	1269	1163	1079	962	896	918	867	822	666	621	1148	
	Carrageenan		97	66(9)	49(9)	41	16(9)	44(9)	71(9)	37	36	56(9)	54	33	23	449	
	PNG(5.0%)	10	7494	6125	5058	4182	4008	3750	3395	3149	3057	2968	2831	2290	2229	3887	
	Carrageenan		236	272	210(9)	165(9)	210(9)	221(9)	133	198	412(8)	217	486(9)	142	220	1474	
	CP (5.0%) Carrageenan	10	6982	6470	5389	4316	4017	3711	3407	3147	3047	2936	2899	2372	2222	3917	
			707	351	202(9)	233(8)	164(9)	149(8)	247(9)	181(9)	161	168(9)	534	182	273	1445	
Female	Vehicle Control	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
	PNG (0.5%)	10	629	560	501	457	417	401	402	372	368	337	336	291	261	410	
	Carrageenan		50	34	30	150	29	23	37(9)	18	15(9)	31	60	36	41	101	
	PNG (1.5%)	10	2018	1765	1523	1389	1315	1321	1206	1190	1173	1885	1028	869	785	1282	
	Carrageenan		169	120	66(8)	60(8)	51(7)	122(9)	64	79(8)	77	97	124	88	47	327	
	PNG (5.0%)	10	6758	5700	5009	4561	4396	4289	4126	3802	3729	3441	3194	2689	2518	4170	
	Carrageenan		470	159	260(9)	369(9)	294(9)	329(9)	706(9)	166	214	146	132	252	26	1133	
	CP (5.0%) Carrageenan	10	6939	5886	5072	4718	4352	4156	4154	3835	3703	3500	3545	2855	2518	4249	
			1169	302	298	497	454(9)	342	293	321	357	343	473(9)	284	253	1154	

a= mean

b=standard deviation

unit: mg/kg BWT/day

Nos. in ( ) indicate the No. of Animals out of 10 whose Daily Intake of Test Materials were computed Based on the Feed Consumption Record. If ( ) is not present, all 10 animals were computed.

## B. Clinical Examination

**Hematology.** There were no significant differences in red blood cell count, white blood cell count, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, reticulocyte count (Retics), and differential count among all groups in either sex.

**Blood Coagulation System.** There were no significant differences in prothrombin time, activated partial thromboplastin time among all groups in either sex.

**Blood Serum Biochemical Examinations.** There were no significant differences in the total protein (TP), albumin, creatinine (Creat.), albumin and globulin ratio (A/G), alkaline phosphatase (ALP), cholinesterase (ChE), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic oxaloacetic transaminase (SGPT), phospholipid (P lipid), total cholesterol (T Chol.), triglycerides (Trigl), blood urea nitrogen (BUN), total bilirubin (T Bil), glucose (Gluc., ) inorganic phosphorus (in P), chloride (Cl), and potassium (K) among all groups in either sex (Table 3).

In female rats fed at dietary level of 0.5, 1.5 and 5.0% PNG carrageenan and CP carrageenan, the levels of serum sodium were significantly lower than the vehicle control and in male rats fed with CP carrageenan, the level of serum calcium was significantly higher than the vehicle control and the 5.0% PNG carrageenan group. In the absence of any abnormal findings related to serum sodium and calcium level abnormalities, the values were considered normal and of no toxicological importance.

**Urinalysis.** There were no significant differences in fresh urine pH, protein, ketone bodies, glucose, absence of occult blood, and urobilinogen observations and 24-hour urine volume specific gravity, color and turbidity observations (Table 4) among all groups in both sexes. However, three female rats fed with carrageenan were found positive for bilirubin, but no indications of intrahepatic or extrahepatic lesions were noted (Cornelius 1970). The concentration of this bile pigment in the urine is directly proportional to the degree of biliary obstruction whether intrahepatic or extrahepatic. Certain substances might have interfered with the semi-quantitative test.

Apparently, there was a tendency of urine volume to increase in animals administered with 5.0% PNG carrageenan with correspondingly lower specific gravity and pale urine color compared with the vehicle control group.

No carrageenan was detected in the urine excreted by all animals administered with PNG and CP carrageenan using the urine metachromasia test with the detection limit of 10 ppm.

**Fecalalysis.** Fecal samples collected on the 6th and 13th of administration from all groups of either sex were negative for fecal occult blood.

Table 3. Results of Serum Biochemical Examination

Sex	Dietary Groups	No. of Animals	T. Prot g/dL	Albumin g/dL	Gluc. mg/dL	A/G	Creat. mg/dL	BUN mg/dL	T. Bill. mg/dL	ALP IU/L	ChE IU/L	AST IU/L	ALT IU/L	Pilipid mg/dL	T. Chol mg/dL	Trigl mg/dL	Ca mg/dL	In. P mg/dL	Na m	Cl mol	K /L
Male	Vehicle	10	6.22 <sup>a</sup>	3.67	165.3	1.4	0.62	43.0	0.09	161.9	179	130	55.2	182	77.5	174	11.0	8.1	152	112	5.1
	Control		0.50 <sup>b</sup>	0.21	30.1	0.1	0.04	4.2	0.05	46.0	43	22	11.6	38	15.1	71	1.8	1.7	11	7	0.5
	PNG (5%)	10	6.42	3.73	200.9	1.3	0.63	39.4	0.10	168.8	254	143	93.6	184	76.9	197	12.0	8.8	155	114	5.6
	Carrageenan		0.80	0.42	50.6	0.1	0.08	8.9	0.05	41.5	191	29	63.4	36	10.3	130	2.7	2.2	19	15	0.9
	PNG (1.5%)	10	6.09	3.70	171.7	1.4	0.67	44.6	0.09	183.2	201	141	69.1	183	80.1	164	11.2	8.8	157	117	5.3
	Carrageenan		0.66	0.41	22.2	0.1	0.08	6.3	0.04	50.1	49	36	32.6	20	11.3	40	1.8	1.5	15	10	0.4
	PNG (5.0%)	10	5.55	3.52	181.7	1.4	0.65	41.4	0.10	146.9	185	139	72.7	166	76.7	117	10.3	8.1	156	116	5.2
	Carrageenan		0.78	0.38	26.9	0.1	0.08	5.3	0.03	28.6	44	43	55.9	18	11.2	21	1.7	1.0	15	9	0.4
	CP(5.0%)	10	6.74	3.96	192.4	1.4	0.70	41.2	0.11	172.2	236	168	72.2	167	78.7	121	13.4	9.2	156	117	5.3
	Carrageenan		0.99	0.43	19.9	0.1	0.08	8.4	0.02	52.9	53	61	37.4	33	12.9	49	2.7	1.9	18	14	0.9
Female	Vehicle	10	6.63	4.14	159.9	1.6	0.65	43.4	0.08	127.1	1708	147	53.7	203	87.7	130	11.9	6.9	150	113	5.1
	Control		0.49	0.20	28.6	0.1	0.05	7.2	0.05	34.8	350	30	12.5	28	12.2	52	0.9	2.1	2	3	0.9
	PNG (0.5%)	10	6.29	3.89	145.6	1.6	0.64	39.2	0.08	121.2	1370	125	43.2	170	81.1	81	11.0	6.1	145	112	4.7
	Carrageenan		0.32	0.20	17.3	0.1	0.07	6.3	0.04	41.9	279	15	7.7	28	13.0	28	0.9	1.7	2*	2	0.4
	PNG (1.5%)	10	6.10	3.85	150.9	1.6	0.62	41.5	0.08	113.0	1573	134	46.4	184	89.6	99	11.1	6.4	146	112	4.7
	Carrageenan		0.31	0.16	11.2	0.1	0.04	5.5	0.04	50.4	429	25	8.7	26	14.6	40	0.8	1.6	1*	2	0.2
	PNG (5.0%)	10	6.26	3.92	159.3	1.6	0.64	39.7	0.06	145.0	1497	133	56.7	186	79.3	126	11.9	6.8	144	110	4.6
	Carrageenan		0.40	0.20	18.5	0.0	0.03	1.9	0.02	35.4	409	21	14.8	36	8.3	68	1.0	1.8	2*	2	0.4
	CP(5.0%)	10	6.64	4.07	194.8	1.6	0.68	39.0	0.09	132.7	1655	196	83.9	178	82.8	95	12.0	7.7	144	111	5.3
	Carrageenan		0.65	0.36	118.3	0.1	0.05	5.2	0.03	35.1	494	193	105	42	17.0	48	1.6	2.8	2*	4	2.3

a = mean

b = standard deviation

# - p&lt;0.05 vs 5.0% PNG Carrageenan and Vehicle Control Groups

\* - p&lt;0.05 vs Vehicle Control Group

Table 4. Result of 24-Hour Urine Examination.

Sex	Dietary Groups	No. of Animals	Urine Volume mL	Urine Values	Specific Gravity							Color			Turbidity				
					1.010	1.020	1.030	1.040	1.050	1.060	1.070	1.080	LY	Y	DY	clear	slight	turbid	
					1.019	1.029	1.039	1.049	1.059	1.069	1.079	1.089							
Male	Vehicle Control	10	10.6 <sup>a</sup> 4.7 <sup>b</sup>	1.0385 0.0130	0	3	4	0	2	1	0	0	1	5	4	1	2	7	
	PNG (0.5%) Carrageenan	10	11.0 9.8	1.0478 0.0210	1	1	1	3	1	2	0	1	2	3	5	3	3	4	
	PNG (1.5%) Carrageenan	10	12.4 6.4	1.0383 0.0150	0	3	3	3	0	0	1	0	2	7	1	4	1	5	
	PNG (5.0%) Carrageenan	10	17.9 7.8	1.0274 0.0100	2	3	4	1	0	0	0	0	4	4	2	2	0	8	
	CP (5.0%) Carrageenan	10	13.5 4.0	1.0322 0.0120	1	4	4	0	0	1	0	0	0	8	2	3	0	7	
	Female	Vehicle Control	10	10.7 3.8	1.0267 0.0070	1	6	3	0	0	N.A.	N.A.	N.A.	2	7	1	3	0	7
		PNG (0.5%) Carrageenan	10	12.4 4.3	1.0231 0.0080	3	5	2	0	0	N.A.	N.A.	N.A.	2	7	1	0	0	10
		PNG (1.5%) Carrageenan	10	10.0 2.8	1.0295 0.0100	1	5	3	1	0	N.A.	N.A.	N.A.	6	2	2	2	0	8
PNG (5.0%) Carrageenan		10	14.3 6.2	1.0242 0.0100	4	4	1	0	1	N.A.	N.A.	N.A.	5	4	1	4	0	6	
CP (5.0%) Carrageenan		10	8.9 4.8	1.0313 0.0120	2	2	4	1	1	N.A.	N.A.	N.A.	2	3	5	5	0	5	

a = mean  
b = standard deviation

Legend:

LY - Light Yellow  
Y - Yellow  
DY - Dark Yellow

N.A. -Not Applicable

### C. Pathology

**Necropsy.** No gross lesions were observed related to the administration of PNG carrageenan, as well as CP carrageenan, in either sex. The distention of colon may be due to the presence of big-sized stools. Prominent lymphoid tissues in the colon are not unusual in rats.

**Organ Weight.** There were no significant differences in the absolute (Tables 5; 5.A., 5.B. and relative organ weight among all groups in either sex. However, there is a tendency for the cecal content to be heavier in animals which were fed with 5.0% PNG carrageenan compared with the vehicle control group in either sex.

**Light Microscopic Examination.** There were no microscopic lesions as a result of the administration of PNG and CP carrageenan in all tissues and organs examined.

Toluidine blue staining of the liver, spleen, mesenteric lymph nodes, and the gastrointestinal tract revealed no deposition of metachromatic materials. Mast cells, which also stained with toluidine blue, were present in some tissues.

**Transmission Electron Microscope.** The deposits of PNG and CP carrageenan were not observed in the liver, kidney and colon of rats and no toxic changes were also noted in these organs

### Mutagenicity, Clastogenicity and Antimutagenicity Potential of PNG Carrageenan

The results of the batteries of test are summarized in Tables 6, 7, 8, 9 and 10.

## DISCUSSIONS

### Three-Month Toxicity Test of PNG Carrageenan in Dietary Administration to Rats

The results of the three-month subchronic toxicity test in rats of either sex revealed no evidence that PNG carrageenan was absorbed from the gastrointestinal tract and no toxic manifestations or adverse effects were observed as a result of ingesting PNG carrageenan up to 5.0% level in the diet. The only effects observed were physiological adaptations to the orally administered test material consisting of soft consistency of formed feces, a tendency of urine volume to increase with corresponding decrease in urine specific gravity and pale urine color, and a tendency for the weight of the cecal content to increase. The same findings were observed with CP carrageenan.

It was reported that metachromatic materials in urine and in the reticuloendothelial cells of the spleen and liver of rodents were proofs of carrageenan absorption (Anderson and Soman 1966; Eagleton *et al.* 1969). In the present study, metachro-

Table 5. Absolute Organ Weight.  
Table 5.A In Male Rats

Dietary Groups	Body Weight g	Brain g	Hypophysis mg	Submandibular g	Thyroid mg	Lungs g	Thymus g	Heart g	Liver g	Adrenals mg		Kidneys g		Spleen g	Cecum g or content		Prostate g	Epididymis (g)		Testes g	
										L	R	L	R		L	R		L	R		
Vehicle	459	2.30 <sup>a</sup>	12	0.71	29	1.61	0.32	1.27	14.8	24	22	1.20	1.26	0.62	1.82	5.97	1.09	0.67	0.66	1.6	1.6
Control	34	2.28 <sup>b</sup>	3	0.06	7	0.13	0.06	0.09	2.3	4	2	0.16	0.13	0.23	0.23	1.17	0.33	0.06	0.05	1	0
																				0.1	0.0
																				0.	2
PNG (0.5%)	484	2.28	11	0.69	28	1.62	0.33	1.35	15.7	23	20	1.38	1.32	0.62	1.84	5.70	1.05	0.64	0.65	1.5	1.5
Carrageenan	38	0.12	2	0.06	3	0.15	0.06	0.10	1.8	5	5	0.09	0.13	0.29	0.24	1.03	0.25	0.06	0.05	9	8
																				0.1	0.1
																				4	4
PNG (1.5%)	480	2.36	12	0.75	30	1.56	0.32	1.33	15.1	22	20	1.20	1.26	0.68	1.72	5.95	1.21	0.66	0.68	1.6	1.6
Carrageenan	39	0.22	3	0.07	8	0.21	0.07	0.11	1.1	6	3	0.11	0.11	0.10	0.23	1.40	0.41	0.07	0.06	2	1
																				0.1	0.1
																				2	3
PNG (5.0%)	465	2.26	12	0.73	29	1.50	0.30	1.30	14.2	22	22	1.22	1.24	0.66	1.76	7.12	1.20	0.67	0.68	1.6	1.6
Carrageenan	39	0.10	4	0.06	7	0.19	0.05	0.08	1.2	3	2	0.09	0.09	0.07	0.25	1.54	0.33	0.07	0.06	3	3
																				0.0	0.1
																				7	2
CP (5.0%)	457	2.28	12	0.73	29	1.53	0.29	1.28	14.1	22	20	1.29	1.29	0.63	1.91	6.08	1.38	0.60	0.62	1.4	1.5
Carrageenan	37	0.13	3	0.06	7	0.16	0.07	0.10	1.4	4	5	0.11	0.12	0.09	0.26	1.47	0.24	0.11	0.11	8	7
																				0.3	0.0
																				4	8

a = mean

b = standard deviation



Table 5.B In Female Rats

Ditary Groups	Body Weight g	Brain g	Hypo-physis mg	Subman-dibular g	Thy-roid mg	Lungs g	Thy-mus g	Heart g	Liver g	Adrenals		Kidneys		Spleen g	Cecum		Uterus g	Ovary mg	
										mg L	g R	L	R		organ	g content		L	R
Vehicle	257	2.05 <sup>a</sup>	13	0.45	20	1.15	0.2	0.82	8.3	31	28	0.78	0.77	0.42	1.24	3.82	0.68	62	59
Control	20	0.06	6	0.03	2	0.23	0.06	0.07	0.7	5	4	0.07	0.07	0.04	0.14	0.64	0.18	10	6
PNG (0.5%)	250	2.10	14	0.47	20	1.08	0.23	0.78	7.8	30	26	0.77	0.74	0.41	1.19	3.76	0.58	58	60
Carrageenan	20	0.6	6	0.4	5	0.06	0.03	0.06	0.7	4	6	0.07	0.07	0.04	0.09	0.42	0.17	8	8
PNG (1.5%)	266	2.07	15	0.49	20	1.13	0.24	0.85	8.1	30	20	0.80	0.79	0.44	1.28	4.27	0.65	55	54
Carrageenan	35	0.05	6	0.04	4	0.11	0.04	0.09	1.2	3	3	0.10	0.12	0.06	0.18	1.11	0.19	7	9
PNG (5.0%)	266	2.07	13	0.49	22	1.16	0.27	0.81	7.9	28	25	0.72	0.73	0.45	1.31	4.67	0.63	57	53
Carrageenan	26	0.04	5	0.04	6	0.17	0.05	0.08	0.7	4	4	0.06	0.06	0.05	0.14	0.97	0.20	13	9
CP (5.0%)	251	2.05	14	0.48	19	1.11	0.24	0.79	7.9	29	27	0.77	0.76	0.45	1.28	4.32	0.70	55	60
Carrageenan	22	0.10	6	0.03	2	0.13	0.03	0.07	1.0	4	3	0.63	0.07	0.05	0.25	1.55	0.31	10	11

a = mean

b = standard deviation

matic materials were not observed in the liver, spleen, mesenteric lymph nodes, and intestines, as well as in the urine, of the test animals orally administered with PNG and CP carrageenan. Our findings were consistent with the subchronic and chronic oral toxicity tests results of Abraham et al, 1985, wherein native carrageenans from different botanical source composed of lambda kappa and iota, have no evidence of carrageenan storage in rats.

Administration of PNG and CP carrageenan did not result in intestinal ulceration. It was suggested that intestinal uptake and ulceration were related; the presence of intestinal ulcers may indicate absorption of carrageenan. They observed that the carrageenan is taken up and stored by lysosomes of the macrophages at the site of ulcerations (Abraham *et al.* 1947). Our histopathological and histochemical findings revealed no accumulation of macrophages in the lamina propria of the gastrointestinal tract and no metachromatic granules in the subepithelial tissues. In addition, fecal samples were negative for occult blood which indicated the absence of intestinal ulceration.

It was reported that there was an upper limit to the size of carrageenan molecules to be absorbed which ranged from 10,000 to 85,000 (Pittman *et al.* 1976). In the present study, the molecular weight of PNG and CP carrageenan were high, more than 150,000 and up to 800,000, and may be the principal reason why there were no carrageenan absorbed.

The digestion of carrageenan in the gastrointestinal tract of rat was not determined in this study. However, it was reported that when carrageenan was fed in rat at dietary level of 2-20%, it was 90-100% excreted in the feces undigested, and consequently cannot have any direct nutritional value (Hawkins and Yaphe 1965). Analysis of fecal samples of mammals, including rat, fed with a variety of lambda, kappa and iota carrageenan, by gel electrophoresis and showed that degradation of high molecular weight carrageenan had occurred, either in the gut or in the feces (Pittman *et al.* 1976). In our study, the degradation of PNG or CP carrageenan was not known. The absence of any evidence of absorption and ulceration indicated that, if ever there was any degradation, the carrageenans were not degraded to as low as 85,000.

The absence of abnormal clinical signs and behavioral changes, histopathological alterations, and clinical examinations, consisting of hematology, serum biochemistry, coagulation test, urinalysis and fecalysis in rats indicated that there were no functional and morphological manifestations of toxicity or adverse effects related to PNG carrageenan, as well as CP carrageenan administration. Our findings in rat were consistent with other reports (Abraham *et al.* 1985; Nilson *et al.* 1959).

The principal effect induced by orally administered PNG and CP carrageenan in rats was the passage of soft stool. Bead-shaped appearance of feces were also occasionally observed with PNG carrageenan. The consistency of the stool may be attributed to the hydrophylic or osmotic property of carrageenan (Leegwater 1974), a non-nutritive polysaccharide and the bulk of which passes through the gastrointestinal tract unabsorbed (Hawkins 1965; Dewar 1970), which resulted to laxative

effect (aperient). In a 56 days feeding and in a lifespan carcinogenicity study of native carrageenan in rats observed some diarrhea, marked chiefly by feces which were semi-solid in consistency, in addition to the soft stool (Grasso *et al.* 1973; Rustia *et al.* 1980).

The lower frequency and late onset of observation and fewer animals observed with soft stool consistency in the group fed with 5.0% PNG carrageenan compared with the group fed with 5.0% CP carrageenan may be partially attributed to the difference in the cellulose content. Both diets have about the same value in terms of macro nutrients, proteins and fats; micro nutrients, vitamins and minerals and palatability as shown in the tables of body weight, feed consumption and efficiency of feed utilization. Philippine Natural Grade carrageenan contains on the average 12% cellulose, an inert substance and a source of dietary or crude fiber which escapes digestions (Anderson, 1988; (WHO Food Additives Series No. 8, 1975) while CP carrageenan contains less than 1% cellulose. When PNG carrageenan was admixed with the standard laboratory diet with approximately 4% crude fiber, the total dietary fiber increased to about 4.4%; and conversely, when CP carrageenan was admixed with the standard laboratory diet, dietary fiber correspondingly decreased to about 3.8%. The difference in the total intake of dietary fiber was about 12.5%. In the present study, there was a tendency for the weight of the cecal content to increase and beaded appearance of stools were observed with 5.0% PNG carrageenan, which may be due to the higher dietary fiber and correspondingly higher water content. Thus, the differences in the frequency, onset the number of animals affected may be due to the difference in cellulose content between the two diets.

The tendency of the urine volume to increase with the corresponding lower urine specific gravity and pale urine color in the absence of renal abnormalities, such as the inability of the kidney to concentrate urine, indicated slight increase in water intake (Coles, 1980). It was observed that an increase in water retention due to an increase in the amount of osmotically active substances from dietary components which are not completely digested and/or absorbed in the intestine resulted to a tendency of the animals to drink more (Leeqwater, 1974).

The fact that neither functional nor morphologic alteration in all tissues and organs were observed with PNG carrageenan compared with the CP carrageenan is of particular interest, since the animals were exposed to daily oral dose for 90 days up to 80 times the magnitude of the acceptable daily intake (50 mg/kg; 1974) in adult man. The findings suggested that PNG and CP carrageenan have the same toxicological attributes. Furthermore, the high cellulose content of PNG carrageenan may have contributed to the improvement of the consistency of feces excreted.

#### **B. Mutagenicity, Clastogenicity and Antimutagenicity Potential of PNG Carrageenan**

The following were found true for both PNG and CP carrageenan:

Carrageenan does not possess direct DNA damaging potential (Table 6). The data on Rec assay showed that there was no inhibition zone that was observed

with carrageenan test samples. The Rec (-) organism, a mutant of *Bacillus subtilis*, does not have the recombination repair system, while the Rec (+) organism has the recombination repair system.

Mutagenicity before metabolic activation of *S. typhimurium* TA 100 was not observed (Table 7).

The data on the Host-mediated assay suggest that carrageenan is not mutagenic after metabolic activation (Table 8). The mutation frequency of the indicator organism which was injected into the peritoneal cavity was not affected by carrageenan samples which were given by oral gavage. This indicates that

Table 6. Direct DNA Damaging Potential of Carrageenan Using Rec Assay

	ZONE OF INHIBITION	
	Rec (+)	Rec(-)
<b>Carrageenan</b>		
PNG 100 mg/ml	0+ /-00	0+ /-00
50 mg/ml	0+ /-00	0+ /-00
25 mg/ml	0+ /-00	0+ /-00
CP 28 mg/ml	0+ /-00	0+ /-00
<b>Negative Control</b>		
Distilled Water	0+ /-00	0+ /-00
<b>Positive Control</b>		
Quinoline	19.53 +/-0.98	23.58 +/-0.93

Table 7. Mutagenic Potential Before Metabolic Activation of Carrageenan Using Ames Test Without S-9 Mix

	No. of Revertants per Plate <i>S. typhimurium</i> , TA 100
<b>Carrageenan</b>	
PNG, 100 mg/ml	13.36 +/- 4.36
50 mg/ml	13.36 +/-3.38
25 mg/ml	7.89 +/-1.12
CP, 28 mg/ml	10.58 +/-4.36
Negative Control, Distilled Water	18.21 +/-3.13
Positive Control, Quinoline	Too Numerous to Count (Very High)

metabolites of carrageenan did not interact with DNA of the indicator organism.

The data on the Micronucleus test revealed no appreciable amount of micronucleated polychromatic erythrocytes were formed in the bone marrow. This means that no chromosome breaking effects were exhibited by the carrageenan samples in bone marrow of the mice (Table 9).

When carrageenan was administered together with three known genotoxins in mice, the data on the Micronucleus test revealed that the chromosome breaking effects of the three genotoxins were reduced by carrageenan (Table 10). Since the

Table 8. Mutagenic Potential of Carrageenan After Metabolic Activation Using the Host-Mediated Assay

	Mutation Frequency of Indicator <i>S. typhimurium</i> His G 46
<b>Carrageenan</b>	
PNG, 2500 mg/kg body weight	2.09 +/-0.33
PNG, 1250 mg/kg body weight	2.05 +/-0.71
PNG, 625 mg/kg body weight	1.48 +/-0.89
CP, 700 mg/kg body weight	2.16 +/-0.39
Negative Control, Distilled Water	2.36 +/-0.42
Positive Control, Benzo(a)pyrene	11.86 +/-1.36

Table 9. Chromosome Breaking Effect of Carrageenan Using the Micronucleus Test

	No. of Micronucleated Polychromatic Erythrocyte per thousand
<b>Carrageenan</b>	
PNG, 2500 mg/kg body weight	2.33 +/-0.89 (1)
PNG, 1250 mg/kg body weight	2.21 +/-0.49 (1)
PNG, 625 mg/kg body weight	2.06 +/-0.82 (1)
CP, 700 mg/kg body weight	1.86 +/-0.32 (1)
Negative Control, Distilled Water	1.92 +/-0.94
Positive Control, Benzo(a)pyrene	7.94 +/-0.96

Note: (1) No significant differences with Negative Control at 0.05 and 0.01 Probability.

Table 10. Antigenotoxicity of Carrageenan Against Dimethylhydrazine (DMH), Dimethylnitrosamine (DMN) and Benzo(a)pyrene (BP)

	No. of Micronucleated Polychromatic Erythrocytes per thousand		
	DMH	DMN	BP
	7.14+/-1.23	7.86+/-0.67	7.30+/-0.71
<b>Plus PNG Carrageenan</b>			
250 mg/kg body weight	2.43+/-0.73 (2)	2.58+/-0.078 (3)	2.58+/-0.68 (4)
1250 mg/kg body weight	2.64+/-0.98 (2)	2.60+/-0.250 (3)	2.61+/-0.92 (4)
625 mg/kg body weight	2.59+/-0.76 (2)	2.38+/-0.095 (3)	2.66+/-0.83 (4)
Negative Control, Distilled Water	2.12+/-0.95	2.12+/-0.95	2.12+/-0.95

Note 2, 3, 4:

With Significant Difference with DMH, DMN and BP at 0.5 Probability.

three genotoxins are metabolized to alkylating agents of DNA, there is a possibility that carrageenan or its metabolite trap the alkylating species or carrageenan inhibits their metabolism to species reactive with DNA.

Carrageenan, PNG and CP, does not possess direct DNA damaging potential. It is not mutagenic before and after metabolic activation. No chromosome breaking effects were exhibited. However, carrageenan inhibited the genotoxic activity of three known carcinogens.

#### Inference

In the present studies, PNG carrageenan has been shown to be free from any subchronic and genetic toxicities and, on the contrary, possesses antigenotoxic activity against known mutacarcinogens. Unmistakably, PNG carrageenan, just like CP carrageenan, has no food safety concerns as a food additive and an ADI similar to CP carrageenan can be assigned.

#### Achievements and Benefits of the Research

In 1995, the FCA-CCFAC granted PNG carrageenan a classification of E 407a under the FCA International Numbering System. In October 1996, consistent with the new status, the EU through the 313 member European Parliament upheld the recommendation of the EU Council to reclassify PNG carrageenan as a safe food additive. The EU lifted the ban on PNG carrageenan and allowed its entry in the European Single Market, an indication of international acceptance.

In 1994, JECFA calendared PNG carrageenan for another round of review in 1998 to assign a final Acceptable Daily Intake, the theoretical level of PNG carrag-

eenan that can be ingested daily by a 70 Kg person for long period of time without any risk. The Seaweed Association of the Philippines has commissioned a British-based laboratory center to undertake additional tests on PNG carrageenan.

As expected, the success triggered significant growth and development in the seaweed industry in terms of strengthening the economy of the Philippines, expansion of the industry and its global market, technological improvement and employment security to the marginalized fisherfolks.

The seaweed industry is already one of the nation's 14 top export industry and it is still growing. Its total export in 1996 reached US\$ 120 M compared to only about US\$ 16 million in 1990, a 750% increase. PNG carrageenan has annual sale of about US\$ 77 M. With the opening of the European market in 1996, the industry expects an additional US\$ 100 to 200 M worth of PNG carrageenan export in the near future.

The Philippines has remained the No. 1 supplier of dried weeds in the world with US\$ 18.7 M export. The 1996 figure shows that the export of its three main products, namely the dried seaweed, the PNG and refined carrageenan in expanding. PNG carrageenan grabbed 64.18% of the 1996 total sales, while dried seaweed and refined carrageenan followed at 16.46 and 19.36%, respectively.

In 1986, the Philippines was only exporting PNG carrageenan to Japan, Holland, Denmark, Canada, USA, West Germany and the United Kingdom. Today, the market has also included other European and non-EU countries in Western Europe, and South America, China, Australia, New Zealand, the Southeast Asian nations, Africa, and Israel, among others.

The seaweed farm sector has been the most benefited. Seaweed farmers, once marginalized as fisherfolks, have now discovered the value of seaweeds. An annual return anywhere from P104,382 to P125,000 per hectare of seaweed farm can be realized. Seaweeds is now considered more profitable than rice and coconut crops in the Southern and Central Philippines.

In 1990, there were only 75,000 Filipino families engaged in seaweed farming. In 1994, the figure rose to 95,050 families, of which 49,750 families are in Tawi-Tawi and Sulu.

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## HEALTH SCIENCES

### SEROEPIDEMIOLOGY OF HEPATITIS A VIRUS AMONG FILIPINOS LIVING IN SELECTED COMMUNITIES IN AND AROUND METRO MANILA\*

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#### ABSTRACT

*One thousand (1000) Filipinos residing mostly in middle class subdivisions in and around Metro Manila were tested for the presence of antibodies to the hepatitis A virus. Both sexes, ages ranging from one year to seventy nine were included in the study. Students comprised 40% of the participants, 82% of whom were enrolled in private schools. The rest were gainfully employed, mostly office workers, young executives and professionals.*

*Overall, the data show an increasing HAV antibody positivity with age in children less than five years of age; anti-HAV positivity is only 10%. This figure doubles by age ten. By age fifteen, 42% are anti-HAV positive, and before the age of twenty, more than half or 54% tested anti-HAV positive. The positivity continues to increase in 10-15% increments until the age of forty, and from then onwards, positivity ranges from 89-96%. These figures tell us that among adults > 40 years old, some 4-11% have no detectable antibodies to HAV, and are therefore still susceptible to HAV infection. Anti-HAV positivity for all age groups is 62%.*

*Of the HAV antibody positive individuals, only 15% had a history of jaundice, which only confirms that the majority of cases are subclinical.*

*The results of this study show a relatively high endemicity of Hepatitis A infection in Metro Manila. The figures we obtained from this seroprevalence study are comparable with data obtained in Bangkok, Thailand where anti-HAV*

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positivity among children attending primary schools is 22-69%. In urban areas in Malaysia, figures may be in the range of 50-55% for all age groups which approaches that of our data.

These are in contrast to the statistics obtained in Singapore in 1985 where overall anti-HAV positivity was 32%. Obviously, differences in standards of living and general sanitation explain the contrasting figures.

**Keywords:** hepatitis A seroepidemiology, subclinical HAV infection, HAV susceptibility.

Viral hepatitis is a fairly common infection in the Philippines. The term implies the viral etiologic agent to be primarily any of the following: the hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C (HCV) and most recently, the hepatitis E virus (HEV).

With the discovery of these viral agents, it became possible to develop reagents that allowed laboratory diagnosis of the specific agents responsible for the hepatitis infection. Sentinel data from the San Lazaro Hospital (Sy *et al.* 1990) show that among acutely ill hepatitis patients (clinically jaundiced, suspected to have primary viral hepatitis), approximately 30% are due to HAV, 40% due to HBV and the rest are non-A-non-B (NANB) hepatitis cases. The latter may be classified as HCV infection or HEV infection. Most of these cases occur among the lower socio-economic groups.

Among these five viral agents, the hepatitis A virus appears to be the most innocuous as far as symptomatology and outcome are concerned. However, it is probably the most ubiquitous and easiest to acquire, via contaminated food or drinks.

The hepatitis A virus is primarily spread via the intestinal-oral route. Infectivity studies performed in human volunteers have demonstrated fecal shedding of virus from 2-3 weeks before onset (period of greatest infectivity), 1-2 weeks after the onset of jaundice, (Hollinger 1985; CDCMM 1985; Feinstone *et al.* no date) Its infectivity can be preserved for years at -20°C and for at least one month after being dried and stored at 25°C. Because the virus survives in fecal contaminated water, outbreaks had been associated with ingestion of contaminated water, milk or shellfish. Recent studies have reported the concentration of HAV in mussels or "tahong" (Enriquez *et al.* 1992), oysters or cockles (ENB 1992).

Transmission is facilitated by poor personal hygiene, poor sanitation and intimate or intrahousehold contact (Hollinger 1985). It is believed that in areas where overcrowding occurs such as in institutions or in areas where general environmental sanitary conditions are substandard, anti-HAV marker positivity is very high. Among institutionalized children below 10 years of age, around 95% of children were found to be positive for anti-HAV at a very young age (Bile *et al.* 1992). Among children housed in relatively better or cleaner surroundings and less crowded conditions, anti-HAV positivity is markedly reduced.

In our country, type A viral hepatitis occurs as an endemic and epidemic infection. It usually spreads among children, in whom some 90% of the cases are

subclinical or non-icteric. Among adults, anicteric infections range from 25-50% (ICD1975). Severity of infection is related to age.

The illness tends to be more serious or prolonged when the patient is malnourished or pregnant (ICD1975). The Department of Health has reported that Hepatitis A results in more deaths than Hepatitis B during the "more acute stage" of the disease(DOH 1991).

Complete recovery is the usual outcome of infections (Hollinger 1985). IgC class anti-HAV which appears in the convalescent phase of disease and remains detectable in serum thereafter, apparently confers enduring protection against the disease. Past infection or past exposure to HAV may therefore be determined by testing for anti-HAV levels in serum.

So far in the Philippines, we have no population-based data on the prevalence of anti-HAV marker. Such data are necessary for planning a hepatitis A control strategy. This particular study defines the epidemiology of HAV infection through a prevalence serosurvey in selected middle and upper middle income class communities in Metro Manila. These groups are deemed to have a relatively low exposure to HAV. It could well be that inadvertent exposure to HAV contaminated food or drinks would place them at a higher risk for acquiring HAV infection.

Specifically, this study was undertaken to determine the presence of antibodies among various age groups in selected communities in Metro Manila.

## **MATERIALS AND METHODS**

### **Study Population and Study Sites**

A total of 1,000 Filipinos, male and female, of all ages belonging to middle and upper middle income families were recruited into the study. Representative numbers of participants from the following subdivisions in Metro Manila were included: BF Homes, Xavierville Subdivision, PhilAm Life Village, Filinvest Homes, SSS Village, Pacita Subdivision, as well as other middle class residential areas. Office workers, young executives and professionals were recruited, whose children attended mostly private schools.

Each participant was asked to fill up a patient data form relevant to the objectives of the study, as well as a consent form for venipuncture.

### **Blood Collection and Anti-HAV Testing**

Approximately 3-8 ml of blood was collected from each participant by venipuncture, allowed to clot at room temperature for 2-3 hours, and the serum separated by centrifugation and stored at -20°C prior to testing.

HAVAB-FIA from Abbot Laboratories was used to determine the presence of antibodies to HAV. Only a qualitative detection of anti-HAV was performed as specified in the kit brochure.

## RESULTS

### Socio-demographic Characteristics of Study Population

A total of 1,000 Filipinos residing mostly in middle and upper middle class subdivisions in and around Metro Manila were recruited into the study.

Each group included approximately 200 subjects ( $\pm 16$ ), with more females participating, at 56%. This may be due to the fact that there are more females than males in the general population, or that the females have a more positive attitude in participating in researches such as this.

The areas of residence of the participants were divided into four (Table 1). Approximately 39% of participants were from the northeastern part of Metro Manila, an equivalent number from the southwestern part, 22% from the central Manila area and 0.3% commuted daily from Manila to Pampanga.

By occupation, approximately 40% are students, 20% are employees, most of whom hold supervisory or junior executive positions. The professionals (health, academe, business, technical) comprise ~19% of the study population. Only about 2% belonged to the low-income group. Among the student participants, 82% were enrolled in private schools, 11% attended public schools, and ~6% were in state universities. The private schools covered were: Miriam College, Ateneo de Manila University, De La Salle University, Perpetual Help College, Colegio de San Agustin, and many other Catholic schools.

Table 1. Number of Participants by Sex and Area of Residence, 1993

Area	Male	Female	Both Sexes	Percentage
NEastern Metro Manila	172	218	390	39
SWestern Metro Manila	178	210	388	38.8
Central Manila	92	127	219	21.9
Other Areas	2	1	3	0.3
Total	444	556	1000	100

#### Legend:

- I = Quezon City, Marikina, Caloocan, Valenzuela, Bulacan, Makati, Mandaluyong, Pasig, San Juan, Taguig, Rizal
- II = Las Pinas, Paranaque, Pasay City, Alabang, Laguna, Cavite, Batangas
- III = City of Manila
- IV = Pampanga

### Prevalence of Anti-HAV Marker

In the age-group <10 years, anti-HAV positivity being contributed more by males (24%) than females. This positivity (12.5%) starts at 3 years of age onwards, increasing to some 30% by age nine. In the group 11-20 years, overall anti-HAV positivity in 48%, the highest reactivity recorded at 69% in the age group 21-30, 85% in the 31-40 age group, and 90% in those 41 years and above. It must be noted that the age-specific prevalence in certain groups tested reached 100%, in those above 30 years old. The sample size for participants in the age group 41 and above is small, but most subjects were anti-HAV positive. However, in certain groups, small number of adults remain non-reactive to anti-HAV. Anti-HAV positivity for all age groups is 62% for both male and female.

Stratifying the age groups by five (Table 2) shows a more clear cut pattern. In children less than five years of age, anti-HAV positivity is only 10%, and this figure doubles by the time the age of ten is reached. By age fifteen –42% are anti-HAV positive, and before the age of twenty, more than half or –54% tested anti-HAV positive. The positivity continues to increase in 10-15% increments until the age of forty, and from then onwards, positivity ranges from 89-96%. Again, these figures tell us that among adults >40 years old, some 4-11% have no detectable antibodies to HAV, and are therefore, still susceptible to HAV infections.

If one were to look at anti-HAV reactivity among different categories of students in different levels of schooling the trend approximates the five-year age-group specific prevalence shown in Table 2.

Table 2. Age-Group (Five and Ten Years Stratification) Specific Prevalence of Anti-HAV in Filipinos Living and Around Metro Manila, 1993.

Age-Group	No. (+)	% (+)
0 – 5	6/60	10
06 – 10	28/125	22.4
11 – 15	37/88	42
16 – 20	67/124	54
21 – 25	68/112	68.7
26 – 30	78/104	75
31 – 40	173/203	85.2
41 – 50	112/126	88.8
51 – 60	31/34	91.2
61 up	23/24	95.8

### History of Jaundice/Clinical Hepatitis

Among the anti-HAV positive individuals, only 15% had either a personal history of hepatitis or jaundice, or family history of jaundice. Some 47% had no personal or family history of hepatitis or jaundice. There were a few, ~ 6%, who had a history of jaundice, but were anti-HAV non-reactive.

Per five year age-groups, a personal history of jaundice (clinical hepatitis) was elicited in only 2.7% among the 6-10 year old age group, in ~5% among the 11-15 age group, and in ~10% (+/-6) in the age group 16-30. In the age group 31-40, ~40% gave a history of clinical hepatitis acquired before the age of 10 in 23%, between the ages of 1-20 in 33%, between 21-30 in 20% and after age 31 in 23% of cases. From 41 years, 3-9% had a history of jaundice.

### DISCUSSION AND CONCLUSION

The results of this study show a relatively high endemicity of hepatitis A infection in Metro Manila. Overall, the data show an increasing % anti-HAV positivity with age. This presumably reflects increasing exposure to contaminated food and drinks, even in areas where sanitation standards are deemed to be high. Nineteen of the participants in the age-group 1-2 years, were anti-HAV non-reactive. This may be explained by the fact that in this age group, precautions are usually taken to feed the child with sterile milk, or even solid food that has been prepared cleanly. After this age, it becomes difficult to closely monitor and supervise feeding habits of toddlers who are prone to put most things that they pick up from the environment into their mouths. At this time, too, parents in general are more relaxed in choosing and preparing food for their children.

At school age, exposure to food prepared outside the home increases. As each individual ages, exposure may help explain the high anti-HAV positivity in our population. The exposure is certainly possible even in a population of individuals who are presumed to have high standards of hygiene and are more discriminating in their eating preferences and habits.

The figures obtained from this seroprevalence study in Metro Manila are comparable with data obtained in Bangkok, Thailand in 1988 (Poovorawan 1992). In Bangkok, anti-HAV positivity among children attending primary schools, 22% to 69%, the figures increasing with each age group. In urban areas in Malaysia (SBH 1993) figures may be in the range of 50-55% for all age groups, which is similar to what we have obtained in this study. These are in contrast to the statistics obtained in Singapore in 1984-1985 (ENB 1992) where only 4.3% were anti-HAV positive in the age group 15 years and below, and 15.5% positive in the age group 15-24 years. Overall, for that year, Singapore had 31.85% anti-HAV positivity. Obviously, differences in standards of living and general environmental sanitation accounted for the contrasting figures. Worthy to note is that in 1991, the overall anti-HAV positivity in Singapore dropped from 31.8% to 21.4%, and in the age group 10-19

years, seropositivity dropped drastically to a low of 0.9%. This changing pattern of HAV infection in Singapore reflects an even higher standard of living and sanitation that this small country has achieved in only about six years.

The foregoing data are consistent with findings in many other countries on the inverse relationship of anti-HAV positivity and socio-economic status. In an unpublished study among middle and upper middle income medical students from the University of the Philippines in 1986-1987 (Lingao, Liver Study Group, UP-PGH), only 42% of the 20-21 year old medical students were anti-HAV positive. This is a little lower than the seropositivity we obtained for the same age group in our study, which is 50-60%. The figure, however, is still much lower than what has been obtained from two rural areas in the Philippines. Dr. Lingao's group found that in rural areas in Laguna and Batangas, majority of the rural population become infected with HAV early in life. By age five, 90-98% of the subjects were already anti-HAV positive. Thus, it suggests that acute hepatitis cases among adults in the rural areas may not be due to HAV anymore. In contrast, among the higher income bracket population in the urban areas, as we have found here, acute viral hepatitis due to HAV is a distinct possibility. At least half of the adult population remain susceptible to HAV infection. It appears that upper social classes are less likely to be exposed at an early age.

Overall, of the anti-HAV positive individuals, only 15% had a personal or family history of jaundice. Clinical hepatitis in these anti-HAV positive individuals developed in 2.7% among the 6-10 years age group, and in 40% in the age group 31-40 years. This finding confirms that most cases of HAV infection are subclinical. This is especially true among the younger age group, whose infection may only be picked up by mild increases in serum transaminases. They are, however, potential sources of HAV infection through inapparent fecal viral shedding. Among the individuals who developed clinical hepatitis presumably due to HAV, infection was acquired between the ages of 11-20 in 33%.

In summary, hepatitis A infection is highly endemic in our setting. Overall exposure as measured by anti-HAV reactivity is 62% for both males and females, and increases with age. Whereas most adults after the age of 40 are anti-HAV positive, there are some individuals who, even up to 62 remain anti-HAV non-reactive. This may reflect real non-exposure to HAV contaminated food, or may reflect a subset of population who may have been exposed, produced antibodies, but have lost these antibodies with time. This finding may also be explained in terms of immunologic mechanisms at work in certain individuals that make them more resistant or less susceptible to HAV infection. Whatever the mechanism, these seronegative individuals remain susceptible to HAV infection, and are more likely to develop the clinical form of hepatitis A.



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# PRESCRIBING PATTERNS OF FILIPINO PHYSICIANS FOR COMMUNITY-ACQUIRED PNEUMONIA IN CHILDREN

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## ABSTRACT

*In 1989, the Department of Health (DOH) and the World Health Organization (WHO) introduced the Control of Acute Respiratory Tract Infection (CARI) Program to pediatricians and general physicians. The study was undertaken to determine the physician's adherence to the WHO-CARI Program. Questionnaires were served to 250 physicians asking their most commonly used antibiotics for treatment of Community-acquired pneumonia classified as: (1) mild-moderate, (2) moderate-severe and (3) very severe pneumonia. Of the 190 respondents, there were 164 general pediatricians, 17 general practitioners (GPs) and 9 pediatric subspecialists. Eighty-eight percent of the respondents adhered to the recommended drug for mild-moderate pneumonia. Less than half, however, were consistent with the CARI Program for the two other types of pneumonia. Amoxicillin, Pen G and cephalosporin were the empiric drugs of choice for the three respective types of pneumonia. Only 15 general pediatricians and two GPs followed the CARI Program strictly for all types of pneumonia. Thus, there is a need to determine the reasons for the limited use of the WHO-CARI Treatment Guidelines by physicians and to plan strategies for more effective means of dissemination and implementation of the WHO-CARI program.*

*Keywords: Prescribing patterns, childhood pneumonia, CARI, Filipino physicians*

## INTRODUCTION

Acute Respiratory Infection (ARI) is a major cause of death among children in developing countries. Pneumonia in particular accounts for 4 million deaths of the estimated 15 million deaths worldwide in children under 5 years annually. Two-thirds of these deaths occur among infants. About 20-30% of all mortality cases from ARI in children less than 5 years old occur during the first two months of life. According to the Philippine Health Statistics (1988), the morbidity rate of ARI is 3,570 per 150,000 for the less than 1 year old and 1,097 per 100,000 for 1-4 years age

group (PPS 1929). Through the years, it has remained the leading cause of morbidity and mortality among Filipino children.

Among developing countries like the Philippines, studies on etiologic agents of pneumonia are very few. Tupasi *et al.* (1990) and Capending *et al.* (1994) in 1990 and 1992 respectively documented *Streptococcus pneumoniae* and *Haemophilus influenzae* as the most common organisms causing bacterial pneumonia. Diagnostic tools in most hospitals however, may not be readily available and are of limited use in identifying the specific organism involved.

In 1989, the Department of Health together with the Philippine Pediatric Society (PPS) launched the Control of Acute Respiratory Infection (CARI) Case Management Program to provide doctors and health workers a rational approach in the management of ARI, particularly in areas where health facilities and health personnel are limited in number. Also the case management guidelines were intended to recognize the use of the most cost effective drugs and also prevent inappropriate antibiotic treatment for respiratory infections and thus retard the development of antibiotic resistance.

Eight years later, this study was undertaken to determine to what extent Filipino physicians adhere to the WHO Treatment Guidelines and to identify the antibiotics preferred for primary and alternative therapy of pneumonia in children.

### METHOD

Questionnaires were randomly distributed to delegates attending the PPS Biennial Convention in August 1996. They were provided a list of antibiotics for treatment of three types of community acquired pneumonia namely: 1) mild to moderate, 2) moderate to severe and 3) very severe pneumonia.

The list of antibiotics was gathered from a previous pilot survey where respondents were asked to write down drugs they used for pneumonia cases. They were then asked to choose which of these antibiotics were their most commonly used primary drugs. Next, they were asked to indicate their alternative choices for each of the three types of pneumonia. They could also write down other antibiotics if they were not included in the list.

The accomplished questionnaires were then collected that the choices of antimicrobial therapy for pneumonia were compared with the WHO-CARI case management guidelines as shown in the following outline:

#### Recommended Drug Therapy (WHO-CARI Program)

Pneumonia	-	Out-Patient Therapy Cotrimoxazole Amoxicillin
Severe Pneumonia	-	Admit Pen G IM or IV

Very severe – Admit  
Pneumonia Chloramphenicol IM or IV

## RESULTS

A total of 190 respondents out of 250 (76%) returned the questionnaires and were included in the study. There were 86.3% general pediatricians, 8.9% general practitioners and 4.7% pediatric subspecialist. Almost half were in private practice (54.2%) and concentrated at the National Capital Region (NCR). Majority (65.3%) of the respondents conduct their clinical practice in urban areas of their respective regions (Table 1).

Table 1. Characteristics of the Respondents

	Number	Percent
<b>Specialties:</b>		
Pediatricians	164	86.3
General Practitioners	17	8.9
Pediatric Subspecialists	9	4.7
<b>Type of Practice</b>		
Strictly Government	30	15.8
Strictly Private	103	54.2
Both Government and Private	56	29.5
Unknown	1	0.5
<b>Place of Practice</b>		
1	7	3.7
2	7	3.7
3	4	2.1
4	43	22.6
6	17	8.9
7	1	0.5
10	4	2.1
11	5	2.6
13	1	0.5
NCR	83	43.7
Unknown	18	9.5
<b>Area of Practice</b>		
Urban	124	65.3
Rural	26	13.7
Both Urban and Rural	37	19.5
Unknown	3	1.6

Fig. 1 shows that for mild to severe pneumonia, 88% of the 164 pediatricians would adhere to the recommended drugs of the CARI protocol but as the pneumonia becomes more severe and complicated, the percentage of pediatricians following the CARI guidelines decreased from 31 to 20%. Likewise, among general practitioners the same pattern is seen where compliance with the CARI case management protocol decreased from 88% to 29% as the severity of pneumonia progresses (Fig. 2). The pediatric subspecialties group were also seen to have a similar prescribing pattern (Fig. 3).

On antibiotics used for mild to moderate pneumonia, doctors preferred ampicillin/amoxicillin over cotrimoxazole (Fig. 4). But drugs were used either as primary or alternative therapy. Only a small number of practitioners used penicillin VK, cephalexin, erythromycin and chloramphenicol as primary drugs for mild to moderate pneumonia. Erythromycin was used significantly as alternative therapy for mild to moderate pneumonia. It was remarkable that Pen G as a single drug given parenterally was preferred by more respondents as the primary drug while chloramphenicol was chosen as the main alternative therapy (Fig. 5). Ampicillin/amoxicillin, chloramphenicol and cephalosporin were chosen as the next three most common primary drugs after Pen G while cephalosporin was chosen as the next most common alternative drug. Combination therapy was also shown to have a significant following both as primary and alternative therapy.

For very severe and complicated pneumonia, combination therapy is the choice of the majority both as primary and alternative regimen. Combination of B-lactam drugs and aminoglycoside or either one of them plus another drug were the top choices. For single drug therapy, cephalosporin was the top choice followed by chloramphenicol with Pen G, ampicillin and oxacillin as minor choices (Fig. 6).

Only 15 out of 164 (9%) pediatricians and 2 out of 17 (12%) general practitioners adhered to the CARI-Case Management Guidelines for all types of pneumonia. The rest complied with only one, two or none of the recommendations for the various types of pneumonia.

## DISCUSSION

The WHO-CARI Program is expected to decrease mortality from CARI in most developing countries. This has been proven in a study in Bohol, Philippines by Lucero *et al.* where training health workers in the CARI protocol resulted in a significant improvement in mortality rates (Lucero 1976). An increasing number of countries are now adapting WHO's strategy for ARI control (WHO 1995).

Despite global and national support, the CARI protocol is not extensively adopted to in our local setting. The answer is not easy. For one, the prescribing patterns of Filipino physicians are usually influenced by many factor such as the place of practice, type of practice and economic considerations. The major influences come from educational materials and promotional advertisements programs of the drug industry. Perhaps government efforts and influence are not enough to

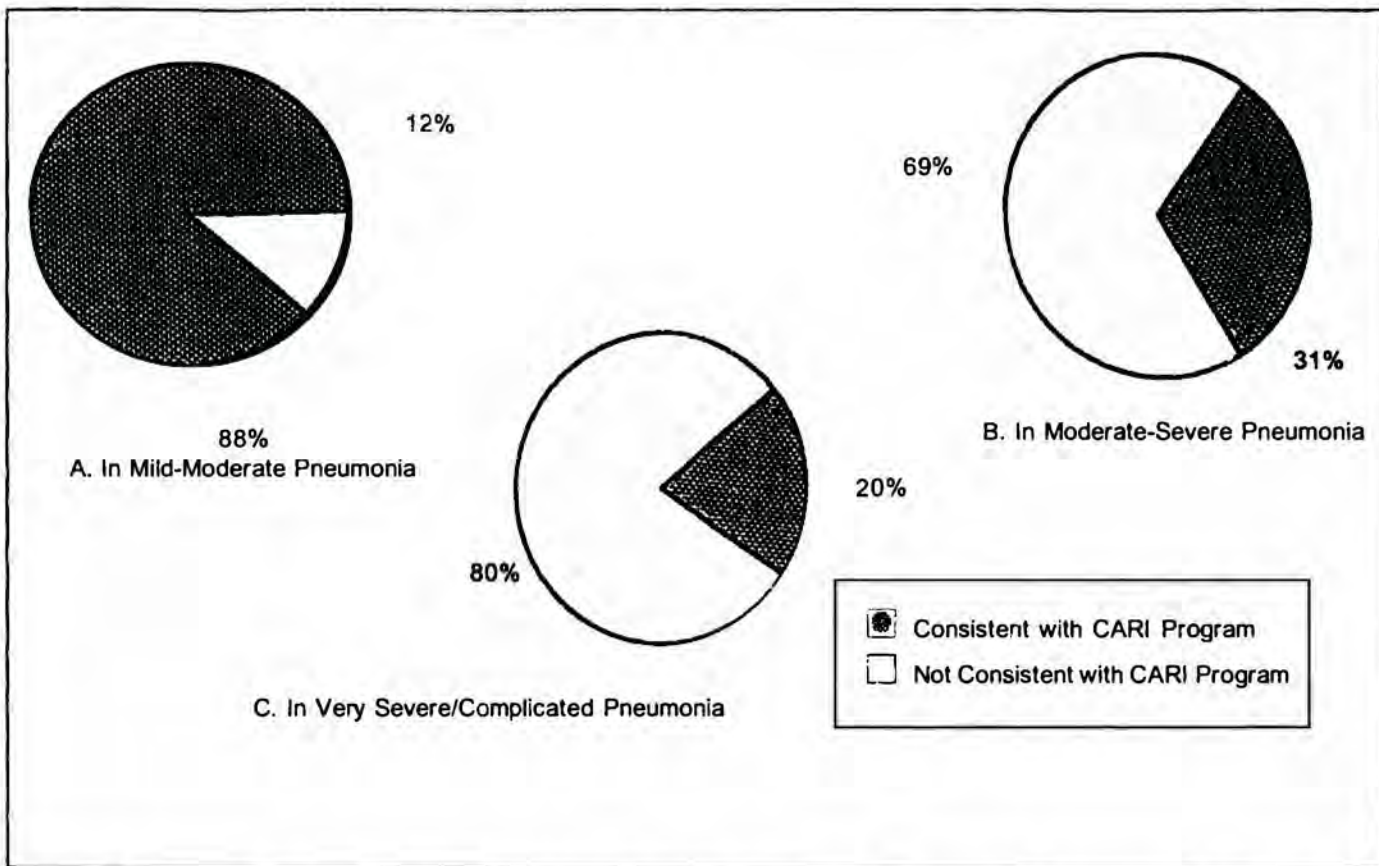


Figure 1. Proportion of 164 Pediatricians Whose Primary Antibiotic Therapy for Pneumonia is Consistent with CARI Program.

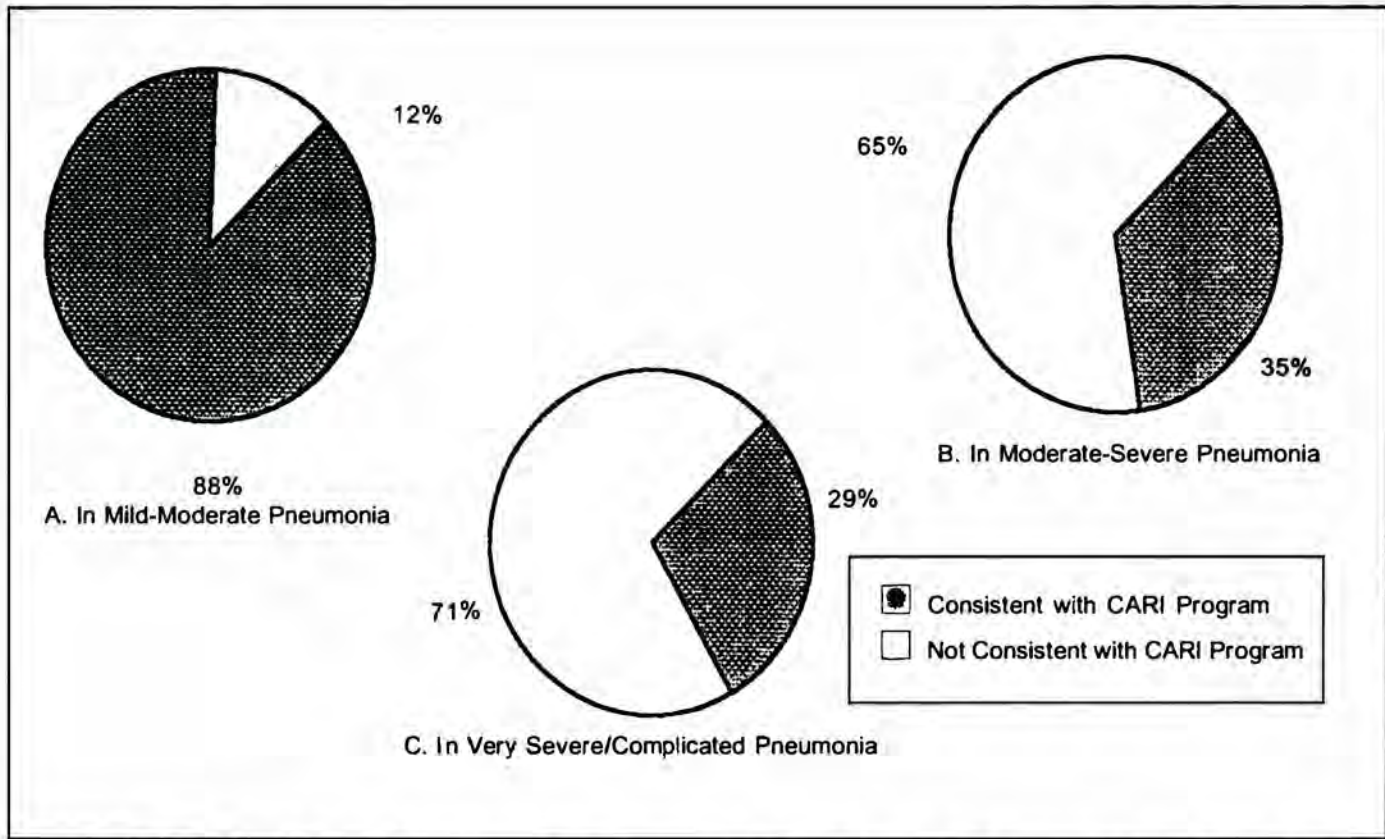


Figure 2. Proportion of 17 General Practitioners Whose Primary Antibiotic Therapy for Pneumonia is Consistent with CARI Program.

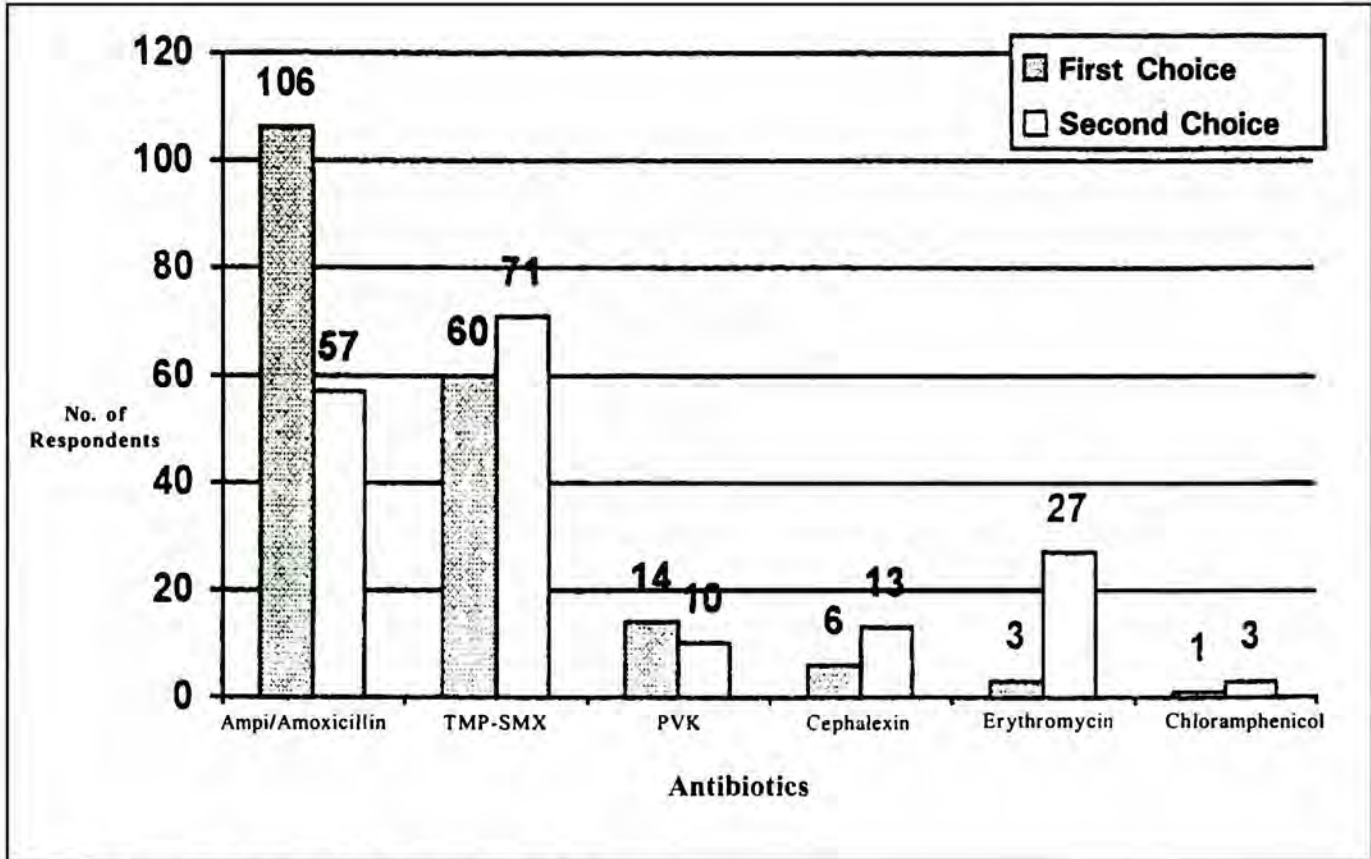


Figure 4. Antibiotics Preferred for Mild-Moderate Pneumonia.



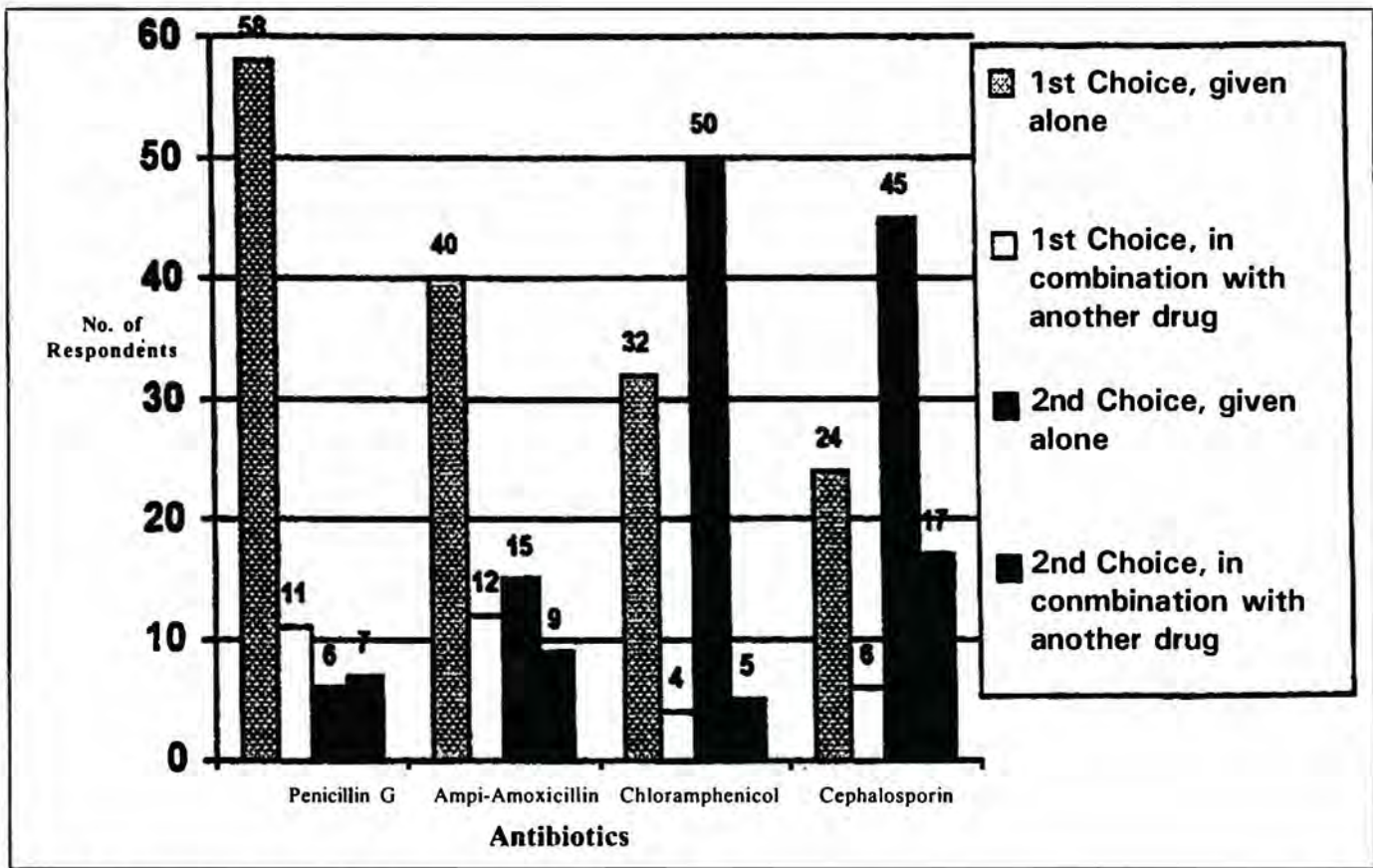


Figure 5. Antibiotics Preferred for Moderate-Severe Pneumonia.

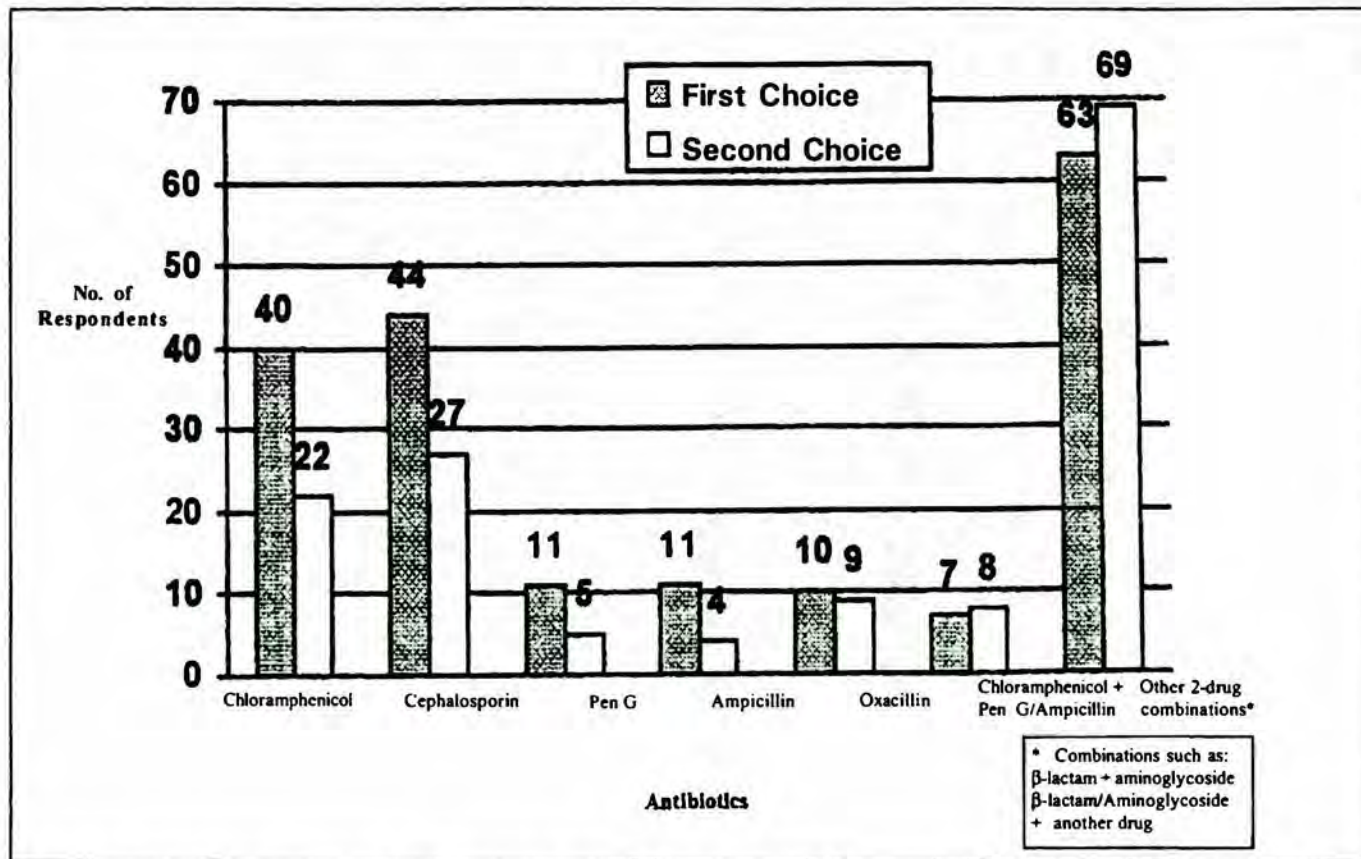


Figure 6. Antibiotics Preferred for Very Severe/Complicated Pneumonia.

promote the CARI program. The pharmaceutical industry may also prove to be a more influential factor.

The Department of Health and the PPS have worked together in the campaign of using the CARI Protocol to provide the most cost effective drugs for pneumonia. It is noteworthy that a significant number (88%) of pediatricians and practitioners adhere to the protocol for mild to moderate pneumonia but for the more severe cases only about one fourth to one fifth would follow these guidelines. Furthermore, only 9-12% of the respondents followed the guidelines for all three types of pneumonia. Clearly there is the need for greater education and promotion of these guidelines by those in the government health policy makers as well as health providers in general.

Understandably, there are many unresolved issues with regard to the use of drugs in the CARI protocol. Among these, are widespread over-the-counter sale of drugs, self medication and emergence of antibiotic resistance for the most common respiratory tract pathogens. These issues sometimes prevent physicians from using the recommended drugs. Another common observation is the inherent resistance of physicians to change their prescribing habits especially when there are no incentives. The incentives may come in various ways, but economic gains, recognition, and self-satisfaction are the most powerful. For private practitioners who make up the bulk of our respondents perhaps the CARI Program does not provide these needs so that there is low compliance to the program.

