Pamaagi:

A HANDBOOK OF SCIENTIFIC PRACTICE FOR YOUNG RESEARCHERS

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**PHILIPPINE SCIENCE HIGH SCHOOL
WESTERN VISAYAS CAMPUS**

DISCLAIMER

The views and opinions expressed by the authors in their articles do not reflect the views and opinions of the Editorial Board, the academic unit, the institution, and the agency.This manual is a compilation of research methods from articles of alumni published across three volumes of Publiscience.

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ABOUT THE COVER
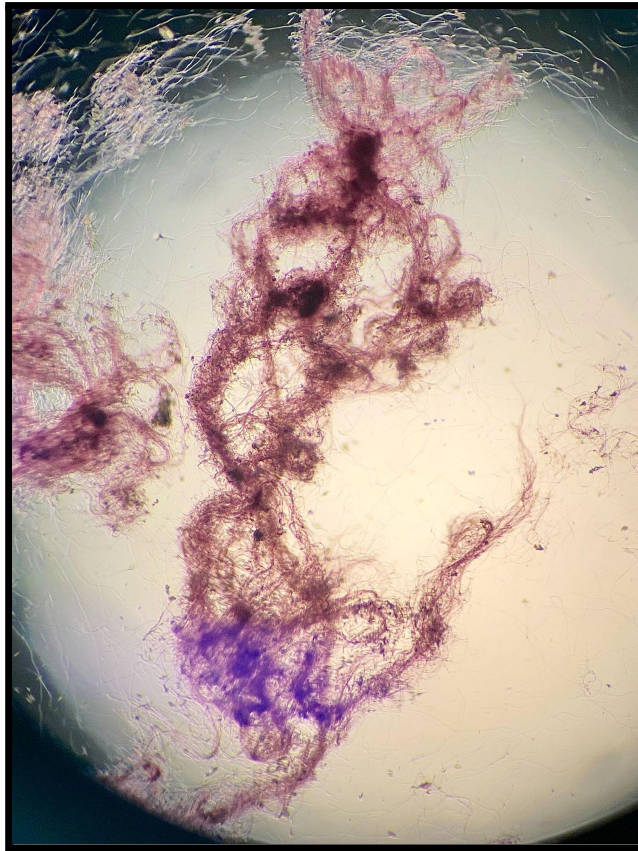
       The cover features an image of *Lyngbya majuscula* (lumot) under a light microscope with 100x magnification. They are unbranched, filamentous cyanobacteria which are single cells, joined together to form thread-like strands. Most people deem this thin, joint, single-celled cyanobacteria to be dangerous as it causes harmful algal blooms. However, when we look beyond its adverse effects, we see its limitless potential. This cyanobacterium is a source of 197 bioactive compounds that have various applications in the aquaculture and pharmaceutical industries. Just like in research, *L. majuscula* represents how we all started as distinct individuals. But with the support and guidance of the PSHS research community – our advisers, teachers, and batchmates, we are coalesced by our scientific ideals. This shows us that through our unified and inquisitive minds, we can continue to serve the people with excellence as we search for the untarnished truth in this ever-changing world.

ABOUT THE BOOK

This book called Pamaagi manifests our desire to help young researchers in searching for appropriate methods in answering their research problem. We decided to collate the methods published in the Publiscience research journal and put it into one reading material. In this edition, we only included the published articles in the first three volumes. Despite our desire to include all articles, we can only publish based on the availability of alumni writers and volunteers in coming up with this first edition. This is our first attempt and so much can be done to improve the handbook.

Unlike in the journal articles, some details of the methods were omitted due to page limitation. In this book, we try to balance detail and brevity. We also provided the link to the published article so that the context of the method can be well understood. Certainly, the details can be requested by requesting for the entire manuscript.

We decided to call this book Pamaagi to honor our Ilonggo heritage. The word Pamaagi means procedure or technique in achieving a desired result. The words support the fact that in the absence of or in a dire situation, one can invoke the pamaagi. The book hopes that readers would also come up with pamaagi as not all methods can be copied exactly. It calls for tweaking and is true to the research aim.

This is our pamaagi.

FOREWORD

Research has long been the flagship program of Philippine Science High School-Western Visayas Campus. PSHSWVC made a lot of firsts in the field of Research: the first campus to have implemented the Science Immersion Program (SIP), which was then still called as Summer Internship Program, the first in conducting the Community Research Program; and the very first in publishing students' research works through "Publiscience."

Now, in its third year, Publiscience is launching "**Pamaagi: A Handbook of Scientific Practice for Young Researchers,**" the compilation of all the research methods done by PSHSWVC scholars, and were presented in the three volumes of the Publication. It is our pride and joy that our scholars and our alumni pursue the untarnished truth, and take on to themselves the responsibility of sharing their research knowledge, experience and expertise through *Pamaagi*.

Kudos to the Research unit, our scholar- researchers and all our Publication Chairpersons and volunteers for this great feat! Congratulations!

SHENA FAITH M. GANELA
Campus Director III

* * *

My warmest congratulations to our Research Unit and the members of the Editorial Board of Pamaagi. My commendation for taking the lead in publishing the first-ever compilation of various essential methods of research called **Pamaagi: A Handbook of Scientific Practice for Young Researchers**. It proudly showcases and reveals the research skills developed by our scholars for the last three years in their research class.

The PSHSWVC celebrates with pride this novel contribution of our select Grade 12 scholars to the body of knowledge through the conduct of research. "Pamaagi" is a clear manifestation of the pure diligence, dedication, and authentic experiences of our scholars in the field of research work. It further highlights their being analytical and critical and strength of character in pursuit of excellence as they perform hands-on research processes amidst this very challenging time.

More importantly, the publication of this handbook supports the school's advocacy in sharing the knowledge and expertise gained and developed by our research students through the guidance and supervision of their research teachers and advisers to the next generation of young researchers of our country. This unselfish effort stems from the core value of service because we believe that knowledge gained and developed if not shared is dead knowledge!

Again, my warmest congratulations and ingat kayo lagi.

ROLANDO LIBUTAQUE
Curriculum Instruction Division Chief

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TABLE OF CONTENTS

PREFACE

Pamaagi: A Handbook of Scientific Practice for Young Researchers is a handbook made by students for students. *Pamaagi* is the culmination of many young researcher's failures and successes - methods that capitalize on their creativity and grit to make the best use out of the limited resources provided to them.

The method is the skeleton of any research study. Through this handbook, *Pamaagi*'s editors and contributors would like to show the capability of young researchers in implementing diverse and technical procedures with proper guidance from experienced adults. With that said, however, failure is always a possible outcome; in fact, it is almost inevitable. It is therefore not only vital to learn from such failures, but to exercise prudence in every step as well. Remember: Research exists solely through the cycle of success and failure. It is, after all, an iterative process. Marginal gains spanning millennia contribute to the vast body of scientific knowledge. It grows as the new generation is taught by the old.

Young researchers, this handbook is for *you*. We hope that you may continue to build upon these foundations, just as the various contributors have in their studies. Through the crests and troughs, keep the fire burning!

THE EDITORIAL BOARD

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DEDICATION

We dedicate this issue to all young researchers
- the future of Philippine science.

BIOLOGY

ASSAYS

OVERVIEW

This chapter expounds on methods for assessing biological and chemical activity of extracts and samples which are mostly organic and plant-based. This includes bioassays and chemical assays that determine antibacterial activity, antimicrobial activity, antibiofilm activity, larvicidal activity, and inhibitory activity. The specific procedures used in the assays from the preparation of materials to the measurement of dependent variables are elaborated in this chapter. These test procedures vary according to the standardized tests employed by the researchers.

A. Antibacterial
(Grantoza, Mationg, Seguano, Oberio)

To prepare the different treatment concentrations, pure extracts were mixed with distilled water to obtain the intended concentrations using the following extract to water ratio: 1:3, 2:2, 3:1and 4:0. The following equation was used for the preparation of concentrations of treatments:

$$volume\ percent\ =\ \frac{volume\ of\ solute\ (mL)}{volume\ of\ solution\ (mL)}\ \times 100\%$$

The medium used for the antibacterial assay was Mueller-Hinton Agar (MHA), a culture media commonly used in antibacterial testing. Nutrient agar and nutrient broth were used for the revival process of the freeze-dried bacteria culture. The bacteria strains were inoculated into the nutrient agar by quadrant streaking to isolate pure colonies which were used for the assay. The media were placed inside the autoclave sterilizing it along with the petri plates, other glasswares and materials used for the experiment. Approximately 10 to 20 milliliters of the media were poured to each petri plate. The plates were cooled while the media solidified at room temperature and were then stored in the refrigerator at about 2- 8°C until use.

McFarland Turbidity Standard was used for adjusting the concentration of bacterial colonies suspended in the inoculum in preparation for inoculation on MHA plates. To make the 0.5 McFarland Turbidity Standard, 9.95 mL of sulfuric acid and 0.05ml of barium chloride were mixed in a test tube. The test tube was agitated well in order to mix the fine white precipitate of barium sulfate in the 0.5 McFarland turbidity standard tube (McFarland Standard 2014). If a dilution was necessary, a sterile pipette was used to add sufficient broth in order to obtain turbidity that would match the standard. A count of 1.50 x 108 colony forming units/mL was produced by the adjusted suspension.

Agar well diffusion method was used to test the antibacterial activity of the *D. alata* ethanolic extracts at different treatment concentrations (25%, 50%, 75%, 100%). Antibacterial assay plates were inoculated with respective bacteria before the agar wells were made. The extracts were then poured into the wells using a micropipette. The cultures were placed in the incubator at 37°C. Plates were initially checked for any zone of inhibition at the 18th hour of incubation, but data was only gathered after 24 hours of incubation. The diameter of the agar wells was 7 mm each. The zones of inhibitions on each plate were measured using a vernier caliper for more precise measurements in millimeters.

There were three readings in each plate with each four bacteria strains having six setups namely, the negative control (water), positive control (co-trimoxazole) and the treatment concentrations (25%, 50%, 75%, 100%). Each bacteria strain had 18 readings and the whole antibacterial assay process had a total of 72 readings all in all.

B. **Anti-Biofilm**
  (Bat-og, Oreta, Villaflor, Jolito)

Antibiofilm activity of the mango peel extracts was determined through Crystal Violet Assay. Six petri plates were prepared and inoculated with the subculture. Approximately 30 mL of Tryptic Soy agar solution was poured into each plate. The petri plates were autoclaved at 121°C, 15 psi for 15 minutes.

From the stored pure culture, the *S. aureus* was transferred to a petri plate with an inoculating loop. Then, it was incubated at 37°C for 24 hours that allowed the bacteria to grow. After which, the culture was inoculated into two separate test tubes containing Tryptic Soy Broth.

Fifty µL of the culture was diluted with 5 mL Tryptone Soy Broth (1:100). 195 µL of the culture dilution were inoculated to individual wells of the 96-well plate underneath a laminar flow hood and left in the incubator to grow for 16 hours. After incubation, the plate was washed and air dried for 30 minutes to remove planktonic cells. 125-µL of the extracts were added after the bacteria formed a biofilm. It was incubated again for 16 hours at 37°C. Three replicates were done for each treatment, distilled water served as the positive control.

After incubation, the plate was washed by pipetting distilled water into each well twice. One hundred twenty-five µL of a 0.1% solution of crystal violet (CV) was added to each well. The plate was incubated again for 15 minutes at room temperature and then washed 4 times with distilled water and turned upside-down, left to dry overnight.

One hundred twenty-five (125) µL of 30% acetic acid was added to each well; then the plate was left to incubate for 15 minutes at room temperature. Absorbance was measured at 590 nm in a plate reader, using 30% acetic acid as a test plate ensuring the accuracy of the absorbance values.

The absorbance value of the blank was estimated to be zero with a range of -0.5 to +0.5. Their difference with the absorbance value of the experimental and positive control wells was equal to the value displayed by the plate reader. Absorbance values indicated the antibiofilm activity of each peel extract. After treatment, the remaining bacterial matter in the well was stained with CV. Relatively low absorbance values indicated that some of the biofilm had been dislodged by the peel extract.

C. Larvicidal

Larvicidal Activity of *Annona muricata* (Soursop) seed and Piper betle (Betel) leaf against *Aedes aegypti*
(Grande, Balmaceda, Taneña, Mediodia)

Larvicidal bioassays were conducted according to standard protocol from the World Health Organization 2005 guidelines for laboratory and field testing of mosquito larvicides in the Standards and Testing Division of the Industrial Technology Development Institute, Taguig, Manila. Preliminary testing of the extracts was carried out to establish the effective range of test concentrations by initially testing a wide range of concentrations which was later narrowed down until the lowest test concentration killed at least 10% of the population while the highest test concentration killed at most 90% of the population. Different concentrations of *A. muricata* extract (5 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm) and *P. betle* extract (300 ppm, 600 ppm, 900 ppm, 1200 ppm, 1500 ppm) were prepared during the study. All experimental exposures were carried out using 200 mL beakers filled with 95 mL of deionized water. Twenty larvae which were a mix of third and early fourth instar were then added into each beaker and subjected to testing of the different test concentrations.

Three replicates were carried out simultaneously for each concentration. Mortality was recorded after 24 hours during which no food was offered to the larvae. The number of dead larvae was counted by transferring the larvae from the used beaker for testing onto a petri dish. The number of dead larvae was counted by transferring the larvae from the used beaker for testing onto a petri dish. A pasteur pipette was used to prod the larvae while observing for signs of movement. Larvae which were unresponsive to the prodding of the pasteur pipette were considered dead, whereas, responsive yet weak larvae were considered to be moribund. Mortality was recorded by counting the total number of dead and moribund larvae in a setup. Negative and positive controls were conducted alongside the established test setups with the negative control being 5 mL of 95% ethanol and the positive control being Abate.