Those who have more advanced training i.e. subspecialists, academicians were observed to deviate more likely from the recommended guidelines and this was also shown in this study. Perhaps they should be encouraged to do more research studies and work on some of the unresolved issues to help them realize the validity and reliability of the protocol. Support for the program must indeed come from all sectors including the paramedical and nonmedical workers with the pediatricians as the lead role.

In summary, the study showed that there is only a small percentage of pediatricians, and general practitioners 9% and 12% respectively who will adhere strictly to the WHO-CARI Case Management Protocol for the therapy of the community acquired pneumonia. The highest compliance (88%) is observed in the therapy for mild to moderate pneumonia but this diminished considerably as the severity of pneumonia increases. The primary choices for the nonsevere pneumonia are ampicillin/amoxicillin and cotrimoxazole with cotrimoxazole and erythromycin being used as alternative for OPD therapy. Pen G is most commonly used in moderate to severe pneumonia. With the very severe cases of pneumonia, a greater use of cephalosporins, aminoglycosides and combination drug therapy is shown. Thus there is a need for better promotion, planning and formulation of strategies to increase physicians' compliance or adherence to the WHO-CARI Case Management Guidelines which is expected to benefit the Filipino child today.

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# DYSLIPIDAEMIA AMONG FILIPINO CHILDREN WITH INSULIN-DEPENDENT DIABETES MELLITUS: PREVALENCE AND RELATION TO GLYCEMIC CONTROL

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## ABSTRACT

*Dyslipidaemia is a major risk factor associated with cardiovascular disease among children with insulin-dependent diabetes mellitus (IDDM). Most studies have demonstrated that with increasing poor control of diabetes as reflected by levels of hemoglobin A1c (HbA1c), total cholesterol, total triglyceride and lipoprotein subfractions are likewise increased. This study aims to determine the prevalence of dyslipidaemia among Filipino children with IDDM and further investigate the relationship of dyslipidaemia with glycemic control (HbA1c) and the different variables such as sex, chronologic age, age of onset, dose of insulin, duration of diabetes and blood glucose (FBS). Among 50 IDDM patients studied, 27 females and 23 males, 62% had dyslipidaemia with increased levels of cholesterol, tryglyceride, LDL and decreased HDL. Females were more affected than males ( $p < 0.02$ ). The most common combined plasma lipid abnormality was hypercholesterolemia with elevated LDL (25.7%). Among the diabetic patients with dyslipidaemia, 76.9% were not in good glycemic control. Dyslipidaemia was positively correlated with HbA1c at  $p < 0.043$ . Cholesterol ( $r = 0.45$ ;  $p = 0.0010$ ), triglycerides ( $r = 0.31$ ,  $p = 0.0269$ ), and LDL ( $r = 0.56$ ,  $p = 0.0001$ ) were likewise positively correlated with glycemic control. However, no significant relationship was noted with HDL and the other variables such as chronologic age, age of onset, dose of insulin, duration of diabetes and FBS.*

*In conclusion, this study showed that dyslipidaemia is prevalent among Filipino children with insulin-dependent diabetes mellitus notably among those who were not in good glycemic control. It underscores the need for regular lipid monitoring aside from glucose monitoring in order to initiate early intervention to prevent the dreaded onset of cardiovascular complications.*

## INTRODUCTION

Insulin-dependent mellitus (IDDM), a common metabolic disorder of children, has been associated with an increased morbidity and mortality from cardiovascular disease (Garcia *et al.* 1974; Kannel *et al.* 1979; Santen *et al.* 1972; Krolewski 1987). Dyslipidaemia is a major risk factor for this condition. The National Cholesterol Education Program (NCEP) in America revealed that elevated cholesterol levels early in life may play a role in the development of adult atherosclerosis (NCEP 1992). The Diabetes Control and Complications Trial (DCCT) showed that younger IDDM patients (ages 13-24) had elevated mean total cholesterol, triglyceride, and LDL cholesterol levels, while HDL was reported to be low (DCCT 1992). Most studies have demonstrated that with an increasing poor control of diabetes as reflected by levels of hemoglobin A1c (HbA1c), total cholesterol, total triglyceride and lipoprotein subfractions are likewise increased (Glasgow *et al.* 1981; Sosenko *et al.* 1980; Semenkovich *et al.* 1989; Asad *et al.* 1994). Glycemic control is significantly correlated with these parameters except for high density lipoprotein Glasgow *et al.* 1981; Sosenko *et al.* 1980; Semenkovich *et al.* 1989; Asad *et al.* 1994).

Thus, a cross-sectoral study was designed (1) to determine the prevalence of dyslipidaemia in Filipino children with IDDM and (2) to further investigate the relationship of dyslipidaemia with each of the different variables such as sex, chronologic age, age of onset of diabetes, dose of insulin (u/kg/day), duration of diabetes, blood glucose (FBS) and glycosylated hemoglobin. (HbA1c).

### Definition of Terms

Dyslipidaemia – abnormal concentration, composition, or metabolism of lipids and lipoproteins (Gang 1994).

Glycosylated Hemoglobin – the quantity of glucose irreversibly attached to the hemoglobin molecule (Sperling 1990).

## SUBJECTS AND METHODS

### Study Population

Our study population consisted of diagnosed cases of insulin-dependent diabetes mellitus (IDDM) based on the criteria set by the National Diabetes Data Group (1979). They included diabetic patients who were on a regular follow-up at the Pediatric Endocrine Clinic of the Philippine General Hospital and private patients under the care of Pediatric Endocrinologists. Subjects were informed by mail on the significance of the study, the scheduled investigation and the necessary preparation for the said examination.

The cross-sectional investigation was conducted from November 1995 to March 1996. A total of 50 patients were included in the study after an informed consent. On the scheduled date of examination patients were made to fast 8-10 hours overnight and blood was extracted prior to the morning dose of insulin.

Questionnaires were filled up to supply data on sex, chronologic age, age of onset, duration of diabetes and dose of insulin.

### Laboratory Methods

A conventional venipuncture was done to withdraw 10 ml of blood. Three ml of blood was placed in an EDTA tube for glycosylated hemoglobin determination. The remaining 7 ml was placed in a plain tube and allowed to clot at room temperature and centrifuged to separate the serum. Two ml was used for fasting blood glucose which was immediately done and the remaining 5 ml was stored at 4°C for the lipid analysis performed at a specified time.

Cholesterol was analyzed by enzymatic colorimetric determination using cholesterol oxidase with a reference value of 200 mg/dl (5.18 mmol/L) as set by the National Cholesterol Education Program for Adolescence (NCEP). Triglyceride was measured by lipase-glycerol kinase method with reference value for males at 60-165 mg/dl (0.68-1.88 mmol/L) and for females at 40-140 mg/dl (0.46-1.60 mmol/L). High density lipoprotein (HDL) is the supernatant after precipitating the serum with phosphotungstic acid-magnesium ions with reference values for males at 35-55 mg/dl (0.90-1.40 mmol/L) and for females 45-65 mg/dl (1.20-1.70 mmol/L). Low density lipoprotein (LDL) was calculated using the Friedewald formula with reference value set by NCEP at 130 mg/dl (3.37 mmol/L).

$$\text{LDL} = \text{Total cholesterol} - \text{HDL} - \text{Triglyceride}/5$$

Glycosylated hemoglobin (HbA1c) was measured by the Eagle Diagnostic Glycohemoglobin (Ghb) procedure which employs a weakly binding cation-exchange resin in a zwitterionic buffer for a rapid separation of Ghb from non-glycosylated hemoglobin. The glycohemoglobin-containing supernatant was poured into a cuvet for measurement of absorbance by spectrophotometer at 415 nm.

Glycemic control using HbA1c was determined as follows:

Good Control	7.5-9.0%
Fair Control	9.0-10.0%
Poor Control	>10%

Blood glucose concentration (FBS) was measured by an enzymatic colorimetric determination using glucose-peroxidase reaction with a reference value of 70-110 mg/dl. (3.9-6.1 mmol/L)

### Statistical Method

There were eleven variables considered in this study namely sex, age in years, age of onset, duration of diabetes, dose of insulin, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), blood glucose (FBS) and glycosylated hemoglobin (HbA1c). Using the Pearson's Correlation Coefficient, relationship among the different variables was determined. Because the expected frequencies were small, the Fisher's Exact Test was used to correlate the

different variables between patients with dyslipidaemia and those without dyslipidaemia.

**Results**

A total of 50 patients with insulin-dependent diabetes mellitus (IDDM), 27 females and 23 males, were included in the study. As shown in Table 1, the patients had a mean age of 14.42 years, with age of onset ranging from 1-19 years (mean=10.70). The mean duration of diabetes was 3.54 years with patients receiving an average daily insulin of 0.98 units/kg body weight. However majority of diabetics were not in good glycemic control with a mean HbA1c of 10.08% and FBS of 223.4 mg/dl. (12.40 mmol/L).

Among 50 patients studied, 31 had dyslipidaemia with a prevalence of 62% as shown in Figure 1. Cholesterol, triglyceride, LDL levels were increased in contrast to HDL which was decreased (Fig. 2).

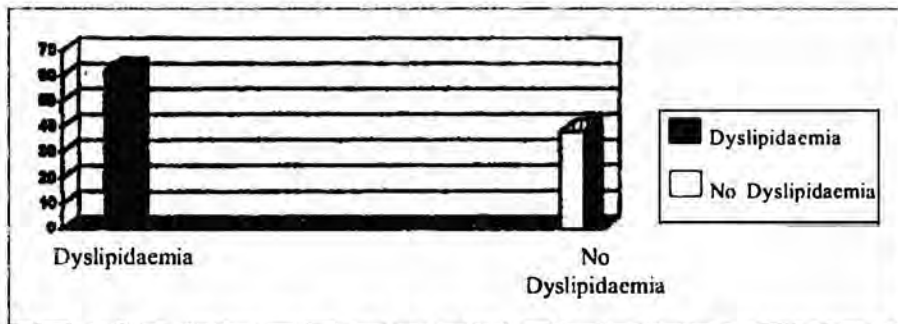


Figure 1. Prevalence of Dyslipidaemia in IDDM Patients

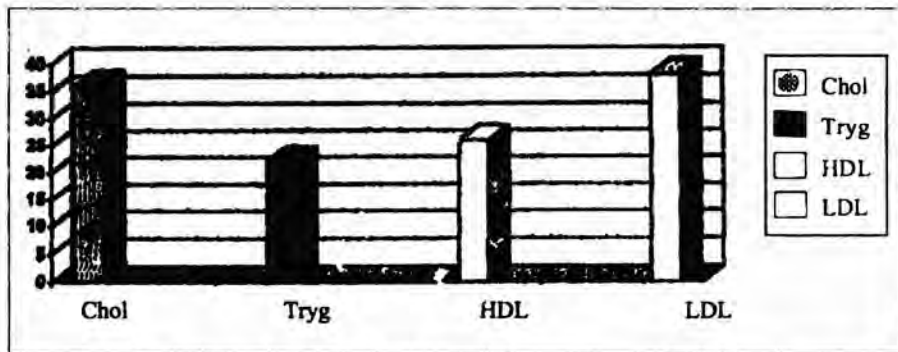


Figure 2. Frequency of Abnormal Lipids and Lipoproteins in IDDM Patients



Table 2 demonstrates the most common combined forms of dyslipidaemia. These were hypercholesterolemia with elevated LDL (25.7%) and hypercholesterolemia, hypertriglyceridemia with elevated LDL (16.0%). Comparing diabetics with dyslipidaemia and those without dyslipidaemia, the Fischer's Exact Test showed a positive correlation  $p < 0.043$  between dyslipidaemia and HbA1c. Significantly less number of patients (22.4%) were observed to have dyslipidaemia in the group with good glycemic control than in the fair and poor glycemic control (76.9%)

Likewise significantly more females were noted to be affected than males ( $p < 0.02$ ) Twenty-one diabetic females (42%) were with dyslipidaemia as compared with only 10 (19.6%) diabetic males. Cholesterol was also positively correlated with HbA1c ( $p < 0.017$ ). Although the chronologic age, age of onset, duration of diabetes, dose of insulin and FBS were relatively higher in patients with dyslipidaemia when compared with patients without dyslipidaemia, these were not statistically significant.

The Pearson's Correlation Coefficient Test revealed a significant correlation between HbA1c and cholesterol, as well as with triglyceride and LDL. However, there was no correlation seen with HDL and HbA1c.

Table 1. Clinical and Biochemical Characteristics of Study Population (N=50)

<i>Characteristics</i>	<i>Mean</i>	<i>Standard Deviation</i>
Chronologic age	14.42	5.86
Agent of onset	10.70	4.73
Duration of diabetes	3.54	3.86
Dose of insulin	0.98	0.43
Blood glucose (FBS)	223.4	107.1
Glycosylated Hemoglobin (HbA1c)	10.08	2.38
Cholesterol	198.7	60.57
Triglyceride	94.12	62.91
High Density Lipoprotein (HDL)	54.16	22.1
Low Density Lipoprotein (LDL)	125.4	51.46

Table 2. Lipid Profile and HbA1c of IDDM Patients with Dyslipidaemia

Variables	Glycemic Control (HbA1c)			
	Good (%)	(Fair %)	Poor (%)	Total (%)
Cholesterol (Chol)	—	3.2	3.2	6.4
Triglyceride (Tryg)	3.2	3.2	—	6.4
HDL	6.4	3.2	9.6	19.2
LDL	—	—	3.2	3.2
Chol + LDL	3.2	3.2	19.3	25.7*
Chol + HDL + LDL	—	—	3.2	3.2
Chol + Tryg + LDL	3.2	3.2	9.6	16.0*
Chol + Tryg + HDL	—	—	3.2	3.2
Chol + Tryg + HDL + LDL	—	—	3.2	3.2
Tryg + HDL	—	3.2	—	3.2
Tryg + HDL + LDL	—	—	3.2	3.2
HDL + LDL	6.4	—	—	6.4
	22.4%		76.9%	

## DISCUSSION

Failure to achieve good glycemic control is highly associated with dyslipidaemia in IDDM.

As reported in the DCCT (1992), elevated total cholesterol, LDL cholesterol, triglyceride, and decreased HDL cholesterol, appear to occur predominantly in younger female diabetic patients (ages 13-24) with relatively higher HbA1c levels. In our study, 76.9% of the 62% IDDM patients with dyslipidaemia were not in good glycemic control especially among females. Several studies have confirmed these findings, showing the importance of diabetic control on serum lipids and lipoproteins in IDDM.

Glasgow *et al* (1981) reported a positive correlation between control and serum cholesterol, triglyceride and low density lipoprotein. High density lipoprotein was not significantly correlated with control. Sosenko *et al* (1980) demonstrated that with increasingly poor control of diabetes, there was an associated statistically significant increase in total cholesterol, triglyceride and lipoprotein subfractions except for HDL cholesterol.

Similarly, Semenkovich *et al.* (1989) revealed that HbA1c was positively correlated with the levels of the total plasma cholesterol, triglyceride, low density lipoprotein and negatively correlated with the level of high density lipoprotein

Table 2. Lipid Profile and HbA1c of IDDM Patients with Dyslipidaemia

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	Good (%)	(Fair %)	Poor (%)	Total (%)
Cholesterol (Chol)	-	3.2	3.2	6.4
Triglyceride (Tryg)	3.2	3.2	-	6.4
HDL	6.4	3.2	9.6	19.2
LDL	-	-	3.2	3.2
Chol + LDL	3.2	3.2	19.3	25.7*
Chol + HDL + LDL	-	-	3.2	3.2
Chol + Tryg + LDL	3.2	3.2	9.6	16.0*
Chol + Tryg + HDL	-	-	3.2	3.2
Chol + Tryg + HDL + LDL	-	-	3.2	3.2
Tryg + HDL	-	3.2	-	3.2
Tryg + HDL + LDL	-	-	3.2	3.2
HDL + LDL	6.4	-	-	6.4
	22.4%		76.9%	

## DISCUSSION

Failure to achieve good glycemic control is highly associated with dyslipidaemia in IDDM.

As reported in the DCCT (1992), elevated total cholesterol, LDL cholesterol, triglyceride, and decreased HDL cholesterol, appear to occur predominantly in younger female diabetic patients (ages 13-24) with relatively higher HbA1c levels. In our study, 76.9% of the 62% IDDM patients with dyslipidaemia were not in good glycemic control especially among females. Several studies have confirmed these findings, showing the importance of diabetic control on serum lipids and lipoproteins in IDDM.

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## QUALITATIVE CHARACTERIZATION OF SOME PATHOLOGIC CORNEAL DISEASES USING CONTACT SPECULAR MICROSCOPY

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### ABSTRACT

*The endothelium is a single layer of hexagonal cells lining the posterior surface of the cornea. It is responsible for the deturgescence state of the transparent cornea. Specular microscopy quantitative analysis of the endothelium is the latest method for its examination though this has not been found to be entirely predictive of corneal conditions that progress to corneal decompensation or edema. This study examined qualitative parameters to determine the association between corneal specular microscopy findings and progression or outcome of trauma (surgery) on the endothelium which causes corneal edema. Using the Konan SP5500 contact type of Specular microscope, the five areas (superior, central, inferior, temporal and nasal) of the corneal endothelium were examined. The following parameters were used: 1) regularity of posterior endothelial surface, 2) clarity of cells and their borders, 3) polymegethism and pleomorphism, and 4) presence of the intra, extra and intercellular bright or dark structures. Results showed that there were some parameters associated with prognosis of the corneal endothelium, namely: 1) the severity of endothelial surface irregularities, 2) the degree of polymegethism and pleomorphism (% of hexagonal cells), 3) the presence of early and late excrescences and inflammatory bodies, and 4) the area with the above findings. Qualitative analysis showed that area characterization is important to predict progression and outcome of corneal disease or conditions which may help in the prognostication of the corneal endothelium.*

**Keywords:** *specular microscopy, cornea, endothelium, corneal disease, qualitative characterization, eye, Konan SP5500, polymegethism, pleomorphism, excrescences.*

## INTRODUCTION

The visual sense (vision) is a result of the eye's proper functioning: 1. optical system (tear film, cornea, aqueous, lens and vitreous), 2. receptor cells (rods and cones of the retina), 3. relay system (bipolar and ganglion cells), 4. conducting system (nerve fibers, optic nerve, chiasma, and radiation), 5. integration and interpretation system (cerebral cortex). For an image to be formed and interpreted as such all these systems must be in proper and optimal condition. Among the systems mentioned, this study is concerned about the part of the optical system that contributes 60-80% of the total dioptric power of the eye, the cornea, specifically its endothelium. The corneal endothelium is a single layer of hexagonal cells lining the posterior surface of the cornea.

The corneal endothelium plays a major role in maintaining the deturgescence state and therefore the transparency of the cornea. Functionally, it acts a leaky barrier but this is obviated by its inherent capability to draw out water from the corneal stroma by its active transport pump sites. A balance between its barrier and pump function is therefore essential and must be maintained to avoid the accumulation of water in the cornea. The loss of this delicate balance or loss of too many of its cells may produce edema which will result in corneal haziness and cause blurred vision. This condition may be produced by an inherent disease of the endothelium or any injury to it usually due to trauma (surgical or nonsurgical).

The evaluation of the endothelium was in the past limited only through the use of high magnification lenses with the slit lamp microscopy. The *in vivo* observations using this method was however limited only to the area being considered and documentation through photography was still of poor resolution. The advent of specular microscopy made possible the detailed observation of the morphology of the corneal endothelial cells in an acceptable resolution.

The latest method of endothelial observation using the specular microscope is based on quantification of morphometric parameters such as cell density, number of cell sides and cell area which can be translated to its coefficient of variation. It, however, was not entirely predictive of conditions in the cornea that will result in corneal edema. This is exemplified by the diabetic endothelium where the morphometric parameters may be normal but cells are hypofunctioning and endothelium in old age where morphometric parameters are low but cells may be normally or hyperfunctioning. These observations have put up the persistent question of whether function is directly related to structure or vice versa.

This study is involved in qualitative characterization of endothelial parameters through the processes of the 3 "Ds": 1. **Describe**, 2. **Define** and 3. **Determine** the functional significance of these structural findings. These parameters may be classified into three groups: 1. **Indicative**, wherein their may be association (indirect relation) of this parameter to a certain condition, 2. **Causative**, wherein a certain parameter results (direct relation) in a certain condition, and 3. **Predictive**, wherein a certain parameter determines outcome or course of a certain condition.

## METHODOLOGY

### Specular Microscopy

Patients undergo contact specular microscopy (Standard Operational Procedure for Specular Microscopy) of both eyes using the Konan (SP 5500 (with standard 40X cone lens) with photographic attachment. The right eye is always done first followed by the left eye. The slit or wide view is utilized depending on the resolution obtained or if a wider area is necessary to characterize the endothelial portion being examined. Scanning of the area is done to determine the most appropriate view to be photographed. Photography of the central, superior, inferior, temporal and nasal areas is done. The film is developed and printed on a standard 3R size.

### Morphometric Calculations

The prints are scanned and the best view of the endothelial area photograph is used for calculations (Morphometric Calculations). Cell density and percent hexagonality is computed per area and is recorded for later reference.

### Qualitative Evaluation

Endothelial area (central, superior, inferior, temporal and nasal) descriptions are done for each cornea according to the following parameters: 1. posterior endothelial surface regularity, 2. clarity of cells and their borders, 3. degree of polymegethism, 4. degree of polymorphism, and 5. presence of bright and dark structures.

The qualitative parameters are defined and classified in the following ways:

**Posterior Endothelial Surface** – transition area of the endothelium and aqueous humor

Degree of Regularity	Percent Regularity
Regular	90-100%
Mildly irregular	75-89%
Moderately irregular	50-74%
Highly irregular	25-49%
Very severely irregular	10-24%
Wavy	0-9%

**Clarity of Cell and their Borders**

<b>Cells</b>	<b>Borders</b>
Clear Granulated Cytoplasm Not visualized	Distinct Blurred Not visualized

**Polymegethism – Variation of cell sizes**

<b>Degree of Polymorphism</b>	<b>Percent Variation</b>
None	0-10%
Slight	11-25%
Moderate	26-50%
Severe	51-75%
Very Severe	76-100%

**Polymorphism – variation of cell shapes (sides)**

<b>Degree of Polymorphism</b>	<b>Percent Hexagonality</b>
None	90-100%
Mild	175-89%
Moderate	50-74%
Severe	25-49%
Very Severe	0-24%

**Bright and Dark Structures**

May be extracellular, intercellular and intracellular

**Bright Structures**

Extracellular: amorphous substances (proteins, fibrin), edema and pigments

Intercellular: edema

Intracellular: lipid, amyloid, cytoplasmic granulations

**Dark Structures:**

Extracellular: depressions, folds, other causes of surface irregularities (endothelial precipitates)

Intercellular: late excrescences (guttata), inflammatory bodies, nucleus

Intracellular: early or late excrescences, inflammatory bodies



## RESULTS

Among 102 eyes observed and analyzed in this preliminary study, some areal parameters and their associated implications found are as follows:

### Severity of Surface Irregularities

It was observed that the more severe the surface abnormalities the more the possibility of endothelial pathology. It is therefore believed that surface abnormalities may be INDICATIVE of a pathological which may be also a result of endothelial insult or degenerative process. These are exemplified by the following conditions: a. post-trauma (insult, physical: as in surgical Descemet's tear or chemical: alkali burns) b. endothelial decompensation [traumatic (iatrogenic or surgical) and non-traumatic (pathologic: Fuch's endothelial dystrophy)] c. chronological degeneration (aged endothelium). The degree of irregularity appears to be related to the severity of the condition wherein pathological process will vary from mild to very severe irregularities, while generative which are chronologically determined varies from mild to moderate irregularities.

### Visualization of Cells and their Borders

The visualization of cells and their borders appears to be important as a status of the endothelial cells and the anterior chamber [aqueous interface, iris (as in iritis) and the chamber angle (the possibility of inflammatory glaucoma). It is therefore thought that their non-visualization is INDICATIVE of resultant endothelial dysfunction when generalized and inflammatory when focal. This is demonstrated by findings in total corneal decompensation where cells are generally nonvisible and keratouveitis where endotheliitis is present and nonvisualization of cells may be only sectoral.

### Degree of Polymegethism and Polymorphism

The changes in cell sizes and shapes are INDICATIVE of pathologic or degenerative conditions that may limit the cell density of the endothelium. It may have a PREDICTIVE value when coupled with a low cellular density count. Cataract patients with moderate to severe polymegethism and polymorphism appear to have more propensity to have corneal edema post-surgery if not properly managed pre-operatively, intra-operatively and post-operatively. The enlargement and metamorphism of cells seems to be related to changes cell stability and perhaps their function wherein changes would probably reflect a compromised state. This may be particularly true if other pathological signs are present such as inflammatory cells or degenerative substances.

### **Presence of Excrescences**

From the observations made of cases of Fuch's dystrophy and other pathological and degenerative corneal conditions, the stage, multiplicity and the aggregation of excrescences are important in prognostication of the endothelium. Pre-operatively they may have an INDICATIVE or PREDICTIVE value while post-operatively they may have CAUSATIVE or PREDICTIVE value.

### **Intra-, Inter-and Extracellular Inflammatory Bodies and Precipitates**

These bodies when present either extra or intracellularly are INDICATIVE of the inflammatory status of the endothelium. When present intracellularly they are also INDICATIVE of cellular degeneration secondary to inflammation. Precipitates similarly maybe present to further indicate the severity of inflammatory reaction in the endothelium and anterior chamber.

## **CONCLUSION**

Descriptive analysis of qualitative parameters used to characterize the corneal endothelium showed the following observations:

1. That area characterization is important to progression and outcome of corneal diseases and conditions which may help in prognostication of the corneal endothelium.
2. Excrescences coupled with inflammatory sides (inflammatory bodies and/or precipitates) seem to be predictive and causative of outcome and progression of corneal diseases and conditions.
3. Surface irregularities, polymegethism and pleomorphism may be indicative of degeneration and hypofunction of the endothelium of some corneal diseases and conditions.

## EVALUATION OF THE HEALTH EFFECTS OF PESTICIDE USE AMONG LAGUNA FARMERS

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### ABSTRACT

*This is a preliminary study that aims to document and compare the health profiles of two groups of Laguna farmers groups of rice farmers which are socioeconomically comparable. The very striking difference between the two groups of Laguna farmers is the history of exposure to pesticides. Morbidity or the occurrence of illness or abnormalities is used to measure health status. Two health surveys were conducted, and the results seem to show that there were significant differences between the two groups in so far as the hematologic, cardiovascular and neurologic health impairments are concerned. It is to be noted that many of the Laguna farmers did not adopt adequate health safeguards. Other factors like smoking and drinking habits of both groups were also explored.*

*Keywords: Pesticides, organophosphates, cholinesterase, pesticide poisoning, polyneuropathy, anemia, adverse health effects, personal, protective equipment.*

### ADVERSE HEALTH EFFECTS OF PESTICIDES – A REVIEW OF LITERATURE

Designed to disrupt vital biological mechanisms, pesticides do not only affect target organisms but nontarget species as well, primarily man.

The adverse health effects of pesticides have been widely reported in professional literature (Lotti 1987). We are particularly interested in organophosphorous compounds which are commonly used by the farmers in the study. When inappro-

priately handled these compounds cause acute and chronic toxicity. Acute toxicity is defined as the "total adverse effects produced by a toxicant when administered as a single dose". On the other hand chronic toxicity can be defined as "total adverse effects produced by a toxicant when administered continuously over a long period of time" (Duffus 1980).

### **Acute Toxicity**

Acute organophosphate poisoning is caused by the phosphorylation of cholinesterase, a substance normally present in the blood. Cholinesterase degrades acetylcholine, the chemical neurotransmitter, to assure the smooth firing of nerve impulses across synapses. The inhibition of cholinesterase by phosphorylation causes acetylcholine accumulation at critical sites, giving rise to clinical manifestations (Corbett 1974). Such manifestations range from non-specific signs and symptoms such nausea and vomiting in mild poisoning, the paralysis in severe cases.

Diagnosis of acute organophosphate poisoning is confirmed by measuring the cholinesterase activity in the blood. Cholinesterase depression as a biological index of toxicity is one of such indices to be proven over time (Duffus 1980). Various studies show that cholinesterase depression correlate well with the development of acute signs and symptoms (Roberts undated).

### **Chronic Toxicity**

Although organophosphates are not known to be highly cumulative there is a group of entities that are due to chronic low dose exposure, that does not apparently affect cholinesterase levels and are therefore called nonanticholinesterase effects. These are the following:

1. Organosphosphate-induced delayed polyneuropathy (OPIDN). Due to damaged myelin sheath of peripheral nerves, this clinical syndrome manifests numbness, pain and paralysis of the extremities developing days and weeks after exposure. Animal experiments have implicated phosphotriesters, cyanofenphos and leptophos. In such experiments, it has been shown that an enzyme other than cholinesterase was depressed. There appears to be no apparent relationship between acute toxicity and the likelihood of the development of a chronic neuropathic effects (Hayes 1982).

2. Chronic exposure may lead to a decrease in platelet. Thrombocytopenia or a decrease in platelet count may manifest as increased bleeding tendency. Thirteen percent of the rice farmers in Castañeda's study and 18% among pesticide formulators in Maramba's study showed thrombocytopenia (Castañeda 1987).

3. Abnormal electrocardiographic (Aldridge and Johnson 1971; Metcalf and Holmes 1969) and electromyographic findings (Jager 1976) in asymptomatic individuals exposed to chronic low doses of organosphosphates.

4. There are reports of changes in the liver function tests, gastrointestinal disturbances and behavioral changes associated with chronic low dose exposure (Levin and Rodnitzky 1976; Lewis *et al.* 1973; Metcalf and Holmes 1969).

5. Hyporeflexia has been considered by some authors to be a highly sensitive indicator of chronic exposure (Roberts 1976; Roberts and Trollo 1979).

6. Anemia has been reported by Maramba in 38% of workers in formulating plants in the Philippines.

7. Other suspected but generally unconfirmed effects include those on the brain, heart and lungs. Hypertension and accelerated atherosclerosis have also been suspected health effects (Hock 1987).

### SPECIFIC OBJECTIVES

1. To identify the health impairment among the farmers.
2. To determine the extent of the health problems and the factors that affect them.
3. To compare the study and control groups as to the differences in their health profile.
4. To document the acute toxic effects of organophosphorous compounds.
5. To identify potential health indices for adverse health effects due to chronic exposure.

### SAMPLING

#### The Exposed Group

Fifty-six farmers from Cabuyao, Calauan, Biñan and Calamba comprised the study group. All 56 had a documented exposure of 15 to 25 years to organophosphorous compounds (44%) and chlorpyrifos (33%). Only 30% of the farmers wore long-t-shirts and 23% wore long pants when spraying pesticide. Thirty four percent took a bath after application.

#### The Unexposed Group

The control group is a group of 40 farmers from barangays Kulapi, Palola Kabatete and Kilib of Lucban, Quezon, who had never used pesticides because pest control was never been a major problem, allowing them to use natural or other cultural pest control measures. Also, their farm animals grazed near their fields.

### METHODOLOGY

The study utilizes a cross-sectional survey which includes interview, physical examination, a battery of laboratory tests and exposure history. The strength of this design lies, however, in its ability to detect chronic and subacute effects. Its

weakness lies in its inherent bias for survivors, that is, the population being studied may be a healthy population. Some of the individuals who were ill may have been excluded in that one slice of time that the survey was conducted. The same set of tests were done for both the control and exposed groups. SAS computer software was used for statistical analysis.

The study was multiphasic.

### **PHASE 1. Exploratory Talks**

A team from the Social Sciences Department of IRRI held exploratory talks with the farmers. In Biñan, Calamba and Cabuyao, there were one-on-one encounters with farmers whom we perceived to be decision makers in that area. The strategy in Calauan was different in that discussions were carried out in groups. In like manner, group discussions were held in the four barangays in Lucban, Quezon, namely Kabatete, Kulapi, Kilib and Palola.

### **PHASE 2. Medical Assessment**

The medical assessment was carried out by a medical team consisting of a physician, nurse, x-ray technician and medical technologists. The nurse interviewed the farmers regarding their personal, family and occupational histories, including their drinking and smoking habits. The doctor performed a complete physical examination on all the farmers using a protocol specially designed for the project. Cholinesterase determinations were done by the medical technologist while chest x-ray and electrocardiogram (ECG) were done by the x-ray technician. The readings for the ECG and the x-ray were done by two cardiologists and a radiologists, respectively.

### **PHASE 3. Lecture on Pesticide Management**

All the farmers were given a lecturette on pesticide management and on health safeguards they can quickly adopt to protect themselves.

### **PHASE 4. Cholinesterase Determination**

Baseline cholinesterase was established for the Laguna and Lucban farmers using the Ellman method (whole blood). The average of two measurements taken not less than 3 and not more than 14 days apart at the time when there was no exposure to organophosphates was considered as "baseline". Repeat cholinesterase was done at two periods that coincide with peaks of pesticide application in Laguna.

### **PHASE 5. Feedback and Referrals**

An immediate feedback of the results were given to the farmers and appropriate referrals to specialists were made.

### PHASE 6. Documentation of Exposure

Exposure to Organophosphates was documented in terms of type, total number of applications and category of pesticides.

### PHASE 7. One-Shot Survey

A one-shot survey was done to determine pesticides management practices, food and feeds taken from the paddy. Knowledge and attitudes concerning pesticide use were also recorded.

### PHASE 8. Walkthrough Surveys

A quantitative evaluation of the work environment and the work processes was conducted.

## RESULTS

### Medical Assessment

Table 1 shows the characteristics of the exposed and unexposed group. Of the 56 farmers in the exposed group, 86% were males while in the unexposed group, 89% were males. Thirty seven per cent of the unexposed group belong to the age group 60 years and above. Seventy seven per cent of the exposed group and 70 per cent of the unexposed groups are smokers. There were more drinkers among the control group (98%) than the exposed group (59%). Forty eight percent of the exposed group users of at least one personal protective gear. Average number of pesticides applications for the past four years in 19 for the study group.

Table 1. Characteristics of the exposed and control groups.

	<i>Exposed (Laguna)</i>	<i>Unexposed (Lucban)</i>
No. of subjects	56	40
Average age (yrs.)	49	56
Age distribution:	%	%
	7	0
	25	0
	18	15
	14	30
	23	18
	13	37
Average income (1990 pesos)	22,522	19,553
Rural Health Unit	4	1
Percentage of smokers	77	70
Percentage of drinkers	59	98

Physical examination revealed that 32% of the exposed group and 45% of the control group had weights appropriate for their height and sex based on the FNRC standards. Average systole and diastole are 120 and 78 for the exposed group, respectively and 129 and 81 for the control group.

Table 2 details the abnormal findings per organ system for both the control and exposed groups. Eye findings in the exposed group were largely due to bilateral pterygium and pale conjunctive. Frequently cited (53.1%) cardiovascular abnormality was high blood pressure while decreased breath sounds (48.7%) was the frequently cited pulmonary abnormality. In comparison, the most frequently cited eye finding in the control group was due to degenerative diseases (48.57%). In the lungs, decreased breath sound (30%) was the most frequently cited abnormality.

Polyneuropathy was noted on physical examination among three farmers in the exposed group, while there was not any indication observed in the control group in spite of the fact that there were more drinkers in this group. Laboratory tests showed normal fasting blood sugar levels for all three farmers with polyneuropathy. Referral to a neurologist one month later showed more severe manifestations of polyneuropathy which was then confirmed by electrodiagnostic studies. Details are shown in Table 3. All were prescribed cyanocobalamine and Vitamin B 1-6-12 for three months and advised to refrain from pesticide application.

Forty one percent of the exposed group had abnormal ECG findings in comparison with 40% among the control group. This is not significantly different. Among those with ECG findings, 44% belonged to the 21-40 age bracket for the exposed group while only 6% for the control group (Table 4). In the 41 to the above 50 age group which are expected to have more cardiac findings, there is no significant difference between the two groups.

There is a significant difference between the exposed and the study group with regard to the hemoglobin levels. Only 5% in the control group had hemoglobin levels lower than normal as compared to 25% in the exposed group. Eighteen per cent in the control group, while only 16% in the exposed group had albumin in their urine, the difference not being statistically significant. Five among the exposed had cholinesterase levels below normal though this is not significantly different from the unexposed.

Table 5 details the abnormal findings read on ECG for both groups of farmers. The authors are well aware of the controversy regarding readings such as "non-specific ST-T changes" which may be read as "normal" by other cardiologists. Two cardiologists were requested to read the ECG and intraobserver variability was not statistically. In both groups the most frequent reading was "sinusbradycardia" which may be expected among manual workers. However, among those exposed, it is to be noted that the second most frequently cited is "nonspecific ST-T wave changes" while in the nonexposed group the second most cited finding is ischemia which may be due to the fact that this is an older age group.



Table 2. Details of abnormal findings per organ system, 56 exposed (Laguna) and 40 unexposed (Lucban) farmers.

ORGAN SYSTEM	LAGUNA			LUCBAN		
	No.	n=abnormal	n=56	Percent	No.	n=abnormal
1. Eye						
a. pterygium	15	30.00	26.79	6	17.14	15.00
b. cataract	3	6.00	5.36	1	2.86	2.50
c. dirty sclerae	3	6.00	5.36	1	2.86	2.50
d. presbyopia	9	18.00	16.07	8	31.43	27.5
e. glaucoma	1	2.00	1.79	0	0.00	0.00
f. icteric sclerae	1	2.00	1.79	0	0.00	0.00
g. pale conjunctivae	13	26.00	23.21	7	20.00	17.50
h. sluggish pupillary reflex	3	6.00	5.36	1	2.85	2.50
i. arcus senilis	1	2.00	1.79	6	17.14	15.00
j. decreased pupillary size	1	2.00	1.79	0	0.00	0.00
k. hemorrhage	0	0.00	0.00	2	5.70	5.00
2. Ear						
a. congestion	1	20.00	1.79	0	0.00	0.00
b. diminished hearing acuity	4	80.00	7.14	0	0.00	0.00
3. Nose						
a. sinusitis	1	100.00	1.79	0	0.00	0.00
4. Throat						
a. tonsillitis	8	61.54	14.29	0	0.00	0.00
b. congestion	3	23.08	5.36	2	100.00	5.00
c. pharyngitis	2	15.38	3.57	0	0.00	0.00
5. CVS						
a. murmur	2	6.25	3.57	0	0.00	0.00
b. high BP	17	53.13	30.36	2	67.00	5.00
c. low BP	13	40.63	23.21	0	0.00	0.00
d. distant heart sound	0	0.00	0.00	1	33.00	2.50
6. Lungs						
a. bilateral harsh breath sound	3	7.69	5.36	0	0.00	0.00
b. wheezing	6	15.38	10.71	3	15.00	7.50
c. rales	2	5.13	3.57	5	25.00	12.50
d. harsh breath sounds	2	5.13	3.57	0	0.00	00.00
e. decreased breath sounds	19	48.72	33.93	7	30.00	15.00
f. decreased chest expansion	6	15.38	10.71	2	10.00	5.00
g. decreased percussion	1	2.56	1.79	0	0.00	0.00
h. basal rhonchi	0	0.00	0.00	2	10.00	5.00
i. apical, bilateral	0	0.00	0.00	1	5.00	2.50
j. dec. b.s.	0	0.00	0.00	1	5.00	2.50

Table 2 (continued)

ORGAN SYSTEM	LAGUNA			LUCBAN		
	No.	Percent <i>n=abnormal</i>	<i>n=56</i>	No.	Percent <i>n=abnormal</i>	<i>n=40</i>
7. GIT						
a. LUQ tenderness	1	20.00	1.79	0	0.00	0.00
b. hepatomegaly	2	40.00	3.57	2	100.00	5.00
c. RUQ tender	2	40.00	3.57	0	0.00	0.00
8. GUT						
a. hypogastric tenderness	1	33.33	1.79	0	0.00	0.00
b. left lumbar tenderness	2	66.67	3.57	0	0.00	0.00
9. Extremities						
a. joint inflammation	1	50.00	1.79	0	0.00	0.00
b. joint pain	1	50.00	1.79	0	0.00	0.00
c. joint tenderness	0	0.00	0.00	5	83.30	12.50
d. + crepitus	0	0.00	0.00	1	16.70	2.50
10. Motor						
a. left gastrocnemius weakness	1	50.00	1.79	0	0.00	0.00
b. weakness of shoulder muscle	1	50.00	1.79	0	0.00	0.00
c. weakness of plantar flexur and dorsiflexur	0	0.00	0.00	1	50.00	2.50
d. weakness of left lower leg	0	0.00	0.00	1	50.00	2.50
11. Reflex						
a. decreased patellar reflex	14	100.00	25.00	5	83.30	12.50
b. negative L reflex	0	0.00	0.00	1	16.70	2.50
12. Sensory						
a. numbness	1	25.00	1.79	0	0.00	0.00
b. sensory deficit	3	75.00	5.36	0	0.00	0.00
13. Skin						
a. papules	7	63.64	12.50	0	0.00	0.00
b. dryness	7	63.64	12.50	4	20.00	10.00
c. erythema	1	9.09	1.79	0	0.00	0.00
d. wounds & scratches in the	1	9.09	1.79	6	30.00	15.00
e. tinea	3	27.27	5.36	10	50.00	25.00
f. eczema	1	9.09	1.79	0	0.00	0.00
g. scars	2	18.18	3.57	0	0.00	0.00

Table 3. Polyneuropathy cases

<i>ITEM</i>	<i>AGE</i>	<i>SIGNS/ SYMPTONS</i>	<i>FBS</i>	<i>ELECTRODIAG- NOSTIC TEST</i>
FARMER A	53	sensory deficit for both upper and lower extremities with associated hyporeflexia and weakness of plantar and dorsiflexors of the ankle.	5.06 (4.18-6.63)	Finding suggestive of sensorimotor polyneuropathy of the lower extremities as evidenced by the delay in the motor stimulation of the posterior tibial nerve and sensory stimulation of both sural nerves.
FARMER B	63	sensory deficit and hyporeflexia for both lower extremities	76 (N:60-100)	Findings suggestive of moderate to severe polyneuropathy. EMG findings showed denervation potentials. Motor stimulation involves both the median nerves and peroneal and tibial nerves. Sensory stimulation involves the sural serves.
FARMER C	49	numbness and paresthesia hyporeflexia and sensory deficit for both lower extremities	66 (N:60-100)	Findings are suggestive of sensorimotor polyneuropathy as evidenced by the delayed sensory nerve conduction velocity of the left common peroneal and the left posterior tibial nerves.

Table 4. Classification by age and sex with abnormal ECG findings among Laguna and Lucban farmers.

<i>AGE</i>	<i>TOTAL</i>		<i>MALE</i>		<i>FEMALE</i>	
	<i>Laguna</i>	<i>Lucban</i>	<i>Laguna</i>	<i>Lucban</i>	<i>Laguna</i>	<i>Lucban</i>
21-30	5 (22)	0	5 (100)	0	0	0
31-40	5 (22)	1 (63)	4 (80)	1 (100)	1 (20)	0
41-50	3 (13)	5 (31)	1 (33)	5 (100)	2 (67)	0
Above 50	10 (43)	10 (63)	9 (90)	1 (10)	1 (10)	1 (10)
Total	23 (41)	16 (40)	15 (94)	15 (94)	4 (17)	1 (6)

Table 5. Details of ECG findings of 56 exposed Laguna farmers and 40 unexposed Lucban farmers.

ORGAN SYSTEM	LAGUNA			LUCBAN		
	No.	Percent <i>n</i> =abnormal <i>n</i> =56		No.	Percent <i>n</i> =abnormal <i>n</i> =40	
Old anteroseptal myocardial infarction	1	3.22	1.79	0	0	0.00
Left ventricular hypertrophy	1	3.22	1.79	1	3.57	2.5
Left ventricular hypertrophy by voltage criterion	0	0	0.00	4	14.28	10.00
Probable right ventricular hypertrophy	1	3.22	1.79	0	0	0.00
LAD	0	0	0.00	2	7.14	5.00
Tendency to LAD	2	6.45	3.57	0	0	0.00
Tendency to LAD	2	6.45	3.57	0	0	0.00
Atrial flutter/fibrillation with controlled ventricular response	1	3.22	1.79	0	0	0.00
ST-T/T wave changes suggestive of inferior wall ischemia	3	9.67	5.36	0	0	0.00
Non-specific ST-T changes	6	19.35	10.71	2	7.14	5.00
Diffuse/inferior/anterolateral wall ischemia	1	3.22	1.79	6	21.43	15.00
Sinus bradycardia	8	25.8	14.29	8	28.57	20.00
Right bundle branch block (RBBB)	1	3.22	1.79	1	3.57	2.50
Widened QRS, wide slurred S at I, V4-6 T wave inversion at I, VI-4 suggestive of RBBB	1	3.22	1.79	0	0	0.00
Presence of U waves	1	3.22	1.79	0	0	0.00
Premature ventricular contractions	2	6.45	3.57	3	10.71	7.50
Sinustachycardia	0	0	0.00	1	3.57	2.50

Table 6 is a summary of the means of the laboratory findings for both unexposed and exposed groups.

The blood urea nitrogen (BUN) and creatinine, both kidney function tests, showed means that were within the normal limits for both the exposed and the unexposed groups.

SGPT is a liver function test and the means for both unexposed and exposed groups were within normal limits. Mean hemoglobin was within normal for both control and exposed groups. The mean white and red blood cell counts were both within normal limits for both unexposed and exposed groups.

Table 6. Cholinesterase and blood chemistry distribution, 56 exposed (Laguna) and 40 unexposed Lucban farmers.

Variable	Lucban	Std. Dev.	Laguna	Std. Dev.	Normal Values
Cholinesterase	4,575.00	888.76	4,978.00	829.60	3,500-4,500
BUN	12.29	3.84	9.07	4.21	6-20 mg/l
Creatinine	1.05	0.18	1.12	0.49	1-2 mg/l
SGPT	19.56	4.39	14.09	3.05	5-30 R-Fruit
Hemoglobin	14.42	1.07	13.73	1.65	13.5-7 m=gms% (M) 12-16 gms% (F)
WBC	7,908.00	1,139.87	7,224.00	1,943.71	5-10,000/cu mm
RBC	4.80	0.32	4.82	0.58	4-6 million/cumm
Platelet	197,750.00	25,164.84	271,607.00	60,265.00	175-350, 000

### Cholinesterase Tests

For the exposed group, the average cholinesterase baseline activity was 4978 with the curve fitting the normal. Most of the values clustered in the middle with a few outliers, the lowest value being 2740, while the highest value being 7526. Both were not within normal limits. The average cholinesterase for the control group was 4575 which is significantly different from the exposed group.

Monitoring was done at two periods coinciding with peaks of spraying. In both monitoring periods there was a significant number of farmers who had a depression in their cholinesterase levels in the first monitoring period, 56% and in the second monitoring period, 61%. All farmers that had a depression of 30% or more in the second monitoring period were examined and all had clinical manifestations such as deminished patellar tendon reflexes, nausea, vomiting and sluggish pupillary reflexes. In comparison, 6.2% of the San Leonardo farmers in Castañeda's study had cholinesterase depression.

There was a continuous fall from baseline, first at 3.52% on peak of spraying, dry season and second at 9.9%, peak of spraying, wet season. Individual farmer graphs were plotted and all showed the same downward trend.

### Smoking and Drining Factors

Among the exposed group, drinkers have a significantly higher occurrence of bronchopneumonia, significant cough, epigastric pain, vomiting ( $\alpha = .01$ ) and headache, nausea asthmatic attacks and diarrhea ( $\alpha = .05$ ).

Among the exposed groups, the smokers have significantly higher occurrence of diarrhea ( $\alpha = .01$ ). There was no significant difference for the occurrence of other abnormalities in the review of systems.

### Use of Personal Protective Equipment

The farmers in the exposed group who used at least one item of personal protective equipment had a lower occurrence of anemia and albuminuria ( $\alpha=.05$ ) x-ray finding ( $\alpha=.05$ ) double vision ( $\alpha=.05$ ) and asthma and dyspnea at  $\alpha=.05$ .

### Intestinal Parasitism versus Low Hemoglobin Level

There was no significant relationship between intestinal parasitism and low hemoglobin level among the exposed group ( $\alpha=.01$ ).

## CONCLUSIONS

The available literature showed that there were indeed acute and chronic effects due to organophosphorous compounds. The acute effect was reflected in the cholinesterase levels, while the chronic effects centered on the lungs, cardiovascular, hematologic and neurologic systems.

**Acute Toxicity.** In the exposed study, there was a depression of the cholinesterase levels at two monitoring periods. This decline during peaks of spraying could be due to the fact that a low number of farmers used appropriate health safeguards.

A comparison of cholinesterase levels for the exposed and control groups showed no significant difference. However, it was noted that there was a higher average among the exposed than the control, and this could be explained by the rebound phenomenon in which after exposure stopped, there was an increase of the cholinesterase levels sometimes as high as twice the normal level.

**Chronic Toxicity.** The chronic effects on the other hand are less directly affected by the environment concentration and are not directly related to the use of protective gear. Rather the chronic effects depend more on accumulation of pesticides in particular target organs over a period of time.

In this study, the following effects were noted to be significantly higher among the exposed group as compared to the control group- hematologic, neurologic and cardiovascular effects.

In summary the differences are as follows:

Exposed group	Control group	z-test
25% Low hemoglobin	5% Low hemoglobin	*
5% Polyneuropathy	No polyneuropathy	*
54% CVS on p.e.	7% CVS on p.e.	*

To establish an association between adverse health effects and a noxious substance, it is necessary to demonstrate a dose-effect relationship (Roberts and Trollope 1979). In this study, we compared two rice farmer groups whose only striking difference was their exposure to pesticides. One group never used pesticides, while the other group had been cocktail of pesticides, mostly organophosphorous compounds. Thus, the effects noted can be associated to the exposure to pesticides of one group and the absence of such exposure in the control.

In a developing country like the Philippines, it is said that concomitant diseases, intestinal parasitism, smoking and drinking make the farmer more susceptible to the effects of pesticides (Copplesstone 1985).

The study showed that indeed drinking and smoking among the exposed group aggravated the occurrence of pulmonary and gastrointestinal symptoms.

It was noted that the exposed group had not adopted adequate health safeguards against pesticide use. This is a practice seen even among Central Luzon farmers as shown by Castaneda's study (1987). This study showed that the use of at least one piece of protective clothing would protect the farmers from hematologic and pulmonary effects.

A typical farmer who uses pesticides can be characterized as a person who suffers from ups and downs of his cholinesterase levels, and is likely to be anemic, hypertensive and runs the risk of having a polyneuropathy.

A high risk farmer is one who does not use at least one form of personal protective clothing, smokes, drinks and applies pesticides 19 times a year, and does not take a shower after spraying.

It is recommended that further studies on the impact of intervention, such as training and the use of personal protective equipment be studied.

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# **MATHEMATICAL, PHYSICAL, AND ENGINEERING SCIENCES**

## **IMPROVEMENT OF BLUM'S ET. AL. SELECTION ALGORITHM**

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### **ABSTRACT**

*Blum, et. al. [1] presented a selection algorithm that finds the  $k$ th smallest element of a set with  $n$  distinct elements using  $5.4305n$  comparisons in the worst case. In this paper, we present an improvement of this algorithm that requires  $5.3975n$  comparisons in the worst case. The contribution of this paper is not on the amount of improvement but rather on the result that the worst case of the best practical algorithm for selection can still be improved. Thus, opening the possibility of further closing the gap between the best practical worst case and the best theoretical worst case for selection.*

**Keywords:** selection, order statistic, analysis of algorithms.

### **INTRODUCTION**

Blum, et. al. [1973] presented an algorithm for selection that requires  $5.4305n$  comparisons in the worst case. This was improved by Schonhage, et. al. [1976] by presenting an algorithm that requires asymptotic to  $3n$  comparisons in the worst case. Unfortunately, the algorithm presented in [4] is much more difficult to implement than the one in [Blum et al. 1973]. From the practical point of view, therefore, the algorithms of Blum, et. al. [1973] remain the best.

One of the algorithms in [Blum et al. 1973] (called PICK1) is known to perform well as the number of elements discarded increases. In this paper, we present a new algorithm called LOCATE, that behaves in the opposite direction, i.e., the algorithm performs well as the number of elements that can be discarded decreases. By combining these two algorithms, an algorithm that requires  $5.4137n$  comparisons is produced. Further refinement reduces this value to  $5.3975n$  comparisons.

**Notations**

For convenience, the notations used in [Blum et al. 1973] will be adopted in this paper. Given a set  $S$  of  $n$  distinct elements,  $k\theta S$  is the  $k$ th smallest element of  $S$ ,  $x_p S$  is equal to the rank of  $x$  in  $S$ . But by arguments of symmetry, the algorithms in this paper assume that  $1 \leq k \leq \lceil n/2 \rceil$ . The small letter  $c$  will consistently be used to represent the size of the columns used in the algorithms presented in this paper and  $h(c)$  is the cost of sorting a  $c$ -column. The term 'c-column' refers to a column with  $c$  elements and the term 'c-sorted' refers to a  $c$ -column that is sorted in ascending order. All sorting steps involved in the algorithms arrange the elements of the set in ascending order and are carried out using the sorting algorithm in [Ford and Johnson 1959]. Further, it should be noted that in the succeeding sections, performance or running time or cost is measured in terms of number of comparisons.

**Blum's et. al. Selection Algorithms**

The most important contributions of their algorithms are the establishment that the selection can be done in linear time. These algorithms are similar with Hoare's selection algorithm (FIND (Hoare 1961)) except that the algorithms ensure that in every iteration about  $1/4$  of the total number of elements being considered are discarded. This somehow explains the reason why the worst case running times of the algorithms are linear.

Their best algorithm, called PICK1, starts by dividing the input into  $c$ -columns which are then  $c$ -sorted. In the succeeding iterations, the retained elements are restored to  $c$ -sorted columns by merging. This means that the columns are  $c$ -sorted only once which explains the cost of  $5.4305n$  comparisons. Since PICK1 will be used as a basis of some improvements to be given later, a slightly reformatted version of it will be given below.

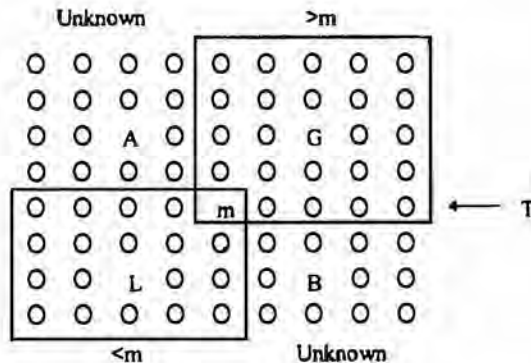


Figure 1. Division of Elements in PICK1

**Algorithm: PICK1**

Comment: Selects  $k\theta S$ , where  $|S| = n$  and  $1 \leq k \leq \lceil n/2 \rceil$ .

1. If  $n \leq 45$ , sort  $S$ , print  $k\theta S$ , and halt.
2. Arrange  $S$  into  $\lceil n/15 \rceil$  columns of length 15, and sort each column.
3. Use PICK1a to select  $k\theta S$ .

**Algorithm: PICK1a**

Comment: Selects  $k\theta S$ , where  $|S| = n$  and  $1 \leq k \leq \lceil n/2 \rceil$  and the set  $S$  and  $T$  are already 15-sorted.

1. If  $n \leq 45$ , sort  $S$ , print  $k\theta S$ , and halt.
2. Arrange  $T$ , the set of column medians (Figure 1), into  $\lceil n/225 \rceil$  columns of length 15, and sort each column.
3. Use PICK1b to select  $k\theta S$ .

**Algorithm: PICK1b**

Comment: Selects  $k\theta S$ , where  $|S| = n$  and  $1 \leq k \leq \lceil n/2 \rceil$ , and the sets  $S$  and  $T$  are already 15-sorted.

1. Select  $m = \lceil |T|/2 \rceil \theta T$  using PICK1a, where  $T$  is the set of all  $c$ -column medians.
2. Compute  $m\theta S$ , by partitioning  $(A \cup B)$  of Figure 1 about  $m$  as follows, stopping as soon as it becomes apparent that  $m\theta S < k$  or  $m\theta S > k$ .
  - (a) Insert  $m$  into each column of  $B$  using binary insertion.
  - (b) Insert  $m$  into each column of  $A$  using linear search technique beginning near the median of the original column.
3. Apply the discard and restore procedure.
4. Decrease  $n$  by the number of elements discarded. If  $n \leq 45$ , sort  $S$ , print  $k\theta S$  and halt, otherwise return to Step 1.

**Algorithm: Discard and Restore – PICK1**

1. If  $m\theta S = k$ , halt. Otherwise, if  $m\theta S > k$ , then discard  $G \cup \{x \mid x \in B \wedge x > m\}$ , else discard  $L \cup \{x \mid x \in A \wedge x > m\}$  and decrease  $k$  by the number of elements discarded.
2. Restore  $S$  to a set of 15-columns.
3. Restore  $T$  to a set of 15-columns.

Let  $P_b(n)$ ,  $P_a(n)$  and  $P(n)$  be the costs of algorithms PICK1b, PICK1a, and PICK1, respectively. These costs were shown in [1] to assume the values

**Proof:** For every pair of  $c$ -columns affected by the discard operation, exactly  $c$  elements are discarded. Since there are  $n/4c$  pairs of affected  $c$ -columns, therefore a total of  $c(n/4c)=n/4$  elements are discarded.  $\square$

**New Selection Algorithm**

Using the simple discard and restore procedure outlined in the previous section, an algorithm that performs well as  $d$  decreases can be constructed. The outline of the algorithm is exactly the same as that of PICK1, except for the discard and restore procedure. An outline is given below.

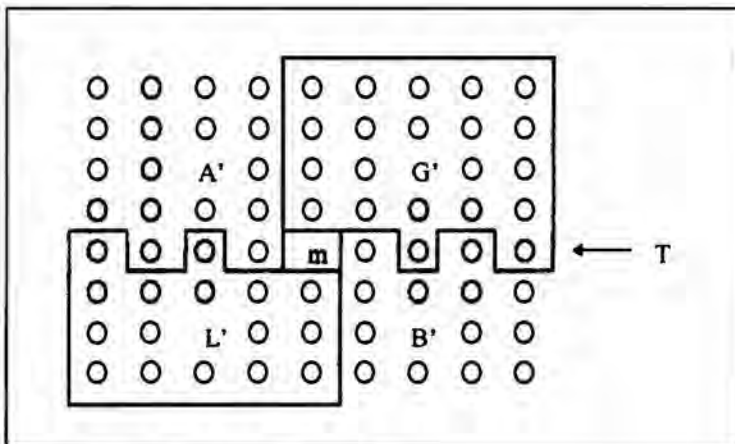


Figure 2. Divisions of Elements in LOCATE.

**Algorithm: LOCATE**

Comment: Exactly the same as PICK1, except that Step 3 uses LOCATEa.

**Algorithm: LOCATEa**

Comment: Exactly the same as PICK1a, except that Step 3 uses LOCATEb.

**Algorithm: LOCATEb**

Comment: Exactly the same as PICK1b, except that Step 3 uses LOCATEa and discard and restore procedure given below.

**Algorithm: Discard and Restore – LOCATE**

1. If  $mpS = k$ , halt. Otherwise if  $mpS > k$ , then discard  $D_g$ , else discard  $D_l$  and update  $k$ .
2. Restore  $S$  to a set of  $c$ -columns as follows:

2. Restore S to a set of c-columns as follows:
  - (a) The c-column medians form  $\lfloor c/2 \rfloor$ -sorted and  $(\lfloor c/2 \rfloor + 1)$ -sorted columns since they were discarded in that form in Step 1 of LOCATEb. This means that  $\lfloor c/2 \rfloor$  pairs, i.e.,  $\lfloor (\lfloor c/2 \rfloor + 1)/2 \rfloor$  and  $\lfloor \lfloor c/2 \rfloor / 2 \rfloor$  pairs from those whose medians are  $(\lfloor c/2 \rfloor + 1)$ -sorted and  $\lfloor c/2 \rfloor$ -sorted, respectively, from out of c affected columns can be merged immediately using the method outlined in the previous section.
  - (b) Combine the extra columns and merge every  $\lfloor c/2 \rfloor$ -column with a  $(\lfloor c/2 \rfloor + 1)$ -column. It should be made sure, however, that  $1/2$  of the extra columns are  $\lfloor c/2 \rfloor$ -columns and the other half are  $(\lfloor c/2 \rfloor + 1)$ -columns. This arrangement can be done easily.
  
3. Restore T to a set of c-columns similarly. Let ZCT be those column medians which were column medians in Step 1 of LOCATEb.
  - (a) Since the elements of Z are arranged in  $\lfloor c/2 \rfloor$ -sorted and  $(\lfloor c/2 \rfloor + 1)$ -sorted columns (elements of Z were discarded in that form when m was computed in Step 1 of LOCATEb), restoring Z can therefore be done by simply merging every  $\lfloor c/2 \rfloor$ -sorted column with a  $(\lfloor c/2 \rfloor + 1)$ -sorted column.
  - (b) Restore the new medians, which were computed after the restoration of S, by sorting them into c-columns.

**Lemma 2.** The cost of restoring S is  $(c-2)((c-1)/c)(n/4c) + (c-1)(1/c)(n/4c)$  comparisons.

**Proof:** The discard operation leaves  $((c-1)/c)(n/4c)$  pairs of adjacent  $\lfloor c/2 \rfloor$ - and  $(\lfloor c/2 \rfloor + 1)$ -columns. Hence, they can be merged using  $(c-2)((c-1)/c)(n/4c)$  comparisons. The unknown columns, whose number is  $(1/c)(n/2c)$  and which are composed also of  $\lfloor c/2 \rfloor$ - and  $(\lfloor c/2 \rfloor + 1)$ -columns can be restored using  $(c-1)(1/c)(n/4c)$  comparisons.  $\square$

**Lemma 3.** The cost of restoring T is  $(c-1)(n/4c)(1/c) + h(c)(n/4c)(1/c)$  comparisons, where  $h(c)$  is the cost of c-sorting.

**Proof:** The restoration of ZCT can be done using the method used to restore S. Since  $|Z| = n/2c$ , therefore there are  $(n/4c)(1/c)$   $\lfloor c/2 \rfloor$ -sorted columns in Z. The same number of  $(\lfloor c/2 \rfloor + 1)$ -sorted columns is present. Hence, Z can be restored to c-columns in  $(c-1)(n/4c)(1/c)$  comparisons. The set of new medians, whose number is  $n/4c$ , can be c-sorted using  $h(c)(n/4c)(1/c)$  comparisons.  $\blacktriangleright$

The costs of LOCATE, LOCATEa, and LOCATEb, which are denoted by  $L(n)$ ,  $La(n)$ , and  $Lb(n)$ , respectively, can be described by the following recurrence relations:

$L(n) \leq nh(c)/c + La(n)$	Cost of LOCATE.
$La(n) \leq nh(c)/c + Lb(n)$	Cost of LOCATEa.
	Cost of LOCATEb.
$Lb(n) \leq La(\lceil n/c \rceil)$	Compute m.
$+ (\lceil \log_2 \lceil c/2 \rceil + 1 \rceil) (n/2c)$	Insert m into B.
$+ (n/2x + d)$	Insert m into A.
$+ (c-2)((c-1)/c)(n/4c)$	Restore S. Step 2a.
$+ (c-1)(1/c)(n/4c)$	Restore S. Step 2b.
$+ (c-1)(n/4c)(1/c)$	Restore S. Step 3a.
$+ h(c)(n/4c)(1/c)$	Restore T. Step 3b.
$+ Lb(3n/4)$	Cost of succeeding iterations.

Given  $c=15$ , the recurrence relations simplify to the following:

$$L(n) \leq 42n/15 + La(n)$$

$$La(n) \leq 42n/225 + Lb(n)$$

$$Lb(n) \leq (60/11)(479n/1125 + d).$$

The algorithm assumes its best performance when  $d=0$ , where

$$Lb(n) \leq 2.3224n$$

$$La(n) \leq 2.5091n$$

$$L(n) \leq 5.3091n$$

The worst performance, on the other hand, occurs when  $6n/30 \leq d \leq 7n/30$ , where

$$Lb(n) \leq 3.4133n$$

$$La(n) \leq 3.5000n$$

$$L(n) \leq 6.4000n.$$

However, the optimum value of  $c$  is  $c=21$ , where  $L(n) \leq 5.2773n$  when  $d = 0$  and  $L(n) \leq 6.3361n$  when  $6n/30 \leq d \leq 7n/30$ . Hence, the following theorem is proven:

**Theorem 1.** There is a selection algorithm that requires  $5.2773n$  comparisons when  $d = 0$  and requires  $6.3361n$  when  $d = 7n/30$ .

We have, therefore, shown the existence of an algorithm whose behaviour is opposite to that of PICK1 as  $d$  increases.

**An Improvement**

Let  $d$  be as described before. As noted earlier, PICK1 performs well as  $d$  increases while LOCATE performs well as  $d$  increases. By combining these two algorithms, the worst case of  $5.4305$  (due to PICK1) can be reduced to a new value of  $5.4137n$ .

Let  $v$  be the value of  $d$  where the performances of PICK1 and LOCATE are equal. A new and better worst case is set by computing for  $d$ , if  $d \leq n$  then apply the discard and restore procedure of LOCATE, otherwise use the discard and restore procedure of PICK1. An algorithm, called LOCATE1, employing this observation is given below. The constant  $d$  and  $v$  are as defined above.

**Algorithm: LOCATE1**

Comment: Exactly the same as PICK1, except that Step 3 uses LOCATE1a.

**Algorithm: LOCATE1a**

Comment: Exactly the same as PICK1a, except that Step 3 uses LOCATE1b.

**Algorithm: LOCATE1b**

Comment: Selects  $k\theta S$ , where  $|S| = n$  and  $1 \leq k \leq n$ , and the sets  $S$  and  $T$  are already  $c$ -sorted.

1. Select  $m = \lceil |T|/2 \rceil \theta T$  using LOCATE1a, where  $T$  is the set of all  $c$ -column medians.
2. Compute  $mpS$ , by partitioning  $(A \cup B)$  of Figure 1 about  $m$  as follows, stopping as soon as it becomes apparent that  $mpS < k$  or  $mpS > k$ .
  - (a) Insert  $m$  into each column of  $B$  using binary insertion.
  - (b) Insert  $m$  into each column of  $A$  using linear search technique, the search starts at elements near the medians of the  $c$ -columns.
3. If  $d > v$ , then use the discard and restore procedure of PICK1. Otherwise, use the discard and restore procedure of LOCATE.
4. Decrease  $n$  by the number of elements discarded. If  $n \leq 45$ , sort  $S$ , print  $k\theta S$  and halt, otherwise return to Step 1.

Let  $L1(n)$ ,  $L1a(n)$ , and  $L1b(n)$  be the costs of LOCATE1, LOCATE1a, and LOCATE1b, respectively. Since the performance of the algorithm is optimum when  $c = 15$ , in order to simplify the succeeding discussions,  $c$  will be set to this value.

When  $d > v$ , obviously LOCATE1 reduces to PICK1. Hence, the cost of LOCATE1 is given by the following:

$$\begin{aligned} L1b(n) &\leq (5n/(n+5d))(13197n/27000 + 3546d/1575) \\ L1a(n) &\leq 42n/225 + L1b(n) \\ L1(n) &\leq 42n/15 + L1a(n). \end{aligned}$$

On the other hand, when  $d \leq v$ , LOCATE1 reduces to LOCATE. Hence, the cost of LOCATE1 is given by the following:

$$\begin{aligned} L1b(n) &\leq (60/11)(479n/1125 + d) \\ L1a(n) &\leq 42n/225 + L1b(n) \\ L1(n) &\leq 42n/15 + L1a(n). \end{aligned}$$

The critical value is equal to the value of  $d$  in the equation

$$(60/11)(479n/1125 + d) = (5n/(n+5d))(13197n/27000 + 3546d/1575).$$

Simplifying the equation will result to  $d = v = 0.01918n$  where with this value of  $d$   $L1(n)$  simplifies to  $L3(n) \leq 5.4137n$ . Since  $\max(h(s)/s) < 5.4137n$  when  $s \leq 45$ , the induction is justified. Clearly, this value is an improvement to the algorithm in [1].

Hence, the following theorem can be stated:

**Theorem 2.** There is a selection algorithm that requires  $5.4137n$  comparisons in the worst case.

### Further Improvement

Let  $l_A$  be the number of elements in  $A$  which are less than  $m$ . The relationship between  $l_A$  and  $d$  is given by the following lemma:

**Lemma 4.**  $l_A \leq d$ .

**Proof:** Two cases must be considered, either  $L$  or  $G$  is discarded.

**Case 1.** If  $L$  is discarded, clearly  $d = l_A$ . Hence, the lemma holds.

**Case 2.** If  $G$  is discarded, then

$$|L| + l_A + l_B = k + 1 < \lceil n/2 \rceil + 1 \leq |L| + |B| \leq |L| + g_B + l_B.$$

From the leftmost and rightmost terms,  $g_B \leq l_A$ . Since  $d = g_B$ ,  $l_A \leq d$  follows.  $\square$

Since  $l_A \leq d$ , Steps 2a and 2b of PICK1b, i.e., the insertion of  $m$  into  $B$  using binary insertion and the insertion of  $m$  into  $A$  using sequential search technique, can be performed in any order without changing the cost of PICK1b as a whole. The lemma also implies that the performance of PICK1 improves as the size of  $l_A$  increases.

Using the approach used in LOCATE1, we can improve the worst case by finding an algorithm that performs well as the value of  $l_A$  decreases. One such algorithm is obtained by making a slight modification on Step 2 of LOCATE1b. The said modification is incorporated into the algorithm below. This time  $v$  stands for the value of  $l_A$  where the performances of PICK1 and LOCATE match.

### Algorithm: LOCATE2

Comment: Exactly the same as PICK1, except that Step 3 uses LOCATE2a.



**Algorithm: LOCATE2a**

Comment: Exactly the same as PICK1a, except that Step 3 uses LOCATE2b.

**Algorithm: LOCATE2b**

Comment: Selects  $k\theta S$ , where  $|S| = n$  and  $1 \leq k \leq n$ , and the sets  $S$  and  $T$  are already  $c$ -sorted.

1. Select  $m = \lceil |T|/2 \rceil \theta T$  using LOCATE2a, where  $T$  is the set of all  $c$ -column medians.
2. Compute  $m\theta S$ , by partitioning  $(A_1 \cup B)$  of Figure1 about  $m$  as follows, stopping as soon as it becomes apparent that  $m\theta S < k$  or  $m\theta S > k$ 
  - (a) Insert  $m$  into each column of  $A$  using linear search technique, the search starts at elements near the medians of the  $c$ -columns.
  - (b) If  $l_A \geq v$  then insert  $m$  into each column of  $B$ , using binary search technique. Otherwise, insert  $m$  into each column of  $B$  using linear search technique, starting from the elements next to the medians of the  $c$ -columns, stopping when the number of elements greater than  $m$  counted so far is equal to  $(l_A + 1)$  or when  $B$  runs out of elements greater than  $m$ , whichever comes first.
3. If  $l_A \geq v$  then use the discard and restore procedure of PICK1. Otherwise, use the discard and restore procedure of LOCATE.
4. Decrease  $n$  by the number of elements discarded. If  $n \leq 45$ , sort  $S$ , print  $k\theta S$  and halt, otherwise return to Step 1.

To simplify the discussion, let  $c$  assume its optimum value which is  $c = 15$ . Let  $L2(n)$ ,  $L2a(n)$ , and  $L2b(n)$  be the costs of LOCATE2, LOCATE2a, and LOCATE2b, respectively.

When  $l_A \geq v$ , obviously LOCATE2 reduces to PICK1 (Lemma 4). Hence, the cost of LOCATE2 is given by the following recurrence relations:

$$\begin{aligned} L2b(n) &\leq (5n/(n+5l_A))(13197n/27000 + 35461_A/1575) \\ L2a(n) &\leq 42n/225 + L2b(n) \\ L2(n) &\leq 42n/15 + L2a(n) \end{aligned}$$

However, when  $l_A < v$  the cost is the same as that of LOCATE1 except for Step 2 of LOCATE2b.

**Lemma 5.** The cost of Step 2 of LOCATE2b when  $l_A < v$  is  $2(n/30 + l_A)$ .

**Proof:** Step 2a of LOCATE2b obviously needs one comparison per column plus the number of elements in  $A$  which are less than  $m$ . Hence, a cost of  $(n/30 + l_A)$ . With regards to Step 2b of LOCATE2b, two cases must be considered. The

first is when  $g_B > l_A$  where  $g_B$  is the number of elements in B which are greater than m. Clearly, this costs  $(n/30 + l_A)$ . The second case is when  $g_B \geq l_A$ , where the cost is less than  $(n/30 + l_A)$ . Therefore, Step 2 of LOCATE2b costs  $2(n/30 + 1)$ .  $\square$

With this step settled, the cost of LOCATE2b when  $l_A < v$  can be written as follows:

$L2b(n) \leq$	$L2a(\lceil n/15 \rceil)$	Select m.
+	$2(n/30 + l_A)$	Compute mrS.
+	$13(14/15)(n/60)$	Restore S. Step 2a.
+	$14(1/15)(n/60)$	Restore S. Step 2b.
+	$14(n/60)(1/15)$	Restore T. Step 3a.
+	$42(n/60)(1/15)$	Restore T. Step 3b.
+	$L2b(3n/4)$	Cost of succeeding iterations.

This recurrence relation simplifies to  $L2b(n) \leq (60/11)(404n/1125 + 2l_A)$ . The critical value  $v$  is equal to the value of  $l_A$  in the equation

$$(60/11)(404n/1125 + 2l_A) = (5n/(n+5l_A))(13197n/27000 + 35461l_A/1575).$$

Simplifying the equation will result to  $l_A = v = 0.04144n$  where with this value of  $l_A$   $L2(n)$  simplifies to  $L2(n) \leq 5.3975n$ . Clearly, this value is an improvement to the previous algorithm. Figure 3 shows the performance of LOCATE2 (line below the intersection) on different values of  $l_A$ , where  $0 \leq l_A \leq 7n/30$ . The theorem below follows:

**Theorem 3.** The selection problem can be solved using  $5.3975n$  comparisons in the worst case.

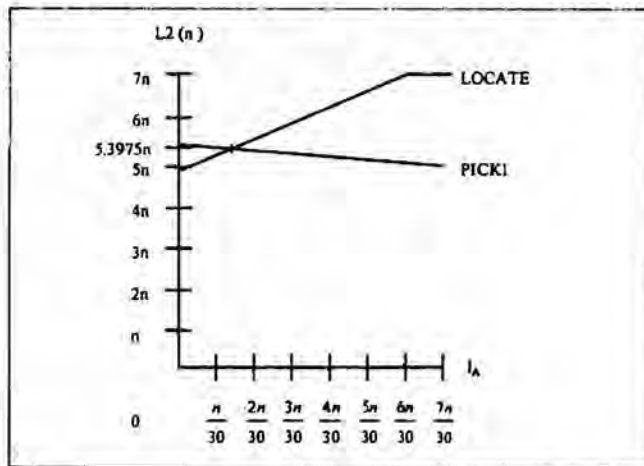


Figure 3. Performance of PICK1 and LOCATE.

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# THE BELLE DETECTOR AT THE KEK B-MESON FACTORY

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## ABSTRACT

*The KEK B-meson Factory is a high energy electron-positron collider being constructed in the 3-km circumference underground tunnel of the National Laboratory for High Energy Physics (KEK) in Tsukuba, Japan. This new accelerator is designed to provide a hundred million pairs of B and B-meson each year. It will start data runs in early 1999.*

*The BELLE Detector is a giant particle telescope to be built at the KEK B-meson Factory by a collaboration of about 250 scientists from 45 institutions in 8 countries. The BELLE Detector will record the collisions of the asymmetric high energy lepton collider: an 8 GeV electron beam onto a 3.5 GeV positron beam. The data are expected to solve the long-standing puzzle of CP violation in elementary particle physics.*

## INTRODUCTION

There are two B-meson factories being built in the world. These new generation machines designed to produce millions of B-mesons per year are called the PEP II collider at the Stanford Linear Accelerator (SLAC) in California, USA and the KEKB located at the High Energy Accelerator Research Organization (KEK) in Tsukuba, Japan. A huge particle detector that will observe the decays of the B-mesons at SLAC is called Babar while it is the BELLE Detector at KEKB. The objective of these experiments is to perform definitive tests of the Kobayashi-Maskawa model for CP violation in the decays of B-mesons. Both are expected to take physics data runs in early 1999.

### The KEK B-Meson Factory (KEKB)

The KEKB is an asymmetric, two ring  $e^+e^-$  collider. The 3.5 GeV  $e^+$  ring and the 8 GeV  $e^-$  ring are placed side by side inside the TRISTAN tunnel at KEK 100m underground which has a circumference of about 3 km. The two rings cross at two points but collide at only one point where the BELLE Detector is located. In each ring, 5000 bunches are stored giving the currents for the electron and positron at

1.1 and 2.6 A respectively. All the ring parameters (some are given in Table 1) were chosen so as to produce the required luminosity of  $10^{34} \text{ cm}^{-2} \text{ sec}^{-1}$ . This goal luminosity will allow the production of  $10^8$  B-meson pairs per year which is the required number for the study of CP asymmetries. One of the most important features of KEKB is the finite crossing angle of  $\pm 11 \text{ mrad}$ , in contrast to PEP II's design of zero crossing angle. Fig. 1 shows the layout of the two rings of KEKB.

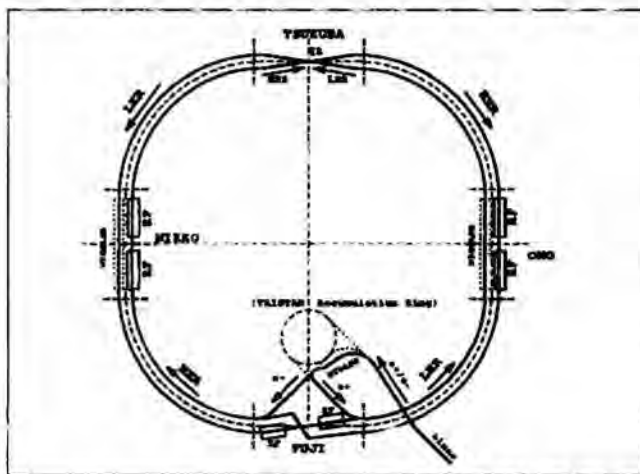


Figure 1. Configuration of the KEKB accelerator system.

Table 1. The Machine Parameters of KEKB and PEP-III high-energy electron-positron colliders.

Physics Start Date	KEKB (KEK) 1999	PEP-II (SCLAC) 1999
Max. beam energy (GeV)	$e^-e^+$ : $8 \times 3.5$	$e^-e^+$ : $9 \times 3.1$
Luminosity ( $10^{30} \text{ cm}^{-2} \text{ sec}^{-1}$ )	10000	3000
Time between collisions ( $\mu\text{s}$ )	0.002	0.0042
Crossing angle ( $\mu\text{rad}$ )	$\pm 11,000$	0
Bunch length (cm)	0.4	1.0
Bunch spacing (m)	0.59	1.26
Beam size ( $\mu\text{m}^2$ )	$260 \times 7$	$155 \times 6$
Bunches per ring per species	5120	1658
Average beam current (mA)	$e^-/e^+$ : 1100/2600	990/2140
Circumference or length (km)	3.016	2.2
Dipoles in ring	$e^-/e^+$ : 116/112	$e^-/e^+$ : 192/192
Quadrupoles in ring	$e^-/e^+$ : 452/452	$e^-/e^+$ : 299/326

### Parity Violation, Charge Conjugation Violation, CP Invariance

Parity (P) is the operation of spatial reflection, effecting the transformation on the position vector:  $\mathbf{r} \rightarrow -\mathbf{r}$ . P invariance means that the laws of physics are the same in a right-handed or left-handed system. For a long time the conservation of parity was believed to be a universal law of nature like angular momentum conservation. In the system of elementary particles, when parity invariance holds, particle production with a left-handed, polarization and that of a right-handed polarization occur at the same rate. However, in a 1957 experiment by Wu et al., parity violation was established in the  $\beta$ -decay of a sample of cobalt-60 nuclei. It was observed that fewer electrons are emitted in the backward hemisphere than in the backward hemisphere with respect to the spins of the decaying nuclei; the so-called forward-backward decay asymmetry.

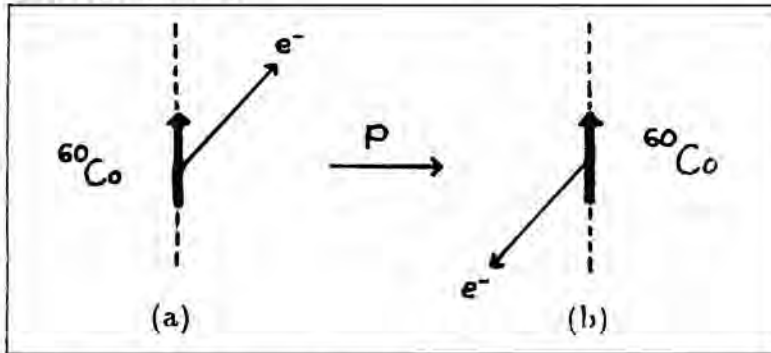


Figure 2. Effect of a parity transformation on  $^{60}\text{Co}$  decay. Parity invariance requires the rates for processes (a) and (b) were equal.

Charge conjugation (C) reverses the sign of charge and magnetic moment of a particle leaving all other coordinates unchanged. Charge conjugation invariance means, as in P, that the same laws of physics is found in the system where one changes the particles to antiparticles. Violations of charge conjugation invariance is exemplified by the neutrinos and antineutrinos emitted in  $\beta$ -decay. Applying the charge-conjugation operation to the  $\nu$ -state in Fig. 3(a), a left-handed antineutrino is obtained, Fig. 3(b). This however does not occur in nature. All neutrinos are left-handed and all antineutrinos are right-handed. But if in addition, we make the spatial inversion of the state in Fig. 3(b), we end up with a right-handed antineutrino, Fig. 3(c), which is observed. Thus the weak interactions are not invariant under C or P separately but they exhibit invariance in the combined operation CP.

CP invariance has been verified in a wide variety of experiments involving weak interactions. It was believed that all types of interaction were invariant under the combined operation CP; like the weak interactions which are known to violate C- and P-invariance separately, but to respect CP-symmetry. In 1964, however, it was discovered by Christenson *et al.* that the long-lived neutral  $K$  meson which usually decays

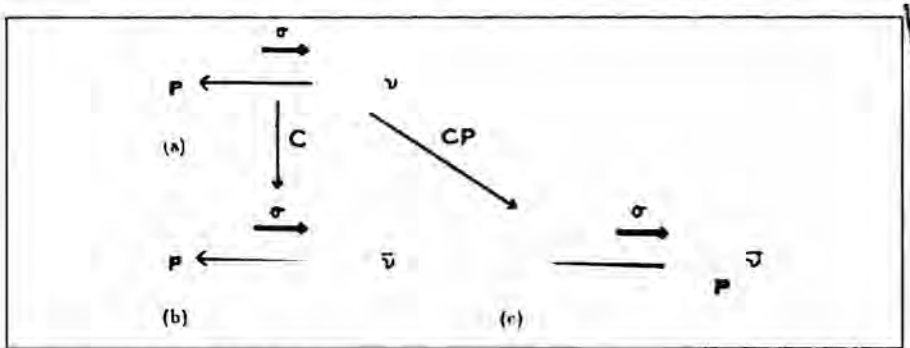


Figure 3. Result of the C- and P-operations on neutrino states.

into three pions, could occasionally decay into two pions which violate CP invariance. CP violation has not been observed outside of the  $K$  meson decay.

**The B-meson, CP Violation**

The bound states of  $b$  quark ( $b$  quark) and a lighter quark (lighter antiquark) are called B mesons ( $B$  mesons). Their masses are about 5 GeV; five times heavier than the protons. The  $b$ -quark is a down type quark of the third generation quark pairs and was first discovered in 1977 at Fermilab in proton-nucleon scattering experiments. In a survey of the  $bb$  system carried out at Cornell's Laboratory of Nuclear Studies, the  $\Upsilon$  ( $4S$ ) is the open  $b$  threshold where the  $b$  and  $\bar{b}$  produced in the  $e^-e^+$  annihilation pick up lighter quark pair from the vacuum and a form a pair of B mesons (Fig. 4).

In 1973, Kobayashi and Maskawa proposed to explain the origin of CP violation of  $K$  meson decays by introducing a third generation of quarks; giving six quarks in total. At that time, only three quarks were known to exist. The Kobayashi-Maskawa model explains the source of the CP violation in terms of the complex phases in the quark mixing parameters. This model also predicts that CP violation should occur in the B system as well.

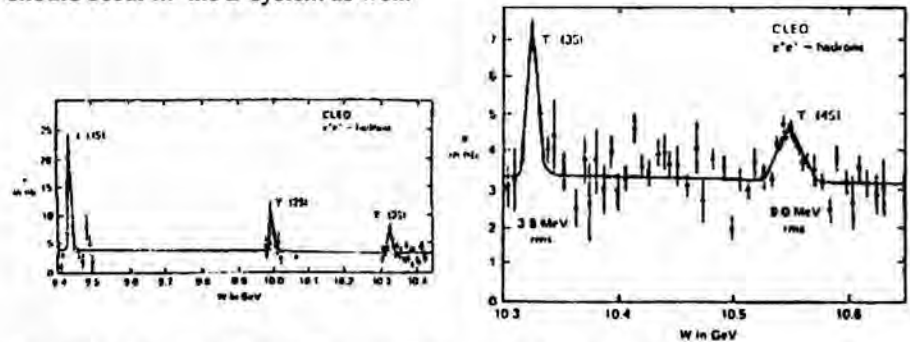


Figure 4. Survey of the  $bb$  system by Cornell's  $e^+e^-$  collider.

The fundamental importance of CP violation is now widely recognized. It may shed light on why the present-day universe is completely dominated by matter when right after the big bang, the universe consisted of exactly half matter and half anti-matter.

### The BELLE Detector

Figure 5 shows the BELLE Detector now being constructed at KEK and which will be installed in the beamline of Tsukuba Hall in 1998. It consists of several tracking devices for charged particles, an electromagnetic shower counter for detecting  $\gamma$ s and electrons and several different devices for identifying the particle types ( $\pi$ ,  $K$ ,  $\mu$ ,  $e$ ).

The BELLE detector was designed based on the following requirements:

- **Angular acceptance**  
The angular acceptance for both charged and neutral particles is  $17^\circ < \theta < 150^\circ$ .
- **Vertex Resolution**  
The accuracy with which the distance between two B meson vertices is measured should be better than 100  $\mu\text{m}$ .  
Silicon Vertex Detector (SVD)
- **Charge particle tracking**  
Detection of low momentum particles with  $p_t > 0.05$  GeV/c is good.  
Central Drift Chamber (CDC) in 1.5 T magnetic field
- **Particle Identification**  
Particle identification capability of  $\pi$ ,  $K$  and  $e$  should be good enough. Aerogel Cerenkov counter (ACC), TOF counters and  $dE/dx$  in CDC
- **Electromagnetic calorimetry**  
High efficiency and good resolution, in particular, for low energy photons are necessary.  
homogenous CsI(Tl) calorimeter.
- **$K_L$  and muon detection**  
High detection efficiency and good resolution in angle measurements for  $K_L$  and muons are needed.  
return yoke instrumented with resistive plate counters

The B-mesons produced in KEKB are boosted along the beam direction and typically travel about 190  $\mu\text{m}$  before they decay. The silicon vertex detector having a cylindrical configuration consisting of double-sided silicon detectors measures the z position with an accuracy of 80  $\mu\text{m}$ .

The central drift chamber (CDC) is a cylindrical device filled with helium gas and contains several thousand very thin wires set at positive voltage. This is placed inside a 1.5 T solenoidal magnetic field. This device measures the position, curvature, momentum and ionization energies of different particles.



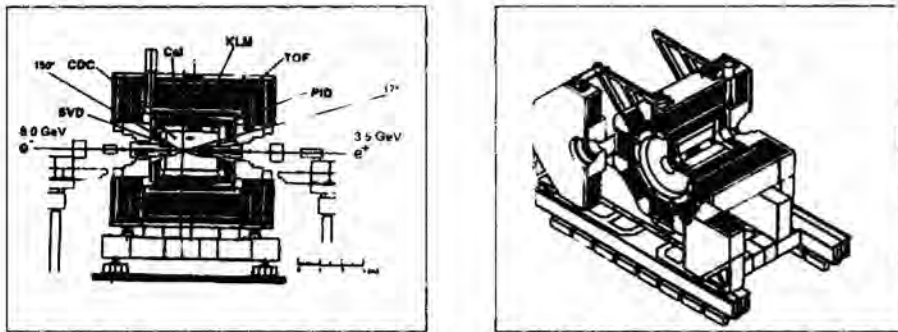


Figure 5. Side view and birdseye view of the BELLE Detector.

Low momentum K mesons can be distinguished from low momentum pions by the different spectra of cerenkov radiation which they generate when they traverse the aerogel cerenkov counter (ACC).

Electromagnetic calorimetry is provided by a highly segmented array of CsI(Tl) crystals about 9000 in number each about 30 cm long. The scintillation light produced when high energy particles traverse the crystals are collected by photodiodes attached to the crystals and the amount of charge collected provides a precise energy measurement of the exiting particle.

The muons and neutral kaons can be identified by the charged particle tracking detectors sandwiched with the segmented iron. The muons travel through the iron without being absorbed unlike pions and kaons.

### The BELLE Collaboration

The BELLE group is an international collaboration consisting of about 250 researchers from 40 institutions in 8 countries. During the March 22, 1997 meeting of the BELLE collaboration held at the University of Hawaii, the author was accepted as one of the collaborating members of the BELLE group.

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## A COMBINATORIAL APPROACH TO SPIN MODELS

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### ABSTRACT

*The construction of new and powerful invariants of knots and links by Vaughan Jones in 1984 and its recent generalizations have led to the discovery of important connections between the theory of knots and other branches of mathematics and sciences. The new knot theory has already been useful to molecular biologists studying the double helices of DNA. In physics, models in statistical mechanics may be defined on a knot or link diagram so that a suitable variation of the partition function of the system is often a knot invariant. In 1989, Jones constructed spin models and posed the challenge of investigating combinatorial structures for sources of spin models. In this paper we present an approach, first observed by Francois Jaeger in 1992, to the study of spin models using a combinatorial object called an association scheme. We outline the background and method of this approach and prove several characterization theorems for spin models arising from some families of association schemes.*

**Keywords and phrases:** association schemes, spin models, knots, links, combinatorics, statistical mechanics.

### INTRODUCTION

In a 1989 seminal paper, Vaughan F. R. Jones introduced the concept of a spin model as a method to construct invariants of links in 3-space (Jones, 1989). The starting point of this new era in the study of knots and links was Jones' discovery in 1984 of his famous polynomial invariant, which was obtained in an entirely different setting, and for which he was awarded the 1990 Fields Medal.

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Jones pursued the connection between knot theory and statistical mechanics via the use of spin models. The spin models he discovered can be defined as pairs  $(W_+, W_-)$  of square complex matrices satisfying certain invariance equations, which then guarantee that the partition function (after an adequate normalization) defines a link invariant. Spin models are used to understand, for instance, phase transitions in statistical mechanics.

Since then the subject has been shown to be related to an amazingly wide variety of subjects such as von Neumann algebras, representations of semi-simple Lie algebras, finite groups, and more generally, statistical mechanics, topological field theory, quantum groups, among others. For instance, see (Jones, 1989), (Jaeger, 1992), (de la Harpe-Jones, 1993) and (Jaeger, 1995).

A major focus of Jones' work was directed towards the combinatorial aspect of the study and he posed the challenge of investigating combinatorial structures for sources of spin models. In his paper, Jones gave two examples of symmetric spin models and raised the question of finding new ones.

In 1992, Francois Jaeger found new spin models for evaluation of the Kauffman link polynomial invariant using special association schemes (Jaeger, 1992). Association schemes are important combinatorial structures and are the main objects of study in algebraic combinatorics. They provide a unifying approach to the study of various mathematical objects such as algebraic graphs, codes, designs, finite geometries, and includes the theory of finite groups.

Indeed the question of finding new spin models has turned out to be intimately connected with the theory of association schemes. Many subsequent works, e.g. (Bannai-Bannai, 1993), (Nomura, 1994), (Jaeger, 1995), and (Bannai-Bannai, 1995), confirmed the importance of the following situation: the matrices of a spin model belong to the Bose-Mesner algebra of some self-dual association scheme, and can be obtained by solving a certain modular invariance equation associated with the character table of the scheme.

The simplest case is that of the Potts model for the Jones polynomial, which can be thought of as arising from the 1-class association schemes, i.e. complete graphs. We describe the model later using the language of association schemes.

Important work is now directed at showing that the connections between spin models, including subsequent generalizations like non-symmetric models and four-weight models by (Bannai-Bannai, 1993), (Kawagoe-Munemasa-Watatani, 1994) and (Bannai-Bannai, 1995), and the theory of association schemes is indeed general and non-arbitrary. A goal of this research direction is to obtain a classification of spin models in terms of association schemes.

This paper provides the background and methods of this combinatorial approach to the study of spin models. We also give a direct proof of the characterization of (generalized) spin models arising from non-symmetric conference graphs. This continues the author's work in (Balmaceda, 1993), where the symmetric case is treated.

In Section 2, we give a brief overview of the study of polynomial invariants of links, which was the main motivation of Jones' 1989 paper. We then discuss the

connection between spin models and association schemes in Section 3. In the 4<sup>th</sup> Section we prove the main result characterizing the spin models arising from non-symmetric conference digraphs. We conclude the paper with some remarks about the directions of this research.

### INVARIANTS OF KNOTS AND LINKS

By a link we mean a finite disjoint union of simple closed curves (knots) in 3-dimensional space. A *knot* is a link with one component. Links can be represented by projections onto the plane, called *link diagrams*, which may be viewed as plane graphs whose vertices are the under/over crossings. (Refer to (Rolfsen, 1976) for basic information about knots and links).

The main problems of knot theory are: 1) to decide whether a knot is really knotted, 2) decide when two knots are equivalent, and 3) "classify" all possible knots. We make the notion of equivalent links more precise below.

**Definition 1.** Two links are said to be *equivalent* if there exists an isotopic deformation of the underlying 3-space which carries one link to the other.

In other words, two links are equivalent if there exists an *ambient isotopy*.

**Theorem 1. (Reidemeister)** *Two diagrams represent equivalent links if and only if one can be transformed from the other by a finite sequence of Reidemeister moves and by planar isotopy.*

By planar isotopy, we mean motions of the diagram in the plane which preserve the graphical nature of the underlying universe. Reidemeister moves are explained below.

Reidemeister's theorem is a basis for a combinatorial approach to knot theory. In particular, it allows the definition of an invariant of links as a mapping from the set of equivalence classes of diagrams to a set of values which is invariant under the Reidemeister moves.

The three types of Reidemeister moves are shown below. The Type III move (called a star-triangle move) is related to the quantum Yang-Baxter equations of physics.

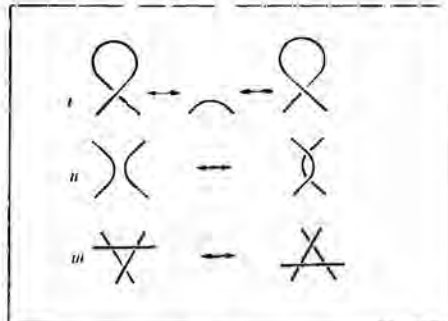


Figure 1. Type I, Type II, and Type III Reidemeister Moves.

An *invariant* is a quantity associated with a link or knot which depends only on the knot in 3-dimensions. For example, the *number of components* is an invariant, although not particularly a strong one. Another simple invariant is the *linking number*, which gives an idea of how two knots wrap around each other, and requires a precise definition of orientation and signs.

An  $\Omega$ -valued invariant for oriented links is a map which associated to each oriented link an element of some ring  $\Omega$ , for example, the complex field, or a ring of Laurent polynomials, in such a way that equivalent links have the same images in  $\Omega$ . Classically, one of the most studied examples of link invariants is the *Alexander-Conway polynomial* defined by J. W. Alexander in 1928. Traditionally, these polynomials were understood only in terms of standard algebraic topology, until the advent of Jones' work.

After the discovery of the Jones polynomial, other polynomial invariants were soon discovered. The new invariants provide powerful methods for distinguishing inequivalent knots. See (Kauffman, 1988) for a survey. The most striking feature of these new invariants, aside from their often mysterious properties, is their (previously unheard of) connection with other branches of mathematics, as well as the natural sciences, in particular, to statistical mechanics in physics, and to molecular biologists studying DNA.

### SPIN MODELS AND ASSOCIATION SCHEMES

We first give the definition of commutative association schemes. For more information on association schemes, see (Bannai-Ito, 1984).

**Definition 2.** A commutative association scheme with  $d$  classes, denoted  $X = (X, \{R_i\} 0 \leq i \leq d)$ , consists of a nonempty finite set  $X$  together with subsets  $R_i \subseteq X \times X$  ( $i = 0, 1, \dots, d$ ) satisfying the following axioms:

1.  $R_0 = \{(x, x) | x \in X\}$ ,
2.  $X \times X = \bigcup_{i=0}^d R_i$ ,  $R_i \cap R_j = \emptyset$  if  $i \neq j$ ,
3.  ${}^tR_i = R_{i'}$  for some  $i' \in \{0, 1, \dots, d\}$ , where  ${}^tR_i = \{(x, y) | (y, x) \in R_i\}$ ,
4. For  $i, j, k \in \{0, 1, \dots, d\}$ , the integer  $p_{ij}^k := \#\{z \in X | (x, z) \in R_i \text{ and } (z, y) \in R_j\}$  is constant whenever  $(x, y) \in R_k$ , and
5.  $p_{ij}^k = p_{ji}^k$  for all  $i, j, k \in \{0, 1, \dots, d\}$ .

In (Jaeger, 1992) the Bose-Mesner algebras generated by the adjacency matrices of some special association schemes were studied and were discovered to be natural places to look for spin models. In particular, Jaeger proves the following result which has since been generalized by others. For instance, see (Bannai-Bannai, 1993).

**Theorem 2. (Jaeger)** Let  $(X, W_+, W_-)$  be a symmetric spin model. Let  $\mathcal{M}$  be the algebra defined by  $W_+$  and the all 1-matrix  $J$  with ordinary matrix multiplication. If  $\mathcal{M}$  is also closed under entry-wise Hadamard multiplication, then  $\mathcal{M}$  is the Bose-Mesner algebra of a self-dual association scheme.

We now give the definition of a spin model. In this paper, we will use the generalized version of a spin model (formulated by Y. Watatani in (Kawagoe-Munemasa-Watatani, 1994) who dropped the symmetry condition upon the suggestion of Jones).

**Definition 3.** Let  $X$  be a finite set with  $n$  elements and  $w_+$  and  $w_-$  be complex-valued functions on  $X \times X$ . Let  $W_+$  and  $W_-$  be the matrices of size  $n \times n$  defined by  $W_+ = (w_+(a,b))_{a,b \in X}$  and  $W_- = (w_-(a,b))_{a,b \in X}$ . If the following conditions are satisfied, the configuration  $(X, w_+, w_-)$  is called a *spin model*:

- (1)  $W_+ \circ W_- = J$ , the matrix whose entries are all 1,
- (2)  $W_+ W_- = nI$ , where  $I$  is the identity matrix of size  $n$ ,
- (3)  $W_+ Y_{ac} = \sqrt{n} w_-(a,c) Y_{ac}$  for any  $a, c \in X$ .

In the above definition,  $Y_{ac}$  is the column vector whose  $x$ -entry is given by  $(Y_{ac})_x = (w_+(a,x)w_-(x,c))_{x \in X}$ . Hadamard or entry-wise multiplication is denoted by  $\circ$ .

This definition generalizes Jones' original definition, where he requires that the matrices  $W_+$  and  $W_-$  are both symmetric. We refer to spin models satisfying this additional condition as *symmetric spin models*.

Jones showed how a (symmetric) spin model may be used to obtain a link invariant. Watatani, Munemasa, and Kawagoe show similarly that the generalized version of a spin model provides an invariant of oriented links. We describe the procedure briefly.

**Invariants of Oriented Links**

For any connected diagram  $L$  of an oriented link, we construct a signed graph as follows. Color the regions in black and white so that the unbounded region is white and adjacent regions have different colors. The set of vertices of the graph is the set of black regions, and the set of edges is the set of crossings. An edge is incident with a vertex if and only if the corresponding crossing is on the boundary of a corresponding black region. We assign signs  $+$  and  $-$ , and an orientation to each edge according to the rules given in Fig. 2 and Fig. 3.

If  $e$  is an edge, then we denote the sign of  $e$  by  $e(e)$ , the initial vertex of  $e$  by  $i_e$ , and the terminal vertex by  $t_e$ . Let  $\nu(L)$  be the number of black regions. If  $(W_+, W_-)$  is a generalized spin model on  $X$ , then the partition function is defined by

$$Z(L) = \sqrt{|X|}^{\nu(L)} \sum_{\sigma} \prod_e w_{e(c)}(\sigma(i_e), \sigma(t_e)),$$

where the product is taken over all edges  $e$ , and the sum is taken over all mappings  $\sigma$  from the set of vertices to  $X$ . It is then shown in (Kawagoe-Munemasa-Watatani, 1994) that the partition function above is invariant under Reidemeister moves of types II and III, as long as the diagrams are connected. Moreover, by a suitable normalization factor (which depends on the writter of  $L$ ), we obtain an invariant of the oriented link  $L$ .

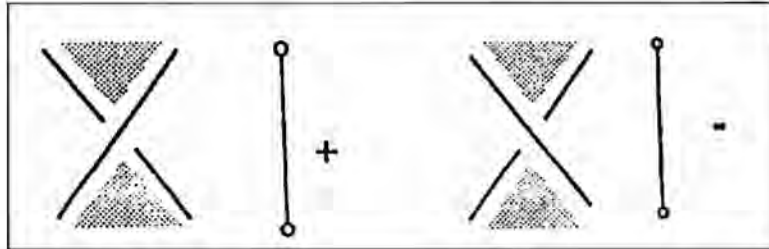


Figure 2. Sign of an Edge

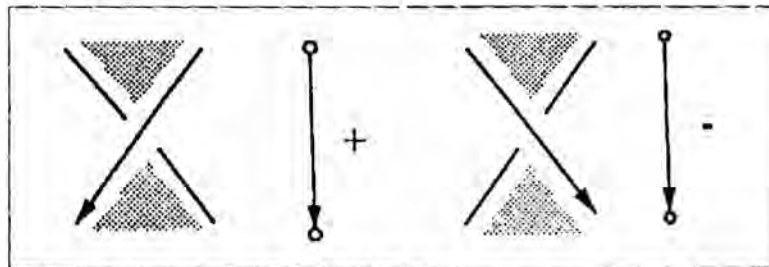


Figure 3. Orientation of an Edge

### Classifying Spin Models Via Association Schemes

As stated in the introduction, the current goal is to obtain a complete classification of spin models in terms of association schemes. The simplest case is that of the Potts model for the Jones polynomial, which corresponds to the 1-class association scheme (i.e., the complete graphs). We describe this below.

**Example.** (*Potts model*, cf (Jones, 1989) and (de la Harpe-Jones, 1993)) Let  $X$  be a set of cardinality  $n$ . Let the relations be given by  $R_0 = \{(x,x) | x \in X\}$  and  $R_1 = \{(x,y) | x \neq y \text{ and } x,y \in X\}$ . Then  $(X, \{R_0, R_1\})$  is a symmetric association scheme of class 1. (Note that  $(X, R_1)$  is the complete graph  $K_n$ ). The Bose-Mesner algebra  $\mathcal{A}$  is given by

$$A = (A_0, A_1) = (I, J)$$

Where  $A_0 = I$  and  $A_1 = J - I$ . Let

$$W_+ = t_0 A_0 + t_1 A_1 \text{ (and hence) } W_- = t_0^{-1} A_0 + t_1^{-1} A_1$$

with  $t_0$  and  $t_1$  satisfying

$$t_1^2 + t_1^{-2} + D = 0 \text{ (} D^2 = n \text{) and } t_0 = -(t_1)^{-3}.$$

Then  $(X, W_+, W_-)$  is a spin model, called the *Potts model*. We note that the link invariant obtained from this model is a special value of the Jones polynomial for oriented links.

Jones' work suggested that the sources of symmetric spin models seem to be the 2-class association schemes some special regularity conditions (e.g. self-dual and locally strongly regular). Contained in this class are the symmetric conference graphs, which include the family of Paley graphs. In the paper (Balmaceda, 1993), the author proves the following theorem:

**Theorem 3.** *Let  $X$  be a set of size  $n$ , and  $(X, W_+, W_-)$  be a symmetric spin model associated with a symmetric conference graph  $G$  with  $n \geq 5$  which is not a Potts model. Then  $G$  is either the pentagon or the lattice graph  $L_2(3)$  with nine vertices.*

By a symmetry conference graph, we mean a strongly regular graph with parameters  $(n, k, \lambda, \mu) = (n, \frac{n-1}{2}, \frac{n-5}{4}, \frac{n-5}{4})$ . The natural extension of the above result would be to consider the non-symmetric conference digraphs (i.e. non-symmetric association schemes of class 2), and to determine if one obtains a generalized spin model from such schemes.

In the next section, we give a direct proof of the following result: there are no such spin models, except when  $n = 3$ . For the case  $n = 3$ , Munemasa and Watatani have previously found some examples (Kawagoe-Munemasa-Watatani, 1994).

### RESULTS AND PROOFS

Let  $X = (X, \{R_i\}_{0 \leq i \leq 2})$  be a non-symmetric 2-class association scheme. Then it is known that  $|X| = n = 4m + 3$ , for some integer  $m$  and that  $X$  is self-dual. Furthermore, if  $A_0, A_1, A_2$  are the adjacency matrices of the scheme,  $A_0 = I$  and  $A_1^t = A_2$ . We display the intersection matrix  $B$  and character table  $P$  of such schemes below.  $P$  is the matrix with entries  $(P)_{ij} = (p_j^i)$ ,  $0 \leq i, j \leq 2$ .

$$B = \begin{pmatrix} p_{10}^0 & p_{10}^1 & p_{10}^2 \\ p_{11}^0 & p_{11}^1 & p_{11}^2 \\ p_{12}^0 & p_{12}^1 & p_{12}^2 \end{pmatrix} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & m & m+1 \\ 2m+1 & m & m \end{pmatrix}$$



$$P = \begin{pmatrix} p_0(10) & p_1(0) & p_2(0) \\ p_0(10) & p_1(0) & p_2(0) \\ p_0(10) & p_1(0) & p_2(0) \end{pmatrix} = \begin{pmatrix} 1 & \frac{n-1}{2} & \frac{n-1}{2} \\ 1 & \frac{-1+\sqrt{ni}}{2} & \frac{-1-\sqrt{ni}}{2} \\ 1 & \frac{-1-\sqrt{ni}}{2} & \frac{-1+\sqrt{ni}}{2} \end{pmatrix}$$

Let  $W_+ = t_0 A_0 + t_1 A_1 + t_2 A_2$ , for nonzero complex constants  $t_0, t_1$ , and  $t_2$ . Following the observation in (Kawagoe-Munemasa-Watatani, 1994), we may assume that  $t_0 = t_1 \neq t_2$ ; otherwise, we will obtain the (trivial) case of the Potts model. Hence we have, by the first condition in the definition of spin models:

$$W_- = t_0^{-1} A_0^t + t_1^{-1} A_1^t + t_2^{-1} A_2^t = t_1^{-1} A_0 + t_1^{-1} A_2 + t_2^{-1} A_1.$$

A useful ingredient in the proof is the result of Jaeger ([6], Prop. 5) which we state below:

**Lemma 4.** *Let  $W_+$  and  $W_-$  be as above. Then  $(X, W_+, W_-)$  is a spin model if and only if the following hold:*

- (1)  $PT = \sqrt{n}T^{-1}$ , where  $T = (t_0, t_1, t_2)^t$  and  $T^{-1} = (t_0^{-1}, t_1^{-1}, t_2^{-1})^t$
- (2)  $E_i Y_{ab} = 0$  for every  $i, j \in \{1, 2\}$  with  $t_i \neq t_j$ , and for every pair  $(a, b) \in R_j$ .

In the lemma above, the matrices  $E_i$  are the primitive idempotents of the scheme chosen in a suitable ordering.

We now state and prove the following result.

**Theorem 5.** *There are no spin models arising from the nonsymmetric conference digraphs, except the ones corresponding to the Potts models and the one on three vertices.*

*Proof.* Since the schemes in consideration have size  $n = 4m + 3$ , the smallest ones correspond to  $m = 0$ , i.e., on  $n = 3$  vertices. And Munemasa and Watatani have found an example corresponding to this case. Moreover, we saw earlier that the Potts models arise (in a trivial way) from the series of graphs being considered.

We now show that for  $m > 0$ , no other spin models can be found. It is enough to show that condition (2) of the lemma is not satisfied. The proof utilizes the algebraic and combinatorial properties of spin models and the corresponding properties of the association schemes involved.

We now proceed with our computations. We assume that  $(a, b) \in R_1$ . Then, since  $t_1 \neq t_2$ , it is enough to show that  $E_1 Y_{ab} \neq 0$ , the zero vector.

Since  $E_1 = \frac{1}{n} \sum_{j=0}^2 \overline{p_1(j)} A_j$ , we see that the vector  $E_1 Y_{ab} = 0$  if and only if  $\sum_{j=0}^2 \overline{p_1(j)} A_j Y_{ab} = 0$ . Let  $(A_j)_{x,y}$  and  $(v)_y$  be the  $(x, y)$ -entry of  $A_j$  and the  $y$ -entry of  $v$ , respectively, where  $v$  is a column vector of size  $n$ , and  $x, y \in X$ .

Then

$$(A_j Y_{ab})_x = \sum_{y \in X} (A_j)_{x,y} (A_{ab})_y = \sum_{y \in X} (A_j)_{x,y} w_+(a, y) w_-(y, b).$$

Since  $w_+(a, y) = t_k$  when  $(a, y) \in R_k$  and  $w_-(y, b) = t_k^{-1}$  when  $(y, b) \in R_k$ , we have

$$(A_j)_{x,y} (Y_{ab})_y = \sum_{k,l=0}^2 p_{jkl}(x, a, b) t_k t_l^{-1},$$

where  $p_{jkl}(x, a, b) = \#\{y | (x, y) \in R_j, (a, y) \in R_k, (y, b) \in R_l\} := p_{jkl}$ .

Suppose that  $x \in X$  satisfies the following:  $(x, a) \in R_2, (x, b) \in R_2$ , with  $(a, b) \in R_1$ . We now show that the equation  $E_1 Y_{ab} = 0$  is not satisfied for the above choice of  $x$ . For this we need to compute all the values of the intersection numbers  $p_{jkl}, 0 \leq j, k, l \leq 2$ .

*Case  $j = 0$ :* It is straightforward to compute the following values:  $p_{100} = p_{101} = p_{002} = p_{101} = p_{011} = p_{020} = p_{021} = p_{022} = 0$ . The only nonzero value is  $p_{012} = 1$ .

*Case  $j = 1$ :* Again we can compute directly the following values:  $p_{100} = p_{101} = p_{102} = p_{110} = p_{120} = 0$ . We now compute the rest of the values by analyzing the parameters of nonsymmetric conference digraphs.

Suppose  $p_{111} = r$ . Since  $p_{111}^2 = \#\{y | (x, y) \in R_1, (y, b) \in R_1\}$  for  $(x, b) \in R_2$ , we get:

$$m + 1 = \sum_{i=0}^2 p_{1ij} p_{i01} + p_{111} + p_{111}.$$

Thus,

$$m + 1 = 0 + r + p_{121} \quad \text{i.e., } p_{121} = m - r + 1.$$

On the other hand, we also have

$$\begin{aligned} p_{11}^2 &= \#\{y | (x, y) \in R_1, (y, a) \in R_1\} \quad \text{for } (x, a) \in R_2, \\ &= \#\{y | (x, y) \in R_1, (y, a) \in R_2\} \quad \text{for } (x, a) \in R_2. \end{aligned}$$

Thus

$$p_{11}^2 = \sum_{i=0}^2 p_{12i} = p_{120} + p_{121} + p_{122}.$$

Or,

$$m + 1 = 0 + m - r + 1 + p_{122}, \quad \text{i.e., } p_{122} = r.$$

Finally,

$$p_{12}^2 = \#\{y|(x, y) \in R_1, (y, b) \in R_2\} \text{ where } (x, b) \in R_2.$$

So,

$$m = \sum_{i=0}^2 p_{1i2} = p_{102} + p_{112} + p_{122}.$$

Or,

$$m = 0 + p_{112} + p_{122} \quad \text{Hence, } p_{112} = m - p_{122} = m - r.$$

Summing up, we have:  $p_{111} = r$ ,  $p_{112} = m - r$ ,  $p_{121} = m - r + 1$ ,  $p_{122} = r$ , and  $p_{100} = p_{102} = p_{101} = p_{120} = 0$ .

Case  $j = 2$ . As before, it is straightforward to compute the following values:  $p_{200} = p_{202} = p_{220} = 0$ . Similarly,  $p_{201} = p_{210} = 1$ . We still need to compute:  $p_{211}$ ,  $p_{212}$ ,  $p_{221}$ , and  $p_{222}$ .

Suppose  $p_{211} = s$ . Then

$$p_{21}^2 = \#\{y|(x, y) \in R_2, (y, b) \in R_1\} \text{ where } (x, b) \in R_2.$$

So,

$$m = \sum_{i=0}^2 p_{2i1} = p_{201} + p_{211} + p_{221} = 1 + s + p_{221}.$$

Hence,

$$p_{221} = m - s - 1.$$

Now

$$\begin{aligned} p_{21}^2 &= \#\{y|(x, y) \in R_2, (y, a) \in R_1\} \text{ for } (x, a) \in R_2, \\ &= \#\{y|(x, y) \in R_2, (a, y) \in R_2\} \text{ for } (x, a) \in R_2. \end{aligned}$$

So,

$$m = p_{21}^2 = \sum_{i=0}^2 p_{22i} = p_{220} + p_{221} + p_{222} = 0 + (m - s - 1) + p_{222}.$$

Thus,

$$p_{222} = s + 1.$$

Similarly,

$$p_{22}^2 = \#\{y|(x, y) \in R_2, (y, b) \in R_2\} \text{ when } (x, a) \in R_2.$$

So,

$$m = \sum_{i=0}^2 p_{2i2} = p_{202} + p_{212} + p_{222} = 0 + p_{212} + (s + 1).$$

Hence,

$$p_{212} = m - s - 1.$$

Finally,

$$p_{11}^1 = \#\{(a, y) \in R_1, (y, b) \in R_1\} \text{ for } (a, b) \in R_1.$$

So,

$$m = \sum_{i=0}^2 p_{i11} = p_{011} + p_{111} + p_{211} = 0 + r + s.$$

Hence,

$$m = s - r.$$

And thus:

$$p_{211} = m - r, \quad p_{212} = r - 1, \quad p_{221} = r - 1, \quad p_{222} = m - r.$$

To complete the proof, we now show that the second condition of the lemma does not hold. Recall that

$$(E_1 Y_{ab})_x = 0 \Leftrightarrow \sum_{j=0}^2 \overline{p_i(j)} \cdot \sum_{k,l=0}^2 p_{jkl} t_k t_l^{-1} = 0$$

Then, substituting the values obtained for  $p_{ijk}$ ,

$$\begin{aligned} \sum_{j=0}^2 \overline{p_i(j)} \cdot \sum_{k,l=0}^2 p_{jkl} t_k t_l^{-1} &= \overline{p_i(0)} t_1 t_2^{-1} \\ &+ \overline{p_i(1)} [r t_1 t_1^{-1} + (m-r) t_1 t_2^{-1} + (m-r+1) t_2 t_1^{-1} + r t_2 t_2^{-1}] \\ &+ \overline{p_i(2)} [t_0 t_1^{-1} + t_1 t_0^{-1} + (m-r) t_1 t_1^{-1} + (r-1) t_1 t_2^{-1} + (r-1) t_2 t_1^{-1} \\ &+ (m-r+1) t_2 t_1^{-1}]. \end{aligned}$$

Since  $t_0 = t_1$ ,  $t_1 = t_2$ , and  $t_2 = t_1$ , and using the values of the  $p_i(j)$  obtained from the character table  $P$  of the scheme, the right-hand side of the above equation simplifies to the following:

$$\begin{aligned} 2m+1 &+ \frac{-1-\sqrt{ni}}{2} \left[ r \frac{t_1}{t_2} + (m-r) + (m-r+1) r \frac{t_2}{t_1} \right] \\ &+ \frac{-1+\sqrt{ni}}{2} \left[ \frac{t_1}{t_2} + 1 + (m-r) \frac{t_1}{t_2} + (r-1) + (r-1) + (m-r+1) \frac{t_2}{t_1} \right] \end{aligned}$$

Collecting terms and simplifying we obtain

$$\left[ \frac{t_1}{t_2} + \frac{t_2}{t_1} - 2 \right] \left[ \frac{-(m+1)}{2} + \frac{(m-2r+1)\sqrt{n}}{2} \right]$$

Since  $t_1 + t_2 \neq 0$ , the factor  $\frac{t_1}{t_2} + \frac{t_1}{t_2} - 2$  will never equal zero. Moreover, the second factor is clearly nonzero. Hence we have shown that  $(E_1 Y_{ab})_x \neq 0$ . From the lemma, we conclude that no spin model arises from this class of association schemes.

Finally, we note that for the second possible ordering of the primitive idempotents  $E_i$ , which results in reversing the second and third rows of the character table  $P$ , a similar argument yields the same conclusion as before. This completes the proof.  $\square$

### CONCLUDING REMARKS

The relation between knot theory and statistical mechanics and the role played by association schemes are not yet very well understood, but the evidence for the connection is substantial. Indeed a real understanding will require the efforts of both mathematicians and physicists, working in diverse areas such as topology, quantum field theory, combinatorics and the geometry of 3-manifolds.

Through the years, many mathematical theories and objects have found relevance and applications in otherwise unrelated areas. The new knot theory, for instance, has been used by molecular biologists in enzyme recognition and the study of DNA, and by chemists studying polymer theory. It is hoped that this article serves as introduction and encouragement for interdisciplinary research.

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## ASSESSMENT OF INFLUENCE IN FACTOR ANALYSIS REGRESSION\*

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### ABSTRACT

*Influence functions are derived for the parameters in the factor analysis (FA) regression and some measures are proposed to evaluate the influence on the estimated regression weights  $\hat{\beta}_F$  and on the squared multiple correlation coefficient  $r^2(\hat{\beta}_F)$ . As an application, a numerical study is conducted to illustrate the present procedure and to show fundamental properties of influence in FA regression from the sensitivity perspective.*

### INTRODUCTION

Sensitivity analysis in factor analysis (FA) has been studied by several authors to evaluate the amount of influence of small changes of data. Among them are works written by Tanaka and Odaka (1989 a,b), Tanaka, Castaño-Tostado and Odaka (1990) and their related papers. For all these various versions of FA model, some measures of influence on the estimates, their precision and the goodness of fit have been used for detecting single or multiple influential observations. In the present paper, we try to develop a method of sensitivity analysis in FA regression based on the influence function derived by Tanaka and Odaka (1989b).

FA regressions is a statistical method which can deal with the problem of linear prediction where the independent variables are subject to errors. There have been some authors who devoted to study on FA regression. Among them include Horst (1941), Scott (1968), Lawley and Maxwell (1973), Isogawa and Okamoto (1980) and Browne (1988) who discussed this topic. FA regression was first proposed by

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Horst (1941) as a method for improving the predictive validity when the given number of prediction variables is large compared to the number of cases. It was suggested by Scott (1966) as a method of linear prediction which is an alternative estimation procedure to the classical least squares regression. This works for situations in which the independent variates are subject to measurement errors and when there are high intercorrelations that cause the least squares procedures to break down. Furthermore, Scott (1966) points out that the method used in FA regression explicitly accounts for the errors as the "unique factors" in the FA model. More directly, the factor model has been employed by Isogawa and Okamoto (1980) as a basis for a prediction model in an effort to elucidate the effects of measurement errors in prediction. Lawley and Maxwell (1973) formulated an optimality problem for the factor analysis model and obtained the optimal biased and unbiased predictors as the solutions of the unrestricted and restricted problems, respectively.

Our main concern in this study is to develop a method of sensitivity analysis in FA regression for detecting influential observations. Here we consider situations where the problem is to predict an observable dependent variate from observable independent variates with measurement errors and where a factor analysis model holds for both of independent and dependent variates. The theoretical formulation given below is closely patterned after the work of Browne (1988). Detailed properties of the underlying model and the maximum likelihood estimates are studied by Lawley and Maxwell (1973) and Browne (1988). First, we derive the empirical influence functions  $\hat{\beta}_F^{(1)}$  and  $[r_2(\hat{\beta}_F)]^{(1)}$  for the FA regression weights  $\hat{\beta}_F$  and the squared multiple correlation  $r_2(\hat{\beta}_F)$ . As an application of the proposed procedure, we try to conduct a numerical study to illustrate some fundamental properties of the influence in FA regression.

### Linear Prediction

Let us consider a sample of  $n$  observations  $x_i = (x_{1i}, x_{2i}^T)^T$ ,  $i = 1, \dots, n$ , on a  $p \times 1$  vector variable  $\bar{x}_i = (\bar{x}_1, \bar{x}_2^T)^T$ . Our concern is to predict the first variable  $x_1$  by means of a linear function of the remaining variables represented by the  $(p-1) \times 1$  vector:  $x_2$ . Let  $x = (x_1, x_2^T)^T$  and

$$S = \begin{pmatrix} s_{11} & s_{12}^T \\ s_{21} & S_{22} \end{pmatrix}$$

be the sample mean vector and covariance matrix. If  $\hat{\beta}$  is a  $(p-1) \times 1$  vector of weights, the predicted value  $\bar{x}_{1i}$  obtained from  $\bar{x}_{2i}$ ,  $i = 1, \dots, n$ , is given by

$$x_{1i} = (x_1 + \hat{\beta}^T(x_{2i} - x_2)). \quad (1)$$

The usual sample multiple regression weights derived by the ordinary least squares method (OLS) are obtained as

$$\hat{\beta} = S_{22}^{-1} s_{21} \quad (2)$$

These weights yield the minimum

$$s_e^2(\hat{\beta}_F) = s_{11} - s_{22}^T S_{22}^{-1} s_{21} \quad (3)$$

of the residual variance and the maximum

$$r^2(\hat{\beta}_F) = 1 - s_e^2(\hat{\beta})/s_{11} \quad (4)$$

of the squared multiple correlation coefficient  $r^2(\hat{\beta})$ .

### Factor Analysis Model

Now consider a factor analysis (FA) model for a  $p \times 1$  observation vector  $x$  given by

$$x = \mu + \Lambda f + e, \quad (5)$$

where  $\mu$  is the mean vector,  $\Lambda$  is a  $p \times k$  ( $k < p$ ) factor loading matrix,  $f$  is a  $k \times 1$  common factor score vector, and  $e$  is a  $p \times 1$  unique factor score vector. And also, we assume

$$E(f) = 0, \quad E(e) = 0, \\ E(ff^T) = I, \quad E(ee^T) = \Psi, \quad E(fe^T) = 0,$$

$\Psi$  denoting a diagonal matrix. Let us partition a  $p \times k$  factor leading matrix  $\Lambda$  as  $\Lambda = (\lambda_1, \Lambda_2^T)^T$ . The common factors  $f_1, \dots, f_k$  form a vector variate  $f$  where  $\lambda_1^T$  is a row vector whose elements  $\lambda_{11}, \dots, \lambda_{1k}$  are the loadings of  $x_1$  on the factors in  $f$ . The elements of the  $(p-1) \times k$  matrix  $\Lambda_2$  are the loadings of the variates in  $x_2$  on  $f$ . Also, we express the corresponding diagonal matrix of unique variances in the form of  $\Psi = \text{diag}(\psi_{11}, \Psi_{22})$ . Then, the covariance matrix derived from the FA model is expressed in terms of the loading matrix  $\Lambda$  and the unique variance matrix  $\Psi$  as

$$\Sigma = \Lambda \Lambda^T + \Psi \quad (6)$$

which is known as the common factor decomposition.

Using the factor decomposition the matrix  $\Sigma$  may be partitioned in the form of

$$\Sigma = \begin{pmatrix} \sigma_{11} & \lambda_1^T \Lambda_2^T \\ \Lambda_2 \lambda_1 & \Sigma_{22} \end{pmatrix}$$



where

$$\sigma_{11} = \lambda_1^T \lambda_1 + \psi_{11} \quad (7)$$

represents the variance of  $x_1$  and

$$\Sigma_{22} = A_2 A_2^T + \Psi_{22} \quad (8)$$

is the covariance matrix of  $x_2$ .

### Factor Analysis Regression

Consider the case in which it is not possible to measure the independent variates  $x_2$  without error in which the dependent and independent variated  $x_1, x_2$  follow a factor analysis model (5). Then, it is derived by Lawley and Maxwell (1973) and Browne (1988) that the regression weights of the best predictor in the sense to minimize  $E(x_1 - \hat{x}_1)^2$  is given by the least squares estimate as

$$\hat{\beta}_F = \hat{\Sigma}_{22}^{-1} \hat{\sigma}_{21}^T \quad (9)$$

where  $\hat{\Sigma}_{22}^{-1}$  and  $\hat{\sigma}_{11}$  are obtained from equations (7) and (8) with the parameters replaced by their estimates.

Suppose that  $\hat{\Lambda}$  and  $\hat{\Psi}$  are obtained by the standard maximum likelihood method under the inequality constraints that the diagonal elements of  $\hat{\Psi}$  are non negative and that the condition  $\hat{\psi}_{11} \neq 0$  is satisfied. Then the residual variance is given by

$$s_e^2(\hat{\beta}_F) = \hat{\sigma}_{11} - \hat{\sigma}_{21}^T \hat{\Sigma}_{22}^{-1} \hat{\sigma}_{21} \quad (10)$$

and the squared multiple correlation coefficient between  $x_1$  and  $x_2$  is expressed in the form of

$$r^2(\hat{\beta}_F) = 1 - \frac{s_e^2(\hat{\beta}_F)}{\hat{\sigma}_{11}} \quad (11)$$

### Sensitivity analysis

For sensitivity analysis, we make use of the influence functions related with FA regression. Our concern is to focus our attention on the two aspects of influence, namely influence on the FA regression weights  $\hat{\beta}_F$  and influence on the squared multiple correlation coefficient  $r^2(\hat{\beta}_F)$ . Let us consider a perturbation on the empirical distribution  $\hat{F}$  to  $(1 - \varepsilon)\hat{F} + \varepsilon\delta_x$  where  $\delta_x$  is the cdf of a unit point mass at  $x$ . Then, using the so called chain rule, we can obtain the empirical influence function for,  $\hat{\beta}_F$  which is equivalent to the first derivative of  $\hat{\beta}_F$  with respect to  $\varepsilon$ , as

$$\widehat{\beta}_F^{(1)} = - \widehat{\Sigma}_{22}^{-1} \widehat{\Sigma}_{22}^{(1)} \widehat{\Sigma}_{22}^{-1} \widehat{\sigma}_{21} + \widehat{\Sigma}_{22}^{-1} \widehat{\sigma}_{22}^{(1)}, \quad (12)$$

where  $\widehat{\Sigma}_{22}^{(1)}$  and  $\widehat{\sigma}_{22}^{(1)}$  are obtained from the corresponding parts of  $(\widehat{\lambda}\widehat{\lambda}^T)^{(1)} + \widehat{\varphi}^{(1)}$ ,  $(\widehat{\lambda}\widehat{\lambda}^T)$  and  $\widehat{\varphi}^{(1)}$  being the influence functions in the maximum likelihood factor analysis derived by Tanaka and Odaka (1989b). As a measure of the influence on the estimate  $\widehat{\beta}_F$ , the vector-valued influence function  $\widehat{\beta}_F^{(1)}$  should be summarized into a scalar-valued quantity. The simplest way to summarize it is to take the Euclidean norm of a vector  $\widehat{\beta}_F^{(1)}$ , i.e.,  $\|\widehat{\beta}_F^{(1)}\|$ . Instead of taking Euclidean norm without any scaling we may scale or standardize before taking the norm. Here we consider two kinds of scaled norms such as univariately-scaled squared distance  $D_{us}$  and the multivariately-scaled squared distance  $D_{ms}$ .

$$D_{us}(\widehat{\beta}_F) = (n-1)^{-1} \widehat{\beta}_F^{(1)T} [\widehat{V}_D(\widehat{\beta}_F)]^{-1} \widehat{\beta}_F^{(1)}. \quad (13)$$

$$D_{ms}(\widehat{\beta}_F) = (n-1)^{-2} \widehat{\beta}_F^{(1)T} [\widehat{V}_D(\widehat{\beta}_F)]^{-1} \widehat{\beta}_F^{(1)}. \quad (14)$$

The matrices  $\widehat{V}$  and  $(\widehat{\beta}_F)$  and  $\widehat{V}_D$  are an estimated asymptotic covariance matrix of  $\widehat{\beta}_F$  and a diagonal matrix whose diagonal part consists of the diagonal part of  $\widehat{V}(\widehat{\beta}_F)$ , respectively. Actually, the quantity  $D_{ms}$  is a generalized version of the Cook's distance which is frequently applied in regression diagnostics. The asymptotic covariance matrix of  $\widehat{\beta}_F$ , i.e.,  $V(\widehat{\beta}_F)$  can be derived from the asymptotic variances and covariances of  $\widehat{V}$  and  $\widehat{\varphi}$ , which are evaluated in the paper of Tanaka and Watadani (1992), using the delta method. However, for simplicity to obtain  $\widehat{V}(\widehat{\beta}_F)$  we utilize the jackknife technique in which  $\widehat{\beta}_{F(i)}$ , the estimate based on the sample without the  $i$ -th observation, is replaced by its linear approximate using the empirical influence function. The covariance matrix based on the standard jackknife technique is given by (see, e.g., Fox, Hinkley and Larntz, 1980)

$$V_p = n(n-n)^{-1} \sum_{i=1}^n (P_i - \bar{P})(P_i - \bar{P})^T, \quad (15)$$

where the matrix  $V_p$  is the jackknife estimate of  $V(\widehat{\beta}_F)$  and  $P_i$  are the vectors which represent the pseudo-values expressed in the form of

$$P_i = n\widehat{\beta}_F - (n-1)\widehat{\beta}_{F(i)} \quad (16)$$

with an average

$$\bar{P}_i = \widehat{\beta}_j = n^{-1} \sum_{i=1}^n P_i, \quad i = 1, 2, \dots, n. \quad (17)$$

As a measure of the influence on the goodness of fit, we consider the influence function for squared multiple correlation coefficient  $r^2(\hat{\beta}_F)$ , which is derived in the following form.

$$[r^2(\hat{\beta}_F)]^{(1)} = - \frac{[s_e^2(\hat{\beta}_F)]^{(1)}}{\hat{\sigma}_{11}} + \frac{s_e^2(\hat{\beta}_F) \hat{\sigma}_{11}^{(1)}}{(\hat{\sigma}_{11})^2} \quad (18)$$

where  $\hat{\sigma}_{11}^{(1)}$  is obtained from the (1, 1) element of  $(\hat{\Lambda}\hat{\Lambda}^T) + \hat{\Psi}^{(1)}$  and

$$[s_e^2(\hat{\beta}_F)]^{(1)} = \hat{\sigma}_{11}^{(1)} - 2\hat{\sigma}_{21}^{(1)T} \hat{\Sigma}_{22}^{-1} \hat{\sigma}_{21} + \hat{\sigma}_{21}^T \hat{\Sigma}_{22}^{-1} \hat{\Sigma}_{22}^{(1)} \hat{\Sigma}_{22}^{-1} \hat{\sigma}_{21}. \quad (19)$$

### Numerical study

To investigate the performance of our sensitivity analysis procedure, we apply our procedure to an artificial data set, which was generated on the basis of an example given in the paper of Lawley and Maxwell (1973). The example discusses the analysis of a sample of 200 students taking certain public examinations. The independent variables relate the examination obtained in the six subjects namely Gaelic, English, History, Arithmetic, Algebra and Geometry. The dependent variable represents the algebra examination score taken after two years. So the main purpose of the analysis is to see how this final algebra examination score (dependent variable) can be predicted from the scores obtained in the six earlier examinations (independent variables). The data of 100 individuals were generated by using the result of analysis with a two factor model of the correlation matrix given in the example of Lawley and Maxwell as

$$\begin{bmatrix} x_1 \\ x_2 \\ x_2 \\ x_4 \\ x_5 \\ x_6 \\ x_7 \end{bmatrix} = \begin{bmatrix} 0.6929 & -0.2855 \\ 0.5405 & 0.4490 \\ 0.5576 & 0.3103 \\ 0.3825 & 0.4474 \\ 0.6971 & -0.1852 \\ 0.7376 & -0.2049 \\ 0.6411 & -0.1697 \end{bmatrix} \begin{bmatrix} f_1 \\ f_2 \end{bmatrix} + \begin{bmatrix} 0.4384e_1 \\ 0.5062e_2 \\ 0.5928e_3 \\ 0.6535e_4 \\ 0.4797e_5 \\ 0.4140e_6 \\ 0.5602e_7 \end{bmatrix}$$

where each of  $(f_1, f_2, e_1, \dots, e_6)$  follows independently  $N(0, 1)$  distribution. Note that all of the variables have positive loadings on the first factor. This factor reflects the overall response of the students to instruction and might be labeled as "general intelligence" factor. Half of the loadings are positive and half are negative on the second factor. This factor is not easily identified, but is such that the individuals who get above-average scores on the verbal tests get above-average scores on the factor. Individuals with above-average scores on the mathematical tests get below-average scores on the factor. This may be labeled as "math-nonmath"

factor. The correlation matrix of examination scores in six subject areas and the result of FA regression are shown in Table 1 and 2, respectively. It is clearly indicated from Table 2 that the first three regression weights are negligibly small, and therefore the examination scores on the three nonmathematical subjects do not contribute much towards the prediction of  $x_1$ .

To analyze some fundamental properties of the influence in FA regression,

Variables	$X_1$	$X_2$	$X_3$	$X_4$	$X_5$	$X_6$	$Y$
$X_1$	1.000	0.439	0.410	0.288	0.329	0.248	0.249
$X_2$		1.000	0.351	0.354	0.320	0.329	0.284
$X_3$			1.000	0.164	0.190	0.181	0.146
$X_4$				1.000	0.595	0.470	0.495
$X_5$					1.000	0.464	0.572
$X_6$						1.000	0.539
$Y$							1.000

Table 2. Result of the FA regression.

Variables	Data of f1-perturbation	St. error	Data of e6-perturbation	St. error
	$\widehat{(\beta_F)}$	$SE(\widehat{\beta_F})$	$\widehat{(\beta_F)}$	$SE(\widehat{\beta_F})$
1	-0.0521	0.0674	-0.0561	0.0726
2	0.0670	0.0378	0.1218	0.0444
3	0.0339	0.0426	0.0586	0.0406
4	0.1622	0.0444	0.2449	0.0954
5	0.5026	0.0973	0.2225	0.1419
6	0.1470	0.0443	0.2238	0.0741

we put into two types of unusual data and make two artificial data sets with different types of perturbation. The first data set contains 99 ordinary observations and one observation (No. 70) with extraordinarily large value of  $f_1$  ( $f_1 = 10$ ). On the other hand, the second data set is composed of 99 ordinary observations and one observation (again No. 70) with extraordinarily large value of  $e_6$  ( $e_6 = 10$ ).

At first, we applied FA regression and the sensitivity analysis procedure to the first data set with an observation of large  $f_1$ . To investigate the influence of every individual, we have computed influence measures  $\|\widehat{\beta_F}^{(1)}\|$ , scaled distances  $D_{us}$  and  $D_{ms}$  as well as the measure on the squared multiple correlation coefficient

$[r^2(\hat{\beta}_F)]^{(1)}$ . The index plots of those measures are shown in Figure 1. As seen from the figure, individual No. 70 which possesses an extraordinarily large value of  $f_1$  is not at all influential to the regression coefficients  $\hat{\beta}_F$ , but it has extremely large influence on the squared multiple correlation coefficient. It is noticed that the effect of  $f_1$  perturbation appears only in the  $[r^2(\hat{\beta}_F)]^{(1)}$  not in  $\|\hat{\beta}_F^{(1)}\|$ ,  $D_{us}$  and  $D_{ms}$ .

Next, we analyzed the second data set with an observation of large  $e_0$ . The index plots in Figure 2 show the influence measures of every observation. As shown in those index plots, there exists one observation, the 70-th individual, which has much extremely large influence on  $\hat{\beta}_F$ , but the influence of the same observation on the squared multiple correlation coefficient is not so extreme. We also analyzed the case of  $e_3$ -perturbation, the results of which are not shown here for saving space. The effect of  $e_3$ -perturbation has appeared more strongly in  $\|\hat{\beta}_F^{(1)}\|$ ,  $D_{us}$  and  $D_{ms}$  and not so much in  $[r^2(\hat{\beta}_F)]^{(1)}$ .

### Discussion

For detecting influential observations, we have derived influence functions for the parameters in the FA regression and proposed some measures of influence on the estimate of regression weights  $\hat{\beta}_F$  and of influence on the squared multiple correlation coefficient  $r^2(\hat{\beta}_F)$ . In the present paper, we illustrated the present procedure when some perturbations are introduced on the data set. In the numerical study, we have considered two types of perturbation to characterize the fundamental properties of influence from the sensitivity perspective.

In the case of  $f$ -perturbation the effect appeared strongly in the measure of influence on  $r^2(\hat{\beta}_F)$  but not at all appeared in the measures of influence on  $\hat{\beta}_F$ . This can be explained by the model as follows. The observation with extremely large  $f_1$  fits the FA regression model well. So as the correlation structure of the variables does not change essentially, the residual variance, the numerator of the second term of the right side of (11), is not affected much. But  $\hat{\sigma}_{21}^{(1)}$ , the denominator of the same part, obviously increases. Therefore,  $r^2(\hat{\beta}_F)$  improves very much if this type of perturbation is introduced.

On the other hand, the effect of  $e$ -perturbation appeared strongly in the measures of influence on  $\hat{\beta}_F$  and also appeared, though not so strongly, in the measure of influence on  $r^2(\hat{\beta}_F)$ . In this case, the perturbed observation is located far from the regression hyperplane because of the large  $e$ -value. So it is obvious that the regression weights  $\hat{\beta}_F$  are affected strongly by this observation.

Now, among the possible sample versions of the influence functions the empirical influence function *EIF* is the easiest to compute. To check the validity of the proposed procedure based on *EIF*, we investigate numerically its relationship to the sample influence function *SIF*. For this purpose, we omit a single observation in turn and perform the FA regression analysis  $n$  times. Then, we calculate the following sample influence function *SIF* which is defined as

$$SIF_i(\hat{\beta}_F) = (n-1) \hat{\beta}_{F(i)} - \hat{\beta}_F \quad i = 1, 2, \dots, n$$

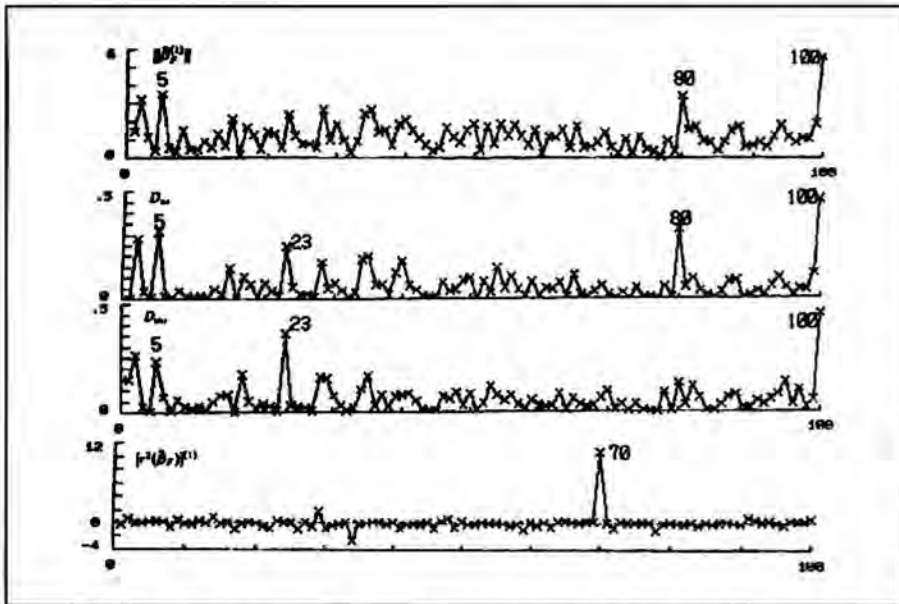


Figure 1. Index plot of four influence measures (Artificial data set of  $f_1$ -perturbation.)

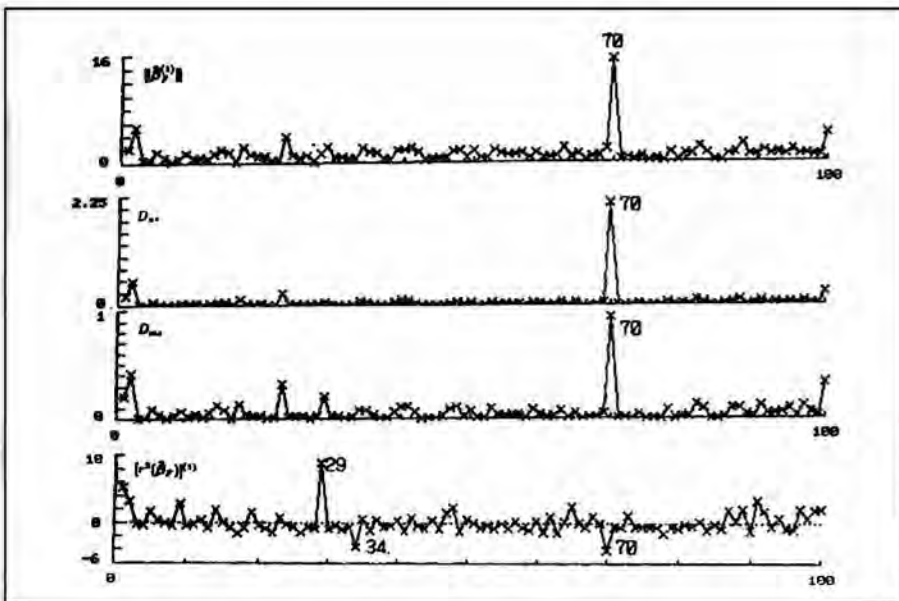


Figure 2. Index plot of four influence measures (Artificial data set of  $e_6$ -perturbation.)

where  $\hat{\beta}_F$  and  $\hat{\beta}_{F(i)}$  denote the estimated parameter vectors based on the sample with and without the  $i$ -th observation, respectively. Scatter diagrams of the statistics  $\|\hat{\beta}_F^{(1)}\|$ ,  $D_{us}$ ,  $D_{ms}$  and  $[r^2(\hat{\beta}_F)]^{(1)}$  versus the corresponding statistics based on *SIF* are given in Figure 3 and Figure 4. In these scatter diagrams, most of the points are located near the straight line  $SIF = EIF$ . These results suggest that the correspondence is good enough to imply that the *EIF*, which is based on the differential coefficient, can be used practically for detecting influential observations instead of the *SIF*. Besides, in terms of computing time, *SIF* requires much time to compute than *EIF*.