Larvicidal activity of *Citrofortunella microcarpa* (calamansi) peel essential oil *Aedes aegypti*

(Carigaba, Leonida, Masculino, Mediodia, Garbo)

In the preliminary testing, the third and early fourth instar *Aedes aegypti* mosquito larvae were exposed to a wide range of test concentrations until there was a set of concentrations established that would give a larval mortality from 10% to 90%. The results were also compared to determine whether the dilution of the extract with the solvents involved had an effect on the mortality of the test organisms. For each concentration, at least four replicates were prepared. The mosquito larvae set-ups contained the appropriate volume of solution concentrations under the test. The same number of controls were set-up simultaneously.

For the final confirmatory test, the test organisms were exposed at concentrations ranging from 8 ppm to 11 ppm. After 24 hours, the mortality of the mosquito larvae for each set up was recorded. The mortality rate was determined by counting the number of deaths upon application of the formulated concentration. It was calculated using the following formula:

$$Mortality\ Rate\ =\ \frac{Number\ of\ dead\ larvae}{Number\ of\ larvae\ introduced} \times 100$$

The larvae were probed with a pasteur pipette and if there was no response from the larvae, which means that it did not move when it was probed, it was considered dead. In calculating the percentage mortality, moribund larvae, which are the larvae that are approaching death, were counted too and were added to the total number of dead larvae. Moribund larvae were qualified as those incapable of rising to the surface. They did not show any reaction when the water was disturbed.

D. **Antimitotic**
   (Abellar, De Juan, Bela-ong)
   After exposure of treatment to onion bulbs for 72 h away from direct sunlight, the number of cells undergoing prophase, metaphase, telophase, anaphase, and interphase was recorded, and the mitotic index (MI) was calculated. Chromosomal aberration was not evaluated due to limited equipment, and unavailability of the certain microscope required in order to perform the task.

After the completion of treatment, the roots (approximately 1-2 mm) were excised and collected, and immediately fixed in 3:1 (ethanol: acetic acid) for 24 hours (Tedesco and Laughinghouse 2012). Root tips were hydrolysed in 1N HCl for 15 min at 60°C, and stained with 2% orcein stain. After the

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removal of root caps from well-stained root tips, a 1 mm of the mitotic zones was immersed in a drop of 45% acetic acid on a clean slide and squashed under a cover glass and examined microscopically (Ozmen and Sumer 2004). Red discoloured roots were observed under 100x magnification for different stages of cell division (Rintelen et al., 2017). Five hundred cells were analysed per root tip (Tedesco and Laughinghouse 2012), summing up to 4500 cells per treatment. The number of cells in each stage of cell division i.e. either prophase, metaphase, anaphase, or telophase, and including the non-dividing cells were counted and recorded. The average mitotic index of 3 root tips for each treatment was determined.

Mitotic index was calculated by using the formula:

$$Mitotic\ index\ =\ \frac{Total\ number\ of\ dividing\ cells}{Total\ number\ of\ cells\ examined}$$

E.   Disk Diffusion

Antibacterial Activity of *Homalomena philippinensis* and *Merremia peltata L. Merr.* against *Staphylococcus aureus*
(Barrientos, Miraflores, Serisola, Mediodia)

Approximately 15 mL of Mueller-Hinton agar was poured into dry and sterile petri dishes. The medium was left to solidify for an hour. One loopful of bacteria was taken from the pure culture then streaked over the entire surface of the agar plate evenly. A pair of forceps was flame-sterilized. Using the forceps, a Whatman # 1 filter paper disc was picked up and immersed into the extracts for one hour. The bacteria was inoculated using an inoculating loop. After about 2 minutes, the moistened filter disc was laid gently on the seeded agar plate. The plates were inverted then incubated for approximately 18 hours.

Antibacterial Activity of Silver Nanoparticles
(Dogeno, Gamboa, Pefianco, Aban, Larroder)

Disk diffusion method was done to test the antimicrobial activity of the silver nanoparticles against *S. aureus*. The first agar plates were divided into four quadrants: each quadrant containing silver nanoparticles, silver nitrate, 15-g *M. oleifera sp.* seed extract, and distilled water. Filter disks were then placed in each quadrant. The petri dish was then incubated for 18 hours to let the bacteria culture grow. After the incubation period, the standard zone of inhibition (ZOI) was measured using a vernier caliper.

Antibacterial activity of copper-chitosan complexes
(Loquias, Placido, Mediodia)

Assessment of the antimicrobial activity of the synthesized copper-chitosan complexes was performed as outlined by the Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard 11th Edition by the Clinical and Laboratory Standards Institute (CLSI) at the Department of Science and Technology VI Regional Standards and Testing Laboratory. Mueller-Hinton (MH) Agar was utilized as a non-selective culture media. The experiment was carried out in triplicates.

The copper-chitosan complexes (0.1, 0.2, 0.5 wt% chitosan content) were suspended in distilled water and loaded onto blank sterilized Whatman No 1 filter paper disks. Ciprofloxacin (5 ug) served as the positive control in the form of commercially available ciprofloxacin-loaded antibacterial discs, while distilled water loaded onto blank sterilized Whatman No. 1 filter paper disks served as the negative control. Chitosan was also loaded onto blank sterilized Whatman No. 1 filter paper disks, totalling 6 treatments.

Prepared plates were incubated for 16-18 hours at $35\pm2°C$ in ambient air as recommended by the CLSI document M45-Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.

The zone of inhibition of each treatment was measured using a Vernier caliper (read to the nearest mm). The susceptibility of *V. parahaemolyticus* to the copper-chitosan complexes was determined based on the breakpoint table provided by the CLSI test interpretation document M45-Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria, wherein a zone of inhibition of > 21 mm indicates susceptibility to a compound tested with ciprofloxacin as the positive control while a zone of inhibition < 15 mm indicates resistance to the compound.

F. **Minimal Inhibitory Concentration**
(Barrientos, Miraflores, Serisola, Mediodia)

Thirteen screw capped 13 mm x 100 mm test tubes were sterilized and numbered accordingly. Using a 1.0 mL serological pipette, 1.0 mL of Mueller-Hinton broth was introduced into the 2nd to the 11th tube. For the 12th tube, 2.0 mL of Mueller-Hinton broth was introduced. Two mL of the prepared plant extract was pipetted into the first and second tubes. The second tube was vortexed for five seconds. Using a sterile 1.0 mL serological pipette, 1.0 mL of the contents of the second tube was aseptically withdrawn and transferred to the third tube, which was vortexed afterwards. The same process was continued until 1.0 mL was withdrawn from the ninth tube and subsequently added to the tenth tube. The contents of the tenth tube were vortexed. From the tenth tube, 1.0 mL was pipetted off the contents and it will be discarded.