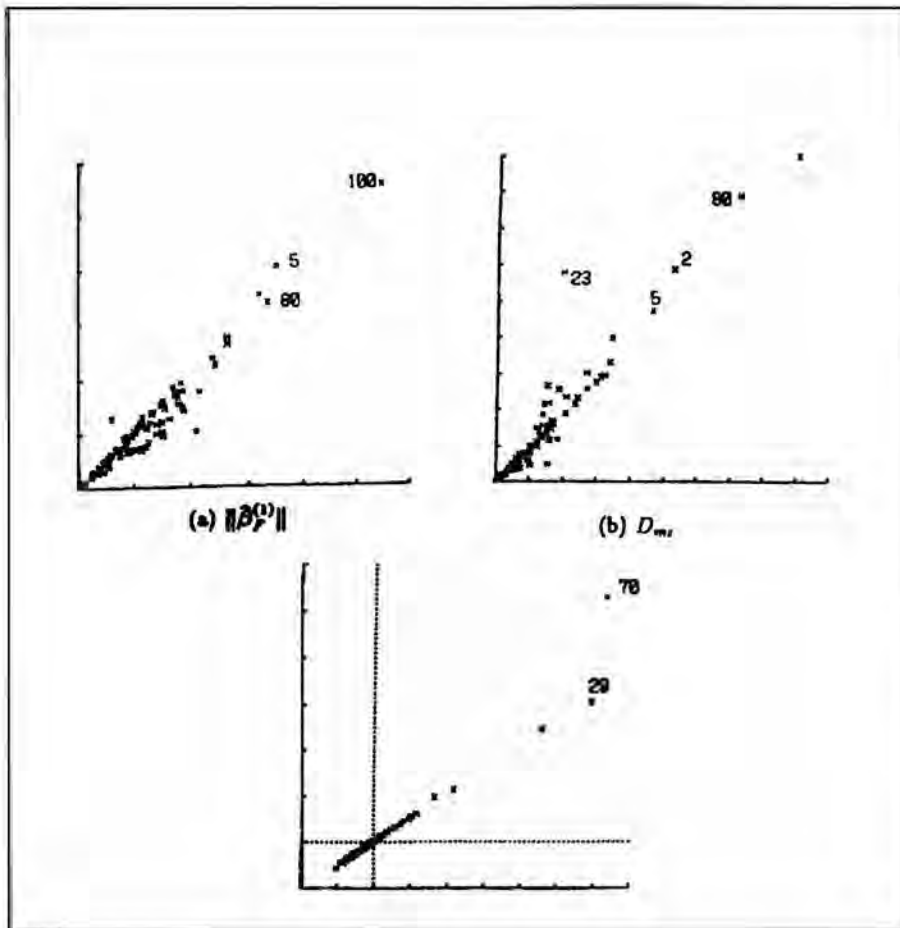


Figure 3. Scatter diagrams of three influence measures based on the EIF (horizontal) versus SIF (vertical) Artificial data set of  $f_1$ -perturbation

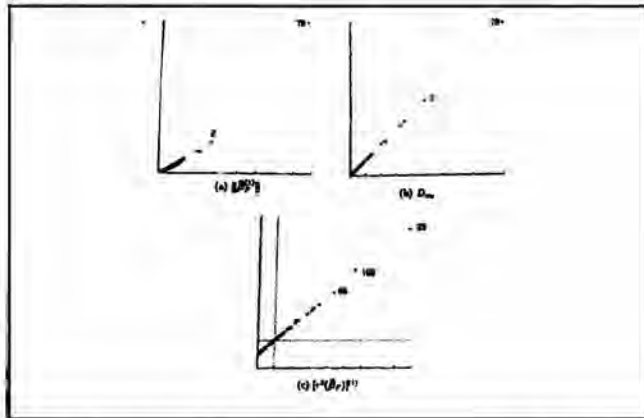


Figure 4. Scatter diagrams of three influence measures based on the EIF (horizontal) versus SIF (vertical) Artificial data set of  $e_6$ -perturbation

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# PREPARATION AND PHOTOELECTROCHEMICAL CHARACTERIZATION OF A CONDUCTING POLYMER (POLYANILINE) DOPED WITH A DYE (COPPER PHTHALOCYANINE TETRASULFONATE)

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## ABSTRACT

*Photoelectrochemistry of polyaniline (PAni), a conducting polymer deposited on an indium-doped tin oxide (ITO)-coated glass substrate, was studied in aqueous medium consisting of 2mM  $\text{Fe}(\text{CN})_6^{3-/4-}$  in 1M KCl. On illumination of the polymer-modified electrode and application of negative potential, cathodic photocurrent densities greater than  $20\text{nA}/\text{cm}^2$  were detected. Doping of the conducting polymer with a dye, copper phthalocyanine tetrasulfonate (CuPcTS) doubled the photoelectrochemical response to about  $40\text{mA}/\text{cm}^2$ . The polymer was prepared by electropolymerization of aniline in 0.1M  $\text{H}_2\text{SO}_4$  via repeated cycling between 0.0 to 0.7v vs. Ag/AgCl. Copper phthalocyanine tetrasulfonate (Na salt) was incorporated by polymerizing the aniline monomer in the presence of the dye. The conducting polymer-dye composite was characterized by IR, UV-Vis, scanning electron microscopy and cyclic voltammetry.*

**Keywords:** Polyaniline (PAni), copper phthalocyanine tetrasulfonate (CuPcTs), photoelectrochemical cell, photosensitizer dye, conducting polymer, electropolymerization

## INTRODUCTION

Interest in conducting polymers has grown steadily in the last two decades commencing with the accidental discovery of polyacetylene possessing high conductivity by Shirikawa et al. In 1977 (1). Polyaniline (PAni), in particular, has been intensively studied due to its inherent chemical stability and the ease at which it can be prepared. It has been used in a wide variety of applications ranging from electronic (2) to electrochemical devices (3). Chemical sensors (4) and batteries (5) have also been fabricated utilizing its special electrochemical properties.

Functionalization of the polymer has also been achieved through incorporation of various molecules including enzymes, redox species and chromophores (6).

Very few however were done on photoelectrochemical characterization of conducting polymers (7, 8, 9) and their photosensitization with dyes like phthalocyanines (10) which are good spectral sensitizer of large band gap semiconductors.

This research, therefore was done to study the photoelectrochemistry of polyaniline and its composite with copper phthalocyanine tetrasulfonate (CuPcTS) (Fig. 1) electrochemically deposited over indium-doped tin oxide (ITO).

### METHODOLOGY

Illumination was done using the light coming from a 150-W tungsten lamp (Hanimes) of a slide projector passed through a 5.5 cm water-filter and a camera UV filter (Astron) to remove the unwanted IR and UV radiation. Potential control and current measurement was done using Bioanalytical Systems CV27 voltammograph coupled to a Hewlett-Packard XYt recorder.

PAni was synthesized by oxidative electropolymerization of aniline in acidic medium using repeated potential cycling. CuPcTS was purchased from Aldrich Chemical Co. as the sodium salt and used as received. The dye-polymer composite of Pani and CuPcTS was prepared by electropolymerization of aniline in the presence of CuPcTS (Na salt or acid form).

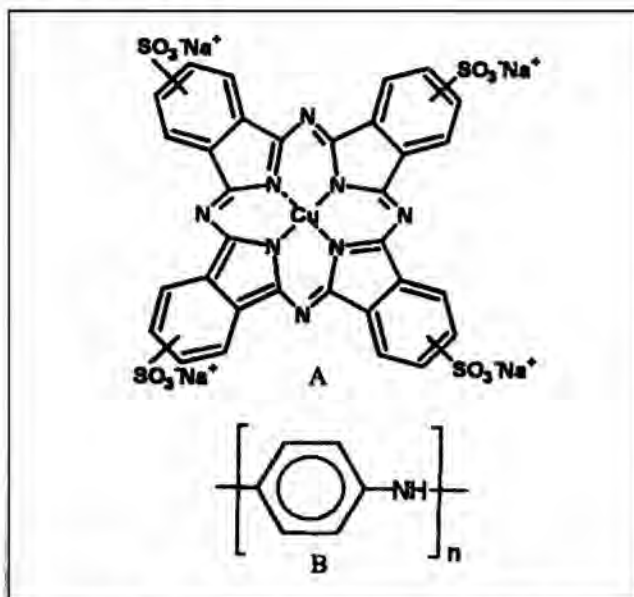


Figure 1. Structures of (A) copper phthalocyanine tetrasulfonate (Na salt) and (B) polyaniline.

All photoelectrochemical experiments were done in aqueous solution of 1M KCl in conventional three-electrode cell fitted with a flat quartz window. Redox couple used was  $\text{Fe(CN)}_6^{3-/4-}$  (potassium salt, J.T. Baker). The reference electrode was positioned close to the working electrode by a Luggin capillary tip. Substrate material was indium-tin oxide (ITO) optically transparent electrode ( $40\text{W}/\square$ , Delta Technologies). All potentials are referred against a Ag/AgCl reference electrode.

UV-Vis spectroscopic characterization of thin films were performed using an LKB Biochrom Ultrospec II spectrophotometer using ITO as blank. Infrared spectroscopy for polymer thin films was obtained from Bio Rad FTS 40 FTIR directly as diffuse reflectance using the DRIFT accessory (Bio-Rad PN 099-0931) and reported as average of 16 scans at  $2\text{cm}^{-1}$  resolution.

Electron micrographs were obtained using a JEOL JSM 5200 Scanning Electron Microscope.

## RESULTS AND DISCUSSION

Our preliminary studies have indicated that a film of polyaniline (PAni) on indium-doped tin oxide (ITO) glass substrate yielded current response on illumination with white light. Shown in Fig. 2 is the photocurrent response of PAni in contact with the redox solution consisting of the  $\text{Fe(CN)}_6^{3-/4-}$  couple in 1M KCl on slow potential cycling at  $20\text{ mV/s}$  and illuminated intermittently. At potentiostatic condition, the photocurrent response was found to be fast in comparison with an ion-conducting polymer-CuPcTS system (11) but rather low at  $3\text{mA}/\text{cm}_2$  (Fig. 3).

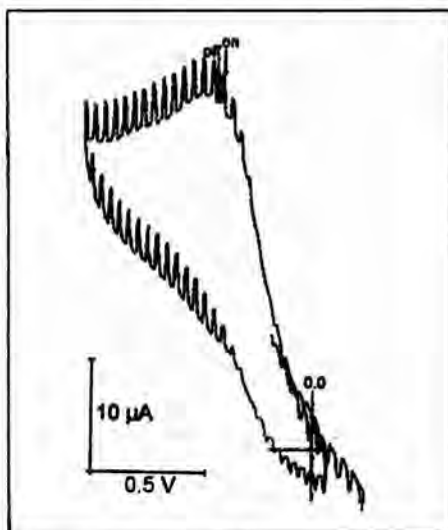


Figure 2. Cyclic voltammogram of PAni in contact with the redox solution and illuminated by a light source chopped at 0.5 Hz.

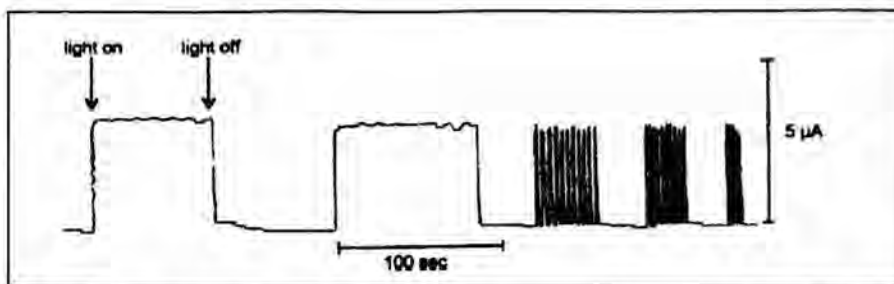


Figure 3. "Square wave" anodic photocurrent response of PANi biased at +0.40 V.

Further studies have shown that the  $\text{Fe}(\text{CN})_6^{3-/4-}$  couple has a supersensitizing effect (12) resulting in an increase of photocurrent response. The cathodic response, in particular, was found to increase rapidly on addition of small amounts of the oxidizing  $\text{Fe}(\text{CN})_6^{3-}$  (Fig. 4).

Also, increasing the amount of PANi deposited (monitored by Absorbance at 370 nm) increased the cathodic photocurrent yield as shown on Fig. 5.

On the other hand, the photocurrent response is also pH dependent as demonstrated by Fig. 6. While large photocurrent response was observed at high pH, the polymer (PANi) was unstable and easily peeled from the electrode. The condition at lower pH (pH 4) was therefore more preferred in this study because of the greater stability of the PANi film.

Fig. 7 below shows the doubling effect of the dopant, copper phthalocyanine tetrasulfonate (CuPcTS) on the photocurrent response of PANi (from 0 to -0.3 v). CuPcTS was incorporated in polyaniline by anodic electropolymerization of aniline (0.1 M) in the presence of  $\text{H}_2\text{SO}_4$  and CuPcTS via potential cycling.

Spectrochemical characterization of the polymer and composite shown in series of figures 8a, 8b, 8c, reveals electrochemic spectral change which could primarily be attributed to the incorporation of CuPcTS in the polymer. The 610 nm absorption of CuPcTS is shown (Fig.8b) present in the composite UV-Vis spectra as the electrode potential is varied. Additional data on CuPcTS incorporation in PANi could be inferred from the DRIFT Spectrum of CuPcTS, PANi, and composite, shown in Fig. 9. The prominent features in the composite spectra are the characteristic bands of PANi (the major component) with additional band at 1026  $\text{cm}^{-1}$  which could be attributed to shift S=O stretch in CuPcTS.

Figures 10a and 10b below are the electron micrographs of PANi and PANi/CuPcTS composite. While the polymer morphology is fibrillar, that of the composite is granular. The morphological change is probably due to aggregation of CuPcTS which is known to occur in dye solution of high concentration. Figure 10c is the micrograph of composite prepared from CuPcTS acid and which also shows granular morphology.

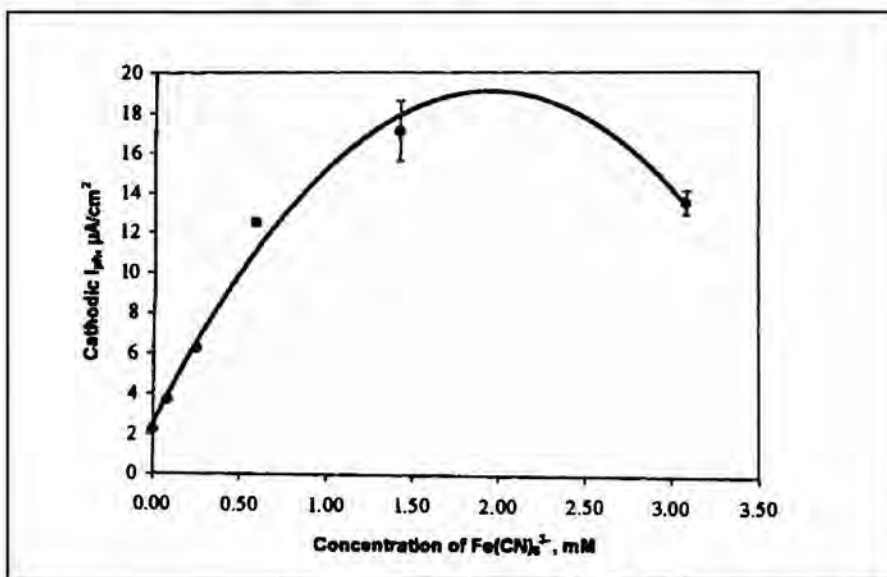


Figure 4. Dependence of photocurrent response of PANi on ITO with increasing concentration of  $\text{Fe}(\text{CN})_6^{3-}$ .

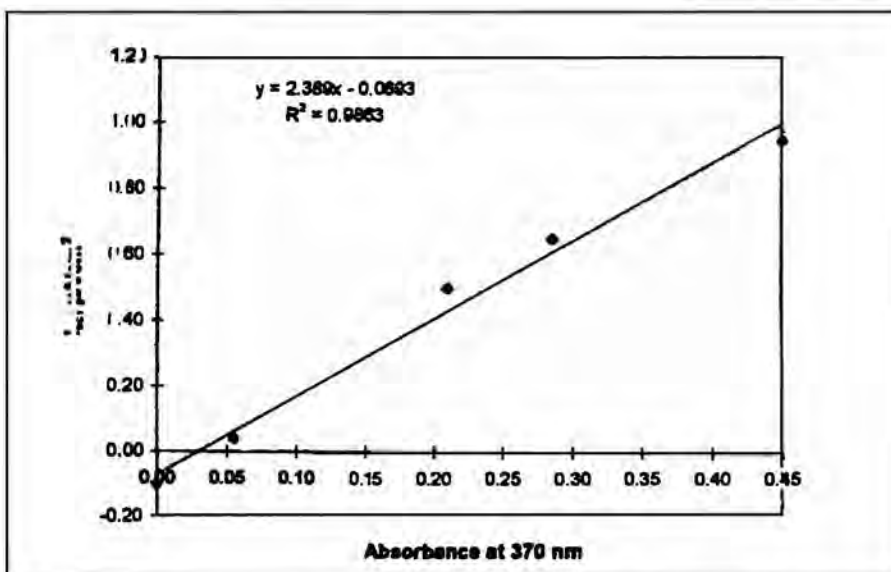


Figure 5. Dependence of short-circuit photocurrent response on film absorbance at 370 nm.

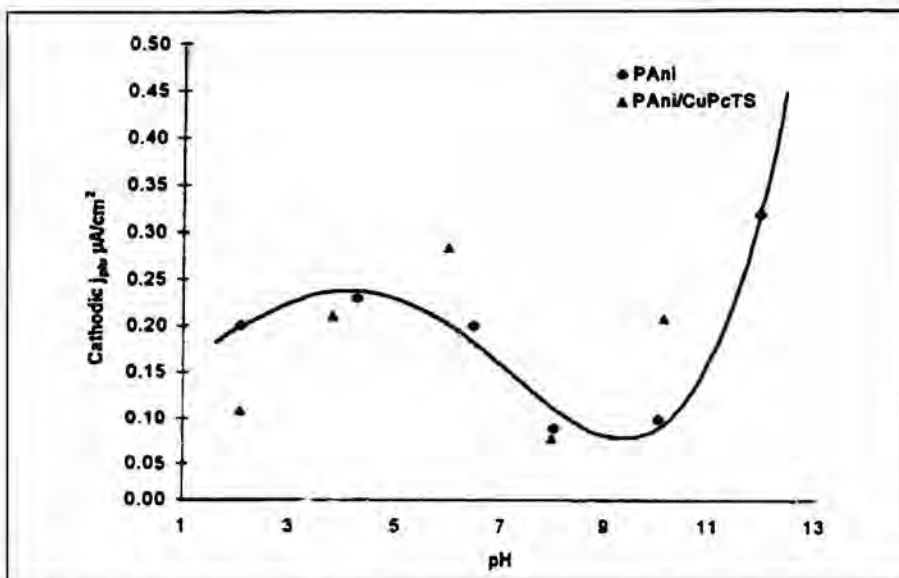


Figure 6. Dependence of the photocurrent on the pH of the electrolyte consisting of 2mM  $Fe(CN)_6^{3-/4-}$  in 1 MKCl; pH was adjusted with HCl and KOH.

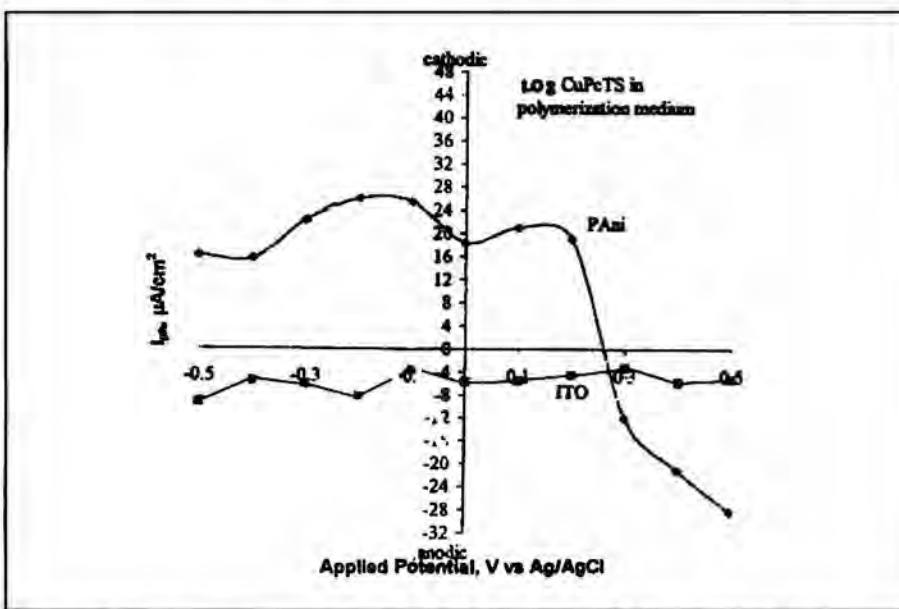


Figure 7. Photocurrent-potential plot of ITO, PANi and PANi/CuPcTS inn contact with the redox couple at Ph 4.0.

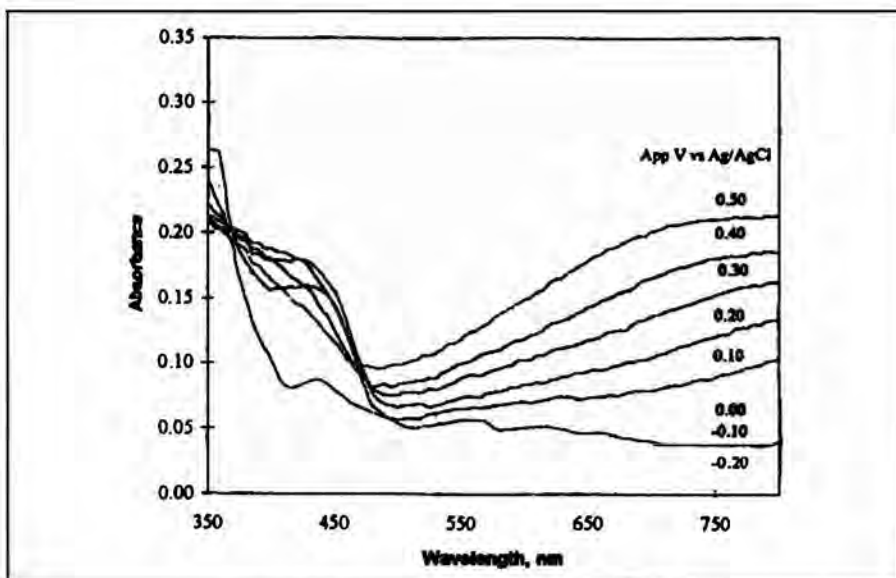


Figure 8.a Spectroelectrochemistry of PANi.

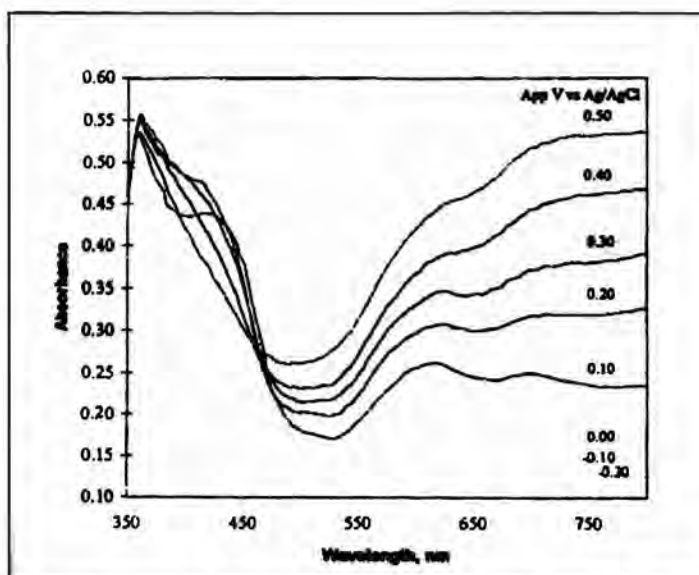


Figure 8.b Spectroelectrochemistry of PANi/CuPcTs in 0.1 M HCl

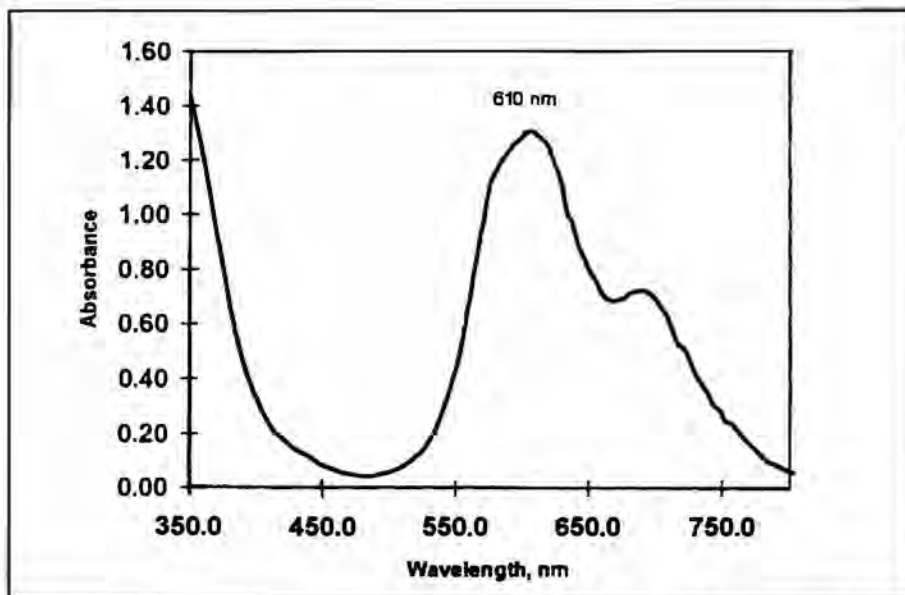


Figure 8.c Spectroelectrochemistry of visible absorbance of a dilute aqueous solution of CuPcTS

Two models to explain the charge generation in PANi and PANi/CuPcTS are being proposed and are shown in Fig. 11a and Fig. 11b, respectively.

In both models, the reduced form of PANi is the photoactive component since it is the major oxidation state of PANi at potentials in which cathodic photocurrents are observed. ITO in these models is simply regarded as an electrical contact. In Fig. 11b, the same mechanism occurs for PANi except that the acceptor here is the ground state CuPcTS<sup>+</sup>.

## CONCLUSION

The preliminary results presented here merit further studies to elucidate the fundamental photoelectrochemical processes involved. Of special interest is the determination of the energetics at the heterojunctions ITO/PANi and ITO/PANi/CuPcTS which can explain the role of the conducting polymer in the charge generation and transport phenomena. So far, it has been confirmed that the polymer is photoelectroactive and that this property could be improved by electrochemical doping with CuPcTS which enhances the light absorbing property of the polymer.

The study showed the photosensitizing effect of the CuPcTS dye on the polyaniline film acting as semiconductor which is at present being considered as a photoactive material for low cost and large area solar cells (13).



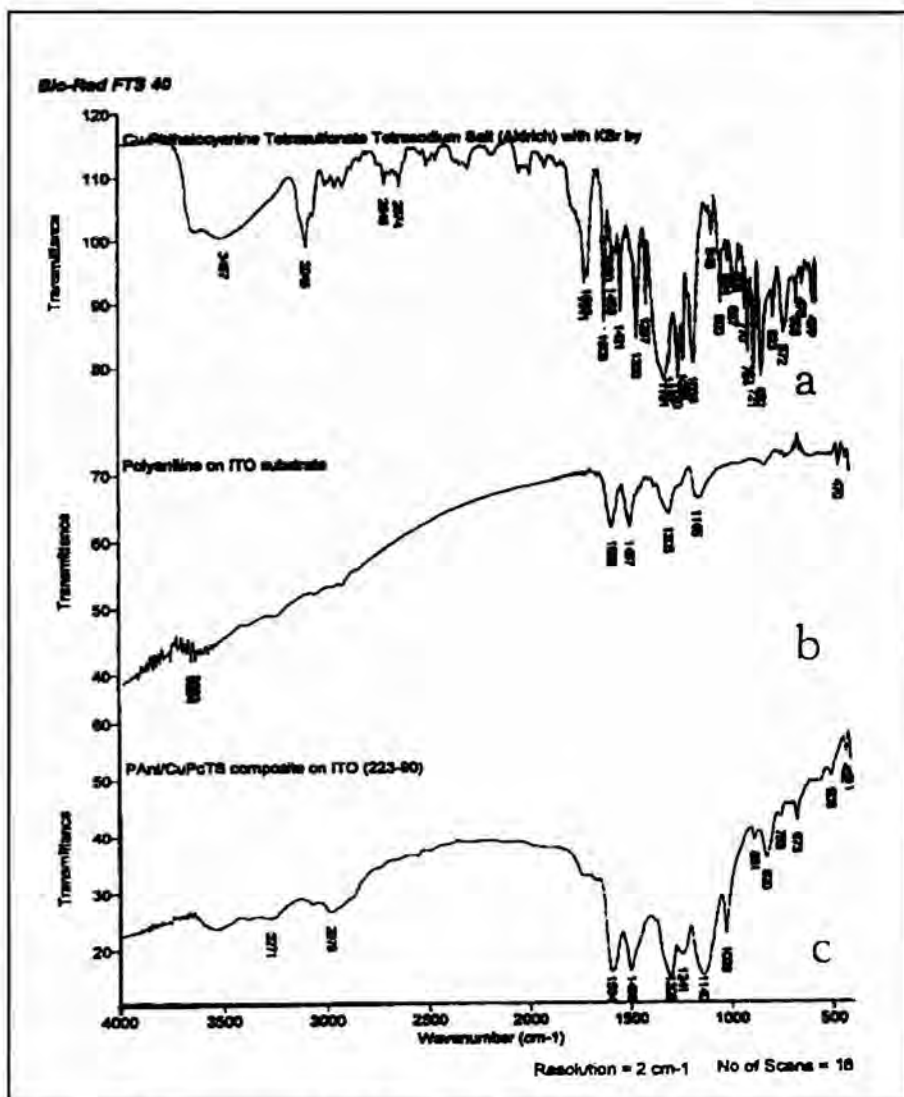


Figure 9. DRIFT spectrum of (a) CuPcTS in KBr, (b) PANi, (c) PANi/CuPcTS between 3500 to 400  $\text{cm}^{-1}$ .

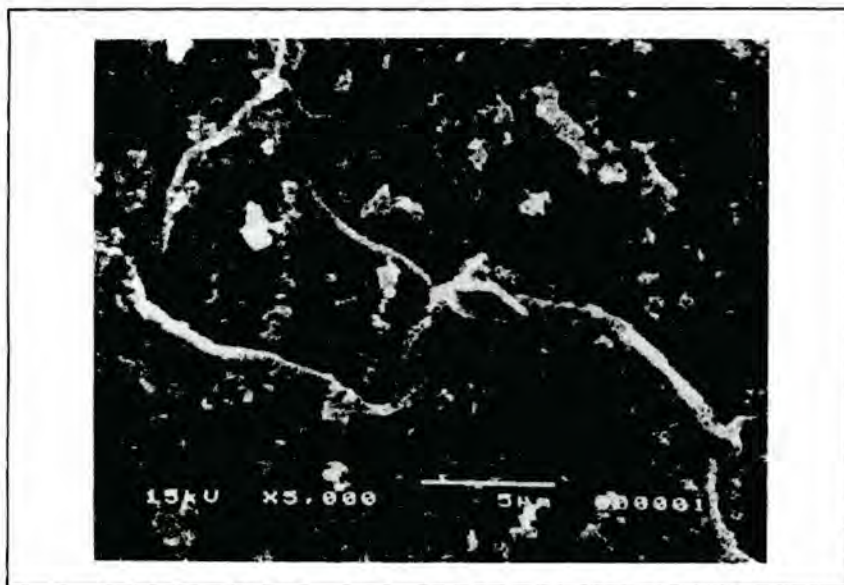


Figure 10.a Scanning electron micrograph of PANi and PANi/CuPcTS on ITO. PANi prepared from a solution of 0.1 M aniline, 0.1 M H<sub>2</sub>SO<sub>4</sub>.

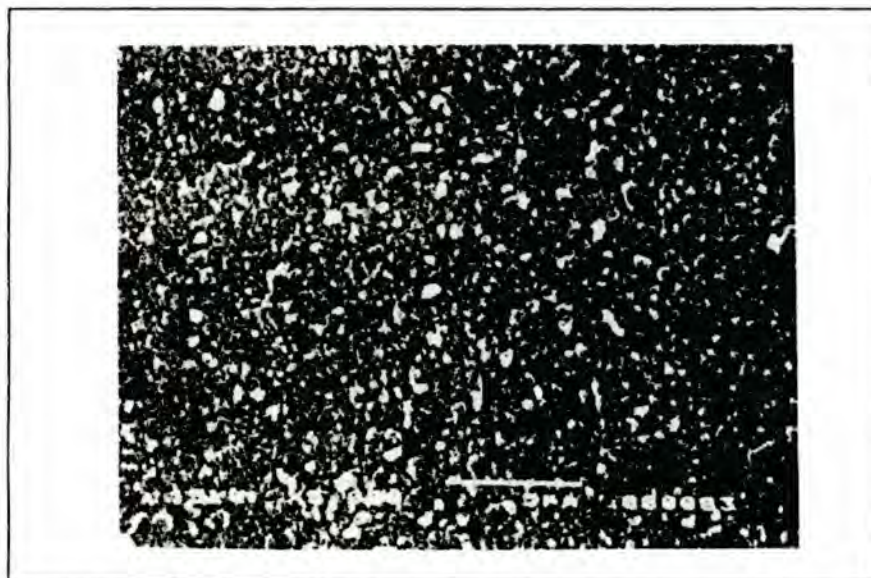


Figure 10.b Scanning electron micrograph of PANi and PANi/CuPcTS on ITO. PANi/CuPcTS prepared from a solution of 0.1 M aniline, 0.1 M H<sub>2</sub>SO<sub>4</sub>, 0.093 M CuPcTS and scanned -0.2 to + 1.0 V for 30 min at 20 mV/s.

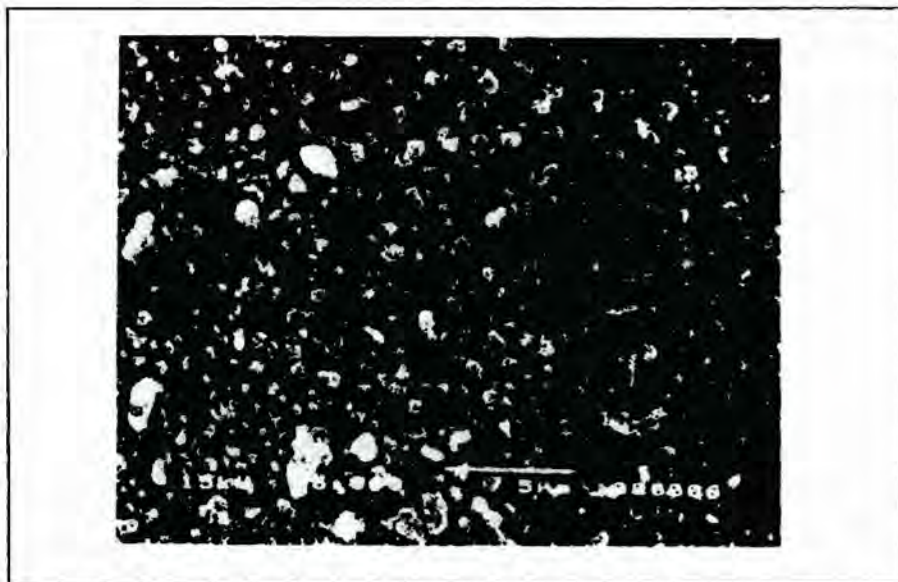


Figure 10.c Scanning electron micrograph of PANi and PANi/CuPcTS on ITO. PANi/CuPcTS prepared from 0.1 M aniline, 0.1 N  $\text{NH}_4\text{CuPcTS}$ , 0.05 N aniline and scanned between 0.0 to +1.0 V for 1 hr with initial 4 scans between 0.0 to 1.2 V both at 50 mV/s.

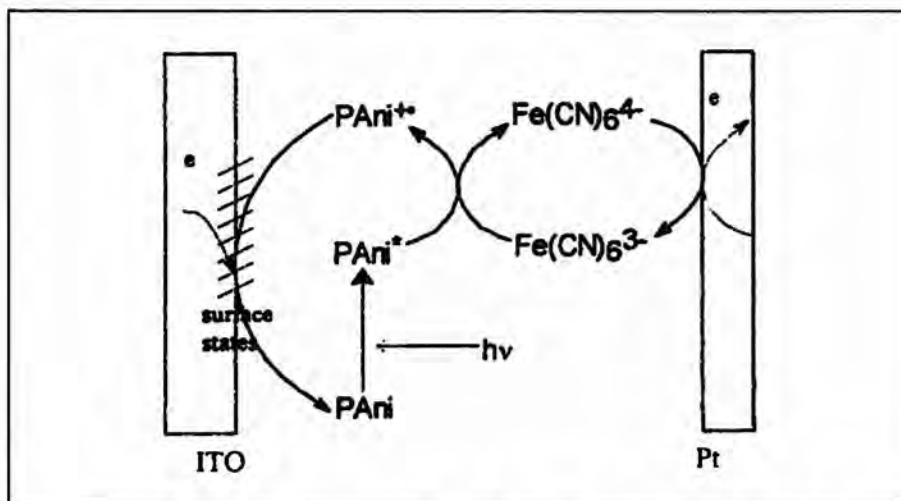


Figure 11.a Schematic diagram to explain the possible photoactivity of PANi.

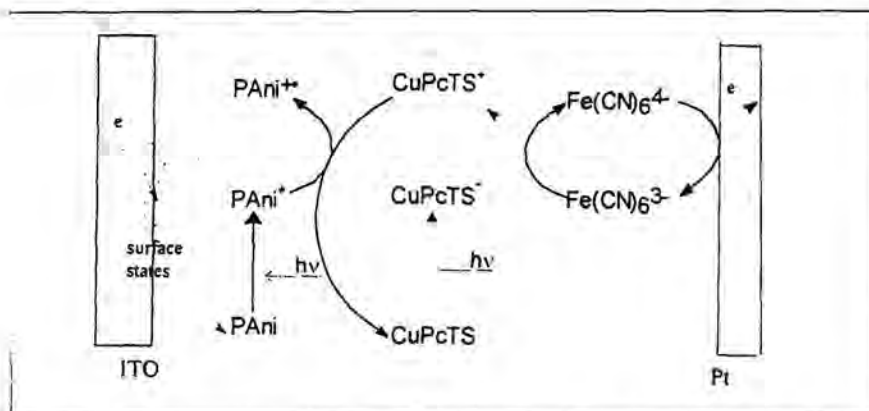


Figure 11.b Schematic diagram showing the possible role of CuPcTS in the charge generation.

### ACKNOWLEDGEMENT

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## DELINEATION OF THE SUBIC BAY FAULT ZONE IN ZAMBALES, LUZON, PHILIPPINES

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### ABSTRACT

*The Zambales ophiolite Complex (ZOC) is a well-studied exposure of upper mantle-crustal sequence in the Philippines. This ophiolite complex is subdivided into three massifs - Masinloc, Cabangan and San Antonio - which are cut by several structures. Reconnaissance gravity and magnetic surveys were conducted in the southern portion of the Zambales Ophiolite Complex to delineate gross structural features. The resulting geophysical anomalies define a major structural feature called the Subic Bay Fault Zone. This confirms the results of earlier field, petrographic and geochemical studies which suggested the presence of a structural boundary between the Cabangan Massif (which represents a mid-ocean ridge mantle suite) and the San Antonio Massif (which represents an arc crust). The results of this work do not only contribute to an understanding of how this ophiolite complex evolved but more importantly identifies a geologic structure which must be taken into consideration in any infrastructure development in this particular area.*

### INTRODUCTION

The Zambales Ophiolite Complex (ZOC), although one of the most studied upper mantle-crust sequence in the Philippines, had most of the studies concentrated on the northern portion (Masinloc Massif) of the ophiolite. The ZOC is made up of three massifs, Masinloc, Cabangan and San Antonio as one goes from north to south (Figure 1). The Masinloc Massif is divided into two blocks, the Acoje and Coto, which on the basis of geochemical and petrological evidences are recognized to preserve island arc and transitional mid-ocean ridge (MOR) - island arc (IA) characteristics, respectively (e.g. Hawkins and Evans, 1983; Geary et al, 1989; Yumul, 1989). This ophiolite complex is an allochthonous terrane bounded on the east and west by the Central Valley Suture and West Luzon Shear, respectively (Karig, 1983). Previous workers have shown that the Masinloc, Cabangan and San Anto-

nio Massifs are cut by very young faults resulting into the formation of grabens in between these massifs (Figure 1). Available paleomagnetic data and palinspathic reconstruction show that the ZOC came from the south. The source is thought to be either the Celebes Basin or the Paleocene-Eocene southwest subbasin of the South China Sea (e.g. Fuller et al, 1983; Karig, 1983; Honza, 1991; Yumul, 1994).

In this paper, additional data will be presented regarding the geology of the central-southern ZOC corresponding to the Cabangan and San Antonio Massifs, respectively. This paper presents geological evidence that will argue that the present-day configuration of the ZOC is a product of the southward translation through the West Luzon Shear/Subic Bay Fault Zone of the San Antonio Massif from the north to its present position. The knowledge that may be derived from this work hopefully may help in understanding how other exposed crust-mantle sequences have evolved through time and space.

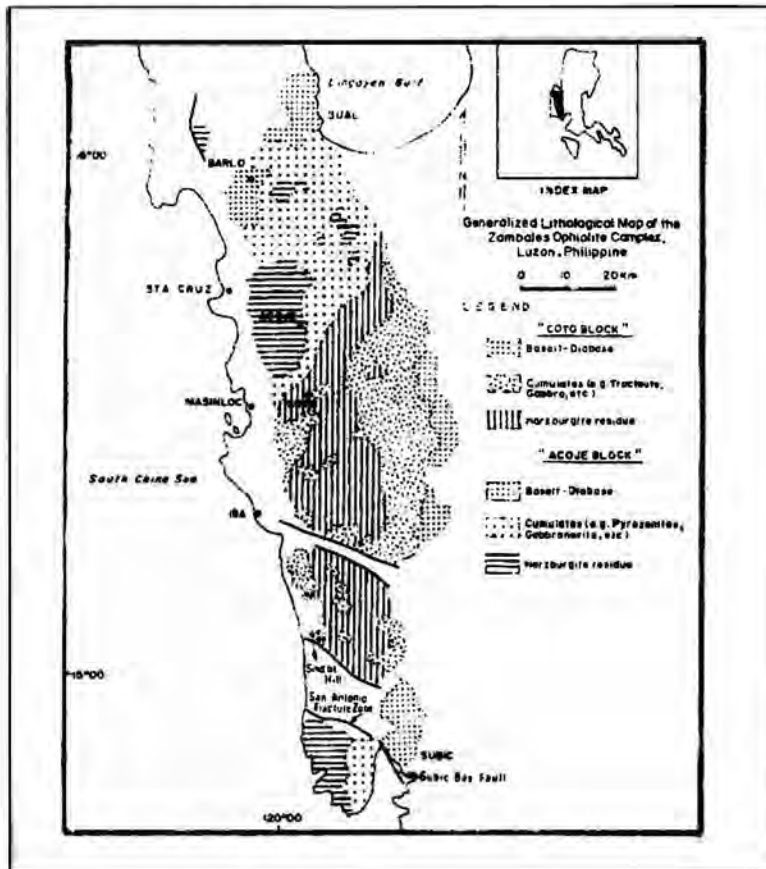


Figure 1.

## GEOLOGY OF THE ZAMBALES OPHIOLITE COMPLEX

The Eocene Zambales Ophiolite Complex is a complete ophiolite suite composed of residual harzburgites and lherzolites, layered to massive ultramafic and mafic cumulates, dike-sill complexes and volcanic rocks. It is composed of three massifs – Masinloc, Cabangan and San Antonio (Figure 1). The Acoje block preserves island (IA) signatures while the Coto block manifests transitional mid-ocean ridge (MOR) – IA characteristics (e.g. Yumul, 1989; Evans et al, 1991). The geology of the Masinloc Massif has been extensively discussed in the literature (e.g. Nicolas and Violette, 1982; Bacuta et al, 1990; Rossman et al, 1989) and what will be presented in the geology of the Cabangan and San Antonio Massifs.

The Cabangan Massif is made up of residual harzburgites, a thin transition zone dunite and gabbros. The sheeted diabase like complex and pillow basalts are found in the southeast portion of this massif specifically in the Subic-Olongapo area (Figure 1). The residual harzburgites, of MOR affinity based on spinel chemistry, are cut by numerous IA diabase - diorite - microgabbro dikes (Yumul et al, 1990). The harzburgites exhibit protogranular to porphyroclastic textures (terms after Mercier and Nicolas, 1975). Harzburgite- and dunite-hosted refractory-type chromitites occur as bands, pods and lenses (Yumul, 1992). The dunites manifest an equigranular texture. The gabbro outcrops encountered are mostly massive and are principally made up of plagioclase, clinopyroxene and olivine which exhibit mesocumulate to orthocumulate textures (e.g. Wager and Brown, 1968; Irvine, 1982).

Intensely tectonized, layered clinopyroxenites and gabbronorites are exposed as hills (e.g. Sindol Hill) along the western edge of the Cabangan massif (Figure 1). These clinopyroxenites and gabbronorites, as will be shown, later, are allochthonous blocks which are not related to the generation of the Cabangan Massif. In the Subic - Olongapo area, sheeted diabase dikes intruded into pillow basalts are exposed. The sheeted diabase dikes are not related to the diabase - diorite - microgabbro dikes intruded into the Cabangan Massif residual harzburgites. The basalts are porphyritic to aphyric and are usually pillowed.

The San Antonio Massif, on the other hand, is composed of harzburgites - lherzolites, a transition zone dunite and a well-developed layered clinopyroxenite - gabbronorite unit distributed from west to east. An east-west traverse from the western most portion of the San Antonio massif to the Cabangan massif Subic - Olongapo area defines a complete ophiolite suite. Geochemical data show that this complete ophiolite sequence is actually a JUXTAPOSED IA terrane (San Antonio Massif) and a MOR/ marginal basin - like crust (Subic-Olongapo area, Cabangan Massif) (Yumul et al, 1990).

The San Antonio Massif harzburgites - lherzolites are fertile and cannot be directly parental to the overlying arc-related dunites and layered ultramafic - mafic sequence (e.g. Yumul, 1992). Textures range from protogranular to porphyroclastic. The transition zone dunite, with equigranular texture, is host to matallurgical-type chromitites. The layered clinopyroxenites, together with the dunites, are well-ex-



posed along the southern coast of the San Antonio Massif. These rocks are characterized by large, equant, euhedral clinopyroxene crystals defining adcumulate textures. The massive to layered gabbronorites have orthopyroxene as an early crystallizing cumulus phase. Textures range from adcumulate to mesocumulate.

A direct comparison of the San Antonio and Cabangan Massifs show their distinct differences:

<b>SAN ANTONIO MASSIF</b>	
Crystallization order	OL $\pm$ SP $\rightarrow$ PX $\rightarrow$ PL
Residual peridotite	Lherzolite-harzburgite
Cumulate sequence	Well-developed transition zone dunite Well-developed ultramafic cumulates-dunite, websterite, wehrlite, clinopyroxenite Orthopyroxene-rich mafic cumulates- gabbronorite, norite, norite-gabbro with gabbro and anorthosite
Chromitite deposits	Metallurgical type
<b>CABANGAN MASSIF</b>	
Crystallization order	OL $\pm$ SP $\rightarrow$ PL $\rightarrow$ PX
Residual peridotite	Harzburgite
Cumulate sequence	Thin transition zone dunite No layered ultramafic cumulate Clinopyroxene - rich mafic cumulates - gabbro, troctolite, olivine gabbro with anorthosite
Chromitite deposits	Refractory type

Available geological and geochemical information show that the the Acoje block of the Masinloc Massif and the San Antonio Massif are correlatable in terms of geologic setting and lithological distributions.

## DISCUSSION

### **San Antonio Massif: terrane translation through the Subic Bay Fault Zone**

A straight east - west traverse from the San Antonio Massif to the Subic - Olongapo area of the Cabangan Massif defines a complete ophiolite sequence (Figure 2). Nevertheless, the sheeted diabase dike complex and pillow basalts exposed in the Subic - Olongapo area have transitional mid-ocean ridge basalt (MORB) - island arc tholeiite (IAT) characteristics. On the contrary, the mineral chemistry of the San Antonio Massif dunites, layered clinopyroxenites and gabbronorites point to an island arc environment of formation similar to that of the Acoje block.

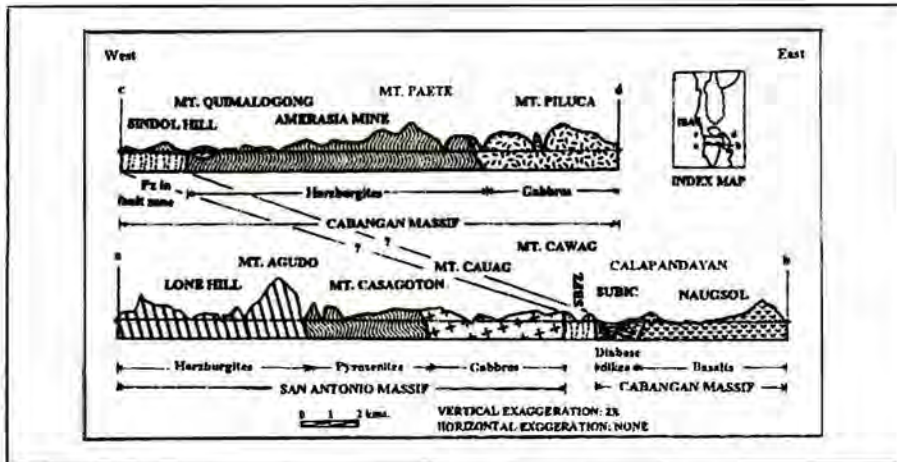


Figure 2.

The difference in the geochemical signature between the San Antonio rock suites of arc affinity and the transitional MORB - IAT diabase - pillow basalts of the Cabangan Massif strongly suggest that a tectonic contact between the two massifs is responsible for their juxtapositioning.

Considering all available information, the San Antonio Massif is believed to have been rifted from the Acoje block and translated southward to its present position. Translation was made possible through movements along the West Luzon Shear/Subic Bay Fault Zone. This will explain the presence of the tectonized layered clinopyroxenite - gabbro-norite hills found along the western edge of the Cabangan Massif. These hills were left during the translation process. A fault zone, which is called here as the Subic Bay Fault Zone, separates the San Antonio island arc terrane from the Subic-Olongapo MOR-like crust.

### Subic Bay Fault Zone: Geological evidence

There are two possibilities that can be thought of with regards to the origin and configuration of the SBFZ. The SBFZ can be considered as a splay of the West Luzon Shear Zone of Karig and others (1986) or that it can just be a splay of the San Antonio Fracture Zone (Yumul et al, 1990). Magnetics and gravity data do not support the presence of the San Antonio Fracture Zone (Dimalanta and Yumul, 1996). It is more viable to model the SBFZ as the southeastern extension of the West Luzon Shear.

Available magnetic and gravity anomaly maps both delineate a distinct linear feature (Figures 3a and 3b). This feature is defined on the basis of nearly linear and parallel contour lines. The rather large and steep magnetic and gravity gradients is associated with the geophysically-defined fault zone which corresponds to the Subic Bay Fault Zone (Dimalanta, 1996).

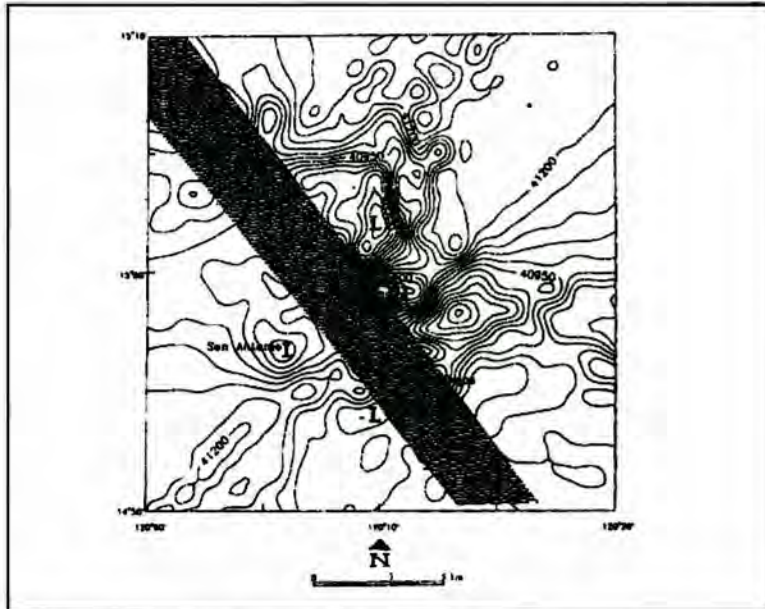


Figure 3a.

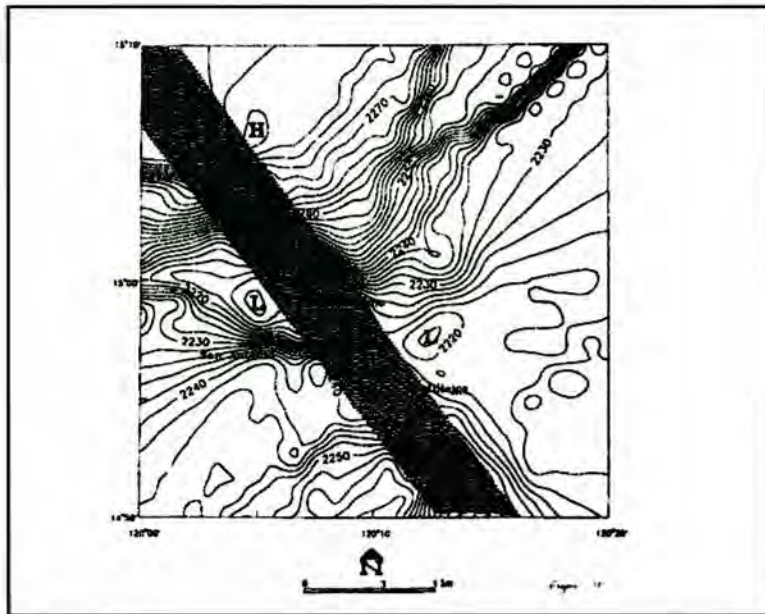


Figure 3b.

More importantly, the recognition of the SBFZ presents a different regional distribution of structures in the ZOC. Our available data do not support the presence of normal faults and a graben in the area between the Cabangan and San Antonio Massifs (Figures 4a and 4b).

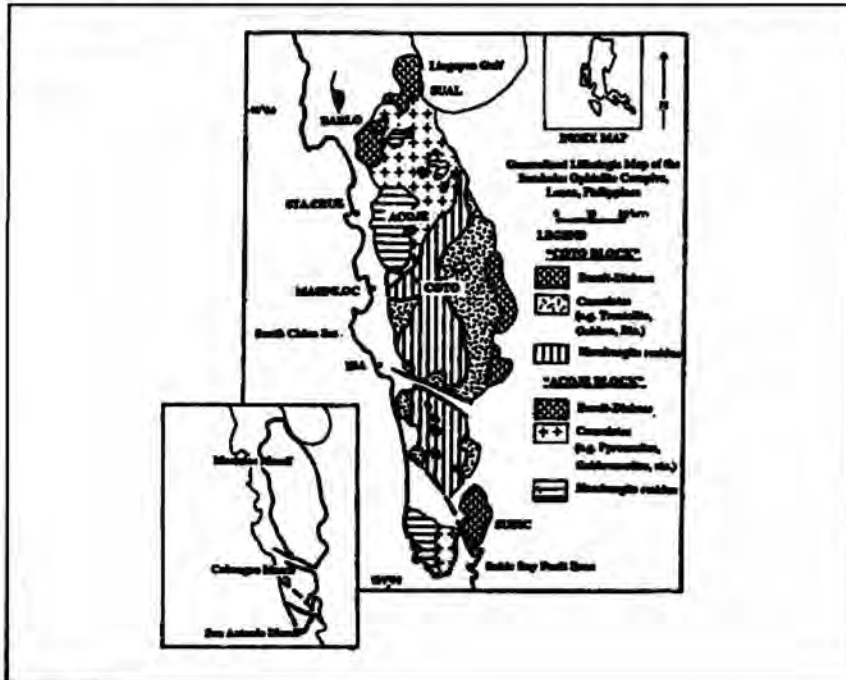


Figure 4a.

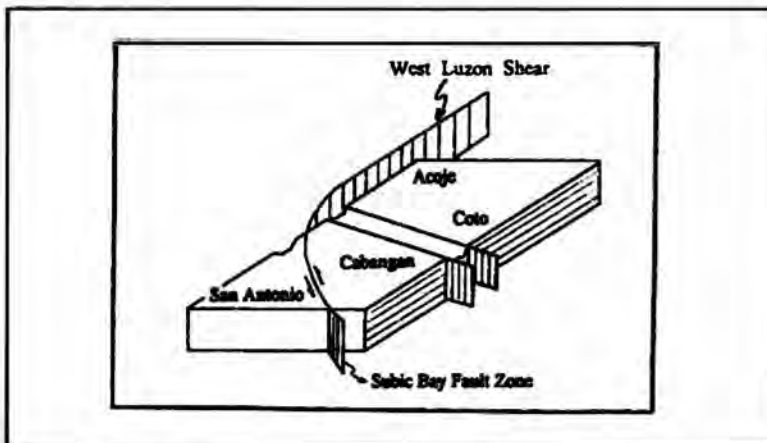


Figure 4a.



## ***SOCIAL SCIENCES***

### **THE EFFECTS OF LANGUAGE FORMATION MATHEMATICAL LEARNING AND PERFORMANCE: AN INFORMATION-PROCESSING PERSPECTIVE**

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#### **ABSTRACT**

*Using an information-processing perspective, the paper addresses the question on the effect of using a bilingual's first or second language on learning and doing math. The perspective assumes that math performance can be analyzed into smaller components, and that language factors can affect these components in different ways. The paper integrates the results of 16 experiments, each of which studies the effects of language format on six components of word problem solving using different experimental paradigms in cognitive psychology. Four possible effects of language format on the components were considered and the results of the experiments were compared to these hypothesized effects. The varying effects of language on word problem solving performance were integrated in a proposed "language-routing model" which specifies how language factors effects specific components of math performance.*

**Keywords:** *Abilities; Bilingualism; Cognition; Education; Information Processing; Language; Learning; Mathematics; Problem-Solving; Thinking.*

#### **INTRODUCTION**

What is the effect of using a bilingual's first or second language on bilingual's mathematical learning and performance? The question about the relationship between language and mathematics has both theoretical and practical significance. At the theoretical level, the question relates to a larger issue regarding the relationship

between language and thought, an issue that has long concerned psychologists, anthropologists, and philosophers, among others. At the time practical level, the question relates to issue regarding the language of instruction especially in bilingual and multilingual communities. In the Philippines, research on the relationship between language and mathematics has been undertaken by researchers in psychology, linguistics, and education. These researches typically involve the comparisons of instructional materials and/or procedures in Filipino or English using unitary measured of mathematical achievement or ability.

In my own research I have been using the information-processing approach to study the effect using Filipino or English on mathematical learning and performance. In this paper, I summarize the findings of a series of 16 cognitive psychological experiments that look into the effects of using either English or Filipino on the operation of specific components of mathematical skills among Filipino-English bilinguals.

### RELATING LANGUAGE AND MATHEMATICS

One of the most compelling hypotheses about the relationship between language and thoughts was articulated by Whorf (1956). He proposed the linguistic determinism hypothesis which states that the structures of language determine the structures of thought. That is, how we perceive, experience, and think about the world depends on the elements of the language we use to talk about the world. This rather strong prescription has long been discredited, particularly with the increase in scientific knowledge about language and about thought. Recent empirical evidence seems to favor two other positions: (a) the structures of thought determine the structures of language (Gerrig & Banaji, 1994) and (b) structural variations in language result to parallel variations in thought; actually, a weaker version of linguistic determinism (Hunt & Banaji, 1991).

The experiments I will describe has been derived from this weaker version of linguistic determinism. In particular, the conceptual framework assumes that using either Filipino or English might result to variations in mathematical performance among Filipino-English bilinguals. The goal in the series of experiments was to determine the specific effects of using either language on particular aspects of mathematical skill.

At a very general level, we can propose four possible effects of using Filipino and English on mathematical performance. First, there could be a *null effect* that is, there is no relationship between the language used and mathematical performance. It is conceivable that mathematical activity is such an abstract process that it is not affected by contextual factors like language. Second, there could also be a *language-proficiency effect*; that is, mathematical performance might depend on the person's proficiency in the language being used. Therefore, mathematical performance is better when the task is rendered in the person's more fluent language. Third, there could be a *language-of-learning effect*; that is, performance

could be better when the task is rendered in the language that was used in acquiring the concepts and procedures. It is possible that math performance is better when the tasks are rendered in the language one has become used to. Finally, there could be a *structural-fit effect*; that is, the structures of one language are more adequate in handling the processing of mathematical concepts and operations. Some people who argue that mathematics should and could only be taught in English are actually implicitly assuming that the English language is structurally more adequate than Filipino for processing mathematical information.

### **THE INFORMATION-PROCESSING APPROACH: DEFINING THE COMPONENTS OF MATHEMATICAL SKILL**

There are many different ways psychologists try to understand and explain cognitive functioning, including mathematical performance. However, cognitive psychologists use an information-processing approach to study the different cognitive functions. This approach assumes that any cognitive activity involves the transformation of information through a series of processes (procedures or operations) that also draw upon existing knowledge representations (or schemata). The analogy is often made between cognitive functions and computer programs. Both include a series of procedures (routines, subroutines, etc.) that operate on information that is encoded or "inputted") and that also draw from internal memory representations.

An important element of this assumption is the notion that any cognitive function could be described as consisting of a complex of smaller functions, which could be further described as having even smaller functions. These smaller elements of a cognitive function are called components and subcomponents. These components and subcomponents are procedures and schemata that operate on specific input. The output of specific components and subcomponents could feed into other components, and so on. The operation of all the component and sub-component parts are related in specific ways that make one cognitive activity.

Using the information-processing approach, we can, therefore, look at mathematical skill as being composed of smaller components and subcomponents. So rather than thinking of mathematical skill as a monolithic, generalized skill, we can think of it as being a composite of several specific but interrelated skills.

If we conceive of mathematical skill in this way, we can then think of language as having a different specific effect on each component, depending on the character of the component. In my research program, I have been working on the assumption that the effect of using Filipino or English in mathematical activities is not a unitary effect. It is conceivable and very possible that the use of either language would have different specific effects depending on the particular component of mathematical skill.

The experiments I describe look into six components of mathematical skill. These are (a) problem-text comprehension, (b) problem-model construction, (c) re-



trieval of specialized meanings, (d) retrieval of number facts, (e) retrieval of relevant analogous problem information, and (f) application of analogous problem information. These six components could be seen as parts of two very related tasks: solving math word problems and transferring information between analogous math word problems. The relationships among the different components are specified in detail theories of word problem solving (see e.g., Kintsch & Greeno, 1985), and analogical transfer (see e.g., Ross, 1984, 1989).

### **The Effects of Language on Components of Mathematical Skill**

To study the effects of language on the six different components of mathematical skill, different cognitive tasks were used to target the particular components. The task was then presented in either Filipino or English to Filipino-English bilingual subjects. The specific effects were then assessed by comparing performance in the Filipino and the English tasks. In this paper, the description of the specific methods used and the results found in the different experiments will be rather brief. I refer the interested readers to the specific papers for more detailed accounts of the methodology and of the results.

#### **Problem-Text Comprehension**

When given math word problems to solve, a person's first task is to read the text and develop an understanding of the information being described in the text. This is the component of problem text comprehension which results to the construction of the text base. In a way, this is a straightforward reading task. The text base is later used as the basis for constructing the abstract problem model.

I studied problem text comprehension in two experiments by using a recall paradigm (Bernardo, 1996a, 1997). Beginning arithmetic students were given simple word problems in either Filipino or English and later asked to recall the problems. The assumption is that the manner of recall will be based on the students' comprehension of the different story elements. I found that recall was reliably better in the students' first language, whether Filipino or English. Error analysis further indicated that students had more gross comprehension errors in their second language. It seems that students comprehend the problem text better in their first language. Moreover, consistent with the view that problem solving begins with this reading process, students were also better in solving the problems when it was in language in which they comprehended it better. Therefore these results show a language-proficiency effect: Students were better at comprehending problems in their first and more fluent language.

#### **Problem-Model Construction**

From the text base, the word problem solver constructs a representation of the structural elements of the problems (i.e., the quantitative relations, etc.). This

representation is called the problem model and is used as the basis for formulating the solution.

To study the effects of language on problem-model construction, I used a problem completion paradigm in two experiments (Bernardo, 1996a, 1997). Proficient problem solvers were given Filipino and English arithmetic word problems without the final question. They were asked to provide a question for each incomplete problem. The assumption was that if the problem solver correctly constructed the problem model, he or she would be able to supply the appropriate question based on the information specified in the problem model and using knowledge about prototypical problem models. I found that there was no difference in the subjects' ability to complete the problems in Filipino and in English. It seems that subjects were able to construct the problem model just as effectively in either language, regardless of what their first language was, and despite the fact that the subjects were taught word problem solving in English and using English word problems.

Therefore, these results show a null effect. It seems that as far as problem-model construction was concerned, language factors have no effect. These results suggest that language does not seem to affect the more abstract elements of math word problem solving.

However, subjects with English as first language performed this task better than those whose first language was Filipino. I think this reflects an interactive effect of language proficiency and language of learning. That is, it may be that those who are more proficient in English learn the process of problem-model construction more effectively because the students are given word problems in English. Even if the process that needs to be learned is essentially abstract, the students can only encounter this information by first routing it through language. Hence, the student who has better facility with the route will learn the abstract processes better. Once they have learned the abstract material, then the language route does not matter anymore, as shown by the earlier results.

### **Retrieval of Specialized Meanings**

One specific subcomponent of constructing problem models in the task of interpreting words and phrases in specialized ways. For example, the phrase, "gave three candies to" can be translated as "plus three" in the problem model. A word like "more" also takes a very specific meaning in problem model construction in math: "more by a specified amount".

In three experiments (Bernardo, 1996b), I studied the extent to which this task was entrenched by comparing interpretations of words like "more" and "less" in mathematical context (i.e., in algebra problems) and a non-mathematical context (i.e., in a story). The results of two experiments showed that the retrieval of specialized meanings was stronger in the language in which subjects learned word problem solving (i.e., English). The results of one experiment showed no difference between English and Filipino, however, this experiment used a procedure quite different from the other two.

These results suggest a language-of-learning effect. It seems the task of retrieving specialized meanings of words is more entrenched in the language in which this task was learned and more often done. Since the subjects encountered math word problems only in English, the specific skill seems to have been better established for English problems. However, the inconsistency in the results warns us to accept this conclusion with some caution.

### **Retrieval of Number Facts**

In computing for the solutions to the word problems, the proficient problem solver has to retrieve basic number facts like " $1 + = 4$ " from memory. Young or novice problem solvers arrive at the answer using strategies other than memory retrieval. However, research (e.g., LeFevre, Bisanz, & Mrkonjic, 1988; Zbrodoff & Logan, 1990) has shown that among people with high numeracy skills, the answers to simple addition and subtraction operations are derived using highly automated memory retrieval processes.

In five experiments (see e.g., Bernardo, 1996c), I studied the effect of language format on the retrieval of number facts using a verification paradigm. Subjects were presented a pair of addends followed by a number. The formats of this information (numerical, verbal Filipino, or verbal English) were varied across blocks of trials for the addends and within blocks for the last number. Subjects were asked to verify whether the number that followed the addends was the sum of the addends. Their verification times were measured. The results show that processing number facts in numerical and verbal-English format was highly automated (fast and flat verification times and low error rates), but processing in verbal-Filipino format was very inefficient (slow and variable verification times and high and variable error rates).

The results show a language-of-learning effect. It seems that the subjects have automated processing number facts in English because that is the language that they have most often processed the information. Again, it is possible that the effect is a structural-fit effect; the English format items actually require shorter articulation time. So it may be that processing in English is faster simply because of the brevity of the items. However, the difference in articulation of the English and Filipino number words does not seem to be commensurate to the difference in verification times nor is it consistent with the error rates. It is possible however, that the brevity of English number words makes it the preferred format in practice, hence the effect is still consistent with a language-of-learning effect.

### **Retrieval of Analogous Problem Information**

One effective strategy for solving a problem is to use a solution that worked in an earlier analogous problem. Since the problems have analogous problem models, the solution that worked in one should work for the other. The first component of this analogical problem solving strategy is the retrieval of the relevant analogous problem.

In two experiments (Bernardo, 1994a, 1996d) I studied the retrieval of analogous word problems in basic probability using a reminding paradigm. Subjects were given a set of word problem to study either in Filipino or English. These problems included explanations about the relevant principles, equations, and solutions. They were then given a set of analogous test problems in Filipino and English, which they were asked to solve after being given some cue about the relevant analogous problem. The subject's solutions were scored in terms of whether they showed the retrieval of the appropriate analogous problem information. The results showed that subjects retrieved the analogous problem better if the language formats of the old and new problems were the same. That is, if they were given a test problem in Filipino, they were more likely to retrieve the relevant problem if this was studied also in Filipino. There was no overall advantage of studying or testing the problems in either language.

The results of the experiments show a language-of-learning effect. In this case, the subjects were more likely to retrieve relevant information when the language used for learning matched the language used for testing.

#### **Application of Analogous Problem Information**

Analogical problem solving does not only involve the retrieval of the relevant analogous information. The problem solver must also be able to correctly apply or instantiate the information from the old problem to the new problem information.

In two experiments (Bernardo, 1996d) I studied whether language affected the application of analogous information by using a modified reminding paradigm. The procedure described in the previous section was used except that the required equations were provided. The task that remained was to apply the information to the new problems. The results showed that subjects performed equally well for the Filipino and English study and test problems. Once they have the relevant information, they can apply the information to any language format. These results show a null effect of language. It seems that the application of abstract concepts and procedures is not affected by language factors.

### **TRENDS AND CONCLUSIONS**

The results of sixteen experiments on six components of mathematical word problem solving reveal different effects of language factors. First, I found null effects on two components that involve abstract concepts and procedures: problem-model construction and the use of analogous problem information. Both these procedures operate at that level where the problem solver already has access to the abstract structural elements of the problem. It seems, therefore, that as regards the use of abstract problem elements, language factors have no effect.

Second, I found a language-proficiency effect in the component of problem text comprehension. The structural information in math word problems is couched in linguistic format, and the problem solver first needs to operate linguistic pro-

cesses before the structural elements can be activated. As far as this process is concerned, the problem solver's effectiveness will depend on his or her proficiency with the language of the problem. These findings are most consistent with earlier research demonstrating the effects of semantic and syntactic factors in problem comprehension (e.g., Cummins, Kintsch, Reusser, & Weimer, 1988; De Corte & Verschaffel, 1987; De Corte, Verschaffel, & De Win, 1985).

Third, I found language-of-learning effects in three components: retrieval of specialized meanings, retrieval of number facts, and retrieval of analogous problem information. Notice that all these components involve the retrieval of information from existing knowledge representations. It seems that the retrieval of this information is very much dependent on some match between the language during retrieval and the language used in the process of developing these representations. This finding is most consistent with research demonstrating how learners use contextual information in developing abstract problem representations (Bernardo, 1994b) and how the activation of such information is hyperspecific to the problem context (Bernardo & Okagaki, 1994).

Finally, I found an interactive effect of language proficiency and language-of-learning in problem-model construction. It seems that students whose first language matched the language in learning or teaching the component of problem-model construction acquired the component skill better than those for whom these two factors did not match (i.e., those whose first language was not English). Again, consistent with earlier research (Bernardo, 1994), the route to learning abstract information involves language processing. The learner's success in learning to some extent depends on his or her facility with language processes.

The most glaring finding in these series of studies is that there is no unitary effect of language factors on mathematical performance. This should serve as a strong cautionary statement for those who have been or who plan to make sweeping statements about the role of language in mathematical performance, mathematical learning, or mathematical teaching. The specific effect language factors depends on the character of the particular components of mathematical performance.

However, I do not wish to give the impression that the varied effects of language on math performance cannot be understood in a coherent frame. For the purpose of providing a tentative organizing theoretical framework, I propose a routing model with the following general and specific propositions:

- (1) Mathematical concepts and procedures are essentially abstract in character, however, most of these concepts and procedures can only be learned by couching them in linguistic structures.
- (2) Components related to learning are affected by language factors, but those which operate independent of the learning components are not.
  - (2.1) Early learning of these concepts and procedures is dependent on the learner's proficiency in the language used to convey these concepts and procedures.