One mL of the diluted bacterial inoculum was introduced into the tubes 1 to 11 and tube 13. In the 13th tube, 1.0 mL of the antibiotic standard was introduced. All tubes were tightly capped then the contents were vortexed. The tubes were incubated at $35°C$ for 16-18 hours. After the incubation period, the tubes were examined for bacterial growth by checking the turbidity in the tube. The tube with the lowest concentration of plant extract at which no growth or turbidity is observed was reported as the minimal inhibitory concentration (MIC) of the plant extract against *S. aureus*.

The MIC Assay was considered valid since the negative control tube has visible growth, the media control tube has no visible growth and the positive control tube has no visible growth.

G. **α-Amylase Inhibitory Assay**
(Alcalde, Tajo, Valencia)

Aloe vera extracts with different concentrations (0%, 20%, 40%, 60%, 80%, and 100% by volume) were first prepared in test tubes The diluting agent used is distilled water. The 0% sample had no aloe vera extract and only distilled water. Total volume of the different concentrations was 2 mL. 200 µL of 0.02 M sodium phosphate buffer, and 20µL of α-Amylase was added in the assay. In a concentration range of 10-100µL/mL, this solution was incubated at room temperature for 10 minutes. 20 µL of 1% starch solution was added on each test tube. 400 µL of 3,5 - dinitro salicylic acid (DNSA) reagent was added. The tubes were incubated in boiling water for five minutes and cooled at room temperature. The reaction mixture was diluted with 5 mL distilled water. The absorbance was measured at 540 nm using a UV-spectrophotometer. The control used is the blank sample and reference concentration is Acarbose, an α-amylase inhibitor.

H. Agar Well Diffusion

(Hembra, Henderin, Pareñas, Sinco)

Leaves of *Mangifera indica* (mango) contain phytochemicals which promote antibacterial activity. This study aimed to determine whether *M. indica* leaves extract can be an alternative antibacterial agent for triclosan.

The streak plate procedure was based on Sanders (2012) plating method. The medium was allowed to reach room temperature. Using an inoculum from the specimen, four-quadrant streak was performed to obtain well-isolated colonies. The specimen was contained on a swab, rolled several times over a small area near the edge of the plate, and streaked on the plate for isolation with a sterile loop starting where the swab was inoculated. The plates were then incubated at $35°C$ and examined after 24 hours.

A 0.5 McFarland standard was prepared by mixing 0.05 mL of 1.175% v/v barium chloride dihydrate ($BaCl_2•2H_2O$), with 9.95 mL of 1% v/v sulfuric acid (H_2SO_4) (Cockerill et al. 2012). Suspension of Staphylococcus spp. of 0.5 McFarland standard turbidity was done by visual comparison of the test tubes. To assist visual inoculum adjustment, bacterial suspensions were compared to McFarland standards against a Wickerham card (Hombach et al. 2015). The Agar Well Diffusion assay was performed in triplicates. Cultures of *Staphylococcus spp*. were inoculated separately on the solidified agar on each petri dish by streaking using a wire loop. About 100 mg/mL-1 of each test liquid soap was dispensed into separate wells using a 100 µg micropipette (Bbosa et al. 2007). The plates were then incubated at $37°C$ for 24 h. The sensitivity of the test organisms to the treatments was determined by measuring the diameter of the zone of inhibition surrounding the wells. After incubation, the diameters of the zones of inhibition were measured with a ruler read to the nearest mm (Bbosa et al. 2007).

COLLECTION

OVERVIEW

This chapter provides methods on the collection of various live and dead organisms as well as biological materials. This category covers factors regarding collection, storage, and transport such as proper timing, temperature, and handling. The conditions provided are necessary to ensure that the samples remain uncontaminated and suitable for use in research.

A. **Mollusk Shells**
(Faciolan, Leonora, Majaducon, Sinco)

Chromium in its hexavalent state, Cr^{6+}, is one of the prevalent heavy metals in aquatic ecosystems with its occurrence primarily attributed to industrial activities such as dye manufacturing and construction run-off. This paper presents the removal efficiency of organo-mineral composites from the shells of three mollusks abundant in the Philippines: *Crassostrea iredalei* (Slipper Cupped Oyster), *Perna viridis* (Green Shell), and *Telescopium telescopium* (Horned Snail).

Shells were obtained from a local wet market and were sent for speciation to Dr. Laureen Manalo, mollusk specialist at the University of the Philippines Visayas for authentication and confirmation of *Crassostrea iredalei* (*C. iredalei*), *Perna viridis* (*P. viridis*), and *Telescopium telescopium* (*T. telescopium*).

Shells were separated by species then boiled at 100°C for 10 minutes. Organic matter contained within the shells were removed prior to fragmentation by shattering. The shell fragments were then rinsed with distilled water. Subsequently, they were then oven-dried at 105°C for a total of 24 hours as prescribed by Zukri et al. (2018). Dried samples were then crushed into fine powder and sieved to particle sizes of ≤ 63 μm.

B. Blood

Sourced from Blood Bank
(Maquiling, Villanueva, Oberio)

The blood samples were acquired from the blood bank at the start of day 0. The blood bank ensured that no contaminants nor pathogens that can affect the results were present in the samples by analysis of Transfusion Transmitted Infections (TTIs). In order to ensure confidentiality, no name, age, sex, or any other personal data of the donor was given to the researchers.

Blood samples
(Alvarez, Oberio)

The blood samples were acquired from a local blood bank agency on Day 0. From each of the three donors, 450 mL of blood was extracted by a phlebotomist in a triple bag. CPDA-1 contained in the bag was used as an anticoagulant after blood extraction. The blood bank ensured that no contaminants or pathogens that can affect the results were present in the samples by analysis of Transfusion Transmitted Infections (TTIs). In order to ensure confidentiality, no name, age, sex, or any other personal data of the donor was given to the researchers.

C. **Phytoplankton**
(Cordova, Demandante, Occeña, Bela-ong)

The vertical tow as described by Milroy (2015) was used as the phytoplankton sampling method. To achieve deeper column sampling, collection of samples was conducted during high tide hours.

Phytoplankton sampling was conducted with the use of a conical plankton net with mesh size of 25 μm, a mouth diameter of six inches, and clamped rubber tube at the cod-end which collects the phytoplankton cells. The plankton net was acquired from University of the Philippines-Visayas (UPV), Miag-ao with a 50-cm mark as graduation. The net was lowered just above the bottom of the water and hauled vertically to the surface at a consistent speed of approximately one meter per second. To achieve a brisk pace while lowering the net, a weight of 2 kg was added to its cod-end. This essentially "flushes out" the net as it is lowered into the water. Once the net has been pulled back to the surface, the content of the cod-end was then transferred to properly-labeled, plastic, screw-cap sample bottles with graduations every 50 mL. The vertical tow was conducted until the phytoplankton sample amounted to over 200 mL in the sample bottle. The depth of each sampling site was determined using a Garmin Fish Finder and recorded.