- (2.2) Because of encoding-specificity in retrieval, activation of learned concepts and procedures is more efficient in the language context in which these concepts and procedures were learned.
- (2.3) The operation or use of concepts and procedures which are separate from retrieval processes are not affected by language factors.

This routing model is an attempt to develop a cognitive psychological framework for understanding the relationship between language and mathematical skill. I am still currently conducting more experimental studies to validate, modify, and fine tune this model. Hopefully, this model will not only be of theoretical interest to those of us who study the human mind, but it should also be of use to those who have a stake in the development of the human mind.

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## LOCALLY-DEVELOPED PSYCHOLOGICAL TESTS: A CRITICAL REVIEW

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### ABSTRACT

*Previous reviews on the status of psychological measurement in the Philippines pointed out the twin-problem of the inapplicability of foreign-made tests and the dearth of locally developed tests. In these reviews, researchers were urged to go into developing original and local psychological tests. The author's current effort to document and organize existing work indicate an upsurge in the development of indigenous psychological measures. This study aims to identify trends in local psychological test development, and to provide an initial evaluation of their reliability and validity. The study is being conducted in two phases: first, locally developed psychological tests are bibliographically documented and annotated; and second, the technical features of the tests are meta-analytically evaluated, based on general information (nature and purpose of test, example of items, scores available, method of scoring, administration time, forms available, and cost), and technical (validity, reliability, and norms) and practical features (ease of administration, scoring, and interpretation, and adequacy of test manual and accessory materials). Preliminary results indicate an increase in measures of a wide variety of Filipino characteristics, using the Filipino language. However, despite the focus on indigenous measures, no follow-up studies on these tests' initial reliability and validity indicators have been done.*

**Keywords:** *local test development, psychological tests, indigenization, Filipino personality, Filipino intelligence, aptitude testing, achievement testing, meta-analysis, test reliability, test validity.*

### INTRODUCTION

In various reviews of the status of psychological measurement in the Philippine setting, the twin-problem of the inapplicability of foreign-made tests and the dearth



of locally developed tests, was pointed out time and again. While some progress has been achieved in test development in the educational setting, a deplorable lag in the development of psychological tests for application in the clinical and industrial settings was observed (Lazo, L., de Jesus, L., and Tiglaio, R., 1976; Ramos, E., 1977; Lazo, L., 1977; Carlota, A., 1980; and Guanzon, M.A., 1985). Carlota (1980) noted several trends in personality measurement, and cited developments in the areas of personality testing, and the measurement of abilities and aptitudes, and of deviant behavior. In all of these reviews, researchers and scientist-practitioners were urged to go into developing original and contextualized psychological tests.

Over the past decade, the authors, together with colleagues handling the psychometrics classes in their respective universities, have asked their students to critique various foreign- and locally- made psychological tests. They noted that in the 1980s and 1990s, more and more efforts were put into local test development. The combined listings that they had as of the end of 1996 came up to close to a hundred studies. These studies included not only test adaptations and translation equivalence studies (as had been the trend up to the 1980s), but also investigations into the construction, validity, reliability and norm development of local tests.

Regular updating of this bibliographic compilation of local psychometric studies is an on-going activity of the psychometric students of the University of the Philippines and De La Salle University. The pace in collecting data was increased in the academic school year 1996-1997, with the undergraduate psychology students of the authors as well as those of Prof. Robert Javier of De La Salle University heavily involved in the first phase of the study.

While a current bibliographic listing of locally developed tests to date is valuable in itself, and evaluation of the scientific features of these tests is even more important. This study therefore aims to evaluate the status of local psychological test development, with the scientific criteria of test reliability and validity applied uncritically to each of these tests. The study will point out gaps in the literature and provide possible directions for future research, pinpointing mediating or interactional trends that may not be too apparent in the individual studies. The authors also hope to identify outlying studies as these may lead to a better understanding of the local test development situation. Subtleties and nuances otherwise not observable in the development of these local tests will be teased out and may thus provide new insights towards the formulation of new hypotheses.

## METHOD

The study is being conducted in two phases.

In the first phase, locally developed tests were bibliographically documented, with research and educational institutions primarily sourced the data. The authors independently located and documented studies on psychological testing and assessment, after which their listings were combined. Attempts were made to scout the resources of other universities and teachers handling psychological measurement

classified, particularly those outside of Metro Manila. The scope of the current study however, includes only the material found in Metro Manila institutions, due to the lack of positive response from institutions outside of Metro Manila. In deciding on the inclusion and exclusion criteria for the study's listing, attention was given to published tests as well as unpublished studies consisting of graduate and undergraduate papers in educational and psychological testing and measurement. Program evaluation studies as well as measures which were developed for highly specific populations and purposes, however, were excluded. For example, training evaluation instruments, assessment of intervention studies in particular institutions, and locally developed admissions examinations of specific schools and universities, were excluded from the current study's listing of locally developed tests.

General and specific information regarding each of the tests were then documented in as follows:

1. Identifying data (test title, author, publisher, date of publication, and location of test)
2. General information (nature and purpose of test, grade or age levels covered, examples of items, scores available, method of scoring, administration time, forms available, and cost)
3. Technical features (validity, reliability, and norms)
4. Practical features (ease of administration, ease of scoring, ease of interpretation, and adequacy of test manual and accessory materials).

A detailed and explicit codebook (see Appendix A) was developed, based on the evaluation outline followed in documenting the various test characteristics. The evaluation outline is reproduced in Appendix B. Both substantive and methodological characteristics of the tests were coded, such as the year when the test was developed and/or published, test's sample characteristics, the research designs pertaining to the establishment of norms reliability, and validity, source of the study, and so on.

At this point of the research, information on 86 of the tests in the listing have already been encoded, while the rest of the data that have been documented are still being independently evaluated by the authors prior to computer encoding.

The second phase of the study is the meta-analytic evaluation of the various features of the tests, focusing on these tests' advantages and limitations for local use. This will be done upon the completion of the data-gathering phase of the study. Stouffer's combined test (in Wolf, 1986), and Cohen's effect size (Cohen, 1965; 1977) using both correlational relationships and group differences, will be the main strategy used in the meta-analysis that will be done. This will entail blocking or grouping the studies into sets with common output measures, and applying the appropriate statistical formulae, i.e., converting the various studies' p values into z values, summing these z values and dividing them by the square root of the number of tests combined for the analysis, and then computing for effect size. Summary

indices of the statistical significance of the various studies' results pertaining to the hypotheses made about the tests' validity, reliability, norming procedures, etc., will therefore be provided.

### PRELIMINARY RESULTS AND DISCUSSION

In the authors' recent effort to document and organize existing work, both published and unpublished, their bibliographic listing of locally developed test indicate an upsurge in academic interest in the development of indigenous psychological measures. A mere listing of locally developed measure over the years demonstrates how this interest has grown by leaps and bounds from the handful of tests in educational psychology which were locally developed in the 1950s. The 1960s listing shows the interest in personality testing of the projective type, the 1970s in creativity, self-perception, personality and vocational testing, and the 1980s an increased interest in personality testing, with a number of researchers doing studies on the Filipino child and the adolescent. In the 1990s, tests were developed to measure a wide variety of Filipino characteristics – *katalinuhan*, *pagkarelihiyoso*, *kaasalang sekswal*, *kakayahang magdala ng tensyon*, *pagkamabahala*, *kahustuhang emosyonal*, *kakayahang berbal sa Filipino*, Filipino management style, dementia screening, empathy, and trustworthiness, to name a few.

The material gathered to date is presented in a series of tables, with locally developed general intelligence (N=13), interest (N=3), aptitude (N=15), achievement (N=47), and personality (N=11) measures listed in Appendix Tables 1 to 5, respectively.

Measures of specific personality characteristics (N=46), aptitudes (N=16), cognitive abilities (N=9), attitudes (N=14), values (N=9), and behaviors (N=13) are listed in Appendix Tables 6 to 11, respectively.

Appendix Table 12 lists those psychological and educational measures which were also locally developed (N=12) but could not be easily categorized into the earlier-mentioned tables.

Appendix Table 13 presents the foreign-made tests that have been studied and adapted for local use (N=18), while Table 14 lists the foreign-made tests which have been translated into Filipino (N=4), with these translations analyzed for translation equivalence with the original English versions.

As can be seen in the various Appendix Tables, the bulk of local test development efforts across the years has been in the areas of the evaluation of achievement, and the assessment of specific personality characteristics. Unfortunately, most of these tests are unpublished, and remain in the archives of university libraries, in offices of private companies providing psychological test develop or program evaluation, and in the personal files of teachers who have encouraged students to pursue original test construction. Considering the voluminous amount of work that has gone into the construction of these local test,

the absence of any systematic documentation of these material has rendered the data-gathering extremely difficult. Consequently, the present study cannot lay claim to the comprehensive of its data.

Results to some of the local psychological tests' scientific usefulness (in terms of the tests; reliability and validity) are encouraging. However, despite the focus on indigenous measures, there were no follow-up studies on the tests' initial reliability and validity indicators, except for the Philippine Mental Ability Test (Padilla, 1947), Philippine Personality Inventory (Gapuz, 1959), the Philippine Thematic Apperception Test (Lagmay, 1965), the Philippine Otis-Lennon Mental Ability Test (1972), the *Panukat ng Ugali at Pagkatao* (Enriquez, 1975), the *Panukat ng Pagkataong Pilipino* (Carlota, 1985), the Filipino Self-Appraisal Inventory (Evangelista, 1986), and the Philippine Aptitude Classification Test (1987). Furthermore, based on the experience of the authors in the collation of the materials for the current study, there is a strong need for a group or a body that will attend to the dissemination and the use of these standardized tests, and to protect them from indiscriminate use and abuse.

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Appendix Table 1. Locally Developed Measures of Intelligence (1947-1994)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1947	The Philippine Self-Administering Test of Mental Ability Test 1963 An analytical study of the PMAT, 1947 reprint, Form II (Antonio Isauro, Xavier University) 1971 A Critical Evaluation (Intong, L., Xavier University)	Sinforoso Padilla	U.P.
1952	Philippine Self Administering Intelligence Test	M.F. Rodriguez	Centro Escolar University
1953	Mental Ability Test for Philippine Secondary Schools	Leila Padilla	U.P.
1954	Test of General Mental Ability for High School Students a.k.a. Ordoñez-Bugarin General Mental Ability Test 1956 A further use and study of the test (Almcdral, R., Nat. Teach. Coll)	Ordoñez and Mena T. Bugarin	National Teachers College
1968	Manila Self Administering Test of Mental Ability	Guidance and Counseling Services for the Secondary Level, Division of City Schools	Department of Education, Culture, and Sports
1969	Philippine Non-Verbal Intelligence Test	G. Guthrie, S. Tayag, and P.J. Jacob	Philippine Normal University
1984	Content-Indigenized Subtests of Mental Ability for Children in a Philippine Barrio	Marcia S. Katigbak	De La Salle University
1984	Adaptive Competencies of 4- to 9-year-old Filipino Children: Alternative to Traditional Intelligence Measurement	Gundelina A. Velazco	De La Salle University
1987	Philippine Mental Ability Test for Use in Business and Industrial Settings	Flordeliza Punongbayan	Philippine Women's University

Table 1 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1990	General Mental Ability Test	Evangeline M. de Jesus	Data being verified
1990	<i>Panukat ng Katalinuhang Pilipino</i>	Aurora R. Palacio	U.P.
1993	Primary Mental Ability Test for Filipino Children	N.T. Vargas-Bcnito	University of Sto. Tomas
1994	Philippine Indigenized Pre-School and Primary School Intelligence Tests	Sr. Ellena Karena Taylor	De La Salle University

Appendix Table 2. Locally Developed Measures of Interest (1977-1991)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1977	Cooperative Vocational Testing Project	Leticia Asuzano, Rose Marie Salazar, and Salud Evangelista	De La Salle University
1986	Philippine Occupational Interest Survey	Center for Educational Measurement (CEM)	CEM
1991	Interest Test on Enterprising and Investigative Occupations	Alvin Vy and Reynaldo Soriano	De La Salle University

Appendix Table 3. Locally Developed Aptitude Tests (1956-1995)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1991	Interest Test on Enterprising and Investigative Occupations	Alvin Vy and Reynaldo Soriano	De La Salle University
date being verified	Panukat ng Oryentasyong Bokasyonal	Glenda Banawa, Ma. Suzanne Bonza, Pamela Gregorio, Rosalyn Reyno, Christopher Marquez, and Mary Rose Tan	U.P. Department of Psychology
date being verified	Philippine Trade Prognosis Test	Bureau of Vocational Education	Department of Education, Culture, and Sports
date being verified	National College Entrance Examination	National Educational Testing Center	Ministry of Education, Culture, and Sports
date being verified	The College Aptitude Examination	M. Ordoñez	Polytechnic University of the Philippines
1956	Philippine Vocational Interests Records	Bureau of Public Schools, Manila	Department of Education, Culture, and Sports
1963	Predicting Freshman College Success at Philippine Universities	L. Q. Arquiza	Data being verified
1965	College Scholastic Aptitude Test	Center for Educational Measurement (CEM)	CEM
1967	Entrance Examination for First Year in Quezon City	Hermenegilda Margate	U.P. College of Education
1973	Measuring Non-Verbal Academic Aptitude at the U.P.	Emeteria Pascual Lee	U.P.
1985	Giftedness Inventory	Child and Youth Research Center	Department of Education, and Sports Division)
Culture, (Testing)			
1987	Scales for Identifying Gifted Children	Floreida Cabrerros	U.P. College of Education
1993	National Medical Admissions Test	Center for Educational Measurement (CEM)	CEM

Table 3 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1987	Philippine Aptitude Classification Test 1987 Occupational Norms (E. Pabualan) 1987 Occupational Norms (E. Valmores)	Center for Educational Measurement (CEM)	CEM
1994	Computerized College Admission Test	Evelyn B. Dacayo	Pamantasan ng Lunsod ng Maynila
1995	A Psychological Test for Medical Representatives in a Pharmaceutical Company	Dolores M. Matias	U.P. Department of Psychology



Appendix Table 4. Locally Developed Achievement Tests (1962-1991)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1962	Freshmen Classification Test in Grammar for the Philippine	Monico Corpuz	Philippine Normal College Normal College
1963	A Written Classification Test in Speech for Freshmen College Students of the Philippine Normal College	Candida Domingez	Philippine Normal College
1964	A Written Classification Test in Listening Comprehension for First Year Students of Public General Secondary Schools	Socorro Mejorada	Philippine Normal College
1964	Written Classifications Test in Grammar for High School Freshmen	Elisa T. Carlota	Philippine Normal College
1964	Achievement Test in English Grammar of Freshmen in Government Schools	Maurital Plaza	Philippine Normal College
1964	Achievement Test in Fourth Year High School English	Teresa A. Carag	U.P. College of Education
1964	Listening Comprehension Test for First Year Students of Public General Secondary School	Socorro F. Mejorada	Philippine Normal College
1965	English and Arithmetic for Intermediate Grades	Jemina Cruzado	Silliman University
1965	Listening Comprehension Test for Secondary School Freshmen	Adoracion M. Halili	Philippine Normal College
1966	A Standardized Achievement Test in Grammar for Grade 4	Felicidad Sibayan	Philippine Normal College
1966	Achievement Test in the Ability to Write in English for Grade 5	Patrocinio Gamelo	Philippine Normal College
1966	Diagnostic Test in English Structure of Coordination for Grades 4 to 6	Concepcion Olbes	Philippine Normal College
1966	Diagnostic Test in Subject-Verb Agreement for Grades 4 to 6	Marietta Parafina	Philippine Normal College
1966	Achievement Test in Reading for Grade 6	Rosario T. Aliño	Philippine Normal College

Table 4 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1967	Diagnostic Test in English Propositions for Grades 4 to 6	Eufemia L. Durian	Philippine Normal College
1968	An Achievement Test in Listening and Speaking for Grade 4	Neonita Dagasun Danao	Philippine Normal College
1968	An Oral Production Test	Lydia Miras Lopez	Philippine Normal College
1970	A Standardized Achievement Test in Health Education for Grade 4	Ceferina Saturnino	Philippine Normal College
1970	An Achievement Test in Elementary Mathematics for Grade 3	Belen Ruiz	Philippine Normal College
1970	An Achievement Test of the Ability to Write in English for Grade 5 Pupils	Purificacion M. Baluyot	Philippine Normal College
1970	Health Knowledge Test for Grade I Pupils	A.N. Capunitan	Philippine Normal College
1970	<i>Isang Pagsusulit ng Panuri sa Wastong Gamit ng Pilipino pura sa Baitang 5 at 6</i>	Candida N. Protacio	Philippine Normal College
1970	Achievement Test for First Year High School Mathematics	Rosita Guzman	Central Escolar University
1971	Mathematical Achievement of a Group of Freshmen in a Manila Public High School	Lourdes P. Gapud	Atenco de Manila University
1971	Achievement Test in Health Education for Grade 4	Ceferina C. Saturnino	Philippine Normal College
1972	English Reading Vocabulary Test for Grades 4 to 6	Melba P. Aguirre Tugade	Sto. Tomas University
1974	A Standardized Health Knowledge Test for Grade 5	Eusebia G. Agduyeng	Philippine Normal College
1975	Achievement Test in Elementary Mathematics for Grade 6	Elsa F. Edaño	Philippine Normal College
1975	Health Knowledge for Grade 6 Pupils	Fermina A. Valdez	U.P. College of Education
1976	Health Test for Grade 5 Pupils	Catalina S. Salazar	U.P. College of Education

Table 4 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1976	<i>Isang Pagsusulit sa Natutunan sa Pilipino ng mga Mag-aaral sa Ikalimang Baitang</i>	Nenita M. Tuazon	Philippine Normal College
1977	<i>Isang Pagsusukat sa Natutunan sa Pilipino ng mga Mag-aaral sa Ikatlong Baitang</i>	Paz Sasuca	Philippine Normal College
1977	Standardized Listening Comprehension Test in English for Intermediate Grades	Loida A. Flores	Philippine Normal College
1981	Achievement Test for Grammar	Maria N. Cusipag	Philippine Normal College
1981	Health Achievement Test for Grade 6	Eusebia G. Agduyeng	Philippine Women's University
1981	Achievement Test in English, Grade 6 Level	Remedios Arabit	De La Salle University
1982	Test on Verbs in Elementary School Graduates	Yolanda Janay	Philippine Normal College
1985	An Achievement Test in Pilipino for Grade 3	Maria G. Manalo	Philippine Normal College
1986	Achievement Test for Grade I <i>Sibika at Kultura</i>	Petrona Agcaoili	Philippine Women's University
1986	Physics Achievement Test for Engineering Students	Bernarda Lita	De La Salle University
1987	University Laboratory School Achievement Test in Arithmetic	Angelina M. Durano	De La Salle University
1987	Language Testing for Hearing Impaired Children	Antonia B. Blanca	Philippine Normal College
1990	An Innovative Achievement Test in College Biology	Nestor Rubenecia	De La Salle University
1991	Integrated Science Process Skills Test	Kenichi Hiura	Data being verified
1991	Achievement Test in Physics for Senior High School Students	Oscar S. Pasagui	Philippine Normal University
1992	Achievement Test in Marine Engineering	Felicito P. Dalaguete	De La Salle University
1995	Instrument to Access Religious Knowledge of Students and Teachers	Josefina Peralta	De La Salle University

Appendix Table 5. Local Measures of Personality (1959-1992)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
Date being verified	Jr. and Sr. High School Personality Questionnaire	Carol Ann Retaña, Joyce Lindaya, Susan Malabanan, Frederick Obligacion, Edith Panay, and Aida Salaguit	U.P. Department of Psychology
Date being verified	Trait Survey Test	Asian Psychological Services and Assessment Corporation (APSA)	APSA
1959	Philippine Personality Inventory, Form I	M.A. Gapuz	In: Philippine Education
1984	Local Norms (Capistrano, N., Cruz, V., David, S., Cachico, L., Liboro, V., and Lucas, D.S.)		
1992	Validity Study (Bolívar, R.W., M.A. Central Phil. Univ., Iloilo)		
1963	Alteration Ranking of Trait Adjectives	Ateneo Central Guidance Bureau	Ateneo de Manila University
1969	Manila Personality Inventory	Petrocinio Toribio	Manila City Schools Guidance, Counseling, and Special Education Services, DECS
1975	<i>Panukat ng Ugali at Pagkatao</i>		
1975	Content domain of "pagkamaramdamin" (Bernardez, E.)		
1975	Kahulugan ng "pakipot" - content domain of the "hirap kausapin" subscale (Pe, R.)		
1975	Validation of the "matulungin" scale (Cipres, S., Paulo, L., & Rustia, J.R.)		
1975	Validation of the "pagkumalikhain" scale (Paler, C.M., Labaycn, G., Dizon, M., Daza, M., Banzuela, F., Bahaor, A., & Adea, V.)		

Table 5 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1979	Reliability of the PUP (Guanzon, M.A.C.)		
1979	Experimental validation of the " <i>matiyaga</i> " scale (Gaffud, M. and de Rivera, L.)		
1980	Correlates of creativity (Socrates, N.)		
1981	Criterion validation and item analysis of the " <i>Katiyagaan</i> " subscale (Collantes, E.M. & Eleosida, I.S.)		
1981	Presentation of the PUP to the international community (Enriquez, V.G. & Guanzon, M.A.C.)		
1981	Translation equivalence of PUP- Cebuano and PUP- Filipino (Alcazar, L.T. & Torotoro, L.B.)		
1981	Validating the " <i>ambisyon</i> " and the " <i>takas ng loob</i> " subscales (Manalo, E.)		
1982	Construct validation indicators for " <i>pagkamapug- bigay</i> " and " <i>pagkamatu- lungin</i> " subscales (De Jesus, A. & Dominquez, L.)		
1982	Construct validation of " <i>pikon</i> " and " <i>pagkapalaaway</i> " subscales (Agbayani, C., Carino, R., Cheung, D., de Joya, & Fernandez, C.)		
1982	Contributing to the construct validation of PUP subscales (Ligeralde, C. and Gaerlan, E.)		
1982	Linguistic equivalence of the Filipino and English versions of the PUP (Canlas, R.P. & Guerrero, R.O.)		
1982	Stubbornness and ambition: Correlation and sex differences/ pre-marital sex (Damian, A. & Gonzales, P.)		

Table 5 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1982	Translation equivalence of PUP-Ilocano and PUP-Pilipino (Jimenez, J.)		
1983	Comparison of the personality of the Filipino across 12 ethnic groups (Guanzon, M.A.C.)		
1983	Positive and negative connotations of the PUP subscales (Guanzon, M.A.C.)		
1984	<i>Pagkamapagsapalaran</i> Scale (Musni, T., Pinto, B., Santiago, P., and Tremo, P.Y.)		
1984	Concurrent validity of the PPP and PUP (Abaya, G.R., Anosan, R., Carsi-Cruz, & Jancian, J.)		
1984	Concurrent validity of the PUP and the PPP (Bautista, A., Dumandan, C., Gutierrez, E., & Limjoco, J. (1984).)		
1984	Parallel scales of the PPP and the PUP (Agana, C., Co, G., Depante, L., Kho, Q. & Taylor, M.)		
1984	PUP-Bata: test development and reliability (Batangan, et al.)		
1985	Male and Female norms for the 14-19 age group reported (Enriquez, V. and Guanzon, M.A.C.)		
1986	Construct validation of " <i>pagkasunud-sunuran</i> " (Alarilla, A. & Ong, J.)		
1987	Convergent and discriminant validity of the PUP and PPP scales on " <i>pagkumalikhain</i> ," " <i>pagkumatiyaga</i> ," and " <i>pagkamaramdamin</i> " (Bernardo, A., Lazo, M.G., & Llamas, J.C.)		
1989	Test-re-test reliability of the PUP (Alfonso, E., Briones, J., Chan, H., Hidalgo, B., Paguio, E., Santos, K., & Santos, M.)		

Appendix Table 5 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1990	<i>Pagsusuri ng Katatagan ng Bersyong Ilokano at ng Katapatan ng mga PUP Subskala ng Pagkamahiyain at Ambisyon*</i> (Bautista, J.G., Guintu, C., & Yu, M.L.)		
1994	Predicting Work Attitude and Interpersonal Skills from the <i>Panukat ng Ugali at Pagkatao</i> (PUP): An Exploratory Study (Susan Theresa G. Montenegro)		
1979	Group Projective Test as a Diagnostic Tool	Josefina A. Limnaco	University of Sto. Tomas
1980	<i>Panukat ng Pakikipagkapwa ng Batang Pilipino</i>	P.O. Abenojar	U.P.
1984	<i>Panukat ng Ugali at Pagkatao para sa Bata</i>	Dennis Batangan	U.P.College of Medicine
1985 1984	<i>Panukat ng Pagkataong Pilipino</i> Concurrent validity of the PPP and PUP (Abaya, G.R., Anosan, R., Carsi-Cruz, & Jancian, J.)	Annadaisy Carlota	UP Department of Psychology
1984	Concurrent validity of the PUP and the PPP (Bautista, A., Dumandan, C., Gutierrez, E., & Limjoco, J.)		
1984	Parallel scales of the PPP and the PUP (Agana, C., Co, G., Depante, L., Kho, Q., & Taylor, M.)		
1984	English Translation of the PPP (Alikpala, E.B. and de los Reyes, Jr.)		
1987	Criterion Validation of the PPP <i>Ang Pagkamalikhain Scale</i> among High School Students (Bondoc, A., Dimaano, T., Guevarra, C., et al.)		
1992	Test on Personal and Social Attributes	Vivian Daroy	Philippine Normal University

Appendix Table 6. Local Measures of Specific Personality Characteristics (1973-1996)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
date being verified	Adjustment Scale for College Students	Lolita Bitanga	
date being verified	Masculinity and Femininity Scale	Marlyn Corpus-Buenaventura	In: Research Journal
date being verified	Parenting Needs Inventory	Francisca Lomotan-Cruz	date being verified
date being verified	Self Rating Scale (of Self-Acceptance, Form A)	Natividad Munnariz	U.P. College of Education
date being verified	Filipino Children's Self Appraisal Inventory	F.A. Vida	date being verified
1973	<i>Ugnayan ng mga Salita</i>	V.G. Enriquez	Philippine Psychology Research and Training House, Inc.
1973	Anagrams Test	V.G. Enriquez	Philippine Psychology Research and Training House, Inc.
1973	<i>Magdrowing ng Bahay Test</i>	V.G. Enriquez	Philippine Psychology Research and Training House, Inc.
1973	<i>Pigura Test</i>	V.G. Enriquez	Philippine Psychology Research and Training House, Inc.
1973	Fear Survey Schedule for Filipino Students	Renato Daquiz	University of the Philippines
1974	Parameters of Aggression	Amelia B. Alfonso	U.P.
1974	The Choice Dilemma Questionnaire and A Measure of Risk-Taking	S. Lao	U.P. Department of Psychology
1974	<i>Sigurista-Bahala Na Complex</i>	Roberto Mendoza	U.P. Department of Psychology
1974	Parental Independence Scale	M.A. Ng	U.P. Department of Psychology



Appendix Table 6 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1975	Crime Picture Interpretation Test: A Measure of Sociopathy	Corazon Lamug	U.P. Department of Psychology
1975	<i>Panukat ng Pakikipagugnayan</i> (Adjustment-Maladjustment Inventory)	R. Castiglioni, A., Cruz, L., Estampador, L., Marcia, F., Lagbao, and Parras, A.C.	U.P. Department of Psychology
1977	Manifestative Factors of Religious Motivation	Cruice G. Sangivi	De La Salle University
1979	Nonverbal Social Adjustment Test for Hearing Impaired Children	Ninta A. Alderete	Philippine Normal College
1979	Fears I Have: A Self-Study Guide	B.C. Elwood	U.P.
1980	Career Maturity Scale for Filipinos	Josefina Santamaria	U.P. College of Education
1981	Pre-Diagnostic Scale for Psychosis	Noemi Catalan	Pamantasan ng Lunsod ng Maynila
1982	The Family Environment Scale: An Initial Diagnostic Tool of Self-Concept and Adjustment of Adolescence	Daniel W. Mitchum	University of Sto. Tomas
1982	Moral Judgment Instrument for Filipino Students	Eva Villanueva	U.P. College of Education
1984	Self Concept Scale for Children	L.U. Agbing	U.P.
1985	Adjustment Inventory Based on Erickson's Stages of Development	Virginia Baes	U.P. College of Education
1986	Security-Insecurity Inventory for Filipino College Students	Salud Evangelista	University of Sto. Tomas
1986	A Construct Validation of a Locally Developed Security-Insecurity Inventory (Pison, R.)		
1986	Sex Norms (Sta. Cruz, S.)		
1988	Test Manual for the Filipino Self-Appraisal Inventory (Security-Insecurity Index) (Evangelista, S.)		

Appendix Table 6 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1990	<i>Paggawa ng Pamantayan ng Filipino Self-Appraisal Inventory</i> (Ligon, M., Uy, C., Vida, F.)		
1986	Teacher Characteristics Inventory	L. Hiceta-Nava	U.P. College of Education
1987	Pasao's Self Concept Scale	Myrna Pasao	U.P. College of Education
1987	Filipino Adolescent Anxiety Scale	Ligaya de Guzman	U.P. College of Education
1988	Filipino Creativity Test	Ana Cojuanco, Cecile Deltette, and Richard Perez	De La Salle University
1988	Maturity of Women Religious	G. Manuyag	De La Salle University
1990	<i>Panukat ng Pagkarelihyoso ng Pilipinong Kristiyano</i>	Josefina R. Cantiller	U.P. Department of Psychology
1991	School Anxiety Scale for Early Adolescence	Norma Cahilig	U.P. College of Education
1991	Mental Health Scale for Filipino College Students in Metro Manila	Imelda C. Labrador	University of Sto. Tomas
1991	<i>Panukat ng Pagkamapagbahala</i>	Marilyn Say, Abigail See, and Shirley Yu	De La Salle University
1992	Anxiety Scale for Children	Carmelita Pabiton	U.P.
1992	<i>Iskala ng Kahustuhang Emosyonal</i>	I. Caldon, R. So, and L. Tolentino	De La Salle University
1992	<i>Iskala ng Iskizophrenia ng mga Pilipino</i>	Donna Capalad, F.A. Diaz, and J.A. Natividad	De La Salle University
1992	Mid-Life Stress of Religious	M.A. Marahay	De La Salle University
1993	<i>Panukat ng Kabuuang Pagkatao</i>	Rowena Ann Garcia, Catherine Leviste, Mary Joyce Mijares	De La Salle University

Appendix Table 6 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1994	<i>Panukat ng Magpapakilala ng Pagkababae at Pagkalalake</i>	M. Chuaansu, M. Talento, and J. Tanghal	De La Salle University
1995	Stress Pictorial Test	Lina Grace Baterna, Ma. Raquel Roxas, and Corazon Toling	De La Salle University
1995	Trustworthiness Scale	F. Garcia, O. Hermando, and J. Samson	De La Salle University
1995	<i>Iskala ng Pakikiisang-Loob</i> (Filipino Test of Empathic Ability)	Raymond Gonzales	De La Salle University
1996	Problem Scale Test	Joanna Bostre, Theresa Canayon, Katherine de Villa, Virginia Macapili, and Christie Tinio	De La Salle University
1996	Spiritual Wellness Scale	Anne Marie Catapang, Kenneth Co, Sheryl Lyn Sarabia, Sharon Marie Tanking	De La Salle University

Appendix Table 7. Local Measures of Specific Aptitudes (1967-1994)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
Date being verified	Work Behavior Profile ( <i>Kaugalian sa Paggawa</i> )	Asian Psychological Services and Assessment Corporation, Inc. (APSA)	APSA
Date being verified	Management Inventory Test	Asian Psychological Services and Assessment Corporation Inc. (APSA)	APSA
Date being verified	Sales Orientation Survey	Asian Psychological Services and Assessment Corporation, Inc. (APSA)	APSA
1967	Entrance Examination for First Year	Hermenegilda Margate	U.P. Counseling
1972	Bilingual Usage Schedule	Amelia B. Alfonso	U.P. Department of Psychology
1973	Non-Verbal Academic Aptitude	E.P. Lee	U.P. College of Education*
1979	Managerial Self-Perception Inventory for Filipinos	Eloisa Ledesma	U.P.
1980	Music Sensitivity Test for Bachelor of Science in Education and B.S. Elementary Education Students	Virginia F. Pabalan	Philippine Normal College
1982	Supervisory Effectiveness Scale	Elmila Ayson-Banico	U.P. College of Education
1982	Pre-School Inventory	R. Ching and K. Ting	data being verified
1982	An Aural Aptitude for Music for Intermediate Grades in Metro Manila	Esther C. Javier	Philippine Normal College
1985	The Metro Manila Developmental Screening Test	Phoebe Williams	data being verified
1989	Motor Development Screening Test	Child and Youth Research Center	Department of Education, Culture, and Sports (Testing Division)
1989	Computer Scholastic Aptitude Test	Normandy T. Nangca and Marivic N. Sandoval	Philippine Women's University

Appendix Table 7 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1993	The De-Westernized Dementia Screening Scale	Lourdes K. Ledesma, B.V. Dipotado, Grace Orteza, and C.E. Santillan	U.P. Department of Psychology, U.P. College of Medicine, and De La Salle University
1994	Photo Career Discovery Kit	Thelma Abiva	Abiva Publishing, Inc.

Appendix Table 8. Local Assessment of Specific Cognitive Abilities (1956-1988)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
Date being verified	Instrument for Assessing Education Numeracy Levels	Pilar Centina	U.P. College of Education
1956	Specific Arithmetic Difficulties in the Intermediate Grader	I.M.a Tudtud	University of San Carlos
1960	Measure of Intellectual Maturation for Filipino Students	Jose M. Icasiano	data being verified
1976	Sample Maze Tests for Assessing the Performance of Grade 3 Pupils	A.D. Marbella	U.P. College of Education
1984	Critical Thinking for Grade 6 Pupils	Danilo S. Gutierrez	Philippine Normal College
1987	Abstract Reasoning Test for Filipino Elementary Children	Child and Youth Research Center	Department of Education, Culture, and Sports (Testing Division)
1988	Cognitive Adaptive Skills Test	Child and Youth Research Center	Department of Education, Culture, and Sports (Testing Division)
1988	Creative Thinking Test for Fourth Year High School Students	Antonio G. Dacanay	Philippine Normal College
1988	Problem Solving Skills of Students in College Algebra	Rosario Leño	De La Salle University

Appendix Table 9. Locally Developed Measures of Specific Attitudes (1969-1994)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
Date being verified	Attitudes towards Effeminate Teachers	Bernadette Abrilla, Cindy Chan, Cristina Go, Shella Guevarra, and Ma. Fe Hernandez)	De La Salle University
date being verified	Supervisory Attitudinal Survey	Asian Psychological Services and Assessment Corporation (APSA)	APSA
1969	Attitudes Towards Birth Control Scale	E. Mamisao, A. Tan, and G. de Vera	U.P. Department of Psychology
1976	Altruistic Attitudes among Teachers	C.A. Ovejera	De La Salle University
1979	An Attitude Scale towards Working Wives	Elizabeth Ventura, Gerda Kochler, Margaret Udarde, and Lou Agbing	U.P. Department of Psychology
1980	Altruistic Attitudes among Teachers	D. Chong	De La Salle University
1980	Philippine Attitudes Scale and Knowledge Orientation	L. Moortgat	De La Salle University
1983	Scientific Attitudes Inventory	E.T. Banon	Philippine Normal University
1986	The Workbook Attitude Scale I	A. Calata, E. Castillo, M. Flores, T. Gola, N. Munnariz, and M. Pasao	NADEC
1987	Attitude Scale Towards Cheating in Business	M. Acosta, J.M. Villanueva, and T. Macalindong	De La Salle University
1987	Attitude Scale on Studying	M. Carrascoso, Cecile Gatchalian, A. Grihalde, J. Inocentes, M.E. Marribay, Juñez, and M. Theard-Johnson	De La Salle University
1988	Attitude towards Female Virginity	K. Tamesi	Assumption College
1989	Mathematics Attitude Inventory	Milagros Ibe	U.P. College of Education
1994	Attitude Scale for Sex Education	Auramar O. de Onon	U.P. College of Education

Appendix Table 10. Locally Developed Measures of Specific Values (1966-1993)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1966	CT Scale or the Traditional Scale	Carmen Alcuaz	Ateneo de Manila University
1982	Counselor Therapeutic Relationship Values Scale	Edilberto Dizon	U.P. College of Education
1984	Values Orientation Scale	Child and Youth Research Center	Department of Education, Culture, and Sports (Testing Division)
1987	Value Development Index	Child and Youth Research Center	Department of Education, Culture, and Sports
1987	Values Inventory for Filipino Children	Evangeline M. de Jesus	U.P. College of Education
1990	Values Orientation Instrument	Camila C. Gonzales	U.P.
1990	Interpersonal Values Scale for Filipino Adolescents	V. Cenal	U.P.
1993	Filipino Work Values Scale	Vicentita Cervera	Abiva Publishing House, Inc.
1993	Values Scale for Filipinos	Domingo Nuñez	Pamantasan ng Lunsod ng Maynila

Table 11. Locally Developed Performance Appraisal Measures (1973-1993)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1973	Instrument for the Assessment and Improvement of Teacher Performance	E.S. Reyes	Ateneo de Manila University
1974	Teaching Effectiveness at the Secondary Level of Instruction	C.G. Estadilla	University of Sto. Tomas
1978	Test of Communicative Competence in Filipino	E. Castillo	Philippine Normal University
1979	Principal's Competencies and Performance	G.G. Par	Ateneo de Manila University
1980	Language Competency Test	Child and Youth Research Center	Department of Education, Culture, and Sports (Testing Division)
1984	Personal-Social Behavior Inventory	Child and Youth Research Center	Department of Education, Culture, and Sports (Testing Division)
1991	<i>Panukat ng Kakayahang Berbal sa Wikang Filipino</i>	J.G. Belmonte and A.C. Carreon	De La Salle University
1991	<i>Pinoy Management Style Inventory</i>	M.C. Benavides, M.R. Reyes, and M.E. Uychiat	De La Salle University
1991	<i>Panukat ng Kakayahang Mamuno ng Pilipinong Mag-aaral sa Pamantasan</i>	Haydee Cayanan, M. Co, and J. Dee	De La Salle University
1991	Interpersonal Skills of Filipino Students in the Health Professions	Cristina Mencias	National Teacher Training Center for Health Professionals
1992	<i>Panukat ng Kakayahang Magdala ng Tensyon</i>	S.J.T. See, M.C.M. Leus, and M.S. de los Reyes	De La Salle University
1992	Laboratory Teaching Effectiveness Scale	Felicito P. Dalaguete	De La Salle University
1993	<i>Panukat ng Kakayahang Makaagapay sa Istres ng mga Tagapangasiwa sa Industriya</i>	A.D. Castillo, S.L. Restubog, and K. Yap	De La Salle University



Appendix Table 12. Other Local Educational and Psychological Measures (1980-1994)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
Date being verified	Manila Counseling Inventory	Guidance and Counseling Special Education Services, Division of City Schools	Department of Education, Culture, and Sports
1980	Problem Checklist for Filipino Children – Intermediate Grades	M. Dimagundayao	Philippine Normal College
1985	Grade 5 Pupils' Major Areas of Concern (using Havighurst's Developmental Tasks)	Maria Encarnacion Tan	Ateneo de Manila University
1987	Human Rights Awareness Scale	PNC Research Center	Philippine Normal College
1990	Philippine Pastor Scale	Roberto B. Betia	U.P.
1991	Filipino Family Relationship Scale	Ester Jimenez	data being verified
1992	The Organizational Assessment Survey	R.H. Alampay	U. P. Department of Psychology
1992	Panukat ng Kaasalang Sekswal	Marfred Lamigo, Teresa Aquino, and Rodel de Guzman	De La Salle University
1993	Multi-Factor Seminarian Scale	Amirtha Raj George, O.P.	U.P. College of Education
1994	A Scale of Post-Disaster Psychosocial Functioning of Children	Josie Y. Rufuerzo	data being verified
1994	<i>Antas ng Pahintulot at Atribusyon ng Paninisi ng Ilang Mag-aaral ng U.P. sa Sekswal na Harassment</i>	K. Doronilla, I. Orolfo, and J. Yacat	U.P. Department of Psychology
1994	Marriage Expectations	Nilo E. Tanalega, S.J.	UGAT Foundation Inventory

Appendix Table 13. Adaptations of Foreign-Made Tests (1958-1991)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
Date being verified	<i>"Ang Palagay ko sa aking Sarili"</i> A Filipino Adaptation of the Piers Harris Children's Self Concept Center	Manila Guidance Testing Scale	MGTC
1958	Wechsler-Bellevue Intelligence Scale Form II for Filipino Use	Prisco S. Matthen	University of Sto. Tomas
1960	The Applicability of the Minnesota Multiphasic Personality Inventory to Filipino Subjects	E. Samson and G. Samson	University of Sto. Tomas
1965	An Item Analysis of the Otis Self-Administering Tests of Mental Ability	Regina Carlos	Manuel L. Quezon University
1965	Philippine Thematic Apperception Test	Alfredo V. Lagmay	U.P. Department of Psychology
1987	Ambiguity Values of the Philippine Thematic Apperception Test (Ventura, E.)		
1987	Scaling the Philippine Thematic Apperception Test (Lazo and Ventura)		
1987	Four Variations in the Administration of the PTAT (Altavaz, M., Divinagracia, G., and Montano, A.)		
1967	Philippine Children's Thematic Apperception Test	Alfredo V. Lagmay	U.P. Department of Psychology
1968	Item Analysis of the Otis Quick-Scoring Test in U.P. Grade 1 Pupils	Gerardo Ang and Agustina Bantigue	U.P.
1968	WISC Factors in Filipino Children	Ma. Reinita Quebral	Ateneo de Manila University
1969	A Sentence Completion Test in Filipino	Allen Tan	Ateneo de Manila University

Appendix Table 13 (continued)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1972	The Philpsycor-Geist Picture Interest Inventory: Male M	Estefania Aldaba-Lim	Philippine Psychological Corporation
1972	Philippine Otis-Lennon Mental Ability Test	Linkurang Pamamatnubay, Pagpapayo at Pantanging Edukasyon	Sangay ng mga Paaralang Lungsod ng Maynila
1978	Predictive Validity (Villa, T.P.)		
1981	The Development of Norms for Public Elementary Schools (Giron, M.F.)		
1974	Local Norms on the 16 PF Test	M.A. Jose, E. Ojano, and Z. Sumagaysay	U.P. Department of Psychology
1974	Admission of Students at the PWU College of Nursing (Alday, S.)		
1975	Philippine Adaptation of the Wechsler Intelligence Scale for Children	Edwin Torres	Department of Education, Culture and Sports
1979	The Development of Philippine Norms for the Gates-McGinntie Reading Test for MM Elementary School Pupils	Liberacion Abarm, Maxima Alburo, Amelita Conp and Florida Ragunton	Philippine Normal College
1979	Diliman Wechsler Adult Intelligence Scale	Crispina B. Gorospe	U.P. College of Education
1981	Cross Validation of the Brainard Occupational Preference Inventory	Domingo B. Nuñez	U.P. Department of Psychology
1982	Wechsler Intelligence Scale for Children - Revised Form for the Filipino-American ESL Students of DODD Schools at Clark Air Base	R.A. Garcia	University of Sto. Tomas
1991	Catell's 16 Personality Factors Profile	M.D. Bantug, J.A. Gochian, and T.M. Tantoco	De La Salle University

Appendix Table 14. Translations of Foreign-Made Tests (1987-1990)

Year Developed	Title of Test or Construct Measured	Author/s	Institutional Affiliation
1987	Desirable-Undesirable Events: Locus of Control Scale	Debbie Ko, Marjorie Sy, and Katherine Tan	De La Salle University
1989	Tennessee Self Concept Scale	M.A. Bautista, A. and Gil, Jr.	De La Salle University
1990	Wechsler Pre-School and Primary School Intelligence - Verbal Scale	S. Anwar, R. Morallo, and C.C. Yu	De La Salle University
1990	Edwards Personal Preference Schedule	A.A. Anabeza, M.F. Cabrera, and R. Rómeo	De La Salle University

## APPENDIX A

## Code Book for the Critique of Locally Developed Psychological Tests

VARIABLE # + DATA FOR ENTRY	COLUMN LABEL	CODES PERCENTENT CATEGORY
1:	studname	<b>Name of Student submitting Critique</b> First eight letters of family name of student s submitting data
2:	studschl	<b>Educational Affiliation of Student submitting Data</b> 1 – U.P. 2 – DLSU
3:	idtest1	<b>Name of Test</b> First eight letters of the label of the primary construct (as found in the test's title) that is being measured
4:	idtest2	<b>Name/s of Author/s</b> First eight letters of test's senior/first/sole author
5:	idtest3	<b>Test Publication Status</b> 1 – published 2 – unpublished
6:	idtest4	<b>Name of Test's Publisher</b> na – not applicable, since test is not yet published First eight letters of test's publisher
7:	idtest5	<b>Year when Test was Developed or Published</b> nr – not reported month/day/year numerically coded
8:	idtest6	<b>Location of Test</b> 1 – with author 2 – with school 3 – with office/institution 4 – with publisher
9:	idtest7	<b>Name of Institution which Supported the Development of the Test</b> First eight letters of the name of the supporting school, office, or organization
10:	geninfo1	<b>Nature of the Test</b> 1 – intelligence 2 – interest 3 – aptitude 4 – achievement 5 – personality 6a... – others 6a creativity 6b comprehension and application analysis 6c attitude 6d Lasallian values 6e teaching effectiveness 6f religious motivation 6g maturity level 6h aptitude and personality

## APPENDIX A (continued)

VARIABLE # + DATA FOR ENTRY	COLUMN LABEL	CODES PERCENTENT CATEGORY
11:	Geninfo2	<b>Purpose of the Test</b> 1 – research 2 – career placement 3 – career counselling 4 – clinical diagnosis 5 – general intelligence/aptitude/personality/assessment 6a ... - others 6a assessment of creativity 6b attitude assessment 6c success appraisal
12:	geninf3a	<b>Lower End of Range of Grades Covered</b> na – grade level of testee is not a salient testing consideration Roman numeral/s representing the lowest grade to which the test-taking may be appropriate
13:	geninf3b	<b>Higher End of Range of Grades Covered</b> na – grade level of testee is not a salient testing consideration Roman numeral/s representing the highest grade to which the test-taking may be appropriate
14:	Geninf4a	<b>Lower End of Range of Ages Covered</b> na – age of testee is not a salient testing consideration nd – age is a salient testing consideration, but no data is provided Youngest age of testee who may appropriately take the test
15:	geninf4b	<b>Higher End of Range of Ages Covered</b> na – age of testee is not a salient testing consideration nd – age is a salient testing consideration, but no data is provided Oldest age of testee who may appropriately take the test
16:	geninfo5	<b>Item Type</b> 1 – written verbal 2 – written numerical 3 – pictorial/non-verbal 4 – audio 5 – written verbal and numerical 6 – written verbal and pictorial 7 – written verbal and audio 8 – written numerical and pictorial 9 – written numerical and audio 10 – pictorial and audio 11 – others
17:	geninfo6	<b>Scores Available I</b> Number of persons who have taken the final test

## APPENDIX A (continued)

VARIABLE # + DATA FOR ENTRY	COLUMN LABEL	CODES PERCENTENT CATEGORY
18:	geninf7a	<b>Scores Available Iib</b> Youngest age in the age range of persons who have taken the final form of the test
19:	geninf7b	<b>Scores Available Iib</b> Oldest age in the age range of persons who have taken the final form of the test
20:	geninfo8	<b>Scores Available III</b> Typology of persons who have taken the final test, e.g., college students, managers, pre-school children, etc. 1 students 2 religious groups 3 counselors 4 teachers 5 deviant vs. Normal groups 6 children
21:	geninfo9	<b>Method of Scoring</b> 1 - hand-scored 2 - machine-scored 3 - hand-and machine-scored
22:	geninf10	<b>Test Administration Time I</b> 1 - test is timed 2 - test is untimed
23:	geninf11	<b>Test Administration Time II</b> nd - information about how long test-taking usually takes was not provided Approximate total time needed for test administration (in minutes)
24:	geninf12	<b>Saleability of the Test</b> 1a - not yet for sale: needs to be studied further 1b - not yet for sale: studies on the test are adequate, but the test developer/s no longer went into the final test production phase 1c - not yet for sale: test is being packaged for final reproduction 2 - definitely not for sale (e.g., it is for institutional use only) 3 - for sale
25:	geninf13	<b>Conditions Set regarding Sale of Tests</b> na - test is not being sold 1 - test is sold only in test packages 2 - test manual, booklets, scoring keys, and answer sheets are sold only as separate items 3 - test may be purchased either as a test package or as separate components of the test package

## APPENDIX A (continued)

VARIABLE # + DATA FOR ENTRY	COLUMN LABEL	CODES PERCONTENT CATEGORY
26:	geninf14	<b>Current Cost of Test Package</b> na – test is not being sold as a text package actual price of test package (in pesos)
27:	geninf15	<b>Current Cost of Test Manual</b> na – test manual may not be purchased separately from the test Package actual price of test manual (in pesos)
28:	geninf16	<b>Current per Unit Cost of Test Booklets</b> na – test booklets may not be purchased actual price of test booklet (in pesos)
29:	pracfea1	<b>Ease of Test Administration</b> 1 – very difficult 2 – somewhat difficult 3 – neither difficult nor easy 4 – somewhat easy 5 – very easy
30:	pracfea2	<b>Ease of Scoring of the Test</b> 1 – very difficult 2 – somewhat difficult 3 – neither difficult nor easy 4 – somewhat easy 5 – very easy
31:	pracfea3	<b>Ease of Test Interpretation</b> 1 – very difficult 2 – somewhat difficult 3 – neither difficult nor easy 4 – somewhat easy 5 – very easy
32:	pracfea4	<b>Adequacy of the Test Manual</b> 1 – no test manual is provided at all 2 – very inadequate 3 – needs major improvements 4 – needs minor improvements 5 – very adequate
33:	pracfea5	<b>Adequacy of Accessory Materials</b> na – the test has no accessory materials 1 – very inadequate 2 – needs major improvements 3 – needs some minor improvements 4 – needs many minor improvements 5 – very adequate
34:	techfea1	<b>Evidence regarding Test's Content Validity</b> 1 – provided 2 – not provided



## APPENDIX A (continued)

VARIABLE # + DATA FOR ENTRY	COLUMN LABEL	CODES PERCENTENT CATEGORY
35:	techfelb	Evidence regarding Test's Criterion-related Validity 1 - provided 2 - not provided
36:	techfelc	Evidence regarding Test's Construct Validity: Factor Analysis 1 - provided 2 - not provided
37:	techfeld	Evidence regarding Test's Construct Validity: Discriminant Data 1 - provided 2 - not provided
38:	techfele	Evidence regarding Test's Construct Validity: Convergent Data 1 - provided 2 - not provided
39:	techfel f	Evidence regarding Test's Construct Validity: Before- and After- Treatment Comparisons 1 - provided 2 - not provided
40:	techflg	Evidence regarding Test's Construct Validity: Contrasted Groups 1 - provided 2 - not provided
41:	techfelh	Evidence regarding Test's Construct Validity: Study of the Test's Internal Structure 1 - provided 2 - not provided
42:	techfeli	Evidence regarding Test's Construct Validity: Study of the Process of Measurement of the Construct 1 - provided 2 - not provided
43:	techfelj	Evidence regarding Test's Content Validity 1 - provided 2 - not provided
44:	techfe2a	Correlation Coefficient for "Criterion-related" Validity Study na0 (if no validity study on the test was reported) na1 (if test was not validated through a criterion-related validity study) actual r value (if test was criterion- related-validated)

## APPENDIX A (continued)

<b>VARIABLE # + DATA FOR ENTRY</b>	<b>COLUMN LABEL</b>	<b>CODES PERCONTENT CATEGORY</b>
45:	techfe2b	<b>Number of Factors Yielded by "Factor Analysis" Construct Validation Study</b> na0 (if no validity study on the test was reported) na1 (if test was not validated through a factor-analytic construct validity study) total number of factors (if test was factor analyzed)
46:	Techfe2c	<b>Correlation Coefficient for "Discriminant Validity" Construct Validity Study</b> na0 (if no validity study on the test was reported) na1 (if test was not validated through a "discriminant validity" construct validity study) actual r value (if test was construct-validated through a discriminant validity study)
47:	techfe2d	<b>Correlation Coefficient for "Convergent Validity" Construct Validity Study</b> na0 (if no validity study on the test was reported) na1 (if test was not validated through a "contrasted groups" construct validity study) actual r value (if test was construct-validated through a convergent validity study)
48:	techfe2e	<b>Outcome of Statistical Test of Hypothesis of Significant Differences between Contrasted Groups for a Construct Validity Study</b> na0 (if no validity study on the test was reported) na1 (if test was not validated through a "contrasted groups" construct validity study) actual statistically significant test of hypothesis value (if test was validated using the contrasted groups comparison validity study approach)
49:	techfe2f	<b>Outcome of Statistical Test of Hypothesis of Significant Differences between Pre-Treatment and Post-Treatment Groups for a Construct Validity Study</b> na0 (if no validity study on the test was reported) na1 (if test was not validated through a "before-and- after-treatment-comparison" construct validity study) actual statistically significant test of hypothesis value (if test was validated using the treatment groups comparison validity study approach)
50:	techfe2f	<b>Validity Coefficient for a "Study of Test's Internal Structure" Construct Validity Study</b> na0 (if no validity study on the test was reported) na1 (if test was not validated through a "study of internal structure" construct validity study) actual internal consistency r value (if test was construct-validated through a study of the test's internal structure)

## APPENDIX A (continued)

VARIABLE # + DATA FOR ENTRY	COLUMN LABEL	CODES PERCENTENT CATEGORY
51:	techfe2g	<b>Assumption Held Regarding the Test's Internal Structure</b> na0 (if no validity study on the test was reported) na1 (if test was not validated through a "study of internal structure" construct validity study) 1 – test items are assumed to be homogenous, i.e., consistently measuring the same construct 2 – test items are assumed to be heterogenous, i.e., the test's different scales measure different constructs that are assumed to be different from each other
52:	techfe3a	<b>Evidence regarding the Test's Reliability: Stability (Test-Retest) Study/Studies Done</b> 1 – provided 2 – not provided
53:	techfe3b	<b>Evidence regarding the Test's Reliability: Internal Consistency (Split-Half) Study/Studies Done</b> 1 – provided 2 – not provided
54:	techfe3c	<b>Evidence regarding the Test's Reliability: Internal Consistency (Inter-Item Correlation) Study/Studies Done</b> 1 – provided 2 – not provided
55:	techfe3d	<b>Evidence regarding the Test's Reliability: Equivalence of Forms Study/Studies Done</b> 1 – provided 2 – not provided
56:	techfe3e	<b>Evidence regarding the Test's Reliability: Other Type/s of Reliability Study/Studies Done (Please specify):</b> 1 – provided 2 – not provided
57:	techfe4a	<b>Correlation Coefficient for "Test-Retest/Stability" Reliability Study</b> na0 (if no reliability study on the test was reported) na1 (if test was not studied for reliability through a "stability" study) actual r value (if the reliability of the test was studied through an internal consistency/split-half correlational study)
59:	techfe4c	<b>Correlation Coefficient for "Internal Consistency: Inter-Item Correlation" Reliability Study</b> na0 (if no reliability study on the test was reported) na1 (if test was not studied for reliability through an "internal consistency: inter-item correlation" study)

## APPENDIX A (continued)

VARIABLE # + DATA FOR ENTRY	COLUMN LABEL	CODES PERCENTENT CATEGORY
60:	techfe4d	<p>Actual r value (if the reliability of the test was studied through an internal consistency/inter-item correlational study)</p> <p><b>Correlational Coefficient for "Equivalence of Forms" Reliability Study</b></p> <p>na0 (if no reliability study on the test was reported)</p> <p>na1 (if test was not studied for reliability through an "equivalence of forms" study)</p> <p>actual r value (if the reliability of the test was studied through an alternate forms correlational study)</p>
61:	techfe4e	<p><b>Correlation Coefficient for a Reliability Study Using Other Methods of Determining a Test's Reliability</b></p> <p>na0 (if no reliability study on the test was reported)</p> <p>na1 (if test was not studied for reliability through a "stability," "internal consistency" or "equivalence of forms" study)</p> <p>actual r value (if the reliability of the test was studied through a study using an approach other than the determining correlations between test-retest, test halves, inter-items, or alternate test forms)</p>
62:	techfea5	<p><b>Time Interval between Test Administrations in Stability Studies</b></p> <p>na – not applicable – no reliability study on the test was reported</p> <p>na2 – not applicable, since test reliability was studied in terms of the test's internal (split-half) consistency</p> <p>na3 – not applicable, since test reliability was studied in terms of the test's internal (inter-item) consistency</p> <p>na4 – not applicable, since test reliability was studied in terms of the test's equivalence of forms</p> <p>Time interval between test administrations (in terms of number of weeks)</p>
63:	techfea6	<p><b>Number of Equivalent Forms Available</b></p> <p>na – not applicable – no reliability study on the test was reported</p> <p>na1 – not applicable, since test reliability was studied in terms of the test's stability (based on test-retest correlational studies' outcomes)</p> <p>na2 – not applicable, since test reliability was studied in terms of the test's internal (split-half) consistency</p> <p>na3 – not applicable, since test reliability was studied in terms of the test's internal (inter-item) consistency</p> <p>Total number of equivalent forms available for use</p>
64:	tyechfea7	<p><b>Type of Norms Available for Use in Test Interpretation</b></p> <p>na – not applicable – no norming study on the test was reported</p> <p>1 – age norms</p>

## APPENDIX A (continued)

VARIABLE # + DATA FOR ENTRY	COLUMN LABEL	CODES PERCENTENT CATEGORY
65:	Techfea8	2 – grade norms 3 – age and grade norms 4 – special groups norms, e.g., managers, religious groups, etc. 5 – others (please specify) <b>Form of Norms Available</b> na – not applicable – no norming study on the test was reported 1 – means/standard deviations 2 – percentiles 3 – stanines 4 – sten scores 5 – others (please specify)
66:	techfea9	5a – percentiles and stanines <b>Adequacy of Available Norms</b> 3 – adequate, given target population for test usage 2 – inadequate, norms available only for some of target groups
67:	techfe10	1 – very inadequate, norms still need to be generated <b>Appropriateness of Norms to Local Situation</b> 1 – appropriate 2 – inappropriate

## **APPENDIX B**

### **Test Evaluation Outline**

- I. Identifying Data**
  - A. Title of Test
  - B. Author
  - C. Publisher
  - D. Date of Publication
  - E. Location of Test
  
- II. General Information**
  - A. Nature and Purpose of Test
  - B. Grade or Age Levels Covered
  - C. Examples of Items
  - D. Scores Available
  - E. Method of Scoring
  - F. Administration Time
  - G. Forms Available
  - H. Cost
  
- III. Technical Features**
  - A. Validity
  - B. Reliability
  - C. Norms
  
- IV. Practical Features**
  - A. Ease of Administration
  - B. Ease of Scoring
  - C. Ease of Interpretation
  - D. Adequacy of Test Manual and Accessory Materials
  
- V. General Evaluation**
  - A. Comments of Reviewer
  - B. Summary of Test's Advantages and Limitations for Local Use

# KNOWLEDGE, ATTITUDES, PRACTICES AND PROBLEMS OF WORKING MOTHERS ON BREASTFEEDING IN THE MUNICIPALITY OF VIRAC, CATANDUANES

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Virac, 4800 Catanduanes*

## ABSTRACT

*Socio-demographic profile of the working mothers and their knowledge, attitudes, practices and problems towards breastfeeding were determined and analyzed in Virac, Catanduanes. The descriptive survey method was used in the study with the questionnaire as the main data gathering tool. The Pearson Product Moment Correlation Coefficient (r), Chi-square ( $\chi^2$ ), Kendall Coefficient of Concordance were the inferential statistics used in the study. The variables used in the study were age, income, number of children, educational attainment and nature of job. Findings showed that working mothers strongly favor breastfeeding, however lack of time to breastfeed infant due to limited maternity benefits, distance of the job from home, stress caused work related problems were the foremost problems encountered. Income was found significantly related to one of the practices, that is, the age of introduction of artificial formula in milk-feeding indicating that the higher the income, the earlier the age for introducing artificial formula. Information dissemination on breastfeeding, strict implementation and strengthen the rooming-in policy and establishment of nursery in working establishment for nursing mother is strongly recommended.*

*(Keywords: breastfeeding, breastfeeding practices, knowledge, attitudes, working mothers, infant, feeding, breastfeeding problems.*

This study aimed to determine and analyze the socio-demographic profile of the working mothers and their knowledge, attitudes, practices and problems towards breastfeeding in the municipality of Virac, Catanduanes from 1990-1995.

Specifically, the study sought to answer the following questions:

1. What is the socio-demographic profile of working mothers in the municipality of Virac?

2. What are the knowledge/concepts of working mothers about breastfeeding?
3. What are the attitudes of working mothers about breastfeeding?
4. What are the practices of working mothers on breastfeeding and other related of feeding?
5. What are the problems met by working mothers regarding breastfeeding practices?
6. Is there significant relationship between the socio-demographic profile of the working mothers and their knowledge, attitudes, practices and problems on breastfeeding.

The descriptive survey method was used in the study with the questionnaire as the main data gathering tool. The study involved 210 employed government working mothers in Virac, Catanduanes.

The Pearson Product Moment Correlation Coefficient ( $r$ ), Chi-Square ( $\chi^2$ ) and Kendall Coefficient of Concordance ( $W$ ) were the inferential statistics used in the study.

The study disclosed the following findings on the profile of the working mothers: (a) The mean age of the working mothers who gave birth from 1990-1995 is 36 years old; (b) The mean number of children of the respondents is 3, (c) The average monthly income of mothers is P5,000 to P6,000.00; (d) Educational attainment of the respondent is Bachelor's degree with masteral units; and (e) Eighty-nine or 42.38% belong to teaching group, sixty-six or 31.43% are in non-teaching, thirty-four or 16.19% are with administrative or supervisory position and the twenty-one or 10% are health workers.

2. The knowledge/concepts on breastfeeding were generally considered by working mothers as "very familiar" while less than half of the concepts were reflected as "moderately familiar." The "very familiar" concept known by the respondents on breastfeeding was "Colostrum is the first milk from the mother's breast after birth that protects the baby from some infection" (97.14%) while the "moderately familiar" knowledge/concepts on breastfeeding declared was that "A mild malnourished mother can lactate successfully for the first few months."

3. With regard to the attitudes towards breastfeeding, majority of the subjects were favorable to breastfeeding since three-fourths of the attitudinal items elicited a "strongly agree" response and almost one third got "moderately agree" response. The strongly agreeable statement was that "Even if a mother can afford to buy canned milk, she should breastfeed her baby" (98.57%). On the other hand, the moderately agreeable attitudinal item was that "Experienced mothers are most likely to be successful in helping a mother to breastfeeding than a medical personnel who has never breastfed."

4. As to the breastfeeding and other related types of feeding practices, most of the working mothers used mixed-feeding (80%); the length of breastfeeding time would last for a year or more (39%) and the usual time of giving breastmilk by



majority of the respondents was in the morning and at night time. Fifty-eight percent of the mothers started to introduce artificial formula in mixed-feeding at the age 0-2 months.

5. The foremost problems encountered by working mothers regarding breastfeeding were the "lack of time to give mother's milk to the baby because of job"; (b) working mothers have very limited days for their maternity benefits, thus breastfeeding is discontinued and the mother's breastmilk starts to dry-up; (c) insufficient flow of breast milk; (d) distance of the house of the office is far, thus the inability to continue breastfeeding after maternity leave; (e) breastfeeding practice is adversely affected due to stress caused by work-related problems.

6. All the tests on the relationship between the socio-demographic profile and knowledge, attitudes, practices, problems on breastfeeding revealed no significant relationship between the variables.

Only income was found significantly related to one of the practices, that is, the age of introduction of artificial formula in mixed-feeding. It indicates that the higher the income the earlier the age in the introduction of artificial formula in feeding.

Based from the findings of the study, the following conclusions were drawn:

1. The typical nursing mother in Virac, Catanduanes is 36 years old, with 3 children and a monthly income of P5,001.00 to P6,000.00; a graduate of bachelor's degree course with masteral units and a teacher.

2. Working mothers were very familiar with the basic concepts of breastfeeding.

3. Working mothers strongly favor breastfeeding.

4. Majority of working mothers practice mixed feeding.

5. The most common problem met by the working mothers is lack of time to give mother's milk to the baby because of job.

6. Knowledge, attitudes, practices and problems are not significantly related with the socio-demographic profile of the working mothers, however, income is significantly related with one of the practices of the working mothers which is the age of introduction of artificial formula in mixed feeding indicating that the higher the income, the earlier the age for introducing artificial formula.

Based on the foregoing conclusions, the study offers the following recommendations:

1. Health Educators should intensify the information dissemination campaign regarding the ways to enhance the flow of breastmilk among lactating mothers and regarding the anti-allergenic substance in human milk by conducting health teaching in the community.

2. Based on observations that hospitals and clinics are not fully implementing the rooming-in policy, there must be strengthening of such policy allowing

mothers and babies to remain together 24 hours a day to permit breastfeeding on demand and to foster a closer mother-child relationship.

3. Strict implementation of the Labor Code of the Philippines stipulates that the management of any organization should provide a nursery in the working establishment for women employees so that lactating mothers can continue breastfeeding the baby in their respective working place.

The study offers the following areas for further study:

1. A similar study to be investigated to the non-working mothers in rural areas.
2. Relationship between breastfeeding knowledge and practices, attitudes and practices, knowledge and attitudes among working and non-working mothers.
3. Impact of Socio-Economic Status on Infant-Feeding Pattern.

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## RESETTLERS IN PAMPANGA: ARE THEY FINALLY SETTLED?

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### ABSTRACT

*Pampanga, the hardest hit province during the catastrophic 1991 Mount Pinatubo eruption, has remained the fragile center/area constantly threatened by the recurrence of lahar flows. This situation has forced many people to leave their homes and seek refuge in resettlement areas - the most immediate solution to their problem. Mount Pinatubo Commission, Government Agencies and Non-Government Organizations have in fact established 19 resettlement sites to accommodate the victims.*

*The process of relocating such displaced population of Pampanga is definitely difficult and costly. To grapple with the reality of complex human needs and nature is inevitable. Now that a great number of victims are resettled, several questions relative to the current status of both resettlers and their sites may still linger in the minds of many. A significant concern may be posed: Are the resettlers finally settled?*

*This research project entitled "Resettlement System in Pampanga" is a rich data source to address the question at hand. It aims to provide both an extensive and an intensive description of each resettlement site, particularly the resettlers' profile (socio-demographic aspects, livelihood and individual household characteristics) and their satisfaction level (to be derived from the data regarding the resettlers' "quality of life" before and after resettlement). In addition, the study also evaluates the economic viability and "total quality management" of each sites.*

*A closer look into the condition/situation of both the resettlers and their sites can be of productive consequence. For one thing, through the available data, insights may be drawn relative to the assessment of the resettlement projects, at least in Pampanga. Major concerns, therefore, including the question previously posed may be dealt with accordingly. For another, conventional answers, particularly by government agencies, which do not really provide exact solutions to problems*

*being encountered in the sites may be re-evaluated, assuming accuracy of the general data and soundness of the analytical framework being followed. Finally, disaster management practice may also be improved as facts and figures gathered can be used to generate systematic and coordinated response among concerned agencies and individuals.*

*The project highlights the strengths and weaknesses of the resettlement program in Pampanga from which policy recommendations regarding resettlement planning may be drawn.*

The study is an assessment of the past and present conditions of the lowland and upland Mount Pinatubo victims presently residing in the nineteen (19) resettlement sites in Pampanga, Philippines. These resettlement sites were established either by the Philippine government and managed through the Mount Pinatubo Commission (MPC) or by the non-governmental organizations (NGOs). The living conditions before and after resettlement were compared to approximate the satisfaction of the resettlers in the four resettlement categories. The assessment of the "quality of life" of the resettlers before and after their resettlement was found to be an important factor in deciding to stay permanently in the site. An estimated test of mean difference of the populations between the present residence (resettlement sites) and their previous residence (hometown) showed highly significant ( $P < .001$ ) difference for all the variables tested in all the resettlement groups. Compared to the previous residence of the resettlers, the houses and homelots are smaller in the resettlement sites. However, in spite of the seemingly lack of space it was found out that both upland and lowland resettlers now have more relatives living with them in their households than before resettlement. In addition, significantly higher participation in community activities in the resettlement could be observed as compared to prior resettlement. However, data show that as a result of dislocation, the unemployed have proportionately increased.

Mean comparisons between permanent and temporary stayers from the lowland and upland significantly showed that temporary resettlers were found to be significantly older, and have lived in previous residence longer, are less satisfied with facilities and services (part of the packaged benefits) in the resettlement sites than the permanent stayers. Comparative findings on income, status of employment, proximity of relatives, organizational membership significantly vary according to the resettlers' type of resettlement.

Based on the variables used in the study, the resettlers housed in the 4 resettlement categories have satisfactorily met their shelter needs (housing, water, light, school buildings and health and recreational services) but the resettlers have yet to satisfy their other basic needs. Poverty is still predominant due to lack of means of livelihood coupled with insufficient income. Partly because of insufficient means and perhaps lack of trade skills, at least a quarter of the resettlement population did not see the resettlement sited as their home in the future.

1. Resettlement Sites
2. Forced Migration

3. Pampanga
4. Mount Pinatubo Victims
5. Philippines
6. Satisfaction
7. Mount Pinatubo Commission (MPC)
8. Non-Government Organizations (NGOs)
9. Quality of Life
10. Lahar, "Mudflows"

### BACKGROUND OF THE STUDY

Since the eruption of Mount Pinatubo 1991, more than a quarter of a million families (384,161) in the province of Pampanga alone have been affected by lahar. This involves some three and a half million persons or 3,538,049 to be exact (Note: the number of families per person may not be mutually exclusive populations). Thus, the figures confirm the claim that the Mount Pinatubo eruption is the "disaster of the century". Table 1 shows the massive loss of property and displaced population in Pampanga and, at the same time reflects the reality of overwhelming rehabilitation problems. In 1991 alone a total of 695,767 people were hit, the figure representing more than half of Pampanga's population in 1990 (1,532,682) (see table 1).

It may be noted that the most number of families affected occurred in 1991 (150,219 – which is 39% of the total number of affected families so far), followed by 1993 (117,609 or 31% of the total number of families affected). The years 1991 and 1993 could therefore be considered as Pampanga's worst based on the large number of population hit by lahar and floods during that period.

Significantly, in the province's only city, Angeles, some 17,305 families have been affected from 1991 to 1995. It was in 1991 when most of these families, involving about 15,864 or 92% of the total number of families during the past four years, were badly hit by the first lahar flow.

In 1995, typhoons Karing to Rosing (a total of eight typhoons) added 20,305 families to the list of mudflow "lahar" victims – mainly coming from barrio Cabalantian, the last barangay in Bacolor to be hit by lahar. Also, during the five years (1991 to 1995), a total of 1,092 deaths, 228 injured and 85 missing have been reported (DSWD, Region III, 1995).

A glance at the data is all that is necessary to pinpoint the Herculean task of both government organizations (GOs) and non-government organizations (NGOs) in addressing the rehabilitation needs of the disaster victims in Pampanga.

It is a common knowledge that the first agency involved in rescuing and caring for the victims is the Department of Social Welfare and Development (DSWD). In the case of Pinatubo affected families, the agency, makes certain that they are first housed in evacuation centers – usually public school buildings or bunk houses and, in some cases, tents.