The 5% Lugol's Iodine solution, purchased from Patagonian Enterprises, Jaro, Iloilo City, was used for the preservation of phytoplankton cells. Five drops of 5% Lugol's Iodine solution were added to each sample bottle.

D. Seaweeds
Handpicking
(Almarza, Gatila, Inosanto)

The three most abundant seaweed samples were collected at the coast of Taklong Island, Guimaras at around 15:00 to 16:00 in three different sampling sites. The seaweed species were handpicked via snorkeling at varying depths within the range of one (1) to two (2) meters. They were placed inside Ziploc® bags filled with seawater and were transported to the laboratory inside an icebox. They were then put inside a freezer with a temperature of -24°C for storage.

The seaweed samples were brought to the laboratory and washed with tap water to remove attached coral parts, stones, and epiphytes, and to thaw ice bits that formed around the samples from being stored in the freezer. The samples were then sun-dried for at least an hour to remove any excess moisture that may affect the measurement of dry weight of the samples.

E. Fish Samples
Clarias gariepinus and *Chanos chanos*
(Constantino, Guillergan, Yabut, Navarro)

Fish samples used for the study were chosen via convenience sampling. *Clarias gariepinus* samples were sourced from Zarraga since, according to Locara (2000), it is the main producer of catfish in Iloilo. Meanwhile, according to White (2013), Dumangas has one of the highest farmed milkfish production in recent years. Collection of ten samples of *C. gariepinus* from Beboy Pantatan in Zarraga, Iloilo was conducted every 4:00 PM, one day before the dissection. The number of fish samples collected per day was determined by the number of fish samples that can be completely dissected and examined in one working day. Fish samples were stored in aerated fry bags, along with water from the pond where the samples were harvested (Truter et al. 2017). Fry bags were placed in large buckets for transportation, with a total number of five (5) fish samples for each bucket. All 30 *Chanos chanos* samples were collected from a fish farm in Dumangas, Iloilo. The fry bags were pumped with oxygen and filled with water from the pond where the samples were harvested. Bags were then placed in large buckets (Villaluz 1984). *Chanos chanos* samples were transported to the Microtechnique Laboratory of SEAFDEC/AQD in Tigbauan, Iloilo for the dissection and examination of the gills and intestines. Different batches of fish samples were dissected on different dates.

Chanos chanos (milkfish)
(Cadorna, Chan, Salmon, Salazar)

Fresh-caught, farmed *Chanos chanos* (milkfish) with fork lengths of 20.2 to 26.8 cm, total lengths of 26.1 to 34 cm, and weights of 123 to 252 g, were collected from the Southeast Asian Fisheries Development Center (SEAFDEC) Brackishwater Station in Dumangas, Iloilo. After capture, the fish were immersed in icy water to shock them. Euthanasia was performed shortly after capture through spiking or iki jime, the physical destruction of the central nervous system. A dissecting needle was inserted into the cranial cavity of the skull found above the eyes and moved in back-and-forth and sideways motions to destroy the fish's brain and spinal cord (Davie and Kopf 2006). The fish were individually placed inside ziplock bags and immediately transported to a laboratory in Philippine Science High School - Western Visayas Campus in polystyrene boxes with 1:1 fish-to-ice volume ratio (Yeasmin et al. 2010). The fish were separated into two groups, one was immersed in 5% formalin for five (5) minutes (Yeasmin et al. 2010) while the other remained untreated. The fish samples from the two groups were stored in a chest freezer at a temperature of $0°C$ to $5°C$ and observed every 24 hours for seven days.

F. Bacterial Sample from Plastic Waste
(Canja, Hilis, Galan, Jolito)

Plastics are known for being durable materials while still maintaining a low cost of production. It is a very important material for commercial use all over the world. However, due to the lack of a reliable method of disposal, the risk of plastic pollution is steadily increasing throughout the years. This study aims to isolate and extract bacteria from the Iloilo City Engineered Sanitary Landfill, in Mandurriao, Iloilo City, and to assess their biodegradation potential on LDPE (Low-density polyethylene), HDPE (High-density polyethylene), and PET (Polyethylene terephthalate).

The bacteria were extracted from plastic waste using swab sampling and from soil samples which were taken from the dumpsite. The plastic wastes were randomly selected and a total of five swab samples were collected. The swabs sealed inside test tubes with tryptic soy broth were transported in a laboratory in PSHS-WVC for testing. For the soil, five samples were collected from a depth of 10 - 20 cm, then placed inside sterile containers and kept at a temperature of $4°C$. (Bolo et al. 2015)

Random sampling was done by having an aerial view of the area (Coordinates: 10°42'34.7"N 122°31'25.8"E) and section it equally into 25 sites. Five randomly selected sites were chosen using a random number generator as shown in Figure 1.

Figure 1. Sites were sampled by random selection.

Site 1 is located in area 7, site 2 in area 6, site 3 in area 13, site 4 in area 18, and site 5 in area 9.

G. *M. Indica* (Mango) Leaves
(Hembra, Henderin, Parenas, Sinco)

Leaves of Mangifera indica (mango) contain phytochemicals which promote antibacterial activity. This study aimed to determine whether M. indica leaves extract can be an alternative antibacterial agent for triclosan. The M. indica leaf samples were collected from Orchard Valley, Tigum Barrio Road, Pavia, Iloilo and were stored in a mesh bag. Prior to identification, the samples were then identified by the Department of Agriculture (DA) in Sta. Barbara, Iloilo.

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DESIGN

OVERVIEW

When designing a device involving biological organisms, it is important to consider mimicking their natural habitats. In the case of Ampunan, Placer, and Robles, the natural habitats of spiders were considered in ensuring that each sector of the device was at least three times the width of a spider leg, the device temperature was regulated, and that the environment was dark. This is to ensure comfort and longevity of the living subjects. It is also important to break down the device into smaller, manageable parts, with specific and accurately measured dimensions . Finally, in the use of software such as CorelDRAW, it is advised to seek out the help of different professionals.

A. <u>UV-Visible</u>
(Ampunan, Placer, Robles, Manalo, Olvido)

The design for the spider web collector consists of three main parts: (1) cover, (2) storage units, and (3) base. In order to ensure accuracy, the design was rendered on CorelDRAW, and the cutting process was done using a laser cutter.

The cover is made up of two layers of clear acrylic sheet. For the top layer, 13 cm by 13 cm squares were cut out. These cutouts were used as coverslips and were bored with 9 holes to serve as ventilation. For the bottom layer, squares of 11 cm by 11 cm were cut out. These cutouts will not be included in the design. The bottom layer was glued with the top layer to act as the holder for the coverslips. A tape was attached to one side of the coverslip to serve as a tab for easier access.

The whole storage unit is composed of a grid of five by five square units. It consists of four outer walls, four inner long panels and 20 smaller panels. These walls were glued together to form storage units measuring 15 x 15 x 15 cm each (recommended area for *P. phalangioides* leg length).

For the base, the area was calculated with the average body length of the spider in consideration in order to avoid harming the spider during web collection. Holes were bored at the center of each storage unit. Wooden rods of three mm-diameter were inserted to these holes, which served as the web collectors. The walls around the base limit the movement of the storage units during web collection.

The spider web collector was assembled by placing the base at the bottom, the storage units in the middle, and the cover at the top. The interior of the storage units including a part of the base below the storage units were then painted black which provided a darker environment which is ideal for the spiders.

DISPOSAL

OVERVIEW

      This chapter includes methods on the disposal of bacteria-related waste. It is important to first ascertain the specific laboratory safety protocols of the particular laboratory the researchers are working in. Waste must always be properly labeled and categorized according to levels of hazard and risk assessment. In particular, glassware must be segregated based on the type of substance they have handled. Once it is time to dispose of these, waste disposal measures must be followed to avoid the spreading of bacteria to different places, and to avoid unnecessary risk towards unrelated individuals.

A. **[Culture](#)**
(Barrientos, Miraflores, Serrisola, Mediodia)

      Proper waste disposal methods were followed for all materials. All used agar and broth cultures were decontaminated immediately after the conduct of the experiment by mixing in hypochlorite for approximately an hour inside the biosafety cabinet. All of the spent agar media and broth were disposed of in the hazardous wastes bin. All glassware, including the culture tubes and dishes cleaned using antibacterial soap.

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DISSECTION

OVERVIEW

This chapter includes methods for dissecting fish samples as preparation for further examination. This covers the extraction of the gill filaments and internal organs of the organism, as well as procedures to ensure optimum sample quantity upon examination.

A. **Fish**
(Constantino, Guillergan, Yabut, Navarro)

Fish samples were dissected by first cutting the left and right operculae open. The gill arches were then cut and removed from the cavity using dissecting scissors and tweezers. The gill filaments were then cut and removed from the gill arches, placed on a glass slide, and covered with a cover slip. An incision was then made from the anus of the fish up to its mouth, exposing the digestive tract (Fish Dissection 2019). The fish samples were then eviscerated. The small and large intestines were removed and placed on a petri dish filled with freshwater for *Clarias gariepinus*, and seawater for *Chanos chanos* to mimic the salinity of the fish pond where the fish species were collected.

ETHICAL CONCERNS

OVERVIEW

This chapter includes ethical considerations in performing specific procedures as well as the appropriate measures to maintain the integrity of the study. Research ethics provides the standards for conducting scientific research. It is important for researchers to adhere to these standards especially when handling living organisms out of respect for their dignity, rights, and welfare.

A. **Human Blood Samples**
(Maquiling, Villanueva, Oberio)
The blood samples were acquired from the blood bank at the start of day 0. The blood bank ensured that no contaminants or pathogens that can affect the results were present in the samples by analysis of Transfusion Transmitted Infections (TTIs). In order to ensure confidentiality, no name, age, sex, or any other personal data of the donor was given to the researchers.

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EVALUATION

OVERVIEW

Evaluation is integral in analyzing and comparing the results of different experimental setups. It is a powerful tool when verifying the validity of test results in comparison to negative and positive controls. In evaluation, young researchers might consider finding related articles with the particular evaluation method that fits their research topic. Using this as a basis, methods may then be adjusted according to the specific needs of the study. One must also consider the software limitations at hand. It is best to identify a free software program which may be learned in a viable amount of time. Finally, in doing statistical analysis, researchers are advised to consult with experts to ensure their methods are accurate.

A. **Fleshy Macroalgal Index**
(Dalabajan, Hilay, Velasco, Navarro, Olvido)

The FMI was measured using the photoquad method. Photoquad method provides visual estimation but in a digital version. The remaining macroalgae attached to the rope were photographed. After taking a picture, the photo quadrant analysis software, photoQuad , quantified the FMI using the grid cell count option. A direct estimate of the species cover was automatically performed. The software offers a more versatile, quick and accurate result. The FMI of each unit will be measured before and after the exposure *T. gratilla* for comparison.

B. **Biodiesel Properties**
(Almarza, Gatila, Inosanto)

Sixteen biodiesel properties were calculated using the Fatty Acid Methyl Ester (FAME) composition of each species. Fuel properties derived from FAME profiles are the following: degree of unsaturation (DU), long chain sat- uration factor (LCSF), cold filter plugging point (CFPP), iodine value (IV), saponification value (SV), cetane num- bers 1 and 2 (CN1 and CN2), saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), kinematic viscosity, density, higher heating value (HHV), amount of C18:3, number of double bonds (Db), and oxidation stability.

C. **Platelet Count and Morphology**
(Maquiling, Villanueva, Oberio)

After extraction, platelet count was measured by running a sample through the hema-analyzer three times and taking the average. Mean platelet volume was measured similarly to the platelet count by running a sample of 1 mL through the hema-analyzer three times and taking the average. The pH was tested using a pH meter by sampling a 10 mL volume of the platelet concentrates and washing the bulb of the pH meter after every measurement. All successive measurements were then taken on Days 3 and 5 of storage.

Platelet morphology was analyzed by photographing microscope smears of the platelets in each setup and manually counting the ratio of activated platelets to the total number of platelets. For a qualitative comparison, microscopic analysis of the samples involving the shape and concentration of platelet change was also done to show their shape and configurations. Discoid and irregular shapes were noted among the platelets. Six photographs were taken from each slide and were then gridded and printed into paper for manual counting. A four-by-four area was then used to count the platelets. The number of irregularly-shaped platelets was then divided by the total number of platelets to obtain the percent change of platelet morphology.

D. **Germination**
(Legurpa Mi, Legurpa Ma, Oberio)

Seed priming is a technique used to improve the overall germination behavior of rice through the imbibition of solutions. This study employed three different priming methods (mannitol, glycerol, and sorbitol) on two local Philippine varieties of *Oryza sativa* (rice) and compared germination behaviors between primed and unprimed seeds.

Eight (8) setups were used for the two varieties (Red and Black) treated with three different priming agents and control of untreated seeds. Twenty-five seeds were sown in one 90-mm Petri dish which contained one layer of Whatman No. 2 filter paper moistened with 10 mL of water which was measured using a syringe. Each setup had three replicates which were made up of six Petri plates for a total of 150 seeds in each replicate and 144 Petri plates for the whole study.

Every day at 7 am in the morning and at 3 pm, seeds were watered using the syringe or as needed to maintain a 100% moisture level. Natural light was allowed to pass through the windows. The temperature was measured and recorded at 4 pm in the afternoon every day using a wall thermometer. Conditions were also observed and recorded such as the presence of insects, animals or any other factor that might affect the study. At 3 pm the number of seeds that have germinated was counted. A seed was deemed to have germinated once it reached a radicle length of 2 mm which was measured using a vernier caliper. These results were then recorded using Google Spreadsheets.