Table I. Number of Affected Families in Pampanga, Philippines

Province/City	1991		1992		1993		1994		1995		TOTAL	
	<i>families</i>	<i>persons</i>	<i>families</i>	<i>persons</i>	<i>families</i>	<i>persons</i>	<i>families</i>	<i>persons</i>	<i>families</i>	<i>persons</i>	<i>families</i>	<i>persons</i>
Pampanga	114,355	632,252	88,284	404,669	114,588	62,804	114.629		57.425		604,276	
Angeles	16,864	63,515	141	3,210	25	113			607		16.537	
TOTAL	159,219	905,767	98,425	407,879	117,808	629,197	114,629		57,922		628,813	

Source: Department of Social Welfare and Development, Region III

## THE PROVINCE OF PAMPANGA: PRIOR TO THE 1991 MOUNT PINATUBO ERUPTION

Pampanga – 60 kilometers north of Manila – has always been considered as a leading growth center in Central Luzon. The Kapampangans are known for their affluence as well as rich in culture. As such, they tenaciously cling to their roots and feel very strongly that they are never “a society of tumbleweeds.” This rootedness both in their soil and in their culture, in fact, is an investment that has yielded them profitable results. To mention some notable places: there is Bacolor, the Athens of Pampanga (as it has produced literary giants), there is Betis, which is known for its artisans, there is Santa Rita, the home of the best pastries and sweets, there is Porac, famous of its Class-A sand. The whole of Pampanga is beautifully symbolized by Mount Arayat: an imposing and enchanting landmark reflecting the proud heritage of the Kapampangans.

### REVIEW OF RELATED LITERATURE/CONCEPTUAL FRAMEWORK

#### A. Issues of Land Settlement Program

Land settlement in the Philippines is perceived as a solution to the social and economic problems of the country. Sparsely populated areas like Mindanao, Palawan and Cagayan Valley had been recipients of settlers from the densely populated areas in the Philippines. The government initiated the movement of population for the purpose of alleviating agricultural production. It cannot be denied that people are important in converting idle lands to productive lands. Human resource, however, may be considered as only one of the main ingredients, the government needs to invest in technology and infrastructure as well (Paderanga in Oberai, 1988). Taking the road to economic prosperity through the land settlement programs has not been smooth sailing. In the past several difficulties were encountered since settlers were looking for non-farm employment opportunities. Settlers and the government set different objectives. There was also the problem of inadequate infrastructure, lack of comprehensive planning in the settlement sites (Arndt in Oberai, 1988). Social tension between settlers and the indigenous population was also an integration problem with no easy solution (Oberai, 1988).

The latest large-scale displacement of human population in the Philippines has been caused by the Mount Pinatubo disaster. The government has already spent P10 billion billion for immediate assistance to the victims, and according to the Mount Pinatubo Commission (MPC) report for 1991-1992, P4.6 billion of the P10 billion spent was expended for infrastructure related to resettlement projects. In addition to the government sponsored resettlement, the NGOs through their collaboratived network, have similar resettlement projects.

Corruption has been noted, however, in the development of government resettlement projects. Banzon-Bautista, (1993) has categorically cited in her book complaints among the settlers. She classifies these complaints into two: First, those



related to the pre-planning stage. Resettlers complained that roads and buildings were constructed before the resettlers' houses. Second, resettlers also complained against the nature of planning and decision-making regarding their resettlement, specifically, their non-participation and non-involvement. This complaint is important considering that popular participation made for successful resettlement (despite insufficient design and material inputs) as experienced in Turkey, Iran and Peru, with cases cited by Oliver-Smith (1991). B. Forced Migration.

The occurrence of unpredictable events, such as war, natural calamities, political upheaval etc. leaves the affected population with no choice but to abandon their abode. Such forced migration has been studied and reviewed in places like Poland (Kostrubeic, 1992), in Central Europe (Stoka, 1992), in Slovakia (Ochovyski, 1989), in the Soviet Union during the Stalinist era (Zeniskov, 1990) and in Ethiopia (Pankhurst-Alulal, 1991).

In forced migration, decision not to resettle is not an option. The forced migrants are given little alternatives. Majority of the above cited studies found that full development of the resettlement sites for these compelled population depends on the willingness of the government to increase the supply of services to the resettlement centers without necessarily increasing the population. Similarly the Pampanga-Pinatubo resettlers never in their lifetime ever dreamed of leaving the comfort of their homes (Nelson, 1995, unpubl.). Moreover, Pampangos, like all Filipinos, have close kin ties and rarely change residence during their lifetime. Filipino mobility, if ever it occurs, is often linked to marriage or job. In contrast, mobility, particularly internal migration is a phenomenon associated with Americans who are obsessed to change residence three to four times during their lifetime (Hansen and Boertbeleen, 1981).

Furthermore, spontaneous resettlers may have to adapt to a new way of life that brings them into direct conflict with some of their most cherished cultural traditions (Turton and Turton, 1984). Also, during this process of rebuilding and relocating, the majority living in several temporary homes, move frequently because of dissatisfaction (Bjorklund, 1981). However, even twenty-one years after the 1964 great Alaskan Earthquake, few alterations – despite material, technological, political and attitudinal changes – in the social structure occurred beyond pre-disaster trends.

The literature cited is far from complete, but it shows that resettlement in the Philippines has occurred in the past through government initiative to relocate the victims of natural hazards but that this relocation is mainly for shelter provisions rather than economic production.

Also the corpus of literature mentioned distinguishes two types of mass migration. The first type is voluntary migration where the migrants themselves decide whether to resettle or not. In this case, the government and the migrants have no high hopes and expectations in making their decisions to resettle. It ought to be pointed out, however, that the resettlement programs in this type are not successful. On the other hand, the second type of mass migration is involuntary where migrants are forced to resettle. Thus, instead of having expectations or hopes for a better future, the resettlers are in most cases, bitter, having suffered stress and strain and may have

aspirations buried in the depths of lahar (Nelson, 1995, unpubl.). Forced migration or forced movement of whole communities from one place to another is difficult to understand and to accept, most especially by affected individuals. A recent study in the Arab Middle East countries focused on the social implications of population displacement and the process of resettlement (Shami, 1993). However, because of its narrow focus, the study failed to provide analytical synthesis nor generalizations based on comparative data. It has also been observed in another study (Pankhurst, 1991) that an increasing number of Ethiopians have been leaving the resettlement areas. One reason is that the collectivized life in the resettlement is not nearly similar to their homeland. For this type of migrants, adjustments can be extremely difficult.

Furthermore, the literature reviewed indicated firstly, that settling or resettling is a slow process and is bound to fail rather than succeed. Success depends upon ways by which the resettled sites are made to be livable, sustainable, that is, in terms of water, education, health services etc. Secondly, it has been noted that people are by nature hard to please. The level of satisfaction depends on the cost in answering their needs or expectations. Thirdly, the cost in putting up infrastructure is bound to have inevitable losses. And there is always a question that remains to be answered: Can one government continue to increase the supply of services?

### THE PRESENT STUDY

Pampanga, the hardest hit province during the catastrophic 1991 Mount Pinatubo eruption, has remained the fragile center/area constantly threatened by the recurrence of mudflow (commonly known in this area as "lahar flows"). This calamitous situation has forced many people to leave their homes and seek refuge in resettlement areas – the most immediate solution to their problem.

The process of relocating such displaced populations of Pampanga is definitely difficult and costly. To grapple with the reality of complex human needs and nature is – inevitable. Now that a great number of victims are resettled, several questions relative to the current status of both resettlers and their sites may still linger in the minds of many. A significant concern may be posed: Are the resettlers satisfied?

This paper underscores the idea that calamity victims go through the dark corridors of loss and grief. For them to recover easily is definitely a matter of perspective. Generally speaking, however, human nature dictates that the ultimate goal after the experience of loss is usually to regain as quickly as possible the lost objects. As victims most of them witnessed the bubble-like disappearance of their houses, occupations, or worse still, loved ones in so short a time. Thus, the socio-psychological disturbance has intensely jarred these people and to restore their equilibrium, so to speak, is what they need most. The cost is, in fact, much greater: "socio-psychological cost" on the part of the resettlers and burdensome "economic cost" on the side of the government.

The success or failure of a resettlement program is primarily indicated by the satisfaction of its beneficiaries – the resettlers. Considering that the beneficiaries are

“forced” by circumstances to resettle in the sites, their level of satisfaction in their new environment tells a lot about how the resettlement program rates.

Being in resettlement areas involved primarily a re-conditioning of the minds of the resettlers to the possible replacement of losses. Their satisfaction level vis-à-vis their present lot is the most important point of consideration in their decision to remain where they are now. A favorable situation will most likely help them get settled easily. To put it metaphorically, the condition of the soil usually determines the success of the planting.

The basic premise is the inverse relationship between the level of comfort experienced by the resettlers before and after their resettlement. In other words, people who had “more” or used to have “much” would never be easily satisfied. Necessarily, those who had “less” will be more open to the acceptance of their fate or will easily reach a particular level of satisfaction and, consequently, take resettlement as a favorable option. A premise, in fact, supported in the case study conducted by NEDA, 1996, 4.1.1,

“Those who were owners of large houses and lots before, were the least satisfied with the resettlement houses. Those with modest property before, if any, expressed general satisfaction. It seems that socio-economic status and prior living conditions combined to determine levels of satisfaction with resettlement housing.”

Ultimately, the question is: How effective are the resettlements in terms of replacing the losses? Specifically, do these resettlements provide an approximation of the amenities, physical comfort and social support of the places of origin of the victims?

### **SIGNIFICANCE OF THE STUDY**

A closer look into the condition/situation of both resettlers and their sites can be of productive consequence. For one thing, through the available data, insights may be drawn relative to the assessment of the resettlement projects, at least in Pampanga. Major concerns, therefore, including the question previously posed may be dealt with accordingly. For another, conventional answers, particularly by government agencies, which do not really provided exact solutions to problems being encountered in the sites maybe re-evaluated, assuming accuracy of the given data and soundness of the analytical framework being followed.

This paper provides an overview of a salient feature of the research project, viz, the socio-demographic profile of resettlers in Pampanga. It is assumed that an assessment of the “quality of life” of the resettlers before and after their resettlement is an important factor in deciding to stay permanently in the sites.

## METHODOLOGY

### Phase I

A preliminary study was done to assess magnitude of the damage caused by the eruption of Mount Pinatubo as well as the extent of population displacement. Secondary data furnished by various government agencies such as the Department of Social Welfare and Development, Mount Pinatubo Commission, and other bodies were examined closely. The available data show that only five percent of the totally affected families are found in the resettlement areas. Moreover, as reflected in the socio-demographic profile in each of the resettlement communities, only the poorest of the poor, the low-income earners, those lacking in marketable skills or human capital such as education are found in these very costly constructed sites. In other words, it may be safe to assume that the rest, 95% of the affected population, have chosen to swim in the vast sea of opportunities rather than wade in pools of government assistance or even patronage.

### Phase II

The revelation that the least productive members of the Pampanga community, a minuscule five percent of affected families, opted to be resettled (a tentative conclusion, at this point), has necessitated the second phase of the research – an actual field research – so that an empirical data base may be established. This phase took two months and used a 17-page interview instrument.

There are currently 19 resettlement sites in Pampanga accommodating the victims. These resettlement sites were built by the Mount Pinatubo Commission (MPC) of the Philippine Government and by the Non-MPC (established by non-governmental organizations). There are separate resettlement sites for the lowlanders and the uplanders hence there are four resettlement areas namely, Lowland MPC, Lowland non-MPC, Upland MPC and Upland Non-MPC. The lowland Non-MPC are 7 in all. There are 3 upland MPC and one Upland Non-MPC. These resettlement sites were established on a staggered basis in the last five years (categorized into Lahar I established in 1992 to Lahar V established in 1996).

Parameters which are deemed critical in determining the approximated satisfaction of resettlers were identified, scaled and validated. With the use of these parameters, the resettlers in 19 resettlement sites were classified according to the sponsoring agencies (the government agencies represented by the Mount Pinatubo Commission [MPC] and non-governmental organizations [NGOs]) and according to location (lowland vs. upland). Thus, the statistical analysis is in reference to the resettlers in these four resettlement categories, namely: a lowland MPC, b. lowland non-MPC, c. upland MPC, and d. upland non-MPC.

The study focused on the resettlers prior to their resettlement and their conditions in the present resettlement sites, their amenities, facilities, services etc. as well as the relevant features of resettlement such as community facilities and services, social attachment and social support. These aspects of resettlement are deemed to be

the significant indicators that determine the satisfaction of the resettlers. A test of difference of means before (their residence prior to resettlement) and after (in the resettlement sites) was computed for the following variables: lot area, floor area, number of rooms, travel time, distance of residence from the town proper, distance of residence from place of work, relatives living in the same household, membership in community organizations and number of organizations. Significant differences in the means using t-test and Chi-square ( $X^2$ ) Mc Nemar on these variables would indicate relative satisfaction of the resettlers. Estimation on the differences of means was done separately for each of the four resettlement categories. Furthermore, test of difference of means was likewise computed for the work status (employed and unemployed) over the past 7 years since 1990-1996. Similarly, test of differences of means was computed using Chi-square, (Friedman) for the monthly income of the resettlers over the same 7 year period (1990-1996). Again, a separate estimation of differences of means was computed for each of the 4 resettlement categories.

Resettlement may be perceived in so many ways. For the resettlers, it may either be a temporary shelter and good only as long as there is a perceived eminent danger in the places of origin or it may be a place to settle down permanently regardless of whatever the future is in their places of origin. Using a one way classification of variance, an attempt was made to differentiate the resettlers' perception on their plans to either stay permanently or not with the following characteristics: age, educational attainment, income, work status, length of stay in previous residence, relatives living with them or nearby, membership in organizations and their perception with regard to the facilities and services rendered to them by the management of their respective resettlement sites.

## RESULTS AND DISCUSSIONS

### A. Socio-Demographic Characteristics of the Resettlers

All data reported are based on estimated population.

Ninety-two percent of the resettlers are the original recipients of the home units which they presently occupy. However, a negligible number (2 out of 439 respondents) indicated that they bought the home unit from a previous awardee.

The computed mean or average age of the resettlers is 25.6 which reflects a young population. Uplanders show a much younger age composition (19.7 for upland non-MPC) as compared to the lowlanders (26.4 for lowland non-MPC).

As to the educational attainment of household heads, the predominant, among lowland resettlers, is high school, followed by those with elementary education. For upland resettlers, most of whom are Aetas (upland minorities in Central Luzon), the highest educational attainment attained by the household heads is grade six. And, in terms of all household members, the most frequent level of education attained is grade six and high school.

When it comes to work status, the upland resettlers have a higher percentage of employment. At least 33% of lowland resettlers are currently unemployed.

In monthly income, forty percent of lowland resettlers have an average income between P2,001 and P3,000. Resettlers from the lowland non-MPC sites have the highest percentage (25%) of household heads earning between P4,001 to 5,000 making them the most well-provided among sites. Income is mostly derived from wages earned from services, production work, and transport equipment operation.

For the household head, the monthly income of all working household members reflected largely similar results. When asked on the sufficiency of their income, regardless of site category, household heads see their incomes as ranging from insufficient to fair. Very few resettlers reported an income that is either very sufficient or highly sufficient.

From 1991 to 1996, there has hardly been any change in the employment status of resettlers. This working sector seem to have retained the same jobs during the five-year period which indicated no improvement in their economic status since their resettlement.

In terms of living conditions, some 10 to 15% of lowland resettlers did not have toilet facilities before being resettled. However, at present, all housing units are provided with private toilet facilities.

For educational institutions, the most predominant is the elementary school. Meanwhile most of the service facilities that the resettlers enjoyed moving are also present in the resettlement sites. Basketball courts, on the other hand, are usually found in lowland sites.

Findings show that the upland non-MPC resettlement sites are the most secure against the threat of mudflows or "lahar".

Compared with the situation before resettlement, a slightly higher percentage of relatives were found living with the family unit after resettlement. This is especially true for resettler families in lowland non-MPC sites and upland MPC sites. However, hardly any difference exists with regard to proximity of relatives to the family unit before and during resettlement.

When it comes to personal or family problems, almost three quarters or 75% of the respondents said they do not have personal or family problems since the time they were resettled.

Seventy-seven percent of respondents indicated that they intend to stay permanently in the site since they have no other choice.

The remaining 23% said that they did not intend to stay permanently and indicated a desire to return to their previous residence as the strongest reason.

## **B. Tests of Significant Differences**

The preliminary data on the composition and perception of the resettlers on various aspects of the resettlement sites show how these conditions compare to the places of origin prior to resettlement. The results, as summarized in Tables 2a to 2e, show highly significant differences ( $p < 0.01$ ) in all the 10 variables without any exception regardless of resettlement categories.

A negative mean value difference between the after (present resettlement) and before (places of origin) was found in the following: lot area, floor area, number of rooms. The present resettlement houses have an average size of homelots of 94 square meters, uniformly built out of galvanized iron roofing and concrete walls with two bedrooms, a small living room and a bathroom/toilet area (see pictures on the next pages). There is no kitchen but most resettlers have added this room at their own cost. It seems that the physical structure of the house and the homelot areas are relatively smaller than their previous residences, thus, discomfort in terms of little physical space may result to some form of dissatisfaction. The variables on the distance of the resettlement sites from their place of work and relative to the nearest town proper and travel time (in Third World countries such as the Philippines, the time spent in travelling is a significant variable since it is affected by the road conditions and the availability of transportation.) There seems to be a big difference between the distance of the present resettlement sites to the town proper compared to their places of origin (see tables). This suggests that most resettlers find the present resettlement areas conveniently closer to the town proper as compared to where they were previously residing. The proximity of relatives, that is whether relatives are staying in their present abode indicated the presence of socio-psychological support, an important aspect during times of crises. In other words, on the top of the physical comfort, resettlers satisfaction also entails psychological support. In a country like the Philippines where close family ties are of great value, proximity of relatives in times of crisis is an indispensable consideration for satisfaction. Information on the composition of the household also indicates that it has become extended which suggests that it has become a mechanism for coping. It also likely indicates that the families have relatives who are also victims of disaster. Results showed significant differences between the after and before conditions of resettlement in this psychosocial variable. The lowlanders have become more extended in their household structure (increased in the number of relatives living in the same household and nearby). The uplanders likewise became more extended although they have always been traditionally larger in terms of their household composition. The family unit is the most basic coping mechanism and is likely to operate in most if not all disaster situations (Kieffer, 1956). Disaster victims do not act as independent individuals but their responses are to a high extent influenced by the families to which they belong. The larger household composition after resettlement may not only be due to coping mechanism strategy but may be attributed to the fact that available home units are much fewer in proportion to those who were rendered homeless and as result those who were awarded home units simply accommodated relatives who are still in queue or are not eligible to be resettled.

One of the more viable programs in the resettlement sites is organizing people to meet their special needs as a group. Resettlers have their own block leaders and cluster leaders who also act as spokespersons and represent them in several occasions during meetings. To be a resettler is almost synonymous to active participation in the self-help programs of the sites. These organizations deal with various projects

Table 2a. Statistics of variables after and before resettlement (lowland, MPC)

Variables	N	Means	Standard Deviation	t-values	Chi-Square
Lot Area	16000	-181.27	246.35	-93.077 ***	
Floor Area	18050	-33.1	31.37	-141.8***	
Number of Rooms	19800	-0.9	0.91	-138.1***	
Length of Time of Travel	19500	4.20	19.03	32.31***	
Distance of Residence (from town proper)	17850	1639.5	4065.11	53.68***	
Distance of Residence (from place of work)	19890	21.71	45.87	66.18***	
Relatives living the same household	19749				503.63***
Relatives living near the household	19749				106.59***
Membership in community organizations	19749				40.33***
Number of organizations	19550	0	0.65	1.09	

\*\*\* p< .001

Table 2b. Statistics of Variables after and before resettlement (lowland, non-MPC)

Variables	N	Means	Standard Deviation	t-values	Chi-Square
Lot Area	1058	-95	117.46	-26.3***	
Floor Area	1242	-36.7	27.78	-46.54***	
Number of Rooms	1380	-0.86	0.84	-38.04***	
Length of Time of Travel	1380	1.36	11.81	4.29***	
Distance of Residence (from town proper)	1380	533.3	2395.07	8.27***	
Distance of Residence (from place of work)	1380	27.33	45.49	22.63***	
Relatives living the same household	1400				92.33***
Relatives living near the household	1400				44.68***
Membership in community organizations	1400				59.14***
Number of organizations	1380	0.23	0.71	12.1***	

\*\*\* p< .001



Table 2c. Statistics of Variables after and before resettlement (upland, mpc)

Variables	N	Means	Standard Deviation	t-values	Chi-Square
Lot Area	47	-95			
Floor Area	235	-17.2	6.22	-42.37***	
Number of Rooms	611	-0.07	0.26	-7.12***	
Length of Time of Travel	611	1.36			
Distance of Residence (from town proper)	564	4583.33	16428.28	6.62***	
Distance of Residence (from place of work)	611	36.92	41.29	22.1***	
Relatives living the same household	612				45.09***
Relatives living near the household				ns	
Membership in community organizations	612				245.75***
Number of organizations	611	0.61	0.73	20.59***	

\*\*\*  $p < .001$ 

Table 2d. Statistics of Variables after and before resettlement (upland, non-MPC)

Variables	N	Means	Standard Deviation	t-values	Chi-Square
Length of Time of Travel	170	17	32.28	6.88***	
Distance of Residence (from town proper)	170	700	1169.83	7.8***	
Distance of Residence (from place of work)	170	27.2	42.34	8.37***	
Membership in community organizations	173				0.01
Number of Organizations	170	0	0.63	0	

\*\*\*  $p < .001$

Table 2c. Statistics of variables after and before resettlement (all resettlement sites)

Variables	N	Means	Standard Deviation	t-values	Chi-Square
Lot Area	17105	-175.44	241.12	-95.16***	
Floor Area	19527	-33.14	31.03	-149.21***	
Number of Rooms	21661	-0.86	0.9	-141.18***	
Length of Time of Travel	21711	4.18	18.58	33.14***	
Distance of Residence (from town proper)	19654	1638.18	48.16	47.81***	
Distance of Residence (from place of work)	21711	22.6	45.86	72.64***	
Relatives living the same household	21934				620.52***
Relatives living near the household	21934				131.9***
Membership in community organizations	21934				0.03
Number of organizations	21711	0.03	0.68	8.1***	

\*\*\*  $p < .001$

that are either self-initiated or established by various sectors. Thus, resettlers are shown to be more actively involved in many organizations than before. This shows a significant difference in the membership and number of organizations of resettlers especially among the lowlanders. Organizations can also act as support groups to augment kin and family relations.

### C. Findings on Income and Work Status of Resettlers

Table 3 shows the proportion of employed resettlers in various resettlement categories from 1990 to 1996.

A decrease in the proportion of those employed is evident in all categories except for upland non-MPC. In 1990 prior to the Mt. Pinatubo eruption, 80% of the lowlanders were employed. The proportion of those employed in 1991 immediately after the Mt. Pinatubo eruption was lowered remarkably to 66% and 70% for lowland MPC and lowland non-MPC respectively. This proportion has been maintained until 1996 which only attests to the fact that a good proportion of the resettlers did not only lose their houses but their means of livelihood as well. The computed Chi-square showed significant differences in employment status experienced by the resettlers during the 7-year period. Moreover, the unemployed settlers are not even qualified in many training programs for livelihood purposes. Examples of which are the "Victims Training Scholarship Program" and the "NGOs and Cooperative Capability Build-up Program" which requires trainees to be at least high school graduates and require an age limit of 32 years old and younger. The unemployed settlers are ineligible for livelihood training program mainly because they are mostly 35 years old and over and have not even reached high school education.

Differences in the mean monthly income of the resettlers during the period prior to resettlement in 1990 and the succeeding 6 years after the eruption is shown in Table 4.

Highly significant differences may be observed among all the resettlers in various resettlement sites. An increase in monthly income was found solely for the

Table 3. Work Status of Resettlers from 1990-1996

Year	Proportion of Employed				
	all	lowland mpc	lowland non-mpc	upland mpc	upland non-mpc
1990	80.34	80.05	76.67	92.31	100
1991	67.41	66.24	70	92.31	100
1992	64.7	62.92	73.33	92.31	100
1993	65.21	64.19	63.33	92.31	100
1994	65.67	66.75	70	92.31	100
1995	67.94	66.5	66.67	84.62	100
Chi-Square	4213.82	4907.63***	244.75***	282.00**	n.s.

\*\*\* p<.001

uplanders in the non-MPC sites. The rest of the resettlers experienced a decrease in income although the difference is just slightly over P200.00. The devaluation that has occurred during the last 7 years in fact meant that the income of the resettlers in 1990 had far more value compared to the income earned in 1996. Thus, the resettlers suffered from loss of income as a result of their dislocation and have not reached the income level they had in 1990 prior to the eruption of Mount Pinatubo.

#### D. Permanently Stayers vs. Temporary Stayers

Perception of the resettlers with respect to their plans of staying permanently was asked. As reported earlier, some 23% did not see the resettlement as a permanent place of residence. The ANOVA was computed to test differences in means of some relevant variables on their plans (permanent and temporary stayers). Findings are shown in Tables 5a to 5e.

The older the resettlers, the longer they have lived in the previous residence the less likely for them to favor staying permanently in the resettlement sites. The temporary stayers as shown in the tables have also consistently given lower ratings on the different facilities and services in their respective resettlement sites. This stems from the attachment, including sentiments they have had in their places of origin. Additional findings vary by resettlers' resettlement categories. For example, in terms of income, the high income earners from the lowland MPC are not looking forward to staying permanently in the resettlement sites whereas it is the high income earners in the lowland non-MPC who desire to stay permanently. A high earner as in the case of the lowlanders in the MPC may have the resources to afford to move elsewhere or to reestablish in their respective places of origin. The high income earners among the lowlanders in non-MPC who desire to stay permanently is a sign of stability and perhaps perceived themselves fortunate and therefore are less likely to move elsewhere. It can also be noted that there is a high proportion of the employed in lowland non-MPC resettlement category who are permanent stayers. Relative to other resettlers, the lowlanders in the non-MPC resettlement sites are better-off. This finding is also true to the uplanders in the MPC managed resettlement sites.

Table 4. Mean Monthly Income of Resettlers from 1990-1996

Year	All	lowland mpc	lowland non-mpc	upland mpc	upland non-mpc
1990	2315.18	2330.68	2913.2	907	739.4
1991	2045.86	2041.95	2766.53	907	739.4
1992	1993.65	1983.31	2803.2	683.76	1319.4
1993	2156.62	2186.24	2346.5	660.69	1319.4
1994	2218.25	2276.77	2518.83	660.69	1318.4
1995	2113.48	2154.28	2276.53	660.69	1319.4
1996	2112.78	2158.05	2263.2	545.3	1319.4
Chi-Square		251061.57***	816.46***	1470646.94**	191.61***

\*\*\* p<.001

The effect of the presence of relatives within and near the sites has varying results. Permanent stayers in the lowland MPC have few relatives living in the same household although 90% of their relatives are in the neighborhood. This is contrary

Table 5a. Comparison between permanent and temporary stayerson some variables (lowland MPC)

Independent Variable	Permanent Stayers	Temporary Stayers	F-value	Z-value
<b>Socio-demographic Variables</b>				
Age	45.75	46.45	133.5***	
Income	1974.94	2720.75	309.57**	
Work Status	57.45%	73/54%		5.01
Length of Stay in Previous Residence	19.91	26.41	420.01***	
<b>Socio-psychological Variables</b>				
Relatives living in the same household	13.59%	29.16%		24.90***
Relatives living in the near household	90.84%	95.83%		11.16***
Membership in community organizations	24.40%	28.12%		5.17
Number of organizations	0.29	0.41	113.29***	
<b>Infrastructure, Facilities and Service Variables Perceived Ratings of Infrastructure, Facilities and Services</b>				
	74.79	67.61	1358.76***	

\*p<.05 \*\*p<.01 \*\*\*p<.001 (one-tailed test)

Note: Comparison are in means and proportions

Table 5b. Comparison between Permanent and Temporary Resettlers on some variables (lowland non-mpc)

Independent Variable	Permanent Stayers	Temporary Stayers	F-value	Z-value
<b>Socio-demographic Variables</b>				
Age	44.44	47.33	6.51**	
Income	2507.4	65.3	164.50***	
Work Status	70.37%	33.33%		8.81***
Length of Stay in Previous Residence	16.44	20	12.74***	
<b>Socio-psychological Variables</b>				
Relatives living in the same household	25.92	0	6.88	
Relatives living in the near household	55.55%	66.66%		2.51***
Membership in community organizations	33.33%	66.66%		7.76***
Number of organizations	0.88	0.33	66.25***	
<b>Infrastructure, Facilities and Service Variables Perceived Ratings of Infrastructure, Facilities and Services</b>				
	55.55	37	693.70**	

\*p<.05 \*\*p<.01 \*\*\*p<.001 (one-tailed test)

Note: Comparison are in means and proportions

Table 5c. Comparison between Permanent and Temporary Stayers on some variables (upland mpc)

Independent Variable	Permanent Stayers	Temporary Stayers	F-value	Z-value
<b>Socio-demographic Variables</b>				
Age	33.91	50	66.80***	
Income	582.58	98	40.18***	
Work Status	16.66%	83.33%		3.04***
Length of Stay in Previous Residence	27.72	50	70.17***	
<b>Socio-psychological Variables</b>				
Relatives living in the same household	16.66%	16.66%		3.04***
Relatives living in the near household	n.a.	n.a.		
Membership in community organizations	66.67%	66.67%		9.03***
Number of organizations	75	0.06%	74.40***	
<b>Infrastructure, Facilities and Service Variables Perceived Ratings of Infrastructure, Facilities and Services</b>				
	49.16	58	71.95***	

\*p&lt;.05 \*\*p&lt;.01 \*\*\*p&lt;.001 (one-tailed test)

Note: Comparison are in means and proportions

Table 5d. Comparison between Permanent and Temporary Resettlers on some variables (upland non-mpc)

Independent Variable	Permanent Stayers	Temporary Stayers	F-value	Z-value
<b>Socio-demographic Variables</b>				
Age	na	na		
Income	na	na		
Work Status	na	na		
Length of Stay in Previous Residence	na	na		
<b>Socio-psychological Variables</b>				
Relatives living in the same household	na	na		
Relatives living in the near household	na	na		
Membership in community organizations	na	na		
Number of organizations	na	na		
<b>Infrastructure, Facilities and Service Variables Perceived Ratings of Infrastructure, Facilities and Services</b>				
	na	na		

\*p&lt;.05 \*\*p&lt;.01 \*\*\*p&lt;.001 (one-tailed test)

Note: Comparison are in means and proportions

Table 5e. Comparison between Permanent and Temporary Resettlers on some Variables (all sites)

Independent Variable	Permanent Stayers	Temporary Stayers	F-value	Z-value
<b>Socio-demographic Variables</b>				
Age	43.38	46.51	192.61***	
Income	1960.86	2622.51	267.40***	
Work Status	68.54%	63.04%		7.29
Length of Stay in Previous Residence	19.25	25.49	470.78***	
<b>Socio-psychological Variables</b>				
Relatives living in the same household	14.65%	28.03		21.90***
Relatives living in the near household	88.41%	95.06%		13.76***
Membership in community organizations	29.13%	28%		1.54
Number of organizations	0.41	35%	21.9	
<b>Infrastructure, Facilities and Service Variables Perceived Ratings of Infrastructure, Facilities and Services</b>				
	72.15	66.67	652.10***	

\*p&lt;.05 \*\*p&lt;.01 \*\*\*p&lt;.001 (one-tailed test)

Note: Comparison are in means and proportions

to the lowlander non-MPC where the permanent stayers have more relatives living in the same household but proportionately fewer relatives living in the neighborhood. Membership in organizations did not differ among the temporary stayers and permanent stayers in the lowland MPC but this variable is significant for lowlanders in the non-MPC and the uplanders in the MPC managed resettlement sites. The socio-psychological variables, as the presence of relatives and organizational membership ought to promote the well-being of the resettlers. It is assumed that community involvement factors for the resettlers to stay. The results are not consistent for all resettlers in various resettlement categories.

## E Categories

From the foregoing tentative data analysis, several conclusions may be drawn. At this point however, it will suffice to say that the various resettlement sites in the 4 categories have fulfilled their primary objective, that is to provide homes to the homeless victims of Mount Pinatubo. They have addressed the need for shelter and have provided the much-needed housing to those with little resources and have no other resource but to depend upon government and private agencies for assistance. The resettlers' little resources stem from inadequate skills as a consequence of limited education (most of them are elementary and high school graduates). Thus, with the resettlers' little income and chronic unemployment, poverty is a reality in the resettlement sites. It is this aspect of providing livelihood to the resettlers that is yet to be addressed by the resettlement organizers. Resettlement, therefore to be fully successful should have two-pronged objectives, that is provide housing and means of

livelihood. These are two aspects that a victim of a calamity has lost due to dislocation. The second aspect, which is providing livelihood is more complex for it entails providing skills for a certain trade. There are however some agencies who have answered the call for this particular need. But those who are most in need (unskilled, unemployed resettlers) are left out simply because they cannot meet the required qualifications of the trainers. Until this need is met, the desire of the resettlers to move will always be there. Only when resettlers are satisfied could resettlement be considered successful.

In ascertaining whether the Mount Pinatubo resettlers are satisfied, an important observation may be cited. Statistical data reveal that the "quality of life" of most resettlers at present is primarily the same, if not better than what they used to have. The strong significant positive difference (after-before) in the variables concerning accessibility of the resettlement sites and psycho-social support from kins given in the tables also suggests a strong reason for these displaced Kapampangans – who are mostly poor – to find their present homes a place of security.

Since their lack of other options forces the resettlers to stay in the sites permanently, perhaps, they are now prepared to begin driving their "tent pegs," so to speak, deeply into the ground and really begin reconstructing their lives.

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## INDIVIDUALISM VS. COLLECTIVISM: IMPLICATIONS FOR HEALTH PROMOTION

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### ABSTRACT

*This paper examines the strengths and limitations of various health promotion strategies as they relate to individualism-collectivism. Individualism-collectivism is discussed in relation to the types of strategies adopted for improving health; active vs. passive measures; and the strategies of education, engineering and enforcement. Findings on individualism-collectivism in the area of communication research are applied to the health promotion domain. It is hoped that the conceptual framework presented in this paper would contribute to the development of culturally-appropriate health promotion interventions.*

**Keywords:** *individualism, collectivism, health promotion*

The most successful health promotion efforts are likely to be those that are sensitive to the culture context of health (e.g., Braithwaite and Lythcott, 1989; U.S. Department of Health and Human Services, 1985). It is therefore important to select health promotion approaches consistent with the cultural orientation of a community. Individualism-collectivism is perhaps the broadest and most commonly studied dimension of cultural variability (Gudykunst and Ting-Toomey, 1988). The purpose of this paper is to present particular approaches to health promotion as they relate to individualism-collectivism. A brief overview of the individualism-collectivism dimension of culture is presented followed by a discussion of its potential influence on the effectiveness of health promotion strategies.

### INDIVIDUALISM AND COLLECTIVISM

Hofstede (1980) describes individualism-collectivism as the relationship between the individual and the individual and the collectivity that prevails in a given

society. In individualistic cultures, individuals tend to prefer independent relationships with others and to subordinate ingroup goals to their personal goals. In collectivistic cultures, on the other hand, individuals are more likely to have interdependent relationships to their ingroups and to subordinate their personal goals to their ingroup goals. Individualist cultures are associated with emphases on independence, achievement, freedom, high levels of competition, and pleasure. Collectivist cultures are associated with emphases on interdependence, harmony, family security, social hierarchies, cooperation and low levels of competition. Collectivist cultures are assumed to be exemplified in mainstream American and Western European cultures. Individualist cultures are exemplified in Asian, African, Latin-American and Southern European cultures.

A concept similar to individualism-collectivism is the independent vs. Interdependent construals of the self. Markus and Kitayama (1991) have suggested that people hold divergent construals of the self—an independent view of self as opposed to an interdependent view of the self. The independent view of the self considers the self as separate from the social context. Individuals seek to maintain their independence from others by expressing or emphasizing their unique inner attributes (abilities, thoughts and feelings) and by promoting their own goals. In contrast, the interdependent view of the self assumes a fundamental interconnectedness among individuals. The self is defined in relation to others in specific contexts (statuses, roles, relationships). The emphasis is on "fitting in", promoting others' goals, and maintaining harmony with the social context. The independent-interdependent self-construal has been conceptualized as an individual difference factor while individualism-collectivism has been conceptualized as a cultural factor (Singelis, 1994).

### **INDIVIDUALISM-COLLECTIVISM AND HEALTH PROMOTION STRATEGIES**

A particular approach to health promotion may be more appropriate for individualist cultures while other approaches may be more appropriate for collectivist cultures. A key issue in health promotion policies and programs is how much constraint the collectivity can impose on individuals to accomplish its health-related goals. If group goals are more important in collectivist cultures than in individualist cultures, then collectivist cultures may allow a greater degree of constraint on individual behaviors than individualist cultures. For example, health promotion that deals with matters of lifestyle such as overeating and smoking may be seen by some individuals in an individualist cultures as an effort to interfere with their right to privacy. From a collectivist perspective, however, collective health is seen as outweighing the individual's loss of freedom. Varying degrees of constraint on the individual are reflected in the types of strategies adopted for improving health: active measures vs. passive measures; and the strategies of education, engineering and enforcement.

### **Active vs. Passive Measures**

"Active" and "passive" are opposite poles of a dimension that along which measures to prevent disease and injury can be usefully classified (Haddon, 1974). The dimension is defined in terms of the amount of action required by individuals in order for them to be protected. Active measures emphasize the role of the individual in adopting healthy behaviors (e.g., eating a balanced diet, regular exercise). In contrast, passive measures are techniques that protect individuals automatically without any cooperation or action on their part (e.g., fluoridation of water supply, automatic seatbelts). Historically, major health gains have been more the result of implementing passive measures at the community level (e.g., improved sanitation, pasteurization of milk), or relatively passive actions on the part of individuals (e.g., immunizations), rather than actions that require frequent actions on the part of the individual to be protected (e.g., vigorous exercise; Williams, 1982). Although the adoption of passive measures may be applicable to both individualist and collectivist cultures, it may be especially useful in individualist cultures. The use of passive measures may be a subtle way of overcoming the resistance of individualism. For example, at the societal level, changing food in ways that would result in healthier diets (e.g., low-fat diets) may be more effective than encouraging individuals to adopt and maintain healthy diets.

### **Education, Engineering, and Enforcement**

The "Three E's" program of education, engineering and enforcement has been examined within the context of health promotion by Alonzo (1993) based on the U.S. Forest Service's "Three E's" program to protect public forests from misuse. This program as applied to health behavior further illustrates the issue of constraint in health promotion. Education is viewed as a solution to a health problem when it is perceived that a lack of information is inhibiting individuals from behaving in their own best interests (e.g., providing information on the modes of HIV transmission). Engineering is an effort to design or manipulate the environment to reduce risk and avoid harm to societal members (e.g., increasing taxes on alcohol and cigarettes). It is somewhat similar to the concept of passive measures discussed earlier. Enforcement refers to the creation of rules and regulations regarding individual and collective behaviors and enforcing them (e.g., banning smoking in public places). Education presents the least constraint on the individual while enforcement imposes the greatest constraint. Thus, for individualist cultures, education may be a more culturally appropriate means of enhancing health when compared to enforcement. Conversely, for collectivist cultures, efforts aimed at enforcement, as well as education and engineering, may be more acceptable. However, it is important to note that the success of any one approach depends on certain considerations. For example, within an individualist culture, it may be necessary to go beyond educational strategies and address social and environmental barriers in order to improve health. Within a collectivist culture, enforcement as-

sumes that there is a consensus as to the appropriate course of action. In both individualist and collectivist cultures, enforcement can raise ethical issues such as coercion, although presumably less in collectivist cultures. McLeroy (1988) suggests that in order to minimize the problems of coercion, the active involvement of the community members in problem definition and the selection of appropriate interventions is necessary.

In sum, health promotion strategies that are least restrictive for the individual (e.g., passive measures, education) may be more suitable to an individualist culture while more restrictive strategies (e.g., enforcement) may be more appropriate for collectivist cultures. However, the use of any one strategy will have to take into consideration important issues such as social-environmental support for individual health change and ethical issues concerning civil liberties.

The next section focuses on persuasive communication used in health promotion. Findings on individualism-collectivism in the area of communication research will be applied to the health promotion domain.

#### PERSUASIVE COMMUNICATION IN HEALTH PROMOTION

Research in the field of communication has shown that cultural differences in individualism-collectivism play an important role in persuasion processes both at the societal and the individual level, influencing the prevalence and effectiveness of different types of persuasive appeals. Two studies have examined the extent to which individualism-collectivism is reflected in the type of persuasive appeals that tend to be used in the United States, an individualist culture, and Korea, a collectivist culture (Han and Shavitt, 1994). Study 1, a content analysis of magazine advertisements, demonstrated that advertisements in the U.S. employed appeals to individual benefits and preferences, personal success and independence to a greater extent than did advertisements in Korea. Korean advertisements employed appeals emphasizing ingroup benefits, harmony, and family integrity to a greater extent than U.S. advertisements. Study 2, a controlled experiment conducted in the two countries, demonstrated that in the U.S., advertisements emphasizing individualistic benefits were more persuasive, and advertisements emphasizing family or ingroup benefits were less persuasive than in Korea. In both studies however, product characteristics played role in moderating these overall differences. Cultural differences emerged strongly for in Studies 1 and 2 for advertised products that tend to be purchased and used with others (e.g., over-the-counter medicines, groceries, insurance, cameras) but were much less evident for products that are typically purchased and used individually (e.g., cosmetics, jeans, greeting cards, credit cards). In other words, for shared products, there were strong differences between U.S. and Korea in the prevalence and effectiveness of appeals. For personal products, however, individualistic appeals were generally favored in both countries. This distinction between health behaviors that affect only the individual vs. health behaviors that affect other people as well. The implications of this distinction for health promotion will be discussed in detail later.

Persuasive communication, often in the form of media messages, can play various roles in health promotion. It can be the primary agent for change in a community health promotion. It can be the primary agent for change in a community health promotion program or solely as a means of complementing or promoting existing services (Flora and Cassady, 1990). One component that is often included in media messages is a statement of the benefits and/or the costs of engaging in a preventive behavior. How individuals perceive the benefits relative to costs of changing behavior may depend, in part, on their cultural orientation along the individualism-collectivism dimension. For instance, if smoking is viewed as having high costs and low benefits for others, such as one's family, and is considered as potentially threatening to one's relationships with others, then individuals in collectivist cultures might be more encouraged to stop smoking. Alternatively, if smoking is viewed as a character weakness (e.g., as reflecting poor will or lack of personal control) and the costs of continuing to smoke (e.g., loss of self-esteem) outweigh any benefits, then individuals in individualist cultures may be more likely to stop smoking. Thus, framing the costs and/or benefits of behavior change in more culturally meaningful ways (i.e., in collectivist cultures, in terms of relationships; in individualist cultures, in terms of inner attributes) may increase the effectiveness of media messages.

For some health behaviors, however, cultural orientation may not necessarily influence the effectiveness of the health message. For example, in individualist culture, it is expected that the likelihood of adopting a regular exercise regimen would increase when the message is framed in terms of benefits vs. costs *to the individual*. However, since the adoption of an exercise regimen does not directly involve others, then the same kind of message-benefits compared to costs to the individual may be equally effective in increasing the likelihood of action in collectivist cultures. In contrast, the use of condoms to prevent sexually transmitted diseases, when framed in terms of the possibility of infecting a loved one, might be more likely to be effective for collectivist cultures than for individualist cultures since the use of condoms has direct implications for the health of others. Thus, the effectiveness of framing a health message in terms of individualism-collectivism would tend to depend on whether the adoption of the health behavior has a direct effect on others or an exclusive effect on oneself. This notion is similar to the findings in advertising research discussed earlier in which personal products, which offer predominantly individually experienced benefits, were unlikely to be convincingly promoted in terms of personal and group benefits. Thus, although individualism-collectivism may have a significant main effect on the effectiveness of health messages, it is necessary to identify possible moderators of this relationship.

## CONCLUSION

This paper examined the effectiveness of various health promotion strategies as they relate to the individualism-collectivism dimension of culture. It is suggested that empirical research be conducted to test the proposed relationships between individualism-collectivism and health promotion strategies. Hopefully, this research would lead to the development of guidelines which could be used in the design and implementation of culturally-sensitive health promotion interventions.

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## POSTER ABSTRACTS

### *AGRICULTURAL SCIENCES*

#### **1. COMBINATORIAL EFFECT OF RICE BLAST RESISTANCE GENES TAGGED USING RESTRICTION FRAGMENT LENGTH POLYMORPHISM**

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Restriction fragment length polymorphism (RFLP) mapping using greenhouse phenotypic data of recombinant inbred lines (RILs) identified four major genes for blast resistance against five races of rice blast pathogen in Southern USA. Using the markers bracketing the gene or closely linked to the resistance gene as tag for each gene, lines with different gene and their combinations were evaluated at the blast nursery. Analyses of the performance of the lines using three parameters: area under disease progress curve (AUDPC), percent disease leaf area (%DLA), and IRRI standard Evaluation System for blast (SES rating) indicated the presence of direct gene effect and gene x gene interaction. In particular, Pi-q6 significantly interacted with other tagged genes while Pi-q1, Pi-b2 did not. Regression model revealed that the best fitting combinations were Pi-q5, and Pi-q6 for %DLA and AUDPC and Pi-q5, Pi-q6, and Pi-b2 for SES rating. Results further revealed that the genes and their interaction can explain only 20-25% of the variation observed at the blast nursery and the remaining differences may be attributed to the minor genes or unidentified major genes in the RIL population.

**Keywords:** rice blast, RFLP, gene expression, major gene, gene interaction, gene mapping, disease resistance, *Oryza sativa*



## 2. GENETIC MAPPING WITH AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) IN RICE

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Genetic map construction and tagging of genes for qualitative and quantitative traits in rice have been conducted mainly through the use of restrictive fragment length polymorphism (RFLP) and random amplified DNA (RAPD) markers. These markers, however, are limited in the number of loci they can assay per reaction and amplified fragment length polymorphism (AFLP) has been proposed as a valuable tool for gene mapping in many plant species. To test the usefulness of AFLP's for genetic mapping in rice, 54 AFLP bands using seven *EcoRI* +3: *MseI* +3 primer combination on an F<sub>2</sub> population derived from an indica/japonica cross were assayed. Only 4 of the 54 bands scored deviated from the expected 3:1 Mendelian ratio for dominant markers (P<0.05). Using the program Mapmaker and a framework map of RFLPs and RAPDs, 50 AFLPs were mapped to rice chromosome except chromosome 12. The AFLPs located on each chromosome varied from 1 to 8 and appeared to be randomly distributed. Using the resulting genetic map, quantitative trait loci (QTLs) for seed and reproductive traits were identified with 7 QTLs bordered by AFLP markers. These results indicate that AFLP technology could be a tool for genetic studies in rice.

**Keywords:** AFLP, QTL, mapping, rice, PFLP, RAPD, genetic

**3. RESTRICTION FRAGMENT LENGTH POLYMORPHISM  
ANALYSIS OF GENETIC VARIATION IN STRAINS  
OF *Pseudomonas* (RALSTONIA) *solanacearum*  
INFECTING *Musa* and *Heliconia* FAMILIES**

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The Philippine population of banana-infecting strains of *Pseudomonas solanacearum* was analyzed in relation to foreign strains infecting *Musa* and *Heliconia* families through restriction fragment length polymorphism (RFLP). Probes which harbor the genes for extracellular polysaccharides (E36, 07 050), endoglucanase (pHE3), tryptophan biosynthesis (p T161), a random clone from Z04 library (p Z1217) and the repetitive element (pM114) were used for the purpose, RFLP patterns were affected by the kind of probes used. Different RFLP types were noted among strains using different probes. Composite RFLP analysis, however, resolved 16 haplotypes grouped into five clusters at 65% similarity level.

**4. FACTORS INFLUENCING GENE EXPRESSION AND  
T-DNA TRANSFER IN *A. tumefaciens*-MEDIATED  
TRANSFORMATION OF RICE**

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Rice has been routinely transformed using the direct DNA uptake methods. Recent developments in rice genetic transformation using *A. tumefaciens* as a natural vector necessitate the investigation of factors that will promote optimum gene expression and T-DNA transfer in the recalcitrant indica rice varieties. Activities of three different promoters were investigated transiently using *gusA* after particle bombardment of suspension cells. Actin and ubiquitin promoters were found to

induce higher GUS activity. A binary vector pCGN1558 was modified to contain these promoters in front of the *gus* A-intron. *A. tumefaciens* strain EHA 101 and A136 were transformed with the derivatives of the binary vector and transformed colonies were tested for infectivity to coleoptile bases of IR54 and IR72 seedlings. GUS activity of the infected coleoptile bases showed that the *A. tumefaciens* colonies were comparable but not better than the positive control At656. Possible reasons for the results were discussed with emphasis on the importance of the compatibility of the *A. tumefaciens* strains with the binary vector, the border sequences, and the Ti-plasmid present.

**Keywords:** gene expression, I-DNA transfer, *A. tumefaciens*, rice

### 5. IDENTIFICATION OF RAPD MARKERS LINKED TO A MAJOR BRUCHID RESISTANCE GENE IN MUNGBEAN (*Vigna radiata* L.)

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Random amplified polymorphic DNA (RAPD) was used to identify markers linked to major bruchid resistance gene in mungbean. Four pairs of near-isogenic lines-----(*V. radiata* var. *sublobata*), a wild relative of mungbean and the source of resistance to bruchid (*genus Callosobruchus*) of the resistant NILs, were used. RAPD reactions were based on the protocol by Williams et al. (1991), with modifications. Eighty-four Operon primers were screened, of which 66 were successful in amplifying loci from the NILs DNAs. Twenty markers were found polymorphic in at least 2 pairs of NILs. Two markers, S01<sub>946</sub> and Q14<sub>695</sub>, were found polymorphic in all pairs of NILs. Segregation analysis and subsequent mapping of the putative markers were done using a set of recombinant inbred lines from the cross Pagasa 7 XTC 1996.

**Keywords:** RAPD, mungbean, *V. radiata*, insect resistance, *Callosobruchus maculatus*, near isogenic lines, recombinant inbred lines.

## 6. RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS OF PAPAYA RINGSPOT VIRUS-RESISTANT INTERSPECIFIC HYBRIDS

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Papaya production worldwide has been markedly reduced by papaya ringspot virus type-P (PRSV-P). Interspecific hybrid of papaya (*Carica papaya* L.) and a wild relative, *Carica cauliflora* Jacq. were produced to develop resistance to PRSV-P. An improved embryo rescue technique was used for the production of interspecific hybrid plants. Resultant hybrid embryos were of (two) types (single and multiple) and were variable in size, colour, and developmental stages. Regeneration via embryogenesis variable F<sub>1</sub> phenotypes. Random amplified polymorphic DNA (RAPD) markers were used to identify hybrids. Genomic DNA of parents and a hybrid was screened using 72 decamer primers of which 57 showed polymorphism between the two parents. On the hybrid, 17 primers revealed polymorphic banding patterns (1-2 bands) of both parents. Five (OPA-07, OPA-9, OPA-19, OPB-12, OPC-06) out of the 17 primers which consistently resolved polymorphisms of the parents were used to assess hybridity of 120 interspecific hybrid. All plants analysed contained at least 1 band from *C. cauliflora* as shown by any of the 5 primers, indicating that they are hybrids. In addition, all plants produced had some intermediate morphological traits of papaya and *C. cauliflora*. Pollen fertility of the hybrid is low (0.6-130%), in contrast to the high (93-100) fertility of the parents.

**Keywords:** *Carica papaya* L., *Carica cauliflora* Jacq., embryo rescue, interspecific hybrids, papaya ringspot virus type-P, primers, random amplified polymorphic DNA, resistant

## 7. STRUCTURE AND EXPRESSION OF A $\beta$ -GLUCANASE MULTIGENE CLUSTER IN RICE

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Plants produce high levels of  $\beta$ -glucanases in roots during seed germination and flower formation and response to invasion by pathogenic microbes. To understand the role of these enzymes in the defense response and various developmental stages, 3  $\beta$ -glucanase genes in rice were cloned and characterized in structure and expression. *Gns2*, *Gns3*, and *Gns4* were tandemly arranged within a 9 kb region in the same orientation of transcription. These genes encoded a mature peptide of 3.2-3.3 kD in size. Both *Gns2* and *Gns3* specified an acidic mature peptide, in contrast to *Gns4* which encoded a basic mature peptide. The *Gns2*, *Gns3*, and *Gns4* isozymes likely had a  $\beta_{1,3}$ -glucanase activity, and may be extracellular because the genes specify a signal peptide but not a C-terminal extension. *Gns2* was expressed at low levels in germinated seed and mature leaf. Neither *Gns2* nor *Gns3* was induced by exogenous and stress treatments. *Gns4* was found to be the most active gene. It may have exogenous hormones important growth and defense-related functions. It was expressed substantially in germinated seed, and in response to exogenous GA, auxin, ABA, and the defense signal salicylic acid.

**Keywords:** ABA, auxin,  $\beta$ -glucanase, calli, coleoptide, ethylen, GA, protein, seed germination

## 8. STRUCTURE AND EXPRESSION OF A NOVEL DEFENSE-RELATED GENE IN *Oryza sativa* Linn

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Knowledge about molecular mechanisms underlying the plant defense response is still fragmentary. To help better understand this process, the genes

encoding the  $\beta$ -glucanases in rice, which are among the pathogenesis-related proteins produced by plants in response to pathogen invasion were A  $\beta$ -glucanase gene, *Gns9*, that has structural and expression properties was cloned. The *Gns9* mature protein products was unusually long, consisting of 322 amino acids (34.5 kDa), about 15 residues longer than the typical higher plant  $\beta$ -glucanase. Its preprotein also contained an N-terminal signal peptide and a C-terminal extension, presumably for vacuolar transport. *Gns9* consisted of two exons and usually short intron (99bp). Analysis of transcript (message) levels showed that unlike many plant  $\beta$ -glucanase genes, *Gns9* was only weakly induced by a fungal elicitor and wounding, and was not stimulated by salicylic acid. However, it was strongly activated by the stress hormone ethylene, and thus may function in an ethylene-dependent defense response. *Gns9* was highly expressed in calli, immature seed, root, and coleoptile, and was strongly responsive to exogenous GA, auxin and ABA, indicating a possible role also in growth and development.

**Keywords:** ABA, auxin,  $\beta$ -glucanase, calli, coleoptile ethylene GA, protein, root, seed

## 9. TRANSIENT ACTIVITY OF THE CAMV35S PROMOTER LACKING THE METHYLATION TARGETS IN RICE AND EFFICIENT SELECTION OF TRANSFORMED RICE CELLS USING THE BAR GENE

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Gene silencing is usually caused by promoter or gene methylation. The activity of a cauliflower mosaic virus (CaMV)35S promoter with lesser number of methylatable cytosine on gene expression was studied. A plasmid (pSan9) with the CaMV35s promoter mutated at the TGACG motifs (methylation target free (MTF 35S) and CaM35S plasmid (pSan2) driving the *gusA* gene was used. The activity of the MTF 35S and CaMV35S promotes to drive the expression of *gusA* reporter gene was determined transiently in rice variety Radon using the polyethylene glycolmediated transformation. Transient GUS activity of two-day DNA-treated protoplast showed an expected lower activity in the protoplast treated with pSan9. Radon protoplasts were co-transformed with the selectable marker *bar* gene and with either pSan2 or

pSan9. Plants were regenerated from the DNA-treated protoplast at a high selection and co-transformation efficiencies. Two transgenic plants produce with minimum integration events shown in the southern blot analysis exhibited high GUS activity using a fluorimetric assay. However, most of the plants regenerated contained high multiple integration events resulting to each inability to determine the activity of the MTF35S promoter in stable conditions.

**Keywords:** cauliflower mosaic virus, methylation, promoter, GUS activity, B-glucuronidase, polyethylene glycol, protoplasts, transformation, *Oryza sativa*, transgenic, *bar* gene

### 10. MOLECULAR MAPPING OF RESISTANCE TO *Thrips palmei* IN POTATO: I. POLYMORPHISM SURVEY AND INHERITANCE STUDY OF RAPD MARKERS

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Random Amplified Polymorphic DNA (RAPD) marker technique was employed to find additional polymorphic DNA markers between *S. tuberosum* and *S. berthaultii* parents of the two mapping populations (BCT and BCB) used in this study. A total of 115 short, random oligonucleotide primers from Operon Technologies were surveyed for differences between the parental genotypes used. Eighty (80) RAPD bands were found polymorphic between the parents. Segregation analyses of all polymorphic RAPD bands identified were conducted in the two backcross populations. Chi-square test suggest that only 14% of the segregating RAPD bands observed followed the expected Mendelian ratio. The mapped portion of the RAPD markers identified in this study is currently being located into the existing RFLP map of potato. Ultimately, the combined RFLP/RAPD map will be used to map the position of the genes for resistance to *Thrips palmei*.

**Keywords:** potato, *S. tuberosum*, *S. berthaultii*, RAPD, DNA markers

## 11. MOLECULAR MAPPING OF RESISTANCE TO *Thrips palmei* IN POTATO: II. EVALUATION OF GLANDULAR TRICHOME TRAITS AND THRIPS RESISTANCE REACTION IN TWO BACKCROSS POPULATIONS

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Parent and progenies of two backcross population, BCT and BCB, derived from a cross between a diploid *Solanum tuberosum* and a wild diploid species, *S. berthaultii*, were evaluated for glandular trichome traits and resistance to *Thrips palmei* under Philippine conditions. Both parental genotypes exhibited the expected variation for glandular trichome traits and contrasting resistance reaction to thrips infestation. *S. berthaultii* expressed both trichome types, A and B, and highly resistant reaction to thrips infestation. Evaluation of the BCT population indicated that the progenies showed a wide range of variation in trichome density and MEBA scores. However, only type A trichomes were found in all the 147 BCT progenies tested. Results of the thrips resistance evaluation showed that all BCT progenies succumbed to thrips damage before the final damage rating could be conducted. On the other hand, the BCB population showed a wide range of variation in resistance reaction to thrips infestation. Trichome traits analyses of the BCB are currently underway. Ultimately, result of this study will be used in mapping the location of thrips resistance gene(s) in potato.

**Keywords:** potato, glandular trichomes, MEBA, *Thrips palmei*, insect resistance, mapping

## 12. DEVELOPMENT OF RINGSPOT VIRUS RESISTANT PAPAYA

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Papaya ringspot virus (PRSV) is a serious problem in papaya production worldwide. It is likely that the planting of resistant papaya cultivars will overcome this



problem. Interspecific hybridization between papaya (*Carica papaya* L.) and the resistant wild species, *Carica cauliflora* Jacq., has been done with the aid of embryo rescue to produce interspecific hybrids. An improved embryo rescue protocol was adapted and subsequently utilized for efficient production of interspecific hybrid between papaya and PRSV-resistant *C. cauliflora* from 90- and 120-day-old embryos. Pre-incubation of hybrid embryos for 5 days on either liquid or solid germination medium full-strength De Fossard nutrients plus 10  $\mu$ M BAP 3 0.25  $\mu$ M NAA, and 58  $\mu$ M sucrose induces germination of hybrid embryos. Subsequent transfer of germinated embryos to a hormone-free nutrient medium allowed good growth of hybrid seedlings in-vitro. Hybrid seedlings are now flowering in the glasshouse and are currently being backcrossed with papaya. This wide hybridization work is an attempt to transfer PRSV resistance from the wild to cultivated species.

**Keywords:** *Carica papaya*, *C. cauliflora*, embryo rescue, inter-specific hybridization, papaya ringspot virus

### 13. TEMPORAL EXPRESSION OF QUANTITATIVE TRAIT LOCI FOR BLAST RESISTANCE IN RICE

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Thirteen quantitative trait loci (QTLs) in 9 chromosomes were identified for blast resistance using phenotypic data from the blast nursery. Eight of these loci were located using IRRI's Standard Evaluation System for blast (SES rating) at 41 days after seeding (DAS) while 6 loci were found for percent diseased leaf area (%DLA) at 40 DAS. The markers closely linked to each QTL were analyzed using data gathered at 19, 26, 33 and 47 DAS to determine if the same QTLs were being expressed at different ages after seeding. Analyses showed that all marker x phenotype associations except for I were not significant at 19 DAS for %DLA but not for SES rating. Most associations were found significant at 26 DAS. Putative QTLs near the major gene(s) were consistently identified at 26 and 33 DAS but not at 19 and 47 DAS for both %DLA and SES rating.

**Keywords:** rice blast, RFLP, gene expression, quantitative traits locus (QTL), minor gene, gene mapping, disease resistance, *Oryza sativa*

**14. TRANSFER OF TUNGRO VIRUS RESISTANCE FROM THE WILD RICE *Oryza rufipogon* GRIFF. TO THE CULTIVATED RICE *O. Sativa L.***

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Rice tungro disease is the most severe and widespread viral disease of rice bringing about substantial losses in many rice-growing areas especially in South and Southeast Asia. Strategies and current status of work on the development of tungro-resistant varieties by transferring resistance genes from the wild relatives of rice to the cultivated rice through wide hybridization was presented. Accession 105908, 105909 and 105910 of *Oryza rufipogon* were subjected to enzyme-linked immunosorbent assay (ELISA) and found to be resistant to the rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV). Those were crossed to IR64, a susceptible commercial variety. The F1 hybrid *O. rufipogon* (100909) x IR64 was backcrossed to IR64 producing 279 BC1F1 plants. Selected BC1F1 plants were further backcrossed to the recurrent parent producing 1650 BC2F1 plants which constituted the mapping population. Backcrossed plants were selected based on morphological similarity to IR64 and resistance to the two viruses. Genetic similarity to IR64 based on RFLP and RAPDS parental served as basis for further backcrossing to reconstitute the genetic background of IR64. RFLP and RAPDS parental survey revealed high level of polymorphism between *O. rufipogon* and IR64.

**15. ASSESSMENT OF GENETIC DIVERSITY IN THE PHILIPPINE ABACA *Musa textilis* Nee. GERMPLASM COLLECTION USING DNA MARKERS**

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The technique of Random Amplified Polymorphic DNA (RAPD) analysis was employed to assess the extent of genetic diversity among abaca accessions collected

and maintained in existing germplasm banks. A total of 104 random, short oligonucleotide primers from Operon technologies were tested to reveal DNA polymorphisms among selected abaca accessions. Fifty-six out of 104 primers screened showed intense and well resolved amplification products. RAPD analysis was subsequently conducted on DNA samples of 106 abaca accessions obtained from existing gene bank collections. Cluster analysis and principal component analysis (PCA) of the RAPD data were analysed using NTSYS-pc computer software. Correlation between RAPD markers and morphological traits were likewise analysed.

**Keywords:** abaca, *Musa textilis*, DNA markers, RAPD, genetic diversity, germplasm collection

## 16. APPLICATION OF DNA FINGERPRINTING IN RESOLVING THE IDENTITY OF A POPULAR FARMERS' VARIETY

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The study aimed to illustrate the usefulness of simple PCR-based markers in establishing the identity of rice varieties with uncertain origin. The study used Banay-banay 7-Toner, a high quality rice variety in Mindanao, which has an uncertain origin. Compared to IR64, both are agro-morphologically very similar. Results of the study showed no significant difference between Banay-banay and IR64 in 18 quantitative and 8 qualitative agro-morphological traits observed. Amylose contents of the two varieties were also found to be comparable and both contain intermediate amylose (21-22%). In the absence of Banay-banay pedigree information randomly amplified polymorphic DNA and simple sequence length polymorphisms (SSR) to fingerprint Banay-banay 7-tonner and IR64 were used. From a total of 360 loci generated by RAPD analysis the 2 samples were different in 8 bands only (2.27) or a similarity of 97.87. SSR analysis also indicated that both varieties had almost the same set of alleles in most of the 24 loci assayed. Compared with other high quality varieties like PSB Rc14, BPI-76 and C4-63G. Banay-banay clustered with IR64. Thus, DNA fingerprinting results strongly supported the earlier observation that Banay-banay 7-Tonner is very similar if not the same as IR64.

**Keywords:** DNA fingerprinting, rice, Banay-banay, IR64

## 17. INFLUENCE OF HOST SELECTION ON THE POPULATION STRUCTURE OF *Xanthomonas oryzae* PV. *oryzae*

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The influence of host genotypes on the genetic structure of the populations of *X. oryzae* pv. *oryzae* was evaluated in plots established in the Philippines over a two year period. Bacterial populations were from three near-isogenic rice cultivars containing the *Xa4*, *Xa7* or *Xa10* genes for resistance to *X. o. pv. oryzae* and the susceptible parent IR 24. The genetic structure of the pathogen population was monitored by using two polymerase chain reaction-based assays. Two genetic lineages (B and C) of *X. o. pv. oryzae* were detected. Lineage C, composed of races, 3 (virulent to *Xa4* and *Xa10*) and 9 (virulent to *Xa4* and *Xa10* and moderately virulent to *Xa7*) dominated the population at both sites. In all cultivars, there was a reduction in the population diversity over the two years. After 2 years selection, populations on the cultivar with *Xa10* consisted of lineage C haplotypes (C-01 and C-05) that were races 3 and 9. Populations on the cultivar with the *Xa7* gene were race 9 and were mostly of strains from the common lineage C, haplotype C-01 and a rare population from C-05. Molecular changes in strains from *Xa7* that were adapted on *Xa7* were compared. Four distinct groups with increased virulence were defined, indicating that virulence to *Xa7* occurred through 4 different evolutionary pathways.

**Keywords:** host selection, *xanthomonas oryzae*, rice genetic structure

**18. ESTERASE ISOZYME ANALYSIS OF ASIAN CORN  
BORER (*Ostrinia furnacalis* GUENEE) POPULATIONS  
FROM THE PHILIPPINES AND JAPAN**

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Thirteen genotypes of esterase-1 were observed in three populations of Asiatic corn borer, 11 from the Laguna population with 5 allele (t, u, v, w, x) 7 from Leyte with 6 alleles (t, u, v, w, x, y) 7 from Cotabato with 5 alleles (t, u, v, w, x). Only the Leyte population had allele. The Asiatic cornborer populations from Laguna and South Cotabato had heterozygosity values of 0.202 and 0.200 higher than that of Leyte with 0.162. The Laguna population was significantly different from that of Leyte and South Cotabato although the latter two were not significantly different from each other. The Japanese population of corn borers from one site had six genotypes of esterase-1 with 4 alleles (t, u, v, w) and heterozygosity value of 0.286 and which were different from the genotypes of the Philippines populations.

**Keywords:** Asiatic corn borer, *Ostrinia furnacalis* Guenee, isozymes, esterase, insect biotypes

**19. KINETIC PROPERTIES AND ACTIVITIES OF CORN LEAF NITRATE  
REDUCTASE AND ROOT PHOSPHOENOLPYRUVATE  
CARBOXYLASE AT DIFFERENT LEVELS OF SALINITY**

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With the increasing demand for food, utilization of marginal lands like the saline areas become important. Breeding for salt tolerant varieties of otherwise salt sensitive crops is one of the strategies undertaken in crop improvement. The biochemical mechanism of salt tolerance in corn using enzyme markers was, therefore, investigated. The kinetic studies revealed that leaf NR was stable at 0-6°C and showed maximum activity at 29°C. Both pH optimum and pH stability were observed at 7.8 Root PEPC was

stable at 30-50°C and showed maximum activity at 30°C. Its optimum was 8 and stable at pH 6-8 its Km for PEP was 16 μM. Salt level of 25 mM significantly decreased leaf NR and root PEPC activities of both the salt-sensitive (P11) and salt-tolerant (P31) corn inbreds. Leaf NR and root PEPC of P31 showed higher activities than that of P11. The activity of root NR of P31 showed sensitivity to high salt levels, while P11 root NR activity significantly increased at salt levels >75 mM. Leaf PEPC of P31 showed greater tolerance to high salt concentrations than P11 leaf PEPC. Results suggest that NR and PEPC might play significant roles in salt tolerance in corn.

**Keywords:** kinetic properties, nitrate reductase, phosphoenolpyruvate carboxylase, salt tolerance, corn

## 20. PHENYLACETIC ACID-ENHANCED ANTHR CULTURE RESPONSE IN INDICA RICE (*Oryza sativa L.*)

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Anther culture is employed to complement conventional rice breeding approach for development of breeding lines tolerant to abiotic stress (cold and saline). The standard protocol utilizes 2,4-D at 2 mg/L level, as the main plant growth regulator for callus induction and formation. Efficiency of this protocol with *indica* rice is far below the targeted 3% green plant regeneration based on number of anthers plated. Hence the effect of phenylacetic acid (RAA) at 10 mg/L in combination with 4 mg/L NAA and 0.5 mg/L of either kinetin or 6-benzylaminopurine, in improving anther culture response of *indica* rice was investigated. Nine out of 17 parental genotypes formed more calli in PAA-enriched callus induction medium compared to 2,4 D-enriched medium. Likewise, with 4F<sub>1</sub> crosses, an increase of 2 times callus formation was obtained in PAA-enriched medium. No significant enhancement in green plant regeneration, however, was observed with the treatments employed. In some genotypes, especially those which usually form calli but never differentiate and regenerate green plants, such as Arkansas and C22, PAA treatment induced green callus differentiation and green plant regeneration. Direct plant regeneration from callus, that is, without transferring from callus induction into regeneration medium, was also obtained. Further investigation on optimizing the culture condition for enhancement regeneration of PAA-treated calli will be conducted.

**Keywords:** anther culture, phenylacetic acid, 2,4-D, NAA, kinetin, 6-benzylaminopurine, callus induction, callus formation, plant regeneration, abiotic stress

## 21. BIOLOGICAL CONTROL OF GINGER RHIZOME ROT USING TRICHODERMA SP.

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A study to evaluate the efficacy of *Trichoderma sp.* at different time of application against *Sclerotium rolfsii* and *Fusarium sp.* causing rhizome rot ginger was conducted in Kidapawan, Cotabato from July 1992 to 1994. Laboratory tests revealed that the introduction of *Trichoderma sp.* simultaneously and one week ahead of the pathogen significantly inhibited the growth of *Sclerotium rolfsii* and *Fusarium sp.* at a spore density of 29,000/10ml of distilled water. In a greenhouse experiment, the introduction of *Trichoderma sp.* one week ahead of the pathogen (*S. rolfsii* and *Fusarium sp.*), introduced simultaneously, and pathogen introduced one week ahead of the *Trichoderma sp.* gave a percentage infection of 58.33%; 39.58%; 66.67% and severity of 59.5%; 71.66% and 66.67%, respectively. Verification in farmers field was conducted using *Trichoderma sp.* in CFA form at 50 gms/hill as basal and 50gms two months after planting as experimental practice and no *Trichoderma* application for farmers practice. Results showed a percentage infection of 8.5% with 12.0% severity while farmers practice with 12.5% and 18.5% infection and disease severity, respectively. *Trichoderma sp.*, applied in spore suspension and CFA form is, therefore, a potential biocontrol agent (BCA) against *S. rolfsii* and *Fusarium sp.* causing rhizome rot in ginger.

**Keywords:** biological control, ginger rhizome rot, *Trichoderma*, *Sclerotium rolfsii*, *Fusarium*

## 22. VARIETAL DIFFERENCES IN THE CELLULOSE, LIGNIN AND SILICA CONTENTS OF THE DIFFERENT PLANT PARTS OF RICE (*Oryza sativa L.*)

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The amount of variation and of cell-wall constituents i.e., cellulose, lignin, and silica in the different parts of the rice plants were investigated. A total of 53 varieties (47 indica and 6 japonica) was used. There were highly significant varietal differences in the cell-wall constituents of the different rice varieties. Cellulose was the most abundant component of the cell wall followed by lignin and then silica. Downward from leaves to the roots, lignin and cellulose contents increased but the silica content

decreased. There was insignificant amount of silica in the roots and the absorbed silica was mainly accumulated in the shoots particularly in the leaf blade. Several patterns of distribution of cellulose, lignin and silica contents within the different plant parts were observed. Majority of the varieties have low amounts of cellulose, lignin and silica. In the varietal classification, Pokkali, salt tolerant, contains high amount of cellulose in the roots but low cellulose and lignin in the shoot. Salt tolerant IR 4595-41-13, had low cellulose and silica in all parts but with high lignin in the leaf blade and root, and high cellulose content with low silica in the leaf sheath and stem.