On the 7th day of the study, the seedling shoot and root length were measured. Two of the longest germinated seeds in each Petri plate were chosen to be measured. This was done using a Vernier caliper. These results were then recorded using Google Spreadsheets.

The germination percentage, germination rate index and shoot/root ratio were calculated using the formulas stated in Salah et al. 2015 and Awasthi et. al 2016.

Germination Percentage

$$GP = \frac{\text{Number of Total Germinated Seeds}}{\text{Total Number of Seeds Tested}} \times 100$$

Germination Index

$$GI = \sum (G_t/T_t)$$

Where the number of germinated seeds on Day t and Tt is time corresponding to Gt in days.

Shoot/Root Ratio

$$SL: RL$$

Where SL is the length of the shoot and RL is the length of the root.

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EXTRACTION

OVERVIEW

This chapter provides different methods on how to prepare an extract from raw materials. Determining the type of method or solvent to be used based on the polarity of the compound to be extracted is essential in attaining a high extraction efficiency. Other factors that should be considered include particle size, temperature, duration, etc.

A. Ethanolic

Dioscorea alata (purple yam) peels

(Grantoza, Mationg, Seguano, Oberio)

The powderized samples were mixed with 95% ethanol and soaked for 48 hours (Khonkarn et al. 2010) in a 500-mL Erlenmeyer flask. Flasks were sealed with cling wrap and covered with black paper to prevent light from affecting the compounds present during the soaking process. The flasks were set aside at a safe place at room temperature. After soaking, the mixture was filtered using Whatman no. 1 filter paper into another erlenmeyer flask. Solid residues from the first extraction were further pressed for additional extracts and the products obtained from pressing were mixed with the earlier filtered extract. Filtered plant solution was set up for rotary evaporation (Eyela Autojack NAJ-100) at 40°C with 100 rpm to remove solvent from the solution. Temperature used during rotary evaporation was decided using the Δ 20° Rule commonly used by scientists for effective extraction. Acquired crude extracts were contained in a sterilized erlenmeyer flask and was then sealed with a cling wrap. The flask was again covered with aluminum foil in order to prevent light from passing through it.

Clitoria ternatea (Asian pigeonwings)

(Triol, Dionela, Ecube, Mediodia)

The collected _Clitoria ternatea_ flowers were washed with distilled water and oven-dried at 75°C for 24 hours (Wang et al. 2014). The dried petals were ground into a fine powder using a NutriBullet blender. Amber bottles were filled with 125 g of powdered _C. ternatea_ flowers, and the powdered petals were macerated in 95% ethanol with 1:10 mass to volume ratio for 24 hours. The mixture was filtered using No. 41 Whatman filter papers and was subsequently filtered using No. 1 Whatman filter papers (Suebkhampet and Sotthibandhu 2013). The filtered extract was then subjected to rotary evaporation (Ika Hb Digital) to obtain the crude extract, which was dissolved once again in 95% ethanol in a 1:1 mass-of-extract-to-volume-of-solvent dilution ratio.

Mangifera indica (mango) leaves

(Hembra, Henderin, Parenas, Sinco)

Leaves of _Mangifera indica_ (mango) contain phytochemicals which promote antibacterial activity. This study aimed to determine whether M. indica leaves extract can be an alternative antibacterial agent for triclosan.

The _M. indica_ leaf samples were washed with tap water twice (Krishnanda and Shabaraya 2016) then rinsed with distilled water to remove unwanted residues. Based on the methods of Zakaria et al. (2006) the leaves were then oven-dried, however, the drying period was extended to four days because the leaves were not crisp dry after 24 hours of oven drying. The leaves were then cut into small pieces using a pair of scissors and were powdered using a Hanabishi kitchen blender. The powdered samples were then macerated for 72 hours using 95% ethanol as the solvent and the resulting mixture was filtered using Whatman no. 1 filter paper (Diso et al. 2017), properly positioned inside a glass funnel. After which, the remaining solvent was then evaporated using the Biobase RE100-Pro rotary evaporator at 40°C with 100 rpm (Zakaria et al. 2006).

B. Methanolic

(Venturina, Comuelo, Samaniego, Jolito)

Extracts from plants such as Bixa orellana, commonly known as Annatto, presents a potential to be an alternative to the commonly used synthetic stains. Thus, this study aimed to evaluate Bixa orellana methanolic extracts as a substitute for safranin in Gram Staining.

Bixa orellana extracts were used to stain *S. aureus*, *E.coli* and mixed bacteria smears. The powdered seeds (100g) were added to 1 L of methanol (CH_3OH) and stirred for 12 hours using a magnetic stirrer and stored without sunlight at room temperature for 12 hours. The extract was then filtered using Whatman Filter Paper No. 1 and was placed in an IKA RV10 Rotary Evaporator at 40°C until all methanol had evaporated. The extract was then stored in a refrigerator prior to staining.

As a confirmatory test, the crude extract (0.25g) was dissolved in 10 mL methanol (CH_3OH) and its pH was then measured using a pH meter. A 50 ppm solution of the reconstituted solution was prepared to find its absorbance which was measured with a UV-Vis spectrophotometer at 300-650 nm and compared to the graph of pure bixin.

C. Hot-water

(Yap, Bungay, Alfonso, Libo-on, Cordero)

Gracilariopsis heteroclada samples were sun-dried for 48 hours. After, it was powderized in a corn mill grinder and was sifted using a 250 um mesh. Thirty (30) grams of powder were boiled in 900 mL of distilled water for three hours at 100°C. The solution was filtered using a cloth and a sieve of 250 μm mesh size into the metal trays. The trays were frozen overnight at -80°C then lyophilized for 24 hours thereafter at 2 mmHg.

D. Crude Extract

(Elizalde, Herida, Jaudian, Mediodia)

Prior to extraction, the collected samples were sundried for 6 hours under sunlight and air dried in the laboratory at night for two days to greatly reduce the moisture of sponges. They were then minced into the finest possible particles. The dried sponges were stored in the refrigerator to prevent any contamination prior to extraction. The extraction process involved creating a mixture using 200 mL of methanol for every 25 g of dried *Callyspongia sp*. The mixture was agitated for two minutes every 30 minutes for two hours in a 500 mL Erlenmeyer flask. The mixture was brought to the Department of Science and Technology (DOST) Region 6 to collect the extract by evaporating the methanol using a rotary evaporator. The resulting extract mixture after evaporation was approximately 80 mL and was stored in an Erlenmeyer flask. The remaining evaporation was done in Philippine Science High School – Western Visayas Campus using a water bath at 40°C. The final crude extract collected weighed 0.53 grams and diluted in sterile distilled water to obtain an initial concentration of 4.8×10^{-2} g/mL.