**Keywords:** acid-detergent fiber, cell-wall constituents, rice, salt tolerant, varietal differences

### 23. INSECTICIDAL ACTION OF PLANT VOLATILE OILS ON SEVERAL INSECT PESTS

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Volatile oils derived from four plant species, namely *Blumea balsamifera*, *Vitex negundo*, *Ageratum conyzoides* and *Chrysanthemum indica*, were evaluated for toxicity against insect pests of different orders. Generally, all the volatile oils were highly toxic to *Spodoptera exempta* and *Nilaparvata lugens*, moderately toxic to *Plutella xylostella*, *Dysdercus cingulatus* and *Musca domestica*, and slightly toxic to *Spodoptera litura* and stored grain insects such as *Callosobruchus chinensis*, *Rhizopertha dominica*, *Sitophilus zeamays* and *Tribolium castaneum*. No toxicity was noted for *C. indica* to *T. castaneum*. Based on the mean LD<sub>50</sub> values from all test insects, the order of decreasing toxicity of the volatile oils was as follows: *V. negundo* > *B. balsamifera* > *A. conyzoides* > *C. indica*. The insecticidal potential of these plant species therefore warrant further investigative study toward a practical agricultural application.

**Keywords:** plant volatile oils, insect pests, biopesticides



**24. *Chalcomyza ipomoeaphaga* MARTINEZ: A NEW  
LEAFMINER PEST OF SWEET POTATO  
(*Ipomoea batata* LAMARK)**

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Dipterous leafminers in sweet potato create conspicuous tunnels in the leaves that can hamper photosynthesis during serious infestation. Sweet potato leaf miner collections from Pinaod, San Ildefonso, Bulacan yielded a new insect pest which has never been recorded in the Philippines. Diagnostic works showed *Chalcomyza ipomoeaphaga* Martines from Guadaloupe to be present. This pest is known to attack sweet potato in Americas. The bioecology of this pest should be studied closely since it poses a potential threat to the production of sweet potatoes in the Philippines.

**Keywords:** leaf miner, pest, Diptera, sweet potato *Chalcomyza ipomoeaphaga*

**25. FIELD PERFORMANCE OF THERMALLY SELECTED  
POPULATIONS OF *Diadegma semiclausum*, A LARVAL  
PARASITOID OF DIAMONDBACK MOTH**

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*Diadegma semiclausum* is suited to cold areas at temperature lower than 24°C. In view of the desire to release this parasitoid in the lowland because of its higher rate of parasitism (90%) than *Cotesia plutellae* on diamondback moth, *Plutella xylostella*, *Diadegma* was selected for resistance to high temperature.

The *Diadegma* population collected from Benguet was reared for three generations in a screenhouse at 29 to 34°C prior to selection and sensitivity of the F<sub>4</sub> adult *Diadegma* to different temperatures was determined by exposure in thermo-regulated incubator (TRI). The adult LT<sub>50</sub> (Lethal Temperature) was exposed for 6 hrs. at 35°C. The selection was made by rearing the parasitized DBM at 35°C inside the TRI at intervals of three generations between selection pressure. Percent developed *Diadegma* cocoons gradually increased from 17 to 55% (F<sub>38</sub>) while the percent adult emergence was relatively stable from F<sub>5</sub> to F<sub>38</sub> ranging from 85 to 95%. The sex ratio was not affected. Fifty-pairs of the heat-resistant *Diadegma* (HRD) and original heat-susceptible *Diadegma* (OSD) were released once in the field on separate plot of cabbage infested with DBM and enclosed within a mosquito net (two trails). The number of cocoons and the rate of parasitism of HRD was higher than OSD. HRD parasitism was comparable to *C. plutellae* (CP). The additive parasitism of HRD + CP was higher than HRD or CP only. The OSD cocoons were much lower than HRD or CP only. Six weeks after harvest of the experimental cabbage, the *Diadegma* parasitism on nearby crucifer fields was 33%. The results showed the possibility of releasing HRD in the lowland.

**Keywords:** *Diadegma semiclausum*, parasitoid, diamondback moth, moth

## 26. LABORATORY SELECTION OF *Diadegma semiclausum*, A LARVAL PARASITOID OF DIAMONDBACK MOTH, FOR RESISTANCE TO HIGH TEMPERATURE

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*Diadegma semiclausum* is suited for the management of diamondback moth (*Plutella xylostella*), the most destructive pest of crucifers, in higher elevation with temperature below 25°C. In view of the desire to release this parasitoid in the lowland because of its higher rate of parasitism (90%) than *Cotesia plutellae*, *Diadegma* was selected for resistance to high temperature. The *Diadegma* populations were collected from Benguet and reared for three generations in a screenhouse at 29 to 34°C prior to selection. The sensitivity of the F<sub>4</sub> adult *Diadegma* to different temperatures was determined by exposure in thermoregulated incubator (TRI). The adult LT<sub>50</sub> (Lethal Temperature) was exposed to 6 hrs. at 35°C. The selection was made by rearing the parasitized DBM at 35°C inside the TRI at intervals of 3 generations between selection pressure. The developed *Diadegma* cocoons gradually increased from 17 to 55% (F<sub>38</sub>) while the adult emergence was relatively stable from F<sub>5</sub> to F<sub>38</sub>, ranging from 85 to 95%. The sex ratio was not affected. A 48% survival of the laboratory-selected heat resistant *Diadegma* was observed when initially exposed in the field at 35°C and RH of 66%. This

indicates that a heat-resistant *Diadegma* can be mass-reared and released in crucifer-growing areas in lowland elevation to supplement *C. plutellae*.

**Keywords:** *Diadegma semiclausum*, larva parasitoid, laboratory-selected, high temperature resistance, diamondback moth, crucifers

## 27. OCCURRENCE, MORTALITY FACTORS AND WITHIN-PLANT DISTRIBUTION OF BOLLWORM, *Helicoverpa armigera* (Hubn) ON COTTON

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The seasonal occurrence of cotton bollworm (*Helicoverpa armigera*), mortality factors, and egg and larval distribution on cotton were determined.

The peak of occurrence of the pest was recorded at 57, 78 and 106 days after emergence (DAE) on cotton planted within the season, in Guimba, Nueva Ecija; Pozorrubio, Pangasinan; and Polomolok, South Cotabato, Philippines, respectively. The pest occurred earlier in late-planted cotton than in the early-planted ones, with peaks of occurrence at 54 and 75 DAE, respectively. Rainfall, relative humidity, air temperature, and sunshine duration did not significantly influence the population trend of the pest.

The mortality factors on the various insect stages were: physiological defects, diseases, natural enemies, and dispersion.

The eggs were predominantly laid on the top young leaves and growing terminals of the upper third portion of the cotton plant. The larvae were confined on the upper third-portion from 43 to 64 days after emergence (DAE), at the middle third-portion from 78 to 85 DAE and the upper third from 92 to 106 DAE. The availability and type of fruiting forms were the primary cues for the pattern in the egg and larval distribution within the cotton plant.

**Keywords:** occurrence, mortality factors, eggs and larval distribution, bollworm, population trend

## 28. THE NATURAL ENEMIES OF PADDY CLIMBING WORM, *Pseudaletia separata* (WALKER) (LEPIDOPTERA: NOCTUIDAE)

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The paddy climbing worm or rice eating caterpillar can decimate large tracks of harvestable rice overnight. Nevertheless, the present of natural enemies effectively check this pest. One species of predator, 11 species of parasitoids and 1 entomopathogenic fungus were collected and identified. The predator belonged to the order Dermoptera family Labiduridae. The parasitoids were represented by 6 species under the order Diptera families Tachnidae (5 spp.) and Sarcophogidae (1sp.) and 5 species under the order Hymenoptera families Ichneumonidae (1sp.), Braconidae (2 spp.), Eulophidae (1sp.) and Encyrtidae subfamily encyrtinae (1sp.). The entomopathogenic fungus was tentatively placed under the genus *Akanthomyces*. The bioecology of these natural enemies in relation to *P. separata* and other rice pest is being investigated further.

**Keywords:** worm, rice eating caterpillar, rice predator, parasitoids, fungus, natural enemies

## 29. BIODIVERSITY CONSERVATION IN PHILIPPINE MANGIFERAS

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Several species of *Mangifera* and a rich germplasm resources of these species occur in a varied ecosystem in the Philippines. In 1976, a nation-wide biodiversity conservation program to collect these valuable plant resource for long-term safekeeping and for future use was started. To date, 5 *Mangifera* species, namely *M. altissima* (paho), *M. caesia* (bauno), *M. indica* (mango), *M. odorata* (huani), and *M. monandra* (apali). *M. altissima* and *M. monandra* are endemic species. Some 265 access of the mango, the country's third most important fruit, has been collected all over the country.

The Carabao, the premier mango cultivar, is represented by 115 accessions. Of the total accession, 124 were documented for tree and leaf characters and 68 for their fruit characters. Wide variations in tree, leaf and fruit characters were observed among the accessions. Work is continuing to collect 'Carabao' and other local mango strains in other parts of the country. The long-term goal is to keep a representative 'Carabao' mango strain from each of the country's municipalities.

**Keywords:** biodiversity conservation, mangiferas, germplasm resources, field gene bank, 'Carabao' mango

### 30. CONSERVATION OF NATIVE PHILIPPINE ORCHIDS

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Ongoing are efforts to help conserve the floral genetic heritage of the Philippines, specifically native Philippine orchids. Five main projects comprise the conservation program: collection of the native orchids from different provenances, embryo and tissue culture of endangered Philippine orchids, determination and optimization of tissue culture conditions for each native species, development of *in vitro* storage techniques, and identification of DNA markers for native orchids. On the short-term, the program aims to: source and document nursery and herbarium specimens of endangered Philippine orchids; propagate these native species through embryo and tissue culture; determination Optimization of Tissue Culture Conditions for Each Native Species; Development of *In vitro* Storage Techniques, and identification of DNA Markers for Native Orchids. The long-term objectives set are as follows: establish a nursery for and maintain a herbarium collection of endangered Philippine orchids; establish tissue culture as a useful technology for plant diversity conservation; establish a gene bank of Philippine orchids for research and eventually for commercial purposes; and develop a rapid and accurate orchid identification procedure for regulatory purpose. Ultimately, it is envisioned that the program will replenish the native orchid species back to their habitats.

### 31. DIVERSITY WITHIN PHILIPPINE IMPROVED RICE VARIETIES AND THEIR PROGENITORS: A MULTIVARIATE ANALYSIS OF PHENOTYPE TRAITS

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Germplasm characterization enhance the efficiency of varietal improvement towards broadening the genetic base of improved cultivars. The study analyzed the diversity for phenotypic traits of 78 improved rice varieties released from 1965 to 1995 and their 81 ancestral lines. Data on morpho-agronomic traits obtained from field evaluation were analyzed by multivariate analysis. Ancestral lines were found more variable than their cultivated descendants. A total of 41 traits contributed to the total variation in ancestral lines Euclidean distance ranging from 3.970 to 17.389, while only 33 traits contributed to the total variation in improved varieties with Euclidean distances ranging from 2.228 to 16.706. Principal component analysis revealed that 6 qualitative characters significantly influence the variation in released varieties. Parental separation was strongly affected by variation in quantitative characters. Cluster analysis permitted separation of improved varieties into 2 major cluster: cluster 1 consisted of 72 (92%) varieties and cluster 2 consisted of 6 (8%) varieties having purple-colored plant parts. Among ancestral lines, *O. nivara* formed a different cluster from the rest of the *O. sativa* group. Results showed that rice breeders selected phenotypic divergence in improved varieties.

**Keywords:** improved rice varieties, progenitors, multivariate analysis, phenotype traits

**32. SOMATIC EMBRYOGENESIS IN BANANA (*Musa SPP.*)  
C. V. LAKATAN AND LATUNDAN**

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Somatic embryos of banana cv Lakatan were initiated on half strength Murashige and Skoogs (MS) basal medium supplemented with 2,4-dichlorophenoxy acetic acid (5 $\mu$ M), proline (20 $\mu$ M), casein hydrolysate (200 mg/l), ascorbic acid (10 mg/l) inositol (100 mg/l) and sucrose (40 g/l). Direct and indirect pathways of somatic embryogenesis were observed from two different types of tissue explants. Regeneration of plants were obtained by transfer of embryos onto MSA medium supplemented with Zeatin (1 $\mu$ M), ascorbic acid (10 mg/l) and (40 mg/l)

**Keywords:** somatic embryogenesis, Lakatan, Latundan, tissue explants, regeneration

**33. PRELIMINARY STUDIES ON SOMATIC HYBRIDIZATION  
BETWEEN *Carica papaya* AND *Carica* SPECIES**

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Protoplast fusion is an alternative method employed in wide hybridization to effect the transfer of useful gene(s) from the wild to the cultivated species. *Carica cauliflora*, *C. quercifolia* and *C. stipulata* are sources of resistance to papaya ringspot virus but they are incompatible with *C. papaya*. Protoplasts were isolated from somatic embryos of 2 papaya cultivars (2001 and EM 6) and mesophyll cells of 3 *Carica* species by enzymatic digestion of the cell walls. Papaya protoplasts were fused with mesophyll protoplasts of the wild species with polyethylene glycol as fusogen. Fusion products were monitored for cell wall development and cell growth.

**Keywords:** *Carica papaya*, *cauliflora*, *C. pubescens*, *C. stipulata*, fusogen, protoplast fusion

### 34. PURIFICATION AND ANTISERUM PRODUCTION TO PAPAYA RINGSPOT POTYVIRUS

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Papaya ringspot caused by papaya ringspot potyvirus (PRSV) is a serious threat to the papaya industry. Its destructive nature necessitates test and accurate diagnosis. This paper reports the purification and production of antiserum to PRSV the prerequisites for the application of antibody-based diagnosis and in development of PRSV resistant varieties.

Biologically pure IPB-PRSV isolate was propagated and maintained in *Zucchini Blackjack* (ZBJ). Two purification protocols were modified to produce the purified virus particles. Antiserum to the virus was prepared by injecting the virus emulsified with Freund's adjuvant into a rabbit. The antiserum produced reacted to its homologous antigen in ELISA and Western blot.

**Keywords:** antiserum, antigen, ELISA, immunology, papaya, potyvirus, serology  
Western blot



**35. COMPETITIVE INTERACTION OF RICE (*Oryza sativa*)  
AND *Echinochloa glabrescens* AS A FUNCTION OF  
PLANTING DENSITY UNDER ELEVATED CO<sub>2</sub>  
AND HIGH TEMPERATURE**

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Potential competition between rice and *Echinochloa glabrescens* in terms of their vegetative and reproductive characteristics were examined at different planting densities at different CO<sub>2</sub> levels (ambient) Ambient + 200) and temperature regimens (27/221°C; 37/26°C). Increased CO<sub>2</sub> concentration promoted greater biomass, tiller number, grain yield, panicle weight and percentage filled spikelets for yield, panicle weight and percentage filled spikelets for rice relative to *E. glabrescens*. Comparison between the two species showed that the ratio of rice/ for total above ground biomass, leaf area, tiller number and grain yield increased with increasing CO<sub>2</sub> concentration indicating a potential competitive advantage for rice under elevated CO<sub>2</sub>. However, if CO<sub>2</sub> and temperature increased simultaneously the increased in rice biomass declined relative to the elevated CO<sub>2</sub> and condition alone. It was also observed that with increasing CO<sub>2</sub> and temperature for rice either CO<sub>2</sub> concentrations. In contrast, grain yield for *E. glabrescens*, while reduced was still significant at either CO<sub>2</sub> concentrations. Overall, this experiment suggests that relative effect of increasing atmospheric CO<sub>2</sub> and high temperature on reproduction could still C<sub>4</sub> over C<sub>3</sub> with global climate change.

### 36. *Desmanthus* AS HEDGEROWS IN AGROFORESTRY CROPPING SYSTEMS

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The potential of *Desmanthus virgatus*, DS 92803 hedgerows in agroforestry cropping systems in the arid hillylands of Ilocos Norte was evaluated over a four year period. As hedgerows, the plants were set at 7, 10, and 20 plants linear m and lopped at a height of 50, 75, and 100 cm above the ground. The grain yield of agricultural crops planted in between the hedgerows were recorded every harvest period. *Desmanthus* hedgerows established at wider spacing were significantly taller and had larger stem diameter. The fresh biomass yields of the plants was not affected by plant density. However, lopping the hedgerows at 50 cm during the establishment period produced more biomass than those cut higher than 50 cm above the ground. Succeeding observations confirmed that biomass was not critically affected by intrarow spacing. Although cutting the plants at a height of 50, 70, and 100 cm above the ground produced more or less the same amount of biomass, these management regimes did not critically affect grain yield of corn, rice, and mungbean. Continuous lopping every 45 days and use of the fresh biomass as much in alleys contributed to a noticeable increase in the amount of soil organic matter over a four year period. The adaptability and high productivity of *Desmanthus* under very close or dense spacing as well as its long tap root and numerous secondary roots undoubtedly suggest its high potential for erosion control.

**Keywords:** *Desmanthus*, hedgerows, lopping, biomass, organic matter, alley crops, hilly lands, cutting height, grain yield, semi-arid.

### 37. DEVELOPMENT OF LOCATION-SPECIFIC TECHNOLOGIES IN MAJOR CORN GROWING AREAS OF LUZON

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To support the Gintong Ani Corn production Program of the Department of Agriculture, the On-farm Research and Outreach Project (OFRO-Corn) was implemented in major corn growing areas in Pangasinan, Tarlac, and Batangas utilizing the on-farm research methodology to test and generate technologies appropriate to unique requirements and circumstances of these areas. Eight to ten farmer-partners were requested to set aside a particular area in their farms to test two factors, namely variety and fertilizer. Six to eight varieties of corn were tested through trials superimposed on cropping pattern (CP) plots. The fertilizer trial involved the use of various bio-organic (BOF) and inorganic (IOF) combinations. Irrigation management trial was implemented in one of the farmers' field in Tarlac site. Results of 1994-1995 and 1995-1996 dry season cropping showed that all hybrid varieties tested produced better yield and higher net benefit in the three key sites. Planting hybrid and application of combined BOF and IOF appeared to be promising in the three key sites. Application of six times irrigation for corn production after wetland-rice in Tarlac starting from 0 DAP to 70-75 DAP, gave better yield and higher net benefit. These technologies were disseminated through farmers' yield days, farmers, extension workers, researchers, and officials of private, non-government organizations and local government, thus, facilitating transfer of appropriate technologies.

**Keywords:** on-farm research and outreach, corn, bio-organic fertilizer

### 38. EFFECTIVE PLANTING TIME AND ARRANGEMENT OF TRAP CROPS AGAINST COTTON BOLLWORM

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The study was conducted at the Central Experiment Station, Cotton Research and Development Institute, Batac, Ilocos Norte to determine the best planting time and arrangement of promising trap crops that would give the most effective control for cotton bollworm. Native tomato and pigeon pea were found most in tomato alone plant per hill spaced 50 cm between hills, planted 14 days after planting cotton in 15 rows of cotton demonstrated a significant reduction of cotton bollworm population and ultimately a high net income of P 47,861.63 per hectare. On the other hand, planting two rows of pigeon pea at 2 plants per hill spaced 30 cm. between hills in every 10 rows of cotton also significantly reduced cotton bollworm population and consequently higher net income compared to single row cotton monoculture. Planting tomato or pigeon pea as trap crop against cotton bollworm significantly reduced spraying frequency from 7-8 to 3-4 times only during the cropping period.

**Keywords:** trap crops, cotton bollworm

### 39. RELAY CROPPING OF GARLIC WITH TOMATO FOR GARLIC FUNGAL DISEASE CONTROL

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An environment-friendly and effective control of *Cercospora* leaf spot and purple blotch of garlic was generated. This was done by relay-cropping with tomato. Tomato was planted one-month after planting garlic at a distance of 1.5 m between rows and 0.5 between hills. This practice reduced infection of *Cercospora* leaf spot and purple blotch by about 32 and 20%, respectively. Likewise, it significantly increased yield of garlic by 650 kg ha over that of the garlic monocrop. An additional net income of P58,704.54 ha<sup>-1</sup> over that of garlic alone was derived from the garlic and tomato harvests.

**Keywords:** relay cropping, tomato, garlic, fungal disease control, *Cercospora* leaf spot, purple blotch

#### 40. SYSTEM APPROACH IN THE EXTRAPOLATION OF RICE PRODUCTION ENVIRONMENTS

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The study dealt with the assessment and agroecological zonation of rice suitability of an environment, documentation of soil and water management practices in rainfed lowland rice watershed as well as risk assessment and sensitivity analysis of rice productivity based on qualitative and quantitative approaches. It was an attempt to contribute to the long-felt need to strategically extrapolate and zonify rice environments in the country. A case study was conducted in the agroecological environment of the municipality of Batac, Ilocos Norte at the northern part of the Philippines to characterize, describe and extrapolate rice production environments using GIS, simulation modeling, and optimization. A strategic rice on-site (SRO) area of 5,132 ha or 33% of the total land area of Batac was classified as suitable for rice production by overlaying physical maps of land use/land cover, soil type, erosion, slope and agroclimate using the Computer-Aided Mapping Program (CAMP). The contiguous 19-ha rainfed lowland rice growing watershed studied had no access to irrigation systems. However, rainfed farmers performed approved soil and water management practices to provide rain-water conservation facilities and structures to sustain the growth of rainfed rice. Simulated yields of rainfed lowland rice from 1984 to 1995 ranged from 2,7 to 9.2 t/ha using crop growth simulation model *Oryza W* under water-limited condition. Risk analysis of the different land evaluation units (LEUs) showed variation of alternative cropping patterns (rice and diversified crops) using the optimization minimization of total absolute deviations (MOTAD). The study demonstrated that the use of systems research tools such as crop growth simulation models and optimization linked with GIS proved useful to farmers, researchers, extensionists, and planners for land use planning.

**Keywords:** System approach, simulation modelling, geographic information systems, environment, optimization, land use planning, rice, extrapolation, *Oryza W*, MOTAD

#### 41. YIELD AND QUALITY COMPONENTS OF CORN HYBRIDS FOR SILAGE

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Corn producers face many interacting factors such as hybrid selection, planting date, fertilization, among others, for each crop they grow. However, in corn grown for silage, consideration of feed quality is also important in establishing its contribution towards meeting an animals needs. This was conducted to independently determine the effects of hybrid and plant population on yield and yield components, nutritive value, dry matter partitioning and N% of corn hybrids grown for silage under New Zealand conditions.

Hybrids CF1 achieved the highest (20,046 kg/ha) whole crop DM yield at final harvest while Janna produced the lowest (15,776 kg/ha) yield. The 75,000 plant population yielded significantly less than 100,000 and 140,000 plants/ha. There was no difference between the 100,000 and 140,000 plant populations. Dry matter partitioning at final harvest revealed the highest proportion in the grain component, followed by the stem, husk and leaf. Total metabolizable energy (ME) content ranged from 11.3 MJME/kg DM to 10.28 MJME/kg. DM. The ME content of of CF1 was significantly higher than all other hybrids. There were no significant hybrid differences in whole crop N% which ranged from 1.07 to 1.16%.

**Keywords:** silage, corn hybrids, plant population, feed quality, metabolizable energy, nutritive value, dry matter yield, dry matter partitioning, nitrogen percentage, yield and yield components

## 42. EFFECTS OF VESICULAR-ARBUSCULAR MYCORRHIZA INOCULATION ON COTTON GROWN IN NUTRIENT DEFICIENT SOIL

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The study was conducted at the Cotton Research and Development Institute, Batac, Ilocos Norte to determine the effects of inoculating vesicular-arbuscular mycorrhiza on cotton (var. CRDI-1) grown in nutrient-deficient soil. The pot experiment was laid out in Completely Randomized Design (CRD) with four replications with the following treatments; Control; VA mycorrhiza alone; VA mycorrhiza + 50% recommended  $\text{NP}_2\text{O}_5\text{K}_2\text{O}$ ; VA mycorrhiza + 100% recommended  $\text{NP}_2\text{O}_5\text{K}_2\text{O}$ ; and 100% recommended  $\text{NP}_2\text{O}_2\text{K}_2\text{O}$ . Mycorrhiza (Mycovam) from National Institutes of Technology and Applied Microbiology was applied at 5g per seed placed at 1 inch below the cotton seed. Results showed that the addition of VA mycorrhiza to inorganic  $\text{NP}_2\text{O}_5\text{K}_2\text{O}$  fertilizer increased the growth and development of the cotton plant up to the reproductive stage. Plants inoculated with VA mycorrhiza + Inorganic  $\text{NP}_2\text{O}_5\text{K}_2\text{O}$  fertilizer were generally taller, and had higher dry matter yield, and had higher number of bolls per plant resulting to higher seed cotton yield. The addition of VA mycorrhiza to only one-half of the recommended inorganic  $\text{NP}_2\text{O}_5\text{K}_2\text{O}$  fertilizer gave the highest seed cotton yield. This indicates that inorganic  $\text{NP}_2\text{O}_5\text{K}_2\text{O}$  fertilizer inputs can be lessened by as much as 50% and the cost of production may be lowered without altering the natural soil composition and structure.

**Keywords:** mycorrhiza, vesicular, arbuscular, inoculation, cotton

## 43. POLLUTION POTENTIAL OF AGRICULTURAL PESTICIDES IN WATER

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The possible contamination of pesticides in ground water and lakes were determined both in the field and in semi-field model ecosystem. Insecticides were applied in rice paddy 15 and 45 days after transplanting at recommended rates. Horizontal movement of the pesticides was monitored by allowing the paddy water to overflow up to 100 m away from the treated field simulating an actual flood condition.

A 100% fish *T. mossambicus* and frog (*Bufo marinus*) mortality was observed after hours of exposure to BPMC + chlorpyrifos treated run off paddy water at 10 and 25 m distance, respectively. However, there were no fish mortalities observed in methyl parathion and monocrotophos treated paddy water exposed at the same condition. Downward movement of monocrotophos was traced in paddy soil packed in PVC pipes. Movement of monocrotophos reached 490 cm layer depth in 30 days and went down further to 60 cm depth in 60 days. The concentration peaked in 270 days but declined in 360 days. This supported the results obtained in ground waters within the treated rice field. Monitoring of well waters within the vicinity of the treated rice fields showed contamination with monocrotophos, chlorpyrifos and endosulfan residues but at levels within tolerable limits based on actual consumption of glasses of water daily. Actual monitoring of pesticide residues in the lakes (Laguna de Bay and Manila Bay) revealed some residues of DDT, DDE, DDD, HCB and TCB at ppb levels (1980-1994) in spite of the rapid dissipation rate of persistent organochlorines in tropical climate with half life of 8 months (DDT) compared to 10 years in temperate countries. Residues of these organochlorines were not accumulated in oyster and mussels.

**Keywords:** pollution, pesticides, water growthwater, lakes

#### 44. UTILIZATION OF *Trichogramma chilonis* IN COTTON PRODUCTION

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Studies on the use of *Trichogramma chilonis* were undertaken to derive maximum benefits for cotton pest control. From 1986 to 1995, different methods of releasing the parasitoids were evaluated and the appropriate stage when to release and the time and schedule of releases were determined. Six techniques of release were evaluated. Pupae and adults were released at different hours starting in the morning until afternoon. Different parasitoid density and schedule of releases were also evaluated. One release station was composed of halved empty coconut shell tied with a string suspended from a bamboo pole at least 30 cm above the cotton plants. A paper clip tied inside the halved coconut shell was used to hang the strip containing *Trichogramma*. At other times the strip was clipped on the undersides of a leaf located on the upper one third of the plant. These methods protected the parasitoids from intense sunlight and excessive moisture. The study showed that the parasitoids could be released using the pupal stage (about to emerge) in the late afternoon or the newly emerged adults morning. *T. chilonis* parasitized 84.60% of *H. armigera* eggs when released at 49,000/ha per release at 2 days interval under field cage conditions while 33.65% parasitization



was observed when released at 67,500hs release starting at 50 DAE and weekly thereafter.

**Keywords:** cotton, *Trichogramma chilonis* pest control

#### **45. PROCESSING OF A TRIPOLIATE YAM, GAMAT (*Dioscorea* sp.) FROM CATANDUANES**

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A trifoliate yam, (*Dioscorea* sp.) locally known as Gamat in Catanduanes, Bicol Region was processed into flour. Snack items were prepared and evaluated. Results of the objective evaluation showed that the processed products were acceptable to a wide range of consumers in the province. Preliminary results of the nutritive analysis of carbohydrates, fats and proteins were also included.

#### **46. AQUACULTURE ENGINEERING DESIGN OF TILAPIA HATCHERY IN A FRESHWATER RECIRCULATING SYSTEM**

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Production of all male tilapia using hormone has become increasingly popular in the aquaculture industry but its effect on human health has not been known. Waste from traditional fry production in fishponds and cages can affect the quality of the water thereby affecting fry production supply. The tilapia hatchery system was designed and constructed to produce an all-male tilapia fry. The system consisted of an all-male compartment, all-female compartment, fry compartment and fry collectors. The emphasis of the design was the reproduction of handling of breeders and fry to improve quality and survival. The system used recycled water making it environment friendly. The system redesigning and re-engineering could be considered a potential household backyard fry production component of the industry to supply the requirement of the traditional grow-out production system in natural waters.

**Keywords:** aquaculture engineering, tilapia, hatchery system

## 47. LIVESTOCK-AGROFORESTRY PRODUCTION SYSTEM

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In an existing agroforestry production system, the abundance of by-products and crop residues such as shrub/tree fodder, corn stover, soybean/mungbean stover, necessitates more effective utilization aside from the traditional practice of enriching soil quality through mulching. Incorporation of livestock, particularly luminants, which can effectively utilize such resources for conversion into an existing agroforestry production system. The study assessed the quantity and quality of available livestock feed resources and determined animal performance in the system. Feed conservation strategies were developed that would fit into the livestock-crop production system module. Limited area entails an efficient use of the available resource and the incorporation of small ruminants were found to be most suitable in the existing system. The loose-pasture and feathering system were not suitable due to the cost of fencing and the limited open area such that complete confinement is recommended. The animals performed well under such management scheme exhibiting positive average daily gains. Available forage for ruminants were identified as follow, Kakawate (*Gliricidia sepium*), Napier grass (*Pennisetum purpureum*), Vetiber (*Vetiveria zizanioides*), Flemingia (*Flemingia macrophylla*), Rensonni (*Desmodium rensonni*), Atsoan Dilaw (*Cassia spectabilis*), Sesbian (*Sesbania sesban*), Katuray (*Sesbania grandiflora*), Sitaw (*Vigna unguiculata*) and Corn (*Zea mays*). The most suitable silage materials in terms of biomass yield and quality were found to be Napier and Kakawate (with a pH of about 5.03) which could be harvested within 3 weeks of ensiling. Quality analysis such as dry matter yield, ash content, % CP and % IVDMD showed variability within the year and these were incorporated into the livestock-crop production model.

**Keywords:** agroforestry, goats, livestock, napier, silage

#### 48. PLANNING FOR AGROFORESTRY FARM DEVELOPMENT THROUGH ALCAM

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Agroforestry, a land-use management system which utilizes the simultaneous or sequential raising of forest trees, agricultural crops, livestock and/or other resources in the same area, is now recognized globally as one of the best technologies for the cultivated uplands. The study aimed to develop a scheme that will enable the farmers to assess an area's capability for agroforestry using slope, existing vegetation and soil fertility as parameters. The factors enumerated can specify the area's potential for erosion, adaptability to trees and other crops, and capacity for sustainable production levels. The study resolved to the Agroforestry Land Capability Mapping Scheme. The scheme classified the areas into the following classes: I-highly capable for agroforestry; II-marginally capable; III-conditionally capable; and IV-not for agroforestry use.

**Keywords:** agroforestry, farm development, cultivated uplands, and capability mapping

#### 49. LOW-FAT-SALT PROBIOTIC CHEESE FOR HEALTH-CONSCIOUS CONSUMERS

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A soft cheese with the probiotic yogurt starter bacterian *S. thermophilus* and *bulgaricus* was developed to meet the increasing consumers demand for value-added low fat and low salt dairy products which are perceived to lessen the consumers' risk to cardiovascular disorders. Its proximate composition, sensory quality, consumers acceptability, shelf life and yield were assessed. Result gave high acceptable probiotic-containing soft cheese having 50.775 and 43.55% lower fat and salt contents, respectively. It also had a longer shelf life than the conventional soft cheese. Its sensory quality improved on storage. Cheese yield and other components were comparable. Scanning electron microscopy showed that the health promoting yogurt starter bacteria were embedded in the cheese matrix and therefore available when the cheese was eaten.

**Keywords:** cheese, probiotic, low-fat, low-salt

## ***BIOLOGICAL SCIENCES***

### **1. BIRDS OF CENTRAL SIERRA MADRE, AURORA AND NORTHERN QUEZON: A PRELIMINARY REPORT**

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The Sierra Madre mountain range is one of the world's biodiversity hotspots. Its continued survival for the future generations is constantly threatened by habitat destruction, particularly through logging. Yet, its biodiversity remains largely unexplored. This study was conducted to document the biodiversity of the least known areas in the Central Sierra Madre, in Aurora and Northern Quezon. Birds are the most studied group of vertebrates and are highly correlated with the health of ecosystems and thus a good indicator of habitat quality. Three watershed areas of the Central Sierra Madre, namely: along the Umiray River in Dingalan, Aurora and General Nakar, Quezon, in San Luis, and Dilasag, Aurora were surveyed and their biodiversity assessed. A combination of several survey techniques was used. These include: a) a two-kilometer line transect over 40 hours of observation; b) mist netting; and c) general audio visual observations. A total of 130 species of birds were identified, representing 23% of the total number of birds in the Philippines, 57% of which are Philippine endemic. There are 25 species of Luzon endemics, 13 species of which were recorded in the survey. In the lowland forest of Dingalan, Aurora and General Nakar, Quezon, 60 species of all Philippine bird species were recorded, 32 of which are Philippine endemic and 5 species are Luzon endemics. In San Luis, Aurora, 87 species were recorded, 46 of which are Philippine endemic and 10 species are Luzon endemic. In Dilasag, Aurora, 83 species of birds were recorded, 49 are Philippine endemic and 12 are Luzon endemic. Threatened species of hornbills, *Buceros hydrocorax* and *Penelopides manillae*, and the Luzon Bleeding heart pigeon, *Gallicolumba luzonica* were recorded in the area. A restricted range forest species of Pitta, *Pitta kochi*, thought to have been wiped out from its former range, was caught and released. It appeared to thrive in a lowland residual forest in the Amro watershed in Dilasag,

Aurora. The results of the avifaunal survey indicate that the Central Sierra Madre is home to an important group of wildlife, both endemic and threatened. This information warrants that the area should be included as one of the priorities for biodiversity conservation.

**Keywords:** birds, Sierra Madre, Aurora and Northern Quezon, vertebrate

## 2. BRACKISHWATER SHELLS ALONG THE EASTERN PART OF CATANDUANES: AN INVENTORY

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Brackishwater shells in the eastern part of Catanduanes, Bicol region were collected using quadrat techniques- by shoveling and hand picking- from April to December 1996. The shells were identified based on the Treatise of Invertebrate Paleontology adopted by malacologists and guide to Philippine Flora & Fauna by Garcia (1986). Population density and some physicochemical parameters were determined by instrumentation. These shells are very important source of protein. The study, a part of the project Inventory of Brackishwater Shells in Catanduanes, aimed to identify the economically important and available edible species found in this island province. Results of the study showed that the presence of the mollusks was dependent on the soil characteristics, i.e. sandy-silt -clayey; pH which ranged from 6.5 to 7.5 and salinity values, from 1 ppt to 30 ppt. The mollusks found in Eastern Part of Catanduanes belonged to class *Gastropoda* and *Bivalvia*.

*Gastropoda* had three orders: namely *Archeogastropoda*, *Mesogastropoda* and *Neogastropoda*. *Archeogastropoda* had one superfamily, *Trochacea* and one family *Trochidae* with two genera. *Mesogastropoda* had four superfamily: *Littorinaceae*, *Cerithiacea*, *Strombiacea* and *Cypraeaceae*. The three superfamily had one family and one genus each except for *Cerithiacea* which had two families *Cerithridae* and *Polaminadadea* which has six genera. *Order Neogastropoda* had two superfamilies *Buccinacea* and *Murizacea* both containing one genera.

*Class Bivalvia* had three orders: *Taxodonta*, *Isodonta* and *Heterodonta*. *Taxodonta* had one superfamily *Archacea* on family *Archidae* with two genera.

*Isodonta* had two superfamily: *Ptericea* and *Ostreacea*. *Ptericea* has three families: *Pteriidae*, *Isognomonidae* and *Placimidae*. *Pteriidae* had one genus *Isognomonidae* and *Placunidae* had two genera. *Ostreacea* had one family, *Crassostrea* and one genus.

*Heterodonta* had five superfamilies: *Dreissensiaceae*, *Cardiaceae*, *Veneraceae*, *Maत्रaceae* and *Telenacea*. Each of these superfamilies had one family with several genera.

**Keywords:** brakishwater shell, *Gastropoda*, *Bivalvia archeogastropoda*, *Mesogastropoda*, *Neogastropoda*, *Taxodonta*, *Isodonta*, *Heterodonta*

### 3. FOOD COMPOSITION OF SOME FISHES FROM LAKE TAAL, BATANGAS PROVINCE

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An analysis of the stomach content of Lake Taal fishes (obtained from the fish landing in Talisay wet market, Batangas, from January 1995 to March 1996) indicated that both plant and animal food are habitually eaten by *Therapon plumbeus* and *Ophiocara aporos*. Only animal food items were identified from the stomach of *Apogon thermalis*, *Glossogobius giurus*, and *Atherina forskali*. Many stomachs were found empty or contained unidentifiable digested matter. The most common recognizable food items based on the mean values of monthly percentages of occurrence for the five fishes were: *T. plumbeus*, plants (macrophytes); *O. aporos*, gastropods (*Vivipara anguillar*); *A. thermalis*, fish (*Atherina forskali*); and *A. forskali*, chironomid larvae.

**Keywords:** food composition, Lake Taal, Batangas, Taal fishes

### 4. COMPLETE LIFE CYCLE OF PHILIPPINE KING CRAB (*Scylla oceanica* DANA) UNDER CONTROLLED CONDITIONS

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*Scylla oceanica* (Dana), known as king crab ("alimango"), is the largest and fast growing portunid mud crab or mangrove crab in the Indo-West Pacific region. The demand for this species in international markets is very high. However, the production supply is limited due to low availability of seedstock from the wild.

To support the needs of the developing crab grow-out aquaculture in the Philippines, as potential replacement for declining prawn industry, a research to

understand the reproductive and developmental biology including the ecological requirements of the king crab, *Scylla oceanica*, in connection with the development of a less costly and efficient mudcrab hatchery technology in Southeast Asia was undertaken. Gravid spawners were bought live from the provinces of Iloilo, Aklan, Capiz and Antique and were reared in the broodstock tanks for the natural spawning. The development of eggs and larvae of *S. oceanica* was monitored periodically using Nikon microscope interfaced with video camera using Global Lab Image analysis computer software. The fecundity, duration of embryonic and larval development, distinguishing characteristics of larvae, and ecological behavior were closely documented. The larvae hatched within 9-12 dys of incubation depending upon the water temperature and salinity. It took the larvae 21-25 days to metamorphose into the first crab instar in culture medium with recorded physico-chemical parameters of pH 8.0-8.3, 32-34 ppt salinity, and 28-30°C. The early larval stages of mudcrabs were composed of five zoeae and one megalopa. The onset of sexual maturity (berried crabs) was observed after four months of culturing the hatchery produced crab larvae in the ponds. This is the first time that the life cycle of *S. oceanica* has been completed under controlled condition. The data obtained in incubating the eggs and culturing the larvae until the crablet stage became the bases in the development of the first commercial crab hatchery in the Philippines.

**Keywords:** *Scylla oceanica*, crab hatchery, crab larvae, developmental stages, life cycle

## 5. USE OF CERTAIN BACTERIA IN THE CONTROL OF *Vibrio harveyi* INFECTIONS IN PRAWNS

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Luminescent vibriosis caused by *Vibrio harveyi* has recently become a serious problem in the prawn industry. The use of antibiotics has been unsuccessful in controlling the disease mainly because of the limited efficacy of existing and readily available drugs, the possible development of resistant bacterial strains, and the limited tolerance of the shrimp larvae to the drugs. Lately, however, there were reports abroad on the occurrence in natural and rearing waters of certain beneficial bacteria capable of repressing the growth of pathogenic *Vibrio* species. Most often, these so-called 'probiotics' or 'biocontrol agents' increase the survival rates of cultured fish

and crustaceans. In this study, five of the 34 bacterial isolates from marine and freshwater environments were found to inhibit the growth of *Vibrio harveyi* when screened using *in vitro* assays. Results of biochemical tests run to initially characterize and identify these potential biocontrol agents suggested that the five strains belong to the genus *Pseudomonas* with the two of them identified positively as *Pseudomonas aeruginosa* using the API 20 NE test kit (bioMerieux). *In vivo* trials are underway to determine their effects on the survival rates of *Penaeus monodon* larvae, the culture of which is currently beset with luminiscent vibriosis. Tests to elucidate the vibriostatic factor produced by these strains are likewise being worked out. The results of this study are expected to generate relevant data on the possible application of these vibriostatic bacteria in controlling luminiscent vibriosis in hatcheries and in grow-out ponds.

**Keywords:** bacteria, *Vibrio harveyi*, *Pseudomonas* species, *Pseudomonas aeruginosa* probiotics, biocontrol, vibriosis, vibriostatic factor, prawns, *Penaeus monodon*

## 6. UTILIZATION OF A MARINE ALGA FOR HANDMADE PAPER MAKING

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A marine alga known in Bicol as “Mariwbariw” was tested for use in handmade paper making. Results of the study showed effective utilization of this marine alga as a raw material for paper making. Short fibers were greenish, but tending to slight crispness, tight and somewhat dense surface and had moderate absorbency. A high volume of fiber equivalent to 40% could be obtained per kilo of cooked dry leaf. The fiber imparted a distinct sheen to any fiber which it was mixed. The strength was weak due to fiber shortness. With these results, the use of “mariwbariw” seems practical to use due to its high yield and nonlaborious harvesting and cooking. The unusual appearance of the paper does seem to recommend for art uses. Fiber of this alga may be added to any pulp for decorative effects, thus the handmate paper produced in such combinations could be converted into various novelty items.

**Keywords:** Marine alga, handmade paper making, Bicol, “Mariwbariw”



## 7. VARIATIONS IN THE PHYCOBILIPROTEIN PROFILES OF SOME STRAINS OF CYANOBACTERIA ISOLATED FROM PHILIPPINE WATERS

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Taxonomic studies on cyanobacteria had been previously dominated by traditional descriptive analysis. An active field of experimental analysis involves the elucidation of the phycobiliprotein profiles of cyanobacteria. This information can add to the possible characters that can be used for algal classification, physiological, biochemical and ecological studies (Siegelman 1978). The phycobiliproteins were estimated spectrophotometrically using the method of Shibata *et al.* (1954) as modified by Shibata (1958). Results confirmed cyanobacteria absorb maximally at 620 nm. The type of phycobiliprotein present was believed to be phycocyanin in which the chromophore is phycocyanobilin, an open-chain tetrapyrrole (Glazer 1984).

**Keywords:** cyanobacteria, phycobiliprotein, spectrophotometry, phycocyanin, chromophore, phycocyanobilin, algal classification

## 8. SOME ECOLOGICAL, EXPLOITATION AND MANAGERIAL ASPECTS OF BATO RIVER

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Basic surveys to obtain preliminary information on ecological aspects of Bato River were conducted from March to October 1996. Bato River is the longest river of the province of Catanduanes, Bicol Region. The biotic factors examined were benthic animals and fish fauna; the abiotic factors include water, temperature, dissolved oxygen, pH, conductivity, and transparency.

**Keywords:** Bato River, Catanduanes, Bicol Region

## 9. ECOTOXICOLOGICAL ASSESSMENT OF SELECTED SITE IN THE MANILA BAY AREA

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The indiscriminate discharging of wastes into our rivers and oceans has led to a growing concern about the state of our aquatic ecosystems. Unfortunately, the monitoring of these habitats relies heavily on conventional procedures which are time-consuming, costly and require specialized training on the part of the test operators. In particular, only a few biological procedures (mainly microbiological) suited to Philippine conditions, had been developed or adopted. The study, therefore, aimed to determine the potential of the bioassay, the *Artemia Acute Toxicity Test* (Crustacea: Anostraca), for the assessment of the quality of waters in selected sites along Manila Bay. *Artemia* commonly known as the brine shrimp is a popular source of fish food in aquaculture. The bioassay with this organism is based on the determination of the concentration of the sample which kills 50% of the nauplii after 24-and/or 48 hours of exposure known as the 24-and/or 48-h LC<sup>50</sup>. Given the climatic conditions prevailing in the country, the results of toxicity tests with the reference chemical (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) showed that the sensitivity of *Artemia* was within the same range as those obtained in other studies abroad (24h LC<sup>50</sup> = 26.63 - 30.99 mg/L; 95% C.L. = 22.29 - 31.81 and 26.50 - 36.24, respectively). The results of the monitoring study conducted in the various test sites from October to December (1996) showed that the 48-h toxicity was found to be site specific. Samples obtained from sites associated with waste generating activities exerted toxic effects on the nauplii resulting in high mortalities. The study showed that *Artemia* can be useful and sensitive test organism in basic and applied aquatic toxicology. The study, therefore, proposes the use of the *Artemia* Toxicity Test as a useful component in the battery of tests used in the monitoring of our marine and brackishwater habitats.

**Keywords:** brine shrimp, ecotoxicology, Manila Bay, *Artemia* Acute Toxicity Test, *Artemia*

## 10. SALT-INFLUENCED GROWTH, PROTEIN PROFILES AND NITROGENASE ACTIVITY OF A MICROALGA ISOLATED FROM PHILIPPINE WATERS

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Microalgae isolates, collected from various Philippine waters were screened for salt tolerance. This study attempted to establish cultures of microalgae that will be useful as biofertilizers in salt-intruded agricultural lands in the coastal regions. One of these isolates, viz., IB-Btgs O1 was observed to be heterocystous and was grown for 14 days in BG-11 medium modified variably with 60, 120, 180 mM NaCl. Growth response was determined through the cell count method and analysis of chlorophyll a levels. Electrophoretic analysis was adopted for soluble and membrane proteins. Nitrogenase activity was monitored using the acetylene reduction test. Results showed that the plotted growth response mean values derived through the cell count method were not significantly different. Growth responses based on chlorophyll a levels, however, revealed a significant reduction in growth for cultures growth in 180 mM NaCl. Electrophoretic profiles of the soluble proteins extracted from treated cells reflected decrease in intensity of a 35 kDa band at low salt concentrations and its disappearance at high salt concentrations. Profiles for membrane proteins revealed the disappearance of a 46 kDa band in lanes containing extracts from all the salt-treated cultures. Nitrogenase activity of cells grown in medium contain 60 mM Na Cl produced the highest amount of ethylene.

**Keywords:** microalgae, biofertilizers, salt-intruded agricultural lands, salt tolerance, growth, protein, nitrogenase

## 11. TERATISMS IN MILKFISH *Chanos chanos* REARED IN NAVOTAS-MALABON-TENEJEROS-TULLAHAN RIVER, MALABON, PHILIPPINES

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A two-year study on the Malabon ponds showed that water had acidic pH (6-3-6.6), low salinity (3.0-11.5 ppt) and high water temperature (17-29°C). Copper and

zinc levels of 0.065 mg/L and 0.083 mg/L respectively were higher than those set by the National Pollution Control Commission for class C water-water fir for aquaculture. Fish samples (19-30 cm length) showed high copper and zinc content in gills, kidney and liver. Metal content in these tissues were much higher than those in water. Copper values ranged from 0.55-479.8 mg/L; those of zinc were 11.5-22.3 mg/L. Lamellar fusion, clavate lamellae and epithelial lifting were the pervasive gill hispatologies. Other aberrations found were hyperplasia of the gill epithelium at the bases of the gill arches and increases mucus secretions. Hepatocytes exhibited lipid type vacuolations, dilated bile duct and sinusoids and presence of blood in the blood in the blood vessels. Nephropathology included dilation of the Bowman's space, presence of dense bodies and epithelial stratification in the tubules.

**Keywords:** teratism, milkfish, *Chanos chanos*, Malabon

## 12. BIOCHEMICAL AND PATHOLOGICAL CHANGES IN VERTEBRATE ORGANS EXPOSED TO SYNTHETIC AND NATURAL TOXICANTS

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Synthetic pyrethroids, considered as the newest major group of broad spectrum organic pesticides are now manufactured as alternatives to organochlorides, organophosphates and carbamates. A natural product, the neem kernel extract is widely used in some countries like India for antifertility purposes. Any damaging effects of the two toxicants on vertebrate tissues were analyzed separately. Changes in the levels of total lipids and total proteins of the fish brain were determined quantitatively by spectrophotometric assay every seventh day during the 28-day period. An electron microscope study was done on the mouse testes. Brain total lipids and total protein showed general decline. Ultrastructure analysis of the testes showed cellular damage. Synthetic pyrethroids and neem kernel extract have deleterious effects on vertebrate tissues.

**Keywords:** vertebrate organs, synthetic and natural toxicants, synthetic pyrethroids

### 13. EFFECTS OF SUBLETHAL LEVELS OF CADMIUM CHLORIDE ON THE BLOOD VALUES OF NILE TILAPIA

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The effects of sublethal levels of cadmium chloride on the blood values of Nile tilapia were studied. The 96 h LCD cadmium chloride to young *Oreochromis niloticus* (TL =60-115 mm, 4.0-17.7 g) was 53 mg/L. The toxicity of cadmium chloride was affected by the concentration of the metal and the pH of the water. Acute exposure of fish to 37 mg/L and 26.5 mg/L cadmium chloride at pH 7.1-7.8 and 5.0-5.8 induced changes in RBC count, Ht, and Hb concentration and leucocyte counts. Leucopenia mainly due to lymphopenia, and a tendency toward neutrophilia were observed in treated fish.

**Keywords:** Cadmium chloride, Nile Tilapia, sublethal levels, blood values

### 14. EMBRYOTOXICITY IN TILAPIA NILOTICA EXPOSED TO SUBLETHAL DOSES OF ORGANOTIN MOLLUSCICIDE

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Snail infestation in ricefields since 1989 has caused destruction of thousands of hectares of riceland. The use of organotins has successfully reduced golden snail infestation. However, reports on the adverse effects on farmers and freshwater organisms have led to the government ban on the use of these chemicals. This study identified tissue aberrations in the homeostatic organs in embryos. Ninety-day exposure of 21-day old embryos to sublethal doses of 0.2, 0.05 and 1.25 ug/L of trimethyl tin resulted to numerous aberrations. Kiney tubules exhibited cytoplasmic vacuolations, cellular debris and stratified epithelia that resulted to the occlusion of the lumina. The glomerulus showed hypertrophy and thick Bowman's capsule. Hepatocytes showed cytoplasmic vacuolation. Gills exhibited epithelial lifting and lamellar fusion.

**Keywords:** embryotoxicity, Tilapia nilotica, organotin molluscicide

**15. SELENIUM AS AQUATIC POLLUTANT: TOXICITIES  
INDUCED IN THE MOUTH LINING AND PHARYNX OF  
*Arius manilensis***

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Selenium is an essential trace element but is toxic in high concentrations to aquatic organisms as well as humans. It is available in the environment from volcanic sources, combustion of fossil fuels and effluents discharged from industrial processes. Waterborne selenium of 12 mg/L or greater has been considered hazardous to health and long-term survival of fish and wildlife. The effect of selenium on the mouth lining and pharynx of fish was assessed in this study since these areas are in contact with toxicants as water passes from the mouth to the gills. Representative areas from the mouth lining and pharynx of adult fish exposed to 11, 19, and 57 ppm (LD50-96hr) sodium selenate for 96 hours were processed for paraffin sectioning and examined under the light microscope. Thickening of the epidermal layer brought about by proliferation in the number of goblet cells, mucous cells, and epithelial cells were seen in the mouth lining of treated fish. Pharynx of selenium-treated fish exhibited sloughing of epithelial lining the tunica mucosa, epithelial lifting, and localization of mucous cells at the mucosal fold area, epithelial lifting and proliferation of mucous cells have been regraded as mechanisms to remove the metal stressor.

**Keywords:** Selenium, aquatic pollutant, *Arius manilensis*

**16. BIOMAKERS OF EXPOSURE TO NEEM SEED EXTRACT  
IN THE TESTIS OF *Mus musculus***

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The neem tree (*Azadirachta indica*) has been reported to induce sterility. The antispermatogenic activity of neem extract on ICR mice (*Mus musculus*) was investigated after treatment with various doses for 35 days. Potential histological biomarkers of exposure to the extract are: cytoplasmic vacuoles, loosened peritubular tissues, defective cell-to-cell interconnections and multinucleated spermatophyte and spermatids.

**Keywords:** neem tree, *Azadirachta indica*, *Mus musculus*, testis

### 17. GILL LAMELLAR RESPONSES IN *Oreochromis niloticus* LARVAE AND FRY ACCLIMATIZED IN ARTIFICIAL SEAWATER

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Tilapias are essentially freshwater fish, although saltwater living species had been reported. A problem in tilapia aquaculture is the lack of suitable natural freshwater venues for the culture. The prospect of successful culture in seawater is one way of addressing this problem. Survival and growth of fish in seawater have been positively linked to the osmoregulatory mechanisms; and gills play a crucial role in osmoregulatory mechanism. This study evaluated the gill lamellar responses of *Oreochromis niloticus* of various ages (1, 11, 21, 31, and 41 days posthatching) that had been acclimated from freshwater to saltwater of 37 ppt. Lamellar samples obtained were processed for paraffin sectioning and light microscopy. Lamellar sections showed significant enlargement of chloride cells for seawater-adapted groups. Epithelial lifting and hyperplasia of interlamellar epithelium were also observed. Damage to the epithelium caused the loss of cellular integrity of the lamella. Significant correlation between chloride cell enlargement were noted indicating the major role of chloride cell modification in seawater adaptation.

**Keywords:** Gill lamellar responses, *Oreochromis niloticus* larvae, artificial seawater

### 18. PROTECTION OF FISH BY LIMING AGENT AGAINST COPPER-INDUCED TOXICITY

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The extent of lipid peroxidation in the hepatocytes of the African tilapia, *Oreochromis mossambicus* during short-term exposures of the fish to copper sulfate (4 mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) with and without the addition of liming agent, calcium carbonate (50 mg/L  $\text{CaCO}_3$ ), was determined by the thiobarbituric acid reaction test (TBA Method). Hepatic Malondialdehyde levels were assayed at 24, 48, 72, 96 and 168 hours of exposure to the chemicals as well as to  $\text{CaCO}_3$  alone and carbon tetrachloride (0.25 ml/L  $\text{CCl}_4$ ), a known hepatotoxicant provided by  $\text{CaCO}_3$ . The findings also presented implications and the use of calcium carbonate for improvement of metal-contaminated waters for fishery purposes.

**Keywords:** liming agent, copper-induced toxicity, African tilapia, *Oreochromis mossambicus*

**19. IN VITRO STUDIES ON THE ACTIVITY OF  
COMMERCIAL PROBIOTICS AGAINST  
*Vibrio harveyi* and *Vibrio anguillarum***

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The effectiveness of four commercially available probiotics against *Vibrio harveyi* and *Vibrio anguillarum* was examined under three *in vitro* procedures. In the parallel-streak plat technique, only Probiotic A exhibited dominant growth over *Vibrio harveyi* and *Vibrio anguillarum*. In the cross-streaking procedure, there was no apparent macroscopic inhibition or overgrowth of the two *Vibrio* species by any of the probiotics. In the second procedure where equal volumes of a probiotic and a *Vibrio* species were mixed, there were changes in the viable counts of the *Vibrio* species when monitored for 72 hours. At dilution  $10^{-6}$ , *V. harveyi* became undetectable after 48 hours when mixed with Probiotic D and after 72 hours when mixed with Probiotic A or with Probiotic C. On the other hand, only Probiotic A and Probiotic C were found to be effective in repressing the growth of *V. anguillarum*. Results of the experiments suggest that Probiotic A and Probiotic C are effective in repressing the growth of both species while Probiotic D is effective for *V. harveyi* only. Probiotic B was found to be ineffective for both species. This study provides local prawn farmers with relevant information that will guide them in choosing the appropriate probiotics used to control prawn diseases.

**Keywords:** probiotics, biocontrol, *Vibrio harveyi*, *Vibrio anguillarum*, prawns



## 20. INSECT ANTIFEEDANT ACTIVITIES OF SOME PHILIPPINE PLANTS

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The ethanolic extracts of 10 Philippine plants were tested for their antifeedant activities against ants, cockroaches, and houseflies. Using a 10% sucrose solution as food base, the extracts of *Annona reticula*, *Caesalpinia pulcherrina*, *Curcuma longa*, *Mangifera indica*, *Odontonema stricta*, *Pandanus odoratissimus*, *Premna odorata*, *Tinosopora rumphii* and *Tithonia diversifolia* were active against ants. On the other hand, the extracts of *Odontonema stricta*, *Pandanus odoratissimus* and *Tithonia diversifolia* exhibited antifeedant activities against cockroaches using a starchy food base (pre-weighted pieces of cake). The extracts of *Mangifera indica* and *Odontonema stricta* prevented houseflies from feeding on a 10% brown sugar solution.

**Keywords:** insect antifeedants, *Annona reticulata*, *Caesalpinia pulcherrina*, *Curcuma longa*, *Mangifera indica*, *Momordica charantia*, *Odontonema stricta*, *Pandanus odoratissimus*, *Premna odorata*, *Tinosopora rumphii*, *Tithonia diversifolia*

## 21. PRELIMINARY STUDIES ON LECTINS FROM *Dolichos lablab* (BATAO)

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Crude extracts from *Dolichos lablab* seeds tested showed positive for lectin activity using hemagglutination assay with human blood types A, B, AB and O. Strongest activity was observed with blood type AB. Sugar inhibition assays demonstrated that the activity was inhibited by mannose and galNAc. The extracts were passed through mannose and galNAc Sepharose. Native PAGE and gel filtration of affinity purified extracts showed the lectin to have a molecular weight slightly above 30 kD. Affinity purified extracts were observed to be stable up to 60°C. The study also demonstrated that agglutination activity was lowest at pH 4.0 and highest at pH 8.0 and 9.0 when dialyzed against EDTA, agglutinating activity was abrogated. Upon dialyzing against buffer with Ca ++, Mg++, Mn++ and Zu++ activity was

restored indicating a nonspecific dependence on cations. Presently, the test for lectin's ability to induce respiratory burst in human neutrophil is yielding promising results. Respiratory burst is characterized by the release of superoxide anions, an important aspect during infections and in antitumor activities. Other plant lectins have been found to induce respiratory burst. Thus, an investigation on similar potentials in lectins from *Dolichos lablab* offers interesting possibilities.

**Keywords:** lectins, "batao", *Dolichos lablab*

## 22. THE USE OF RECOMBINANT DNA TECHNOLOGY IN RECOVERING A NATURAL PRODUCT FROM A LUMINOUS BACTERIUM

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A lot of gram-negative polarly flagellated bacteria possess the ability to emit light. They are called luminiscent/luminous bacteria. Most of them are marine forms living freely or symbiotically. Luminescence is brought about by the enzyme luciferase, a long chain aliphatic aldehyde flavin mononucleotide (FMN) and oxygen. Recombinant DNA technology plays a tremendous role in the recovery of natural products. This technique was employed in *Xenorhabdus luminescens*, a strain Hm primary, a luminous bacterium that produces a red pigment. This offers the possible exploration of its pigment as an alternative food dye in the industry. This paper presents studies about the genetics of pigment formation. The pigment genes were cloned and expressed in *Escherichia coli*; the recombinant plasmid (pUC 18+ the pigment genes) named pCGLS 100 was screened and isolated and a partial restriction map was constructed. This fragment was translated and subsequently used as a probe for other species.

**Keywords:** recombinant DNA, Natural products, *Xenorhabdus*. luminous dye, genetics cloning, plasmid, nick translation

**23. AMPLIFICATION AND MOLECULAR CLONING OF  
TENEBROSIN GENES FROM THE SEA ANEMONE,  
*Actinia tenebrosa***

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Using degenerate oligonucleotide PCR primers TNC-2 and TNC-3, and genomic DNA as template, a 306 bp amplified band was cloned and sequenced by means of standard protocols. Sequencing of this amplicon yielded two very similar sequences for tenebrosin. The first one was confirmed to be that of tenebrosin-C from the known primary structure of the protein while the second sequence was designated as tenebrosin-X. The sequences, when translated, were found to represent amino acid residues 38 to 139 of tenebrosin. There were no introns, no cysteine residues and sequence comparison revealed changes in some codons leading to both replaced and silent amino acid substitutions. Analysis of the compared sequences disclosed that ten codon changes led to amino acid substitutions most of which were conservative. Five codon changes did not alter the corresponding amino acid. Any of the two-cloned sequences was a good probe to search for and clone other tenebrosins from the same organism or from other marine organisms which produced related toxins or cytolytins. Toxins are important from a biotechnological point of view because they are potential sources of new biopesticides, pharmacological drugs or immunotoxins as cancer chemotherapeutic agents.

**Keywords:** cloning, molecular cloning, tenebrosin genes, sea anemone, *Actinia tenebrosa*

**24. PHYLOGENY OF CONE SNAILS BY NONRADIOACTIVE  
DNA SEQUENCING**

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Venoms from *Conus* snails contain a wide variety of small peptides which are currently used as pharmacological tools for neuroscience and drug development. The *Conus* phylogeny served as a guide for choosing the particular snail to be investigated for a specific peptide. In this study, the DNA was extracted from hepatopancreas of *Conus* species using a rapid technique, a 168 technique using

silver staining for DNA detection. The resulting sequences together with previously known sequences for other species, were used to construct phylogenetic trees.

**Keywords:** cone snails, DNA, DNA sequencing, PCR peptides, phylogeny and silver staining

## 25. MITOCHODRIAL DNA RESTRICTION FRAGMENT LENGTH POLYMORPHISM AS GENETIC MARKERS IN YELLOWFIN TUNA (*Thunnus albacares*)

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The tuna industry is one of the major dollar earners for the Philippine economy. This resource, however, is threatened from exploitation due to inadequate specific management and conservation measures. Genetic population structure studies of Philippine tuna can serve as guiding reference in establishing fishery management policies for sustainable development. This research aims to develop restriction fragment length polymorphism (RFLPs) from the 16S rRNA segment of mitochondrial DNA as genetic markers to analyze yellowfin tuna (*Thunnus albacares*) populations in the Philippines. The total DNA of yellowfin tuna was extracted from liver and muscle tissue through a rapid one-step extraction (ROSE). The 16S rRNA segments were amplified using the polymerase chain reaction (PCR). Preliminary data indicated monomorphism results for 11 samples from five Luzon provinces as shown by three out of six restriction enzymes tested.

**Keywords:** yellowfin tuna, ROSE, PCR, mitochondrial DNA, 16S rRNA, RFLP

## 26. PCR-RFLPs OF THE mtDNA D-LOOP and 16s rRNA GENE SEGMENTS IN MILKFISH, *Chanos chanos*

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The Polymerase Chain Reaction (PCR) is a rapid and simple method of amplifying specific genes or DNA sequences, which can be applied in the study of genetic

variability of marine species such as milkfish. PCR amplification conveniently provides enough copies of the mt DNA D-loop region 16s rRNA gene segment for subsequent restriction with enzymes for the detection of variability in the form of restriction fragment length polymorphisms (RFLPs). RFLPs which characterize certain populations or species can be used as markers in the study of genetic population structure. In this study, a 2Kb fragment of the mtDNA D-loop region and a 600 bp fragment of the 16s rRNA gene was amplified from milkfish DNA. Results of restriction digestion of both PCR products using 12 enzymes showed polymorphic RFLPs in the D-loop region but no variation in the 16s rRNA segment. The data affirm the utility of D-loop RFLPs as genetic markers for milkfish population studies.

**Keywords:** *Chanos chanos*, milkfish, polymerase chain reaction, restriction fragment length

## 27. DNA VARIATION AND GENETIC RELATIONSHIP AMONG FILIPINO TRIBES AND MAJOR/MINOR GROUPS

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The Filipino population is so diverse consisting of more than 100 morphologically and/or linguistically distinct groups. This diversity may be reflected as DNA variation. Data on DNA variation would provide us insights into the genetic relationships among Filipino groups and help resolve issues about our origin. But a more practical application of these data is in constructing the genetic databases needed in DNA typing for forensics. The country's crime agencies, the National Bureau of Investigation, and the Philippine National Police are developing their capability to use DNA typing for forensics. The genetic databases are needed to properly interpret DNA typing results. Human blood/saliva was collected from volunteers with informed consent and hospitals/blood banks. DNA was extracted and analysed for variation in known, discrete regions. The patterns of differences found among population groups were compared statistically. Initial results from Metro Manila population sample showed that the locus D1S80 can be used for forensic DNA typing as well as for analysing genetic variation.

**Keywords:** DNA variation, genetic relationship, forensic DNA typing

## 28. MOLECULAR MARKERS FOR CLONAL FINGERPRINTING IN BANANA AND MANGO

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The more recent development in DNA markers has produced abundant and highly variable new markers that may overcome the limitations of traditional markers used in varietal identification in banana and mango. The study classified banana and mango cultivars using PCR-based random amplified polymorphic DNA (RAPD) and microsatellite markers. Genomic DNA from immature leaf samples of banana and mango were amplified by PCR using a set of single primers of 10 nucleotides or primer pairs of *Musa* sequence-tagged microsatellite sites. Protocols for RAPD analysis for mango and banana were optimized. Preliminary results in both crops indicate that RAPD analysis can reveal significant differences among cultivars tested.

**Keywords:** RAPD, DNA markers, microsatellites, mango, banana, *Musa*, *Mangifera indica*

## 29. OPTIMIZATION OF PCR CONDITIONS FOR THE RAPD ANALYSIS OF SEVEN TREE SPECIES

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The ability to differentiate between cultivars is necessary for breeding work and is just a step towards the eventual genetic engineering of the coconut (*Cocos nucifera* L.) durian (*Durio zibethinus* Murr.) ilang-ilang (*Cananga odorata* Hook.f.) narra (*Pterocarpus indicus*), chico (*Manikara indica* (L.) van Royen), and mango (*Mangifera indica* L.). One method of differentiating cultivars is via RAPD (Random Amplified Polymorphic DNA). But before RAPDs can be used in these three species, it is necessary to optimize the PCR conditions.

High-MW genomic DNA was extracted from the leaves of said tree species using a modified Doyle & Doyle (1990) CTAB Method. Eighteen arbitrary decamers

were then screened and used in the optimization of the PCR conditions. DNA, primer and MgCl<sub>2</sub> concentrations, and annealing temperature were varied to obtain optimum results. Clear and distinct RAPD bands were generated using about 0.8 ng/μl DNA, 0.3 μM primer, and 3mM MgCl<sub>2</sub> in the PCR reaction mixture. The PCR program which gave optimum results (with Amplitron I thermal cycler) were as follows: a 5 min initial dwell at 94°C, followed by 45 cycles of 1 min at 92°C, 1 min at 34-35°C (depending upon primer) and 2 min at 72°C, and a final dwell of 10 min at 72°C.

Primers that gave positive results can then be considered potential RAPD markers for the said tree species. Preliminary tests of said primers generated polymorphism with chico.

**Keywords:** PCR, RAPD, *Cocos nucifera*, *Durio zibethinus*, *Cananga odorata*, *Pterocarpus indicus*, *Manikara indica*, *Mangifera indica*, DNA polymorphism

### 30. NOVEL SOMACLONAL VARIANTS REGENERATED FROM CALLUS OF RICE *Oryza sativa* L.

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Genotypic variability in the occurrence of somaclonal variation in regenerated plants and progenies of regenerated plants of some Philippine rice varieties was evaluated. Mature seeds of varieties Elon-elon, C-4, PARC-3 and Dinorado were induced to undergo callus formation and plant regeneration via somatic embryogenesis *in vitro* on Murashige and Skoog's basal medium supplemented with auxins and cytokinins. In the first generation of regenerated plants, significant variations for plant height, lengths of internode, flag leaf and panicle, number of spikelets, and percentage fertility were observed. Although negative changes in some agronomic traits appeared, two novel somaclonal variants in variety Dinorado having significantly increased panicle length, number of spikelets, increased fertility and reduced plant height due to increase in internode length were isolated and found to breed through for three generations. This improvement in character expression in two isolated lines of Dinorado showed that somaclonal variation is a promising biotechnological technique for crop improvement.

**Keywords:** auxins, cytokinins, callus, *in vitro* culture, plant regeneration, progeny, *Oryza sativa*, somaclonal variation, somatic embryogenesis, tissue culture

### 31. TRANSFORMATION OF *Zea mays* LINNAEUS (LINNAEUS) INBRED LINES IN THE PHILIPPINES VIA PARTICLE BOMBARDMENT: A STATUS REPORT

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Improving crop varieties through transformation process has been one of the most promising applications of biotechnology. While this technology has been successfully documented in developed countries, the Philippines has yet to produce its first transgenic plants. This study is part of an overall effort to improve maize production through genetic engineering of genes conferring effective resistance to the insect pest, Asiatic corn borer. This work is the first attempt to transform Philippine-bred maize lines under local conditions. The modest gains and problems encountered in the conduct of this type of research in the Philippines is presented. Recommendations to better improve the prospects of utilization of this technology are discussed.

**Keywords:** transformation, particle bombardment, genetic engineering, maize, *gus* gene, herbicide resistance, Asiatic cornborer

### 32. MOLECULAR CHARACTERIZATION OF LOCAL *Bacillus thuringiensis* ISOLATES EFFECTIVE AGAINST ASIATIC CORNBORER *Ostrinia furnacalis* (G.) AND DIAMONDBACK MOTH, *Plutella xylostella* LINN.

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Out of of 268 local isolates of *Bacillus thuringiensis* screened against asiatic cornborer (ACB), *Ostrinia furnacalis* and diamondback moth (DBM), *Plutella xylostella*, three were selected. LEP-20 and LEP-3(1) showed high toxicity to ACB but moderately toxic to DBM while LEP-#13 showed high toxicity to DBM but moderately toxic to ACB.

Analysis of solubilized insecticidal crystal protein revealed the presence of proposed protoxin and toxin subunits whith molecular mass of about 130 and 65 kDA



proteins for LEP-20 and LEP-3(1) and an additional 140 kDa protein for LEP-#13. Plasmid DNA profile showed that only LEP#13 is different from LEP-20 and LEP3(1). This is possible since LEP-20 and LEP-3(1) was serotypically identified as *B. thuringiensis* subsp. *galleriae* while LEP-20 and LEP-3(1) were *B. thuringiensis* subsp. *kurstaki*. Scanning electron microscopy presented the different shapes of crystals within each isolate, PCR reveals the presence of cry1Aa, cry1Ab and cry1Ac genes in LEP-3(1) and LEP-20 while cry1Aa, cry1Ab and cry1B genes in LEP-#13.

### 33. DELTA-ENDOTOXINS OF LOCAL *Bacillus thuringiensis* STRAINS: BIOCHEMICAL AND TOXICITY STUDIES AGAINST THE ASIATIC CORN BORER *Ostrinia furnacalis*

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Awareness of the harmful environmental effects of chemical pesticides has led to the search for novel, pest-specific insecticides such as the crystal protein produced by a soil microorganism, *Bacillus thuringiensis* (Bt), against pests such as the Asiatic corn borer (ACB). The biochemical and toxicological properties of the crystal protein/s of the local BT strain LEP 20 in preparation to incorporating their respective Bt genes to corn were studied. The production and solubilization of the protoxin crystals fractionated the protoxin and bioassayed the fractions against the ACB. Sepharose 2B gel chromatography gave a broad band (139 and 140 kD by SDS-PAGE) with three peaks which showed low toxicity. Preparative SDS-PAGE generated major of 133 to 139 kD which had a lower toxicity (13.3%) compared with the crude sample (46.7%). Ultrafiltration yielded high-MW proteins (>100kD) possessing high toxicity (100%) while the low molecular weight fraction (>50kD>MW>10kD) had slight toxicity (13.3%). Specific staining (PAS) indicated that the protein is glycosylated. The ACB exhibited different susceptibilities to protoxins from other local Bt strains and one commercial strain. These results indicate that toxicity against the ACB can be attributed to more than one active fraction and their synergistic interactions.

**Keywords:** Crystal; endotoxin, *Bacillus thuringiensis*, *Ostrinia furnacalis*, corn, toxicity studies, gel chromatography, preparative electrophoresis, ultrafiltration.

**34. BIOMODIFICATION OF COCONUT (*Cocos nucifera*) Linn.  
OIL AND NON-LAURIC OILS FOR THE PRODUCTION OF  
SPECIALTY FATS AND OILS**

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With the great demand for vegetable fats and oils today, coconut oil, the most abundant lauric oil, is a much desired article because of its unusual properties compared to other cooking fats and oils. However, it is susceptible to hydrolysis and blends badly with nonlauric oil foam used in deep fat frying. The present knowledge on foaming during deep fat frying is limited. This research investigated the cause of foaming from the triglyceride distribution pattern of the oils, modified the triglyceride structures through lipase-catalyzed interesterification, developed a technology for the production of coconut and nonlauric blends through enzymatic interesterification, which could provide high value and better health benefits. Using three nonlauric oils namely: cashew, fish and sesame, the foaming activity of their straight blends (0 hr) with coconut oil in different ratios was observed to foam considerably. Lipase-catalyzed interesterification of the oils was carried out and foaming activity of the interesterified blends was decreased by 50%. Generally, modification by the enzyme of the oils decreased foaming. This was found to be accompanied by a significant change in the triglyceride profile which was determined by the equivalent carbon number.

**Keywords:** lipase-catalyzed, interesterification, triglycerides, foaming, fatty acids, lauric oils, non-lauric oils, enzyme, equivalent carbon number



## HEALTH SCIENCES

### 1. ANTITUBERCULAR CONSTITUENTS OF *Lunasia amara* BLANCO

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Alcoholic extracts from 90 species of plants collected from the Subic forest were assayed against five microorganisms: the Gram (+) *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, the Gram (-) *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, and the aci-fast Mycobacterium 607. One of the plants that exhibited promising inhibitory activity was *Lunasia amara* Blanco (Rutaceae). Results showed inhibition to *B. subtilis*, *S. Aureus* and *Mycobacterium* 607. Assay of the crude alcoholic extract against *Mycobacterium tuberculosis* H<sub>37</sub>Rv resulted in 99% inhibition at 1000 mg/mL. A bioassay-directed isolation of the antitubercular constituents of *Lunasia amara* resulted in three crystalline isolates. Thin layer chromatograms of the isolates suggest their alkaloidal nature when sprayed with Dragendorff's reagent. Spectroscopic analysis revealed the alkaloids to be gravcolinine, 4-methoxy-2-phenylquinoline and kokusagine.

**Keywords:** alcoholic extracts, assay, crystalline isolates, alkaloids

### 2. BACTERIAL CARDIOLIPIN FOR SERODIAGNOSIS OF SYPHILIS

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Presently, serological diagnosis of syphilis in the Philippines makes use of commercially-prepared cardiolipin from beefheart. This study seeks to isolate and

identify certain bacteria belonging to *Family Enterobacteriaceae*, *Halobacteriaceae*, *Rhodospirillaceae* and Genus *Thermus* which can be potential sources of lipid antigen and used for serological diagnosis of syphilis. The bacterial lipid was extracted from the microorganisms using chloroform-methanol mixture. Presence of the cardiolipin was determined using thin-layer chromatography and Fiske Subarrow method for inorganic phosphorus. Further purification of the cardiolipin was done using Sephadex LH-20. The purified bacterial cardiolipin antigen was coated onto the surface of 96-well plates. Fifty positive sera (RPR test) and fifty negative sera (RPR test) were used to test for the sensitivity of the isolated bacterial antigen and were ran simultaneously with VDRL test. Plates were read at 492 nm and 620 nm (references) using LP-200/LP 300 Diagnostic Pasteur microplate reader. The absorbance reading of bacterial antigen and VDRL was compared. Results showed a very high correlation with a p value of 0.78. The study suggests the use of bacterial antigen for serological identification of elevated amount of reagin found in cases of syphilis.

**Keywords:** Syphilis, cardiolipin, lipid, antigen, serological diagnosis.

### 3. AN INVESTIGATION ON THE MEDICINAL QUALITIES OF *Moringa oleifera*

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This paper presents the studies on the anti-inflammatory, antimutagenic and antitumor promoting activities of isolates from the extracts of dried seeds of malunggay and the elucidation of the structures of these bioactive isolates. Bioassay results indicated that the isolated sterol reduced inflammation in the hind paw of mice induced by carragenan and reduced the number of micronucleated polychromatic erythrocytes induced by the mutagen, mitomycin C. The glucosylsterol derivative and the acylglucosylsterol were likewise anti-inflammatory, antimutagenic and strongly inhibited the activation of the Epstein-Barr virus-early antigen, indicating strong antitumor promoting activities. A rhamnosyl thiocyanate derivative also exhibited remarkably strong antitumor promoting activity while a rhamnosyl thiocarbamate derivative exhibited antihistamine release activity, another indication of anti-inflammatory activity. Published literature showed that the same rhamnosyl thiocy-

anate derivative is antimicrobial, and the thiocarbamate derivative and a mustard oil glycoside are hypotensive. However, a rhamnosyl acetonitrile derivative is mutagenic.

**Keywords:** *Moringa oleifera*, malunggay, anti-inflammagens, antimutagens, antitumor promoters

#### 4. POTENTIAL ANTI-PLATELET AGGREGATING AGENTS FROM MARINE SPONGES AND TUNICATES

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When stimulated, platelets will aggregate to form a hemostatic plug. This activation causes thromboembolism and inflammation. Acetylsalicylic acid (aspirin) is one of the most important drugs used for the prevention and management of these diseases. An *in vitro* assay was established to screen for potential platelet aggregation inhibitors comparable to that of aspirin. A total of 113 sponges and tunicates collected from Baler, Quezon were screened qualitatively using the giemsa staining method. Ten crude methanol extracts showed 100% inhibitory activity at 2mg/ml against calcium chloride-induced platelet aggregation. Bioassay-guided isolation and purification of the potential compounds and further quantitative testing of the active samples are currently underway. This *in vitro* assay was developed on the microliter scale to allow quick screening of large number of samples of small quantities such as marine extracts.

**Keywords:** sponges, tunicates, platelets, aggregation, aspirin, calcium chloride, inflammation, giemsa, anti-platelet, marine.

#### 5. PURIFICATION AND CHARACTERIZATION OF HUMAN CHORIONIC GONADOTROPIN ALPHA SUBUNIT: A POTENTIAL LUNG CANCER MARKER

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Initial studies on the distribution profile of alpha and beta subunits of urinary human chorionic gonadotropin in lung cancer, using an ion-exchange chromatogra-

phy revealed an unusual increase in concentration of the alpha subunit in the non-small cell lung carcinomas (NSCLC). SDS-PAGE under reducing conditions was performed to assess the purity of the frozen-dried isolated subunits. A distinct band of about 20 kD was significantly observed in the alpha subunits from non-small cell carcinoma of the lung. When subjected to Western blotting, the 20 kD protein band reacted strongly with the monoclonal antibody against the hCG alpha subunit. Further purification of the subunits was performed using repeated reverse phase HPLC (Supelcosil C18) until a single peak reactive against hCG alpha antibody was obtained. The purified and characterized hCG alpha subunit can be used in the production of an anti-hCG immunoassay kit for nonsmall cell lung cancer. The immunoassay kit can serve as an easy, fast and inexpensive screening test for that type of lung cancer.

**Keywords:** lung cancer, urinary human chorionic gonadotropin, cell carcinoma, Western blotting, immunoassay

## 6. STUDIES ON *Prosopis vitaliana* NAVES AS A POTENTIAL SOURCE OF DRUG MATERIAL

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A large number of drugs being used today are derived from plants and the search for bioactive compounds from plants is still an ongoing process. As a result of a field screening in the Zambales rainforest, a plant has been identified to have a great potential as a source of a new drug material. *Prosopis vitaliana* Naves (Leguminosae), locally known as "aroma" was collected from the forest and subjected to chemical and biological studies. Antimicrobial activity of the crude ethanolic extract was evaluated using ATCC bacterial strains and 19 bacterial wound isolates. Results obtained from disc diffusion assay indicate that it inhibited both Gram (+) and Gram (-) bacteria, as well as species of *Staphylococcus* and *Escherichia coli* from wound isolates. Cytotoxicity assay using the brine shrimp assay and showed an LC<sub>50</sub>-35.69 µg/mL. Cytotoxicity assay using the microculture tetrazolium (MIT) method indicated a comparable activity against both the human cancer cell lines and murine fibroblasts. The Ames test showed that it is nonmutagenic against *Salmonella typhimurium* TA 98 and TA 100. No DNA damaging properties were observed up to a dose level of 400

$\mu\text{g}/\text{plate}$ . The crude ethanolic extract was subjected to bioassay-guided isolation. The antimicrobial activity was monitored using disc diffusion method and bioautography and the cytotoxicity was monitored using brine shrimp assay. Two antimicrobial and cytotoxic alkaloids were isolated and their structures were elucidated using different spectroscopic methods.

**Keywords:** aroma, Leguminosae, *Prosopis vialiana*, crude ethanolic extract

## 7. THE ANALGESIC ACTIVITY OF THE ALKALOIDS FROM THE SEEDS OF *Ipomoea muricata* JACQ. CONVULVACEAE

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Indolizidine alkaloids were previously isolated from the seeds of *Ipomoeae muricata* for which analgesic activity of the seeds was attributed to. In separate studies, the analgesic activity was reported for the alkaloid ipomine and ipalbidine. This study compared the analgesic activity of four indolizidine alkaloids from the seeds of *Ipomoea muricata*. The analgesic activity of three tertiary indolizidine alkaloids, ipomine, ipalbidine, ipalbine and a quaternary indolizidine alkaloid designated as EL, were tested on mice by the hot-plate method. All alkaloids showed analgesic effects at dose levels of 1 mg/kg-BW, 5 mg/kg-BW and 10 mg/kg-BW, except for standard drug indomethacin at 5 mg/kg-BW. The order of analgesic activity of the alkaloids was EL. > ipalbidine > ipomine > ipalbine. By relating the structures of the individual alkaloids to their activities, it appeared that the presence of the quaternary nitrogen in the structure of EL contributed to its enhanced activity. EL can be a lead compound in the development of a potent analgesic agent.

**Keywords:** analgesic, alkaloids, *Ipomoea muricata*



## 8. CORRELATIONS OF CYTOTOXIC AND APOPTOTIC RESPONSES DURING GAMMA-IRRADIATION OF MOUSE THYMOCYTES TREATED WITH A CANDIDATE HERBAL RADIOPROTECTOR GC-2112

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Biomedical studies on nuclear fallout effects showed that whole-body exposure to relatively low doses of ionizing radiation (5-10 Gy) induces the clinically broad spectrum of the *Hematopoietic Syndrome* characterized by severe anemia, immunodeficiency, followed by death within 10-30 days. Whole thymus explants derived from intra-familial 30-day old inbred male ICR mice were cultured *in vitro* after enzymatic dissociation and removal of thymic macrophages. This thymocyte model is applied in many cell death researches and had been found to undergo a morphologically and molecularly distinct p53-based cascades of apoptotic events with exposure to DNA-damaging insults, such as some anti-cancer agents, hyperthermia, hypoxia and radiation. Exogenous application in culture of a radiation protecting agent GC-2112, a herbal extract derived from water-soluble components of garlic bulbs (*Allium sativum*), improved total cellular survival for various observation periods concomitantly shifting the LD<sub>50/24</sub> (lethal dose) from 17 Gy (control) to 21 Gy (with radioprotection). Target-based analyses revealed time-dependence of radioprotection process which exhibited shifting in both a and b parameters and clustering of thymocyte deaths into three distinct modes. This increase in total cell survival of GC-2112-treated thymocytes, however, failed to correlate with the prevention of apoptosis-associated internucleosomal DNA scissions. DNA fragmentation in the absence of irradiation and with treatment of GC-2112 was induced despite the overall improvement of total cellular survival of thymocytes exposed to radiation. The mean dose reduction factors (DRFs) ranged from 10.7-1.1. Mechanisms to the observed radiomodification may possibly involve a mixture of bioactive poly-thiols found rich in garlic bulbs. The findings presented a paradoxical correlation between apoptosis, cell survival and radioprotection which may be explained by the differential reactivities of the bioactive compounds in GC-2112 and the variabilities in radiosensitivities of the primary thymocyte culture. The differential effect on radio protection showed potential in the

application of herbal drugs as dietary prophylaxis against clinical morbidities arising from either medical, occupational or environmental exposures to ionizing radiation.

**Keywords:** radioprotectors, apoptosis, thymocytes, radiation-induced death, herbal medicine, target theory, immunity, radiation biology, applications in radiotherapy

## 9. CULTIVATION OF MAMMALIAN CELLS FOR LABORATORY ASSAY SYSTEMS

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The developed techniques of culturing cells *in-vitro* led to the production of specific cells. These serve as tools to the understanding of the cell's biological nature contributing further to the proper diagnosis and search for the therapeutic modalities. The study cultivated mammalian cells for laboratory assay systems. Freshly explanted surgical tissues were immersed in Hank's Balance Salt Solution. The tissue explants were enzymatically digested and cultured in RPMI 1640 culture medium supplemented with 10% fetal bovine serum. Obtained cells were incubated at 37°C in 5% CO<sub>2</sub> and fed every other day until they showed confluent growth and characteristic morphology. Cells were harvested and subcultured for various laboratory assays and cryopreservation. Results of the study showed the culture of the following cells: normal urothelial cells, transitional and squamosal carcinoma of the urinary bladder, sphenoid wing meningioma and tumor cells of the posterior fossa of human brain. Preliminary studies showed that these cells demonstrate variable growth behavior, growth rate, characteristic morphology and viability in culture medium. Laboratory assays also showed promising results for the search of therapeutic agents from natural product extracts and the search of homografts for the reconstructive surgery.

**Keywords:** Mammalian cells, assay systems

## 10. ALBUMIN EXCRETION RATES IN FILIPINO PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS: A CASE COHORT

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Diabetic kidney disease is an important cause of morbidity in insulin-dependent diabetes mellitus (IDDM). The 1991 guidelines issued by the American Diabetes Association included assessment of albumin excretion rate in the urine as a sign of progression of the disease process. The early detection of microalbuminuria [20-200 ug/min] by immunoturbidimetric assay (ITA) can be an important tool for early diagnosis and the intervention to prevent irreversible kidney damage in the population at risk. This study aimed to determine the significant differences in glycosylated hemoglobin levels and albumin excretion rates within 6 months in this study group of IDDM patients. It also aimed to examine the relationship between MA and the following clinical characteristics: age at onset of IDDM, duration of IDDM, height, weight, body mass index (BMI), insulin requirement per day, glycosylated hemoglobin, systolic and diastolic blood pressure. In March 1996, 31 Filipino diabetic children and adolescents were studied for early signs of diabetic nephropathy. Eight patients were identified as having microalbuminuria (MA). All 31 subjects were followed up for six months. In the initial ITA, microalbuminuria was identified in eight patients. On repeat ITA, two of the eight patients showed normal albumin excretion. In six patients in whom the microalbuminuria persisted, the AER had decreased. One patient who had a normal AER initially became microalbuminuric. In the normoalbuminuric group, the male: female ratio and mean age was 1:1.3 and 12.73 +/-5.0 respectively while in the microalbuminuric group, it was 1:7 and 14.5 +/-5.0. The mean difference in AER between the first and second ITA in those with microalbuminuria showed a trend towards a decrease in excretion rates. However, this was not statistically significant ( $p=0.08$ ). Among variables studied, only diastolic blood pressure ( $p=0.05$ ) was found to be significantly correlated with microalbuminuria.

**Keywords:** albumin, excretion, diabetic kidney disease, insulin-dependent diabetes mellitus, and microalbuminuria.

## **11. C-PEPTIDE IN FILIPINO CHILDREN WITH INSULIN-DEPENDENT DIABETES MELLITUS: A MEASURE OF RESIDUAL B-CELL FUNCTION**

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Children with insulin-dependent diabetes mellitus (IDDM) have minimal or absent secretion of insulin, thereby requiring exogenous insulin as therapy. Studies have shown that C-peptide concentration is a valid index for residual B-cell function, unaffected by exogenous insulin and insulin antibodies. This study was done to determine the residual B-cell function as measured by C-peptide in Filipino children with IDDM and to correlate this with variables including sex, weight, age at onset of IDDM, duration of illness, serum insulin, dose of insulin, blood glucose (FBS), and glycosylated hemoglobin (HBA1C). Among 50 IDDM patients studied, 18% (n=9) had residual B-cell function, the age, duration of diabetes, insulin doses of patients and HBA1C were not significantly different from those with abnormal levels. Patients with abnormal C-peptide levels were noted to have significantly lower age of onset ( $p < 0.0015$ ) and weight ( $p < 0.0084$ ). There was no significant correlation between the duration of diabetes and glycemic control.

**Keyword:** Insulin-dependent diabetes mellitus, residual cell function, C-peptide concentration, exogenous insulin, insulin antibodies

## **12. DETERMINATION OF MICROALBUMINURIA (MA) USING SEMIQUANTITATIVE METHOD (MICRAL-TEST) IN FILIPINO PEDIATRIC PATIENTS WITH INSULIN DEPENDENT DIABETES MELLITUS (IDDM)**

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The hallmark of the diabetic kidney is persistent proteinuria and progressive renal impairment. Several years prior to the appearance of proteinuria, subclinical elevation of urinary albumin excretion or microalbuminuria (MA) can be detected.

MA is albumin excretion rate of 20-200  $\mu\text{g}/\text{minute}$ . MA is highly predictive of diabetic nephropathy, hypertension and cardiovascular disease. This study was done to determine the prevalence of MA using Micral-Test (MT) in Filipino patients with IDDM, to determine the diagnostic properties of MT (validity and accuracy) and to compare it with quantitative immunoturbidimetric assay (ITA). MT was determined by semiquantitative dry immunochemical method using a 24 hour urine sample. The same sample was used for ITA. Thirty one diabetics were tested for MT. There were 24 normo albuminuric and 7 microalbuminuric patients. In the former group, the male: female ratio and mean age were 1:2 and 12  $\pm$  5.0 ( $\pm$ -SD) respectively while in the latter group, it was 1:6 and 14  $\pm$  5.3 ( $\pm$ -SD). The prevalence of MA in the study was 22.58%. MA has a sensitivity of 85.51%, specificity of 9%, negative predictive value of 94.11% positive predictive value of 42.68% and accuracy of 71%. The study disclosed 77.42% of Filipino diabetics were correctly classified as either having or not having elevated urinary albumin excretion using ITA as the gold standard. The Micral-Test provided a sensitive and specific test for clinic screening of microalbuminuria.

**Keywords:** Microalbuminuria, semi-quantitative method, Insulin-dependent diabetes mellitus

### 13. A CHROMOGENIC DIAGNOSTIC KIT FOR THE RADIOSENSITIVE TYROSINASE ENZYME IN ORIENTAL FRUIT FLY *Bactrocera philippinensis*

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A biochemical marker for radiation injury in Oriental fruit fly, *Bactrocera philippinensis*, was identified and isolated in denaturing polyacrylamide gel electrophoresis (SDS-PAGE). The biomarker, Gs-protein, is a tyrosinase enzyme whose gene locus is found to be radiosensitive even at a very low radiation dose of 25 Gy as demonstrated by the loss of Gs-protein band from SDS-PAGE gel analysis of pupal homogenates from irradiated larvae. Isolated Gs-protein has a tyrosinase enzyme activity capable of converting tyrosine into an intermediate product, DOPA. It also exhibit the copper-containing property of mushroom tyrosinase as shown in the preliminary TXRF analysis. An apparent molecular weight of 109 kDa was calculated from SDD-PAGE preparation of the Gs- protein with high molecular weight proteins as standards. A chromogenic test based on the reaction of tyrosinase with 2-methyl-DOPA was developed for *B. philippinensis* for use as a field quarantine kit. Irradiated fruit fly has been shown in earlier studies to lose its tyrosinase enzyme. The presence

of tyrosinase in homogenate material of fruit fly at different stages of development starting from late instar into adult fly could be positively identified using this diagnostic test where a dark brown to black coloration is observed when the homogenate is applied to a spot of 2-methyl-DOPA on acetate film. Absence of color development within 5 min. after a homogenate from squashed fly in the water was made to react with two-methyl-DOPA would indicate that the fly had been sufficiently irradiated and therefore will not develop into an adult insect. This simple test was based on the work of Nation *et al.*, on Mediterranean fruit fly and has been modified and developed for *B. philippinensis*. The chromogenic kit is also being tested for wild type *B. philippinensis* which are endemic in Guimaras Province where mangoes are grown extensively for export.

**Keywords:** Tyrosinase, chromogenic, diagnostic, Gs-protein, fruit fly, biomarker, radiosensitive, *Bactrocen philippinensis*

#### 14. CLINICAL AND LABORATORY PROFILE OF PEDIATRIC PATIENTS WITH NONTYPHOIDAL SALMONELLA BACTEREMIA IN PGH AND RITM FROM 1991-1995

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Morbidity and mortality from Salmonellosis rank high among children. The study aimed to describe the clinical and laboratory profile of patients with *Salmonella bacteremia*. Children from 0-18 years old with *Salmonella bacteremia* admitted in PGH and RITN from 1991-1995 were used in the study. Fifty percent of records of 117 patients with *Salmonella bacteremia* were retrieved. Results showed that in infants 1 year and below, common symptoms were watery nonbloody diarrhea, fever and vomiting. Anemia and dehydration were complications noted in 52% and 38% respectively. CHC showed either leukopenia, leukocytosis or thrombocytopenia. Fecal leukocytes was seen in only 28%. Group B *Salmonella*, being the predominant pathogen, was sensitive to Chloramphenicol in 57% of isolates. In older children, abdominal pain together with fever and diarrhea were frequently observed. 32% developed dehydration during confinement. Group C *Salmonella* was the most common isolate, with 69% sensitive to Chloramphenicol. 67% clinically improved after antimicrobial and supportive treatment.

**Keywords:** Nontyphoidal *Salmonella bacteremia*, Salmonellosis, PGH

**15. GENOTYPE ANALYSIS OF HEPATITIS C VIRUS AMONG BLOOD DONORS AND INMATES IN METRO MANILA, THE PHILIPPINES**

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A genotype analysis of Hepatitis C virus among blood donors and inmates in Metro Manila was done. Antibodies against Hepatitis C virus (HCV) were detected in 18 (2.3%) of 800 sera from commercial blood donors and 23 (4.6%) of 502 sera from inmates in Metro Manila. The difference in the antibody prevalence between the two groups was statistically significantly ( $P < 0.05$ ). HCV RNA was detected in 14 (78%) of the 18 antibody-positive sera from blood donors and 19 (83%) of the 23 antibody-positive sera from inmates. Genotype analysis revealed that HCV-1b (50%) was most common among blood donors, followed by HCV-1a (36%) and HCV-2a (7%). Among inmates, on the other hand, HCV-1a (68%) was most common, followed by HCV-1b (11%), HCV-2b (5%). Overall, HCV-1a and HCV-1b appeared to be predominant among them. Thus, the genotype prevalence in the Philippines was distinct from those in other Southeast Asian countries such as Thailand, Vietnam and Indonesia, and also distinct from those in the Far East including Taiwan, Mainland China and Japan.

**Keywords:** Hepatitis C virus, genotype, blood donor, inmate

**16. HEPATITIS C VIRUS INFECTION AND RISK FACTORS AMONG INMATES AT MUNTINLUPA, METRO MANILA: AN EMERGING CONCERN IN THE COMMUNITY**

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The Hepatitis C virus infection and risk factors among inmates at Muntinlupa, Metro Manila were studied. The seroprevalence of hepatitis C virus (HCV) infection was 5.0% in 502 inmates examined at the Camp Sampaguaita, Bureau of Prisons,

Muntinlupa, Metro Manila. The practice of homosexual activities and parenteral drug use were found to be significantly associated with HCV infection a prevalence of 12.9% and 35.0%, respectively. Though the prevalence of anti-HCV was higher among those with tattoos, who had blood transfusion, pierced ears, "bulitas" implants, and self-inflicted wounds or "paandar" these were not statistically significant. Local baseline data of HCV infection in the general population is needed to determine the magnitude of the disease and to determine risk factors.

**Keywords:** Hepatitis C virus, Hepatitis C, Muntinlupa, Metro Manila, homosexual activities

### 17. PULMONARY PARAGONIMIASIS IN NON-RESPONDING TUBERCULOSIS PATIENTS IN IROSIN, SORSOGON

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In the province of Sorsogon, pulmonary tuberculosis remains as a leading cause of mortality and morbidity. In the municipality of Irosin, of the 157 patients listed in the TB registry in 1993, an estimated 40-50% are nonrespondents to short course chemotherapy. As a prelude to a bigger survey of TB patients not responding to chemotherapy and to document pulmonary paragonimiasis among this set of patients, a study of nonrespondents was done. Patients clinically diagnosed to have pulmonary tuberculosis by the Rural Health Unit in Irosin, Sorsogon and nonresponding to at least six months of anti-tuberculosis treatment were studied by the research team in October 1994. Clinical histories were reviewed, patients were examined, and an early morning sputum specimens were collected and examined for the presence of acid-fast bacilli and *Paragonimus westermani* ova. Nine patients were seen and examined, and all had clinical histories consistent with pulmonary tuberculosis. Six had history of hemoptysis, and eight had abnormal pulmonary findings at the time of examination. Four were found to be positive for AFB, while five had *Paragonimus* eggs in their sputum. Three patients with AFB also had *Paragonimus* ova in their sputum. Pulmonary paragonimiasis exists in Irosin and should be considered in patients suspected to have pulmonary tuberculosis. Routine screening for *Paragonimus* in endemic areas may be necessary in patients clinically diagnosed to have pulmonary tuberculosis and in those who are responding to anti-TB treatment.

**Keywords:** pulmonary paragonimiasis, tuberculosis



**18. THE PHILIPPINE GENERAL HOSPITAL EYE INJURY REGISTRY (PRELIMINARY REPORT): DEMOGRAPHICS OF EYE INJURY CASES SEEN FROM JANUARY- JUNE 1996**

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Ocular trauma is one of the major causes of monocular blindness in the Philippines with a 13.6%-28.5% incidence in population-based surveys. In the Philippine General Hospital (PGH), 26% of all new eye cases are due to eye injuries. In an effort to establish a national registry of ocular injuries for all major centers in the country, the PGH Eye Injury Registry (PGH EIR) was initiated. Initial data of 1,071 cases of eye injuries included in the PGH EIR from January-June 1996 were reviewed. Cases were analyzed for demographic information. Mean age of the patients reviewed is 26.99% yrs. 63.3% of the patients are in working age group. Mostly are males (80%). Workplace accounted for 36% of all injuries. Majority of the patients (48.1%) are laborers and technicians; half of whom are metal and building trade workers. Serious eye injury (injury involving permanent and significant structural change) account for 17.0% of all injuries. Visual activity at initial consult was Hand Movement to Light Perception in 38.9% of these patients. The incidence of eye injuries requiring hospitalization 15.1%. Annual medical costs were estimated conservatively at 3.1% million pesos for this hospital alone and approximately 681,000 man-hours were lost. Work-related eye injuries are frequented and may lead to loss of vision and man-hours. The development of preventive programs and the reduction of ocular morbidity need to be addressed.

**Keywords:** ocular trauma, monocular blindness, eye injury

## **MATHEMATICAL, PHYSICAL AND ENGINEERING SCIENCES**

### **1. A STRATEGY TO CLONE A cDNA FRAGMENT CODING FOR A NOVEL METHIONINE-RICH PROTEIN IN SOYBEAN (*Glycine max*)**

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An 8 KD (2D-1) methionine-rich protein (MRP) was purified through a combination of selective enrichment of the albumin fraction through heparin-Sepharose affinity chromatography, 2D-SDS-PAGE and radiolabelling of methionine-containing proteins with [1-<sup>14</sup>C] iodoacetate. The MRP contains 8.6% methionine, 11.4% lysine and high amounts of glutamic and aspartic acids. The N-terminus sequence facilitated the synthesis of degenerate oligonucleotide primers for use in Polymerase Chain Reaction (PCR) to amplify cDNA fragments specifically coding for the gene. The PCR generated fragment was then used to screen a cDNA library and analyze the DNA sequence which confirmed the high sulfur amino content of 17.2% (8.6% methionine and 8.6% cysteine). It was found to be present as two copies in the genome after Southern analysis. A search in the database to elucidate the MRP's biological role showed that it shares homology with DNA binding proteins in *Arabidopsis* and a sulfur-rich protein in lupine seed.

**Keywords:** degenerate oligonucleotide, selective enrichment, heparin-Sepharose affinity, Methionine-rich protein, radio-labelling

**2. BIOACTIVE PRINCIPLES FROM LIBAS (*Spondias pinnata*)  
AND ELEPANTING PUTI (*Heliotropium indicum*)  
AGAINST *Haemophilus gallinarum***

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Potential bioactive components from *Libas* and *Elepanting Puti* were extracted sequentially using solvents of different polarities. Bioassay of the crude solvent extract showed that the acetate extracts of the leaves and bark of *Libas* as well as the leaves and seeds of *Elepanting Puti* were effective in controlling the growth of *Haemophilus gallinarum*. The petroleum ether and ethanolic leaf extracts of *Libas* and *Elepanting Puti* also exhibited antimicrobial activity. The LD<sup>50</sup> of the ethanolic leaf extract of *Libas* and ethyl acetate extract of *Elepanting Puti* were 10.55 g and 11.78 g per kg body weight of albino mice, respectively. The phytochemicals responsible for its antimicrobial properties were found to be the flavonoidal group of compounds for *Libas* and tannins, and polyphenolics for the *Elepanting Puti* ethyl acetate extracts.

**Keywords:** bioassay, antimicrobial, bioactive components, phytochemical, flavonoidal, tannins, polyphenolic

**3. A CANONICAL CORRESPONDENCE ANALYSIS OF  
SOME *IN VITRO* BIOLOGICAL ACTIVITIES OF  
EXTRACTS FROM SPONGES AND  
TUNICATES FROM BALER, QUEZON**

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An important aspect in natural products chemistry is the investigation of factors that influence the production of bioactive metabolites. If known, these factors may help predict the likelihood of finding active compounds, improving the generally low "hit rate" obtained in screening programs. A study was conducted to document the biological activities of natural products extracted from organisms in Baler, Quezon. More than 100 sponges and tunicates were screened for *in vitro* antimicrobial and antifungal activity, brine shrimp toxicity and cancer cell cytotoxicity. Thin layer chromatography profiles of the extracts were made. A canonical correspondence analysis was done to examine the possible relation between biological activity and 254 nm-flourescent spots. The results suggest that activity against *Klebsiella sp.*

accounts best for the occurrence of these spots in various species. The results imply that, to some extent, conditions at the geographical site of sampling influences the production of metabolites. Whether these conditions are due more to biotic than to abiotic factors remain to be confirmed.

**Keywords:** natural products chemistry, sponges, tunicates, biological activity, antimicrobial antifungal, canonical correspondence analysis, bioinformatics, bioactive metabolites

#### 4. EXPRESSION OF THE THIRD ADENYLATE-FORMING REGION OF THE $\delta$ -(L- $\alpha$ -AMINOADIPYL)-L-CYSTEINYL-D-VALINE (ACV) SYNTHETASE FROM *Penicillium chrysogenum* IN THE *Aspergillus nidulans* EXPRESSION SYSTEM

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The penicillin precursor ACV is synthesized from the constituent L-amino acids and ATP by a multifunctional peptide synthetase, ACV Synthetase, (ACVS), Peptide synthesis proceeds via the non-ribosomal thiotemplate mechanism independent of the ribosomes and messenger RNA. The peptide products produced by the various peptide synthetases showed unusual amino-hydroxy- or carboxylic acids and comprised a diverse pool of antibiotics, immunosuppressants; insect-, plant- and animal toxins. The ACVS consisted of three domains, each containing key motifs involved in ATP-binding, acyladenylate formation and aminoacylation of the substrate amino acids. Presumably, one domain was associated with the recognition of one substrate amino acid. The substrate specificity of each domain apparently

resided on its primary structure. This encoded specificity had been proven by limited proteolysis with regard to the middle domain which recognized and activated residue incorporated into the peptide product, cysteine. Confirmation of the substrate specificity of the other domains was deemed useful for the construction of hybrid peptide synthetase genes by mixing domains of known substrate specificity/preference from different peptide synthetases to produce novel bioactive peptides. Determination of the substrate specificity of the third domain of the ACVS was further studied by overexpression of the fragment corresponding to the adenylate-forming region (*domC*). The integration of the fusion gene to the *A. nidulans* genome was targeted via homologous recombination to the *argB* locus yielding stable inte.

**Keywords:** penicillin precursor, peptide synthetase, fusion gene, hybrid peptides

## 5. A FIELD EFFECT TRANSISTOR (FET) SENSOR FOR DISSOLVED SULFUR DIOXIDE

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The measurement of sulfur dioxide is important in food industries and environmental monitoring. Field effect transistor (FET) sensor offers attractive features for the measurement of sulfur dioxide such as miniature size, fast response, low noise and low power consumption. An FET sensor was coated with a thin film of hydrogel containing a hydrogen sulfite electrolyte and found to be sensitive to sulfur dioxide. Sulfur dioxide generated from a pH-4 potassium hydrogen phthalate buffer solution that is monitored by the pH-FET. The sensor exhibited a response time of 2 min. and a very good linearity ( $r=0.9952$ ) in the range of  $5.6 \times 10^{-2}$  to  $1.6 \times 10^{-5}$  M. Its sensitivity was 22 mV per decade of sulfur dioxide concentration. The response of the sensor was affected by factors such as the hydrogel concentration, the electrolyte concentration and the pH of the carrier solution. The study shows that a miniaturized sensor that provides a direct and fast determination of dissolved sulfur dioxide is feasible.

**Keywords:** sulfur dioxide, field sensor, hydrogel concentration, electrolyte concentration field effect.

## 6. AN OPTICAL BIOSENSOR FOR TOXIC METAL IONS BASED ON THE INHIBITION OF IMMOBILIZED UREASE

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The detection of toxic heavy metal ions in aquatic and terrestrial resources has become a major analytical endeavor in environmental control. Optical fiber biosensors based on immobilized enzymes promises a simple, sensitive, rapid and inexpensive approach to the on-line monitoring of these metal ions. The study used a fiber-optic biosensor based on an immobilized urease to detect toxic metal ions. The detection scheme involved the optical measurement of the inhibitory effects of metal ions on the immobilized enzyme. The sensitive optical reagent consisted of a mixture of urease bonded on an activated aminoglass beads and thymol blue indicator linked onto similar aminoglass material. When these glass beads were packed at the tip of a fiber optic bundle then situated in a microflow cell chamber, a convenient metal detection system was realized. Inhibition curves for different metal ions have been successfully obtained, exhibiting the following dynamic ranges:  $5 \times 10^{-3}$  -  $5 \times 10^{-6}$  M Hg<sub>2</sub>,  $5 \times 10^{-7}$  -  $5 \times 10^{-5}$  M Ag-,  $1 \times 10^{-5}$  -  $1 \times 10^{-4}$  M Cu<sub>2</sub>,  $5 \times 10^{-5}$  -  $5 \times 10^{-3}$  M Cd<sub>2</sub>,  $1 \times 10^{-4}$  -  $1 \times 10^{-2}$  M Zn<sup>2+</sup>, and  $5 \times 10^{-4}$  -  $1 \times 10^{-2}$  M Pb<sup>2+</sup>. Various factors affecting the inhibition measurements (e.g. type of buffer, pH, buffer concentration, flow rate, temperature, and sample volume) were also investigated. Similarly, studies on the regeneration of the inhibited enzyme were pursued.

**Keywords:** metal ions, environment control, optical fiber biosensors, urease enzyme, optical measurement.

## 7. SOLID-STATE pH SENSOR BASED ON CONDUCTING POLYPYRROLE

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Interest in the field of coated wire electrode was generated by the desire to miniaturize, simplify and produce cheaper and more robust ion-selective electrodes.

In the study, a solid-state potentiometric pH sensor was fabricated by electro polymerization of pyrrole monomer onto a platinum wire. The conducting polypyrrole was electrodeposited under constant current from a phosphate buffer solution (pH7) containing bovine serum albumin and pyrrole. The sensor exhibited a linear response at the pH range 3 to 10 with a sensitivity of -42 mV per pH. The response which was measured against a Ag/AgCl reference electrode was highly reproducible with a relative standard deviation of less than 3%.

**Keywords:** pH sensor, polypyrrole, ion-selective electrode, electropolymerization

### 8. THE USE OF A LOCALLY ISOLATED LUMINOUS BACTERIUM: *Photobacterium Leiognathi* USTCC 26 AS A BIOSENSOR FOR THE RAPID DETECTION OF TOXICANTS IN WATER SAMPLES

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A total of 28 locally isolated marine bioluminescent bacteria and 2 type strains: *Vibrio fischeri* DSM 7175 and *Vibrio harveyi* DSM 6904 were taxonomically classified. Based on their bioluminescence property and sensitivity to the detergent sodium lauryl sulfate (SLS), a local strain identified as *Photobacterium leiognathi* USTCC 26 from squid (*Loligo sp.*) was chosen as the test strain. Under local condition, the test strain was observed to emit a stable amount of light as a form of metabolic by product. Exposure to a toxicant over a period of 5 minutes resulted in a significant decrease in light output which was directly proportional to the concentration of the toxicant. The light emitted was quantitatively measured using a luminescence fluorometer. A 52.9% reduction in light output was obtained with 50 ppm sodium lauryl sulfate (SLS); a 23.2% reduction with 0.7 ppm phenol, and a 6.7% reduction with 1:2 dilution of MWSS tap water sample.

**Keywords:** biosensor, bacterium, toxicant detector, bioluminescence property

## 9. DESIGN OF AN ELECTRODE-IMMOBILIZED CATALYST INCORPORATING INORGANIC COMPLEXES AS POLYMERIC BACKBONES

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Several immobilized catalysts were found effective in electrochemically reducing carbon dioxide where increased efficiency and selectivity had been achieved compared with their homogeneous analogs. The inorganic complexes of the form,  $\{[(bpy)_2Ru(dpq)]_2IrCl\}^{5+}$  and  $\{[(bpy)_2Ru(dpb)]_2IrCl_2\}^{5+}$  where bpy = 2, 2'-bipyridine, dpq = 2, e-bis(2-pyridyl)quinoxaline and dpb = 2,3-bis(2-pyridyl)benzoquinoxaline, reduced CO<sub>2</sub> to CO with high selectivity. In this study, the substitution of bpy with vbpy (4-vinyl4'-methyl-2, 2'-bipyridine) to yield  $\{[(ybpy)_2Ru(dpq)]_2IrCl_2\}^{5+}$  was achieved. Using this synthetic modification, the complex can be electropolymerized onto an electrode through the vbpy substituent to yield a catalytic surface. In addition, preliminary studies involving the  $[Ru(vbpy)_2(dpq)]^{2+}$  chromophore yielded electrochemically robust polymeric backbone when deposited onto an electrode surface.

**Keywords:** chemically modified electrodes, inorganic complexes, electrocatalysis CO<sub>2</sub> reduction, electropolymerization, metallopolymer, bridging ligands iridium-based catalyst, ruthenium complexes, vinylbipyridine

## 10. MICROPOROUS SiO<sub>2</sub>/VYCOR MEMBRANES FOR GAS SEPARATION

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Porous Vycor glass membranes with 40A initial pore diameter were modified using low pressure chemical vapor deposition (LPCVD) of silicon dioxide (SiO<sub>2</sub>). Diethylsilane (DES) in conjunction with O<sub>2</sub> or N<sub>2</sub>O were used as precursors to



synthesize the  $\text{SiO}_2$  films. Both "single side" (reactions flowing on the same side of the porous membrane) and "counterflow" (reactants flowing on both sides of the porous membrane) reactant geometries were investigated. The flow of  $\text{H}_2$ , He,  $\text{N}_2$ , Ar, and toluene ( $\text{C}_7\text{H}_8$ ) was monitored *in situ* after each deposition period. Membranes modified by the "single side" reactants geometry exhibited good selectivities between small and large molecules. However, cracking in these membranes after prolonged deposition limited the maximum achievable selectivity values. Higher selectivities and better mechanical stability were achieved with membranes produced using the "counterflow" reactants geometry. Pore narrowing rate was observed to increase with oxidant flow ( $\text{O}_2$  or  $\text{N}_2\text{O}$ ). For membranes prepared using both oxidants, selectivities on the order of 1000:1 were readily attained for  $\text{H}_2$  and He over  $\text{N}_2$ , Ar, and  $\text{C}_7\text{H}_8$ . Compared to  $\text{O}_2$ , the use of  $\text{N}_2\text{O}$  caused improvements in both the pore narrowing rate and  $\text{N}_2$ : $\text{C}_7\text{H}_8$  selectivity. Membranes prepared using the "counterflow" geometry showed no signs of degradation or cracking after thermal cycling. The membranes produced can be useful for the improvement of the yield of equilibrium limited reactions such as hydrogenation and dehydrogenation reactions. They can also be useful for the prevention of the emission of hazardous volatile organic compounds which is a common problem in chemical industries.

**Keywords:** gas separation, membrane, chemical vapor deposition, permeability, selectivity, volatile organic compounds, pore size, silicon dioxide, vycor glass, and diethylsilane

## 11. LYOTROPIC LIQUID-CRYSTALLINITY OF ERYTHROCYTE GHOSTS

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Lytotropic liquid crystals are mixtures of amphiphilic compounds and a polar solvent, most often water. An example of such a mixture found in living systems is the cell membrane. A study was made on the mesomorphic properties of membrane preparations from normal and abnormal human red blood cells using optical micros-

copy and differential scanning calorimetry (DSC). Characteristics textures of the lyotropic neat phase were observed in normal erythrocyte ghosts which exhibit the nematic phase similar to those observed in thermotropic liquid crystals. DSC thermograms and optical textures of normal ghosts revealed phase transitions from crystalline gel to smectic to nematic to a homogenous solution at temperature ranges 10°C to 70°C. At the physiological temperature (35°C - 37°C), the membranes exhibited the lamellar smectic phase. Similar tests performed on membranes of Thallasemic (abnormal) red blood cells showed no similarity to the liquid crystalline behavior of normal membranes.

**Keyword:** amphiphilic, crystalline gel, erythrocyte ghosts, liquid crystals, lyotropic, nematic, optical microscopy, smectic, Thallasemic, thermotropic

## 12. CHOLESTERIC LIQUID CRYSTAL-POLYMER COMPOSITE MATERIALS FOR DISPLAY DEVICES

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Liquid crystal science has paved the way for the emergence of liquid crystal displays (LCDs) which has revolutionized the impact of information technology. Unfortunately, available display technologies still lack the capacity to cope with the sophistication of information transfer, hence, development of LCDs and other types of display devices are still limited. The study dealt with the basic preparation and characterization of polymer dispersed liquid crystal (PDLCs). PDLCs are another type of display devices which utilize the electro-optical properties of liquid crystal-polymer composites. With proper refractive index-matching, PDLCs may be switched from an opaque OFF state to a clear ON state upon application of voltage. Liquid crystals (LCs) formulations based on coco-cholesteryl esters (i.e., liquid-crystalline esters derived from coconut oil) were dispersed in commercially available polymethyl methacrylate (PMMA) and in laboratory-synthesized epoxy-based side chain polymers (SCP). Switching properties of the resulting polymer-dispersed liquid crystals (PDLC) were evaluated. PDLCs using PMMA as a binder exhibited distinguishable droplets with bipolar and twisted radial configuration. A maximum voltage of 60 volts was needed to transform a 70:30 (LC:PMMA) mixture into a transparent state. LC formulations dispersed in the epoxy-based SCP bearing cholesteryl and cyanobiphenyl pendant groups were found to exhibit fingerprint texture. Compared to the LC/PMMA composite, the LC dispersion required voltage for switching.

**Keywords:** cholesteric, composite, liquid crystals, liquid crystal displays, polymer dispersed liquid crystals, coco-cholesteryl esters, epoxy-based polymers, finger-print texture.

### 13. PHYSICAL AND CHEMICAL IMMOBILIZATIONS OF QUINHYDRONE IN POLYMER MATRICES

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The physical-chemical immobilization of quinhydrone in polymer was determined. Quinhydrone, a combination of hydroquinone and quinone has found wide application in pH sensing advices. A quinhydrone-based polymer electrodes as pH sensor alternatives were fabricated and characterized. A poly(vinyl chloride) (PVC) membrane was used to physically immobilize quinhydrone (QH) showing attributed as a pH sensor (slope - 44.0 mV pH<sup>-1</sup>). However, there was significant deviation in the range of electrode response due to segregation and variability in the PVC-QH mix and nonreproducible fabrication steps. X-ray photoelectron spectroscopy (XPS), a surface analytical and diagnostic technique, helped determine why the electrode surface possessed the desired of undesired property. The chemical immobilization by covalent bonding of quinhydrone in conducting polypyrrole allowed enhancement of sensor structure and fabrication reproducibility. The potentiometric characteristics of the resulting films showed sub-Nernstian response of 46.0 mV pH<sup>-1</sup> (25°C); a detection limit of  $1 \times 10^{-10}$  mol dm<sup>-3</sup>; potential drift of  $\pm 1.9$  mV potential and low hysteresis with slope  $\pm 1.9$  mV pH<sup>-1</sup>. Quinhydrone functionalized polypyrrole offers potential for pH sensor devices with the electroactive species, conductive support and the mechanical support in an integrated form.

**Keywords:** quinhydrone, polymembrane, pH sensor devices, electroactive species

#### 14. GROWTH OF AMORPHOUS CARBON, DIAMOND-LIKE CARBON AND DIAMOND FILMS VIA PLASMA-ENHANCED AND HOT FILAMENT CHEMICAL VAPOR DEPOSITION

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Amorphous carbon, diamond-like-carbon and diamond films were deposited on silicon (100) substrates via plasma-enhanced chemical vapor deposition (PECVD) and hot-filament chemical vapor deposition (HFCVD). In the PECVD method, depositions were made via a two-step process that involved negatively biasing the substrates in a 1-3% methane in hydrogen discharge followed by growth of the film for at least 10 hours. The substrates were heated to temperatures up to 500°C. They were exposed to the negative glow region of a d.c. discharge. On the other hand, the HFCVD technique involved pretreatment of the substrates and film growth. The registered temperatures of the substrates when deposited occurred within the range of 580-760°C. The total gases used in the process were hydrogen and methane which constituted 0.1-3% of the total gas filling pressure. In the pretreatment stage, a 7-8.3% methane concentration was fed. The total gas filling pressure was varied in the range of  $8-40 \times 10^2$  Pa. A scanning electron microscope (SEM) and reflecting microscope were used in the determination of the films' surface morphologies. X-ray diffraction (XRD) was utilized in verifying the crystal structure of the films grown. Other characterization techniques, like Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy confirmed the grown films.

**Keywords:** chemical vapor deposition, diamond-like films, diamond thin films

#### 15. SOLVENT EXTRACTION OF ESSENTIAL OILS FROM YLANG-YLANG *Cananga odorata*

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The essential oil local industry is restricted to compounders, blenders and other components which are mostly imported requiring large dollar outlays. To

minimize this dependency, the local industry started to tap indigenous sources of essential oils which include our Ylang-Ylang trees. Essential oils in concrete and absolute forms were extracted from mature freshly harvested Ylang-Ylang (*Cananga odorata*) flower using solvent extraction with petroleum ether as extractant. Percent yield, energy requirement and solvent recovery were determined as affected by extraction time or number of siphonings in a modified large volume soxhlet extractor. Results revealed the highest percent yields obtained were 2.18% and 1.37% for concrete and absolute forms, respectively, after ten siphonings (11 hr and 50 min extraction time). Percent recovery of petroleum ether averaged at 72%. Losses were due to solvent evaporation, solvent absorption by the Ylang-Ylang flowers and vacuum pump losses in vacuum evaporation. Power requirement for solvent extraction was determined to be 0.476 kWh per ml concrete essential oil. Average energy efficiency was 61.6%. Energy losses occurred due to lack of insulation and energy inefficiency of equipment. The absolute essential oils produced had an ether number of 188, characteristic of first class quality essential oils.

**Keywords:** essential oils, solvent extraction, Ylang-Ylang, petroleum ether

## 16. DECOLORIZATION OF RAW SUGAR SOLUTION USING STEAM-ACTIVATED CARBON FROM CANE BAGASSE

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In a well-managed sugar factory, bagasse in excess of its fuel requirement may be processed and utilized in many ways, like in the production of activated carbon (AC). AC is an important product in the purification process, e.g. removal of odor and color. Determination of process conditions for AC production is vital for bagasses utilization. Samples of cane bagasses from the Canlubang Sugar Estate, Philippines were carbonized for one hour in a batch reactor (3.0 cm ID x 19 cm L.) inside a horizontal electric furnace, at 300°C, with nitrogen gas pressure maintained at 0.5 kg/cm.<sup>2</sup> and a flowrate 100 cc/min. The carbonized samples were activated for hour at 800°C by steam (3720 cc/min.) Seven batches of carbonization and activation resulted to the production of 10.12 g AC. Based on the air-dried bagasses, the average yield was 15.86% AC which approximately contained 0.89% moisture, 8.1% ash and 81% fixed carbon. The effective contact time (average of 60 min) and effective dosage

(average of 1. g AC/100 ml raw sugar solution of 50°Bx) were determined by measuring percent color removal at 560 mu. The adsorptive capacity of AC was measured by determining its iodine number, the milligram of iodine adsorbed per gram of carbon. The following values were obtained for the steam-activated carbon: 730.32 mg/g (AC from two-year old bagasses samples) and 647.92 mg/g (AC from 1-year old bagasses samples). Compared to the CO<sup>2</sup> - activated carbon of lower iodine number (301.13 mg/g), the steam-activated carbon had higher adsorptive capacity.

**Keywords:** activated carbon, bagasses, decolorization, sugar, raw sugar, steam-activated, carbon, iodine number, cane bagasse

## 17. PERFORMANCE EVALUATION OF A DUAL MODE BIOMASS GASIFIER-COMBUSTOR FOR PROCESS HEAT<sup>1</sup>

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A gasifier-combustor was designed, fabricated and tested for its performance at different air flow rates in updraft (UM) and downdraft (DM) modes of operation.

Results indicated that a defined temperature profile characterized by a sequential surge of temperature starting from the oxidation zone occurred in the various levels during the runs, illustrating the movement of the fire/oxidation zones during the gasification process. In all the runs, the highest level of the gasifier had the lowest temperature. Gas composition varied at different air flow rates and changed with time. The downdraft mode of operation produced more combustible gases (CO, H<sub>2</sub> and CH<sub>4</sub>) than the updraft mode operation. Low airflow rates gave better gasification performance than high airflow rates. Produced gas temperatures for all test ranged from 75-260°C and heating values ranged from 0.1 to 8MJ/m<sup>3</sup>. Mass and energy balance evaluation showed that dry air and clean gas comprised for the material input and output, respectively. The heating value of the output gas and tar made up most for the energy output along with the heat and unaccounted losses.

## 18. DESIGN, FABRICATION AND TESTING OF A COMPUTER INTERFACED PROCESS CONTROLLER FOR A SOLUTION HEATER

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The study conducted in anticipation of the need of village level technologies for small scale continuous solution heaters required in fermentation and similar processes. The study designed, fabricated and tested a computer interface control system as well as developed an automatic controller software. The apparatus had the following components: digitizer board, controller board, sensor transducers and transmitter, comparator, heater, heater sensor, level sensor and retention tank. Turbo language software was used in the system. Setpoints of 60°C, 40°C and 80°C at flowrates 5ml/sec, respectively, were tested for temperature responses. The settling point temperatures were determined from average responses at continuous operation at 60°C with 5ml/sec, a settling point temperature of 60.51°C was closest to the temperature setpoint. at 40°C and 80°C, the settling point temperature were above the setpoint. An empirical model was developed to determine the effective flowrate that will reach the given setpoint. At 40°C the flowrate was 24.026 ml/sec; at 60°C, 17.695 ml/sec; at 80°C, 11.836 ml/sec. Empirical model of effective flowrate and setpoint was also developed. The setpoint range was between 30°C to 73°C. The effective flowrate was between 0 ml/sec to 53.5 ml/sec.

**Keywords:** empirical model, sensor transducer, controller board, digitizer board, comparator, computer interface, process controller.

## 19. EXTRACTION OF ALUMINUM OXIDE $Al_2O_3$ FROM THE PYROCLASTIC MATERIALS OF MT. PINATUBO

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Mineralogical analyses of the volcanic material of Mt. Pinatubo indicated the presence of materials such as silicon oxide, aluminum oxide and lithium oxide in

varied amounts. Activated oxide (alumina) is the main raw material for the production of aluminum. Activated alumina is used in creating artificial jewels, high refractors and other material for optical instruments. Thus, effective and efficient extraction of alumina from pyroclastic materials may change the economic importance of lahar. Aluminum oxide was extracted from lahar using 10, 20, 30 and 40 g of NaOH as extractant using a modified Bayer process. The time of digestion was varied to 1, 2, 4 and 5 hrs. The pH of precipitating aluminate ion to aluminum hydroxide was also varied by adding different amount of HCl acid to produce solutions with a pH range of 11.00 to 8.51, 8.50 to 6.51, and 6.50 to 0.01. The percent aluminum (% Al) composition of the pyroclastic materials and extracted alumina was determined using an Atomic Absorption Spectrophotometer. The aluminum composition of the pyroclastic materials used in this study was 0.32% by weight. The unpurified alumina product was analyzed from 0.01 to 1.8% by weight of aluminum. The average percent extraction yield of alumina ranges from 3.31% using 10 grams of NaOH as extractant at 1 hour digestion, with a pH range of precipitation of 0.01 to 6.50, to 95.83%, using 40 grams of NaOH as extractant at 5 hours of digestion, with a pH of precipitation range of 8.51 to 11.00. The amount of NaOH used, time of digestion and pH of precipitation range of 8.51 to 11.00. The amount of NaOH used, time of digestion and pH of precipitation was found to have significant effect on the percent extraction yield of alumina. An increase in the amount of NaOH used and time of digestion increased the percent extraction yield of alumina. The pH of precipitation that gave the higher extraction yield was found to be in the pH range of 8.51 to 11.00. Using 40 grams of NaOH and 5 hours of digestion time also maximized extraction yields.

**Keywords:** aluminum oxide, pyroclastic materials, extraction, Mt. Pinatubo, alumina extraction

## 20. FORMATION OF TIN ON METAL SUBSTRATES USING A MAGNETIZED SHEET PLASMA

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Numerous titanium nitride (TiN) films were produced on different target materials (Cu or stainless steel) using a magnetized sheet plasma. The use of a magnetized sheet plasma presents a new approach to the production of TiN. The mixed-species plasma was formed from a total initial gas filling pressure of 5.33 Pa consisting of 1%  $N_2$  in Ar. The plasma current was 12.0 A and the potential between the cathode and anode was 150.0 V. A titanium disc placed near the anode is sputtered by the plasma beam. The target substrate was either immersed in the sheet plasma or placed about



4.0 cm parallel to the sheet plasma. In the case of Cu substrate, the to form TiN require heating of the substrate. Both substrates were biased 20.0 V relative to the anode. X-ray fraction (XRD), Raman and microchemical energy dispersive X-ray emission (EDX) spectroscopic characterizations confirmed the produced TiN films. The films were characterized by the golden color attributed to TiN.

**Keywords:** magnetized sheet plasma, titanium nitride thin film.

## 21. FUEL AND COMBUSTION MODELLING OF A NATURAL CONVECTION FLUE-CURING BARN

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The flue-curing of tobacco is energy intensive. Most if not all of it is derived from fuelwood. The existing system result to high energy losses. A fuelwood combustion analysis and modeling were undertaken using a mathematical model. Subsequent testing of the model was undertaken under field conditions. The model illustrated the importance of analyzing the fuel use and combustion process to estimate the energy efficiency of the barns. The fuelwood use rate, combustion processes and products of combustion were determined and analyzed. The analysis indicated that the fuelwood use can be predicted at different stage of the flue-curing process. The products of combustion like carbon dioxide, carbon monoxide and sulfur dioxide were predicted using the model. The result of the study is a very important tool in tobacco barn design and improvements specially in combustion, energy efficiency and emissions control.

**Keywords:** barn, carbon dioxide, carbon monoxide, combustion, efficiency, energy, flue-curing, fuel, fuelwood, sulfur dioxide, tobacco.

## 22. A KNOWLEDGE-BASED SYSTEM FOR SHIP HULL FORM DEFINITION

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The purpose of this research was to develop a software for designing knowledge-based ship hull forms. The study developed an easy-to-use, parameter based, shape selection hull definition computer program which was linked to an advisory component that provided useful, necessary and explainable advice to the user. A combined object oriented and knowledge based systems methodology was employed for development and implementation. System objects, structures and models of activities developed during the analysis and design stage greatly facilitated rapid program writing. The design system generated a hull form by aggregating user-selected shapes representing different geometric boundaries of the hull. Hull variation was accomplished by changing simple parameters such as entrance angles, length-breadth ratios and radii. Hydrostatic calculations were included for analysis and referred to the advise system for evaluation. The advise system consisted of a fishing boat knowledge base implemented in an expert system development environment. Rules for the advise system were elicited from experts using a program written to derive basic decision making elements. Using the software resulted in an easy-to-use process for designing a ship hull.

**Keywords:** *ship, design, hull form, expert system, knowledge based system, artificial intelligence, programming, object-oriented, knowledge acquisition, model*

## 23. A NEW LOOK AT VASOMOTION USING CHAOS ANALYSIS

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The vasomotion observed in rat mesenteric at rest using video-microscopy was analyzed using nonlinear analysis. It appears that the dynamics of vasomotion, as assessed by RBC velocity in single arterioles, is naturally chaotic. Temporal

variations in the RBC velocity were found to be aperiodic and the power spectrum indicated the presence of different frequencies. To differentiate from noise, the phase space plot was graphed revealing that the points are bounded. Although this could either indicate a chaotic system or periodicity, the first return map showed that no two points overlap, thereby indicating a chaotic behavior. Quantitative analysis demonstrated that altering the embedding dimension from values of 4 to 6, 8 and 10 produced the same signature, suggestive of self-similarity. The plateau of the signature corresponded to a non-integer value of approximately 1.2, again characteristic of a chaotic system. The temporal profiles of the blood velocity signals decreased in amplitude as the injected volume of the calcium channel blocker Nicardipine (NIC) was increased from 0.3 ml to 0.9 ml and 1.8 ml. The decrease in blood velocity is expected, considering that the drug produces an increase in arteriolar diameter and a fall in blood pressure. Qualitative analysis of the signals using the phase space plot and the power spectrum indicated the presence of different periods in the signals. The observed multiperiodicity was evident under all concentrations of NIC. Thus it appears that while Nicardipine is able to produce the desired alterations in systemic blood pressure and vessel diameter, it is also able to preserve the naturally chaotic character of the microcirculation.

**Keywords:** vasomotor, rat mesentri arterioles, video-microscopy, temporal variation, Nicardipine, microcirculation, power spectrum, periodicity

## 24. DEVELOPMENT OF THE THEORY OF FINITE PSEUDOGROUPS

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Non-associative finite algebraic structures have many important applications in pure and applied mathematics. In particular, structures belonging to the quasigroup family have been the focus of many studies. Along this line, an interesting structure called the pseudogroup - a not-associative loop in which every element has a unique inverse was studied.

Very little is known about pseudogroups in general but they constitute an important class of loops that includes IP loops and Moufang loops and they are involved in the theories of non associative algebras, loop theory, as well as in theoretical physics. This paper presents some important results of our research program to develop the foundations of the *Theory of Finite Pseudogroups*.

## 25. CHARACTERIZATION OF ABELIAN PSEUDOGRUUPS OF ORDER SIX

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A pseudogroup  $\langle G; \rangle$  is a not associative loop such that every element has a unique inverse. The existence of finite pseudogroups had been established in 1981 by R.E. Cawagas for all orders  $n \geq 5$ . However, the number of non-isomorphic pseudogroups of any given order is not yet known except for order 5. The study determined and characterized all pseudogroups of small order. To achieve this, two computer programs were developed, the *ICONSTRUCT* and *FINITAS* which were used in generating and analyzing all pseudogroups of orders  $n = 5, 6$  and  $7$ . Using these two programs, it was found that a total of  $7$  non-isomorphic abelian pseudogroups of order  $6$  exist.

**Keywords:** pseudogroup, finite, non-isomorphy, Abelian

## 26. SIMULATION OF LAHAR/FLOOD FLOWS OF PASIG-POTRERO RIVER

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A two-dimensional mathematical hydraulic model was developed simulating the dynamics of combined lahar and flood flow events that are initiated by storm rainfall inside a given river basin. Applying the mass and momentum conservation laws, together with the rheological properties of non-Newtonian flowing mixtures of water and non-cohesive sediments (lahar sand), a system of partial differential equations was formulated and coded into a 'FORTRAN Powerstation' microcomputer program. The model predict spatial and temporal variations of flow surface elevations, river bed elevations (deposition and erosion), flow velocity vectors and discharges, sediment concentrations and transport rates, and hydraulic/lahar forces, over a two-dimensional grid of nodes which geometrically represents the river basin. Utilizing as inputs the meteorological, topographical, hydrological, geological, and engineering data from various agencies (DPWH, PAGASA, PHIVOLCS, ZLSMG, and others), the combined lahar and flood flows of the Pasig-Potrero River basin inside the Megadike system were simulated. The simulation outputs consist of computed

discharge and stage hydrographs, sedigraphs, elevation-discharge rating curves, sediment-discharge rating curves, profiles of bed and flow surface along the dikes, and contour maps of deposition and scour pattern. The output was presented to the Mt. Pinatubo Commission to provide critical design criteria in the upgrading/revision of the existing Megadike system which consists of east and west lateral dikes, a transverse dike, two spillways, and several bridges along the Gapan-San Fernando-Olongapo Road.

**Keywords:** lahar flood, mathematical modeling, hydraulic, rheology, simulation

## 27. MOUNT PINATUBO – MOUNT ARAYAT TRANSECT: ALONG-AND ACROSS-ARC VARIATIONS

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Whole rock geochemical analyses confirmed the existence of along and across-arc variations in the Central Luzon area. The variations are basically due to the differences in the degree of partial melting of the source of the regions and amount of magma fractionation. The amount of fluids varied for each volcanic center. The Ba/La versus La/Sm relationship suggested that there appeared to be an increasing amount of fluid from south to north at the same degree of partial melting. At a first order of approximation, there appeared to be high fluid/high sediment correlation from Balungao (highest) - Balaybay; Tarlac; Pinatubo - Arayat - Mariveles; Natib; Samat (lowest). Mount Cuyapo and Simsiman did not follow the trend. The increased Zr/Y content could be due to continental sediments or fluid/melt interaction with the lower crust. Adakite-like rocks (high Sr/Y, low Sc, low HREE) were also observed among the sample population. This could be due to slab melting, lower crust melting, intracrustal fractionation or plagioclase accumulation. An understanding of the spatial-temporal-geochemical relationships of these different volcanic centers is critical in the understanding of the evolution of the Philippine island arc system. It will also help formulate the necessary mitigating and remedial measures with respect to volcano-related hazards.

**Keywords:** arc, volcano Mount Pinatubo, Mount Arayat, geochemistry, hazards, evolution, variations

## 28. LEVELS OF INORGANIC ARSENIC SPECIES IN MANILA BAY USING SELECTIVE HYDRIDE GENERATION-ATOMIC ABSORPTION SPECTROPHOTOMETRY

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The distribution of the inorganic forms of arsenic in the waters of Manila Bay was investigated using selective hydride generation atomic absorption spectrophotometry. An automated hydride generator coupled to an atomic absorption spectrophotometer enabled the determination of As(III) and As(III) + As(V) using the batch extraction method. The distribution of the dissolved arsenic species in the waters of Manila Bay was found to be in the following concentration ranges: <3-7 nmole/l As(III), 5-36 nmole/l As(V) and 8-36 nmole/l total arsenic. The horizontal distribution profiles of As(V) and total arsenic did not vary greatly among stations within the same period. Surface depletion of As(V) and total arsenic was generally observed during the wet season. The concentrations of As(V) and total As during the dry season were higher and more evenly distributed.

**Keywords:** inorganic arsenic species, Manila Bay, selective hydride generation, atomic absorption spectrophotometry, total arsenic, batch extraction, distribution profile

## 29. TRACE METAL SPECIATION OF MANILA BAY WATER SAMPLES USING LINEAR SWEEP ANODIC STRIPPING VOLTAMMETRY

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Voltammetric methods of analysis were developed using the linear sweep anodic stripping voltammetry (LSASV) for the trace metals analysis in seawater. A

rotating gold disc electrode was used for the simultaneous determination of Cu(II) and Hg(II), while a mercury-thin film (*in situ*) on a rotating glassy carbon disc electrode for the determination of Cd(II), Pb(II), and Zn(II) in acetate buffers. Stripping peak potentials observed at +0.51 V, +0.28 V, -0.47 V, -0.65 V and -1.04 V (vs SCE) for Hg(II), Cu(II), Pb(II), Cd(II) and Zn(II), respectively, were used for quantitative analysis. The chemical speciation of some coastal waters of Manila Bay by Showed that, Except, for zinc, which averaged about 40% labile metals, the dissolved metals namely copper, cadmium, lead and mercury were all in the bound or non-labile forms. These non-labile metals were found to be mainly composed of inorganic complexes and/or metals probably adsorbed on inorganic matter, with the exception of Pb(II). The percentage of non-labile inorganics were 65%, 69%, 70%, and 18% for Cu(II), Hg(II), Cd(II), and Pb(II), respectively.

**Keywords:** trace metal speciation, Manila Bay, linear sweep anodic stripping voltammetry

### 30. WATER QUALITY ASSESSMENT OF VARIOUS SELECTED SITES IN MANILA BAY USING PHYSICO-CHEMICAL PARAMETERS

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Manila Bay is one of the centers of shipping, fishing, commercial and domestic activities in the country. It serves as catch basin of the major tributaries in Metro Manila. Despite these activities there are few existing studies on the water quality of the bay. This study aimed to determine the Bay's physico-chemical parameters in sites representing the various activities. Assessment of pH, temperature, DO, salinity, conductivity, and turbidity were done using the Horiba Water Quality Checker. Nitrate content were analyzed using the Cadmium Reduction Method. The amount of orthophosphate was determined using the Vanadomolybdophosphoric acid Method, qualitatively, analysis was performed by FT-IR and NMR. Samples were obtained from October to December 1996. Data gathered showed estuarine and areas with fish markets and domestic settlements had considerably lower values in salinity and DO; and significantly higher values for nitrates and orthophosphates. Extremely high concentration of oil and grease exceeded the standard which is 3 mg/L for shipping, fishing and boating areas. Data obtained can be used as baseline information for the monitoring of pollution trends of various near-shore areas of Metro Manila. It can serve as basis for pollution management policies.

**Keywords:** water quality assessment, Manila Bay, pollution management, physico-chemical parameters

**31. EPITOMORPHIC COMBIPEPTIDES TARGETTING  
THE ANTIGENIC DIVERSITY OF THE VP1  
POLYPROTEIN (135-146) OF THE FOOT-AND-  
MOUTH DISEASE VIRUS SEROTYPE A**

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Epitomorphic mimetics taken as strung-ensemble of peptide sequences are engineered against linearly immunogenic regions of the foot-and-mouth disease virus (FMDV) belonging to the serofamily A. These comprise residues flanking the RGD loop, an important structural motif identified with integrin-binding proteins and CAM families, of the immunodominant epitope VP1 known as the antigen site A. With the current vaccination strategy applied worldwide, such as, with live and killed viral vaccines and monovalent synthetic peptides, the failure to attenuate epidemics in cattle-producing countries have been, to a certain extent, attributed to the impotency of administered vaccines in the presence of antigenic drifts of highly mutating viruses. A biophysical approach involving empirically-guided hyper-mutation of a strong signaling epitope for designing new-generation synthetic vaccines aimed at countering this problem was developed. In the study, the importance of salient molecular endowments of the synthetic combipeptides was emphasized. Assignment of exhaustive functional mutations within the residue 135-146 has been biased by RNA and protein sequence and conformational comparisons between 6 known and antigenically distinct subtypes, A<sup>22</sup>, A<sup>rg79</sup>, A<sup>ven76</sup>, A<sup>24</sup>, A<sup>32</sup> and A<sup>5</sup>. The iterated epitomorphs (altered epitopes) were filtered using an exclusional scanning procedure involving computational algorithms of the antigenicity-determining parameters (hydrophilicity plots; strict (residuewise) and charge palindromicity; secondary structure and its retention states as an intact viral sequence, as an epitopic clustering as "high" and "low-stringency" groupings. The study showed that molecular design of



the anti-viral immunogens, the epitomorphic combipeptides, may be used as fine structured tools for immunologic studies as basis for the development of novel vaccines deemed to capture a strong neutralizing antibody repertoire and a solid cell-mediated protection against FMDV challenge in economically important livestock.

**Keywords:** Synthetic vaccines, antigenic drifts, quasi-speciation, Foot-and-mouth disease virus, vaccine designing, Biophysics, aphovirus, epitopes, epitomorphs, virology and immunology

## ***SOCIAL SCIENCES***

### **1. PARTICIPATIVE CONSTRUCTION OF UPLAND FARMERS CONCEPTS AND EXPECTATIONS OF FARMING SYSTEM IN EASTERN VISAYAS REGION: A CASE STUDY**

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A case study was conducted in the Visayas State College of Agriculture (VISCA) Forest Reservation, Baybay, Leyte to: (1) picture the concepts of upland farmers on their individual upland farms; (2) determine the upland farmers expectation of their farms and what they expect outside interventions; and (3) explore their concepts on environmental issues, laws and community concerns over their upland farms. Data gathering was done through personal and informal interview without the use of a questionnaire and with an indefinite time for conversation. The following conclusions were drawn: 1) They are respondents did not like to be referred to as "Kaingineros"; (2) They are very serious in managing their uplandfarms; 3) The suggested species are giant ipil-ipil, kakawate and pineapple in contour making of their upland farms; 4) Respondents tend to be receptive to upland farming technologies introduced, on issues on upland farm sustainability and did not have specific ideas on forestry laws; and 5) Poverty, lack of employment opportunities, and unavailability of land forming urge the respondents to continue upland farming.

**Keywords:** participative construction, upland farmers, concepts and expectations, upland farming system

## 2. SUCCESSFUL FARMING SYSTEMS IN THE PHILIPPINES A DOCUMENTATION

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Numerous efforts to improve productivity and income of small farmers continuously generated production technologies and developed extension strategies to disseminate proven technologies. Farmers' adoption of recommended technologies however have often been slower and lower than expected, worsening the socio-economic status of the farmers. The study aimed to document success stories of farmers practicing farming systems in attempt to bring to the fore the important role that innovative farmers play in effecting changes in other farmers' practices. The project, which comprehensively documented individual farmers' practices and their farming systems, aimed to analyze and highlight critical factors that contribute to their success as farmer entrepreneurs. Using case analysis, nine models representing specific situations in lowland, upland and hillyland farming systems in the various regions of the country were documented and assessed by a multi-disciplinary technical team from UPLB. The farming systems models include: a) Diversified Vegetable Farming in Hillyland Areas (Atok, Benguet); b) Tomato after Irrigated Rice Production (Batac, Ilocos Norte); c) Diversified Vegetable Production in a Rice-based Farming Systems (Talavera, Nueva Ecija); d) Pineapple and Coffee-based Farming Systems (Tagaytay, Cavite); e) Pond Aquaculture in an Irrigated Lowland Rice-based Farming Systems (Pila, Laguna); f) Lowland Rice-based Farming Systems (Pili, Camarines Sur); g) Irrigated Rice-based Farming Systems (Sibalom, Antique); h) Crop Diversification in Sloping areas (Tabango, Leyte); and i) Fruit-based Systems (Tampakan, South Cotabato). Experiences of Farmers revealed adaptations made on certain recommended technologies based on available resources and situations they are in. Common factors contributory to successful farming systems as highlighted in the project were the following: (1) access to new and appropriate technologies (2) access to government extension support service, product market, availability of resources and (3) personal trait and characteristics.

**Keywords:** farming systems, appropriate technologies, innovative farmers