E. Lipids

(Almarza, Gatila, Inosanto)

The sun-dried samples were minced using a pair of scissors as a means to pulverize them. Triplicates of one-gram minced seaweeds were measured for each species using a triple beam balance. The weighted seaweeds were placed inside test tubes and were added with one milliliter of chloroform and two milliliters of methanol resulting in a 1:2 ratio. The weighted seaweeds were placed inside test tubes and were added with one milliliter of chloroform and two milliliters of methanol resulting in a 1:2 ratio. The mixtures were vortexed for six minutes and an additional one milliliter of chloroform was added again and vortexed for another 30 seconds.

Finally, one milliliter of distilled water was added and vortexed for 30 seconds resulting in a final ratio of 2:2:1 of chloroform: methanol: distilled water mixture.

The mixture was filtered using a glass funnel and a filter paper setup in order to obtain their liquid components. The residues were then transferred into another set of test tubes where the separation of phases was going to take place. In order to do that, the samples were centrifuged at 1000 rpm for five minutes at room temperature. The upper phases (methanol layer) were siphoned out using a Pasteur pipette. The lower phases (chloroform layer) were then transferred to their respective preweighed test tubes.

The filtrates were treated to a hot water bath using a hot plate with the temperature set at around 185°C until the remaining chloroform contents of the solutions were removed completely, leaving the surface of the base of the test tubes clad with lipids only.

F. Chitosan
(Janiya, Lopez, Magtoles)

Five hundred (500) grams of crab shells were washed and dried under direct sunlight. Cleaned shells were crushed and put in a 1L beaker with 4% NaOH to boil at 100°C for one (1) hour. The samples were removed from the beaker and cooled down at room temperature. The samples were again crushed and pulverised to produce a powder using a mortar and pestle. The pulverised crab shells were sieved using a 0.2-mm mesh sieve to acquire finer particles of crab shell powder. The sample powder was then demineralized by adding 1% HCl to the sample powder in a ratio of 1:4 w/v powder to HCl for 24 hours. The HCl was drained from the mixture.

The sample powder was then treated by adding 2% NaOH solution in a 1:2 w/v ratio powder to NaOH solution. This treatment was done for one hour to remove the albumen. The treated powder was washed using distilled water. The sample was then soaked in boiling 50% NaOH for two (2) hours to deacetylate. The powder was filtered from the beaker and cooled down at room temperature for one (1) hour. The powder was washed carefully with 500 mL 50% NaOH. The powder was oven dried at a temperature of 120°C for 24 hours.

G. Hydroxyapatite
(Janiya, Lopez, Magtoles)

Five hundred (500) grams of crab shells were crushed to produce smaller pieces of shells by using a mortar and pestle. The crab shells were boiled in distilled water for two (2) hours to remove adherent meat. The crab shells were filtered from the beaker and air dried. The crab shells were again crushed and pulverised and were then sieved using 0.2 mm sieve to acquire fine particles. The acquired powder was heated in a furnace at 1000°C for five (5) hours for it to calcinate and form Calcium oxide powder. After heating, it was cooled down at room temperature. The powder was then mixed with distilled water in a 1:1 w/v ratio solution.

The phosphoric solution in 0.6M was added dropwise resulting in a 5:3 m_{powder}/v_{H3PO4} solution. The solution was kept at room temperature for 24 hours to precipitate. The precipitate was filtered using a Whatman No. 40 filter paper and washed carefully with distilled water. The precipitate was oven dried at 120°C for three (3) hours and sintered by heating it in a furnace at 900°C for four (4) hours.

H. Aloe Vera Plant
(Alcalde, Tajo, Valencia)

The leaves were carefully removed from the aloe plant. The roots were left in the pot to insure regrow of the plant. The leaves were separated and washed. The aloe gel was separated from the latex and rind by scraping using a stainless steel spoon. The fresh leaf gel was homogenized using an electric blender while the latex and the rind was powderized, also, using a blender.

The aloe gel was tested by the assay first, then the latex and rind were tested by the alpha amylase test two days after. The same aloe plant was the source of the aloe gel and aloe latex with rind. The latex and the rind was sun/shade dried for two days. The dried portions were manually broken to smaller pieces using a mortar and pestle and were further powderized using an electric blender. The aloe vera latex and rind were then placed in solvent-solvent extraction to be able to extract the constituent.

I. Microplastics from Sediment Samples
(Colacion, San Diego, Secondes, Oberio)

The obtained sediment samples were carefully placed onto separate aluminum foils. They were oven-dried at 60°C for 48 hours. Two sieves of 4 mm and 2 mm grid sizes were washed with distilled water and dried using a paper towel. They were stacked together with the 4 mm sieve on top of the 2 mm sieve. The dried sediments were passed through the sieves. The retained fraction on the 4 mm and 2 mm sieve were properly disposed of while those which passed through were temporarily stored for further processing.

Sieved samples of mass 500 g were mixed with 2000 mL of saturated NaCl solution. The mixture was stirred for five minutes. After stirring, the mixture was allowed to stand for an hour. A filtration setup utilizing Whatman No. 41 filter paper was constructed, as illustrated in Figure 2. The supernatant was carefully poured into the filtration setup to acquire the floating particles. The particles retained on the filter paper were allowed to dry inside a drying oven at 50°C for 48 hours.
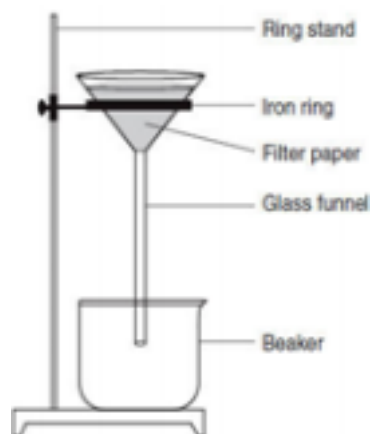

Figure 2. Set-up for density separation of microplastics

The oven-dried particles from the density separation step were transferred into a 50 mL glass beaker through gentle scraping with the use of a spatula. Twenty milliliters (20 mL) of 30% hydrogen peroxide was added to the beaker. The mixture was incubated at 60°C for 24 hours. The mixture was covered with a watch glass for the entire duration. The solution was filtered using Whatman No. 41 filter paper, and the acquired particles were oven-dried at 50°C for 48 hours before being stored inside glass Petri dishes.

IDENTIFICATION

OVERVIEW

This chapter covers methods involving morphological and molecular techniques in the identification of various organisms ranging from the microscopic to the macroscopic level. This category will specifically discuss the use of equipment and identification guides to distinguish species of parasites, seaweeds, and plants based on their form, structure, and appearance and the use of DNA sequence to identify bacterial species.

A. **Bacteria**
(Suarez, Apdon, Balinas, Baldonado, Hernando)

A TCBS agar plate containing the cultured bacteria with isolated colonies was packed tightly then secured to be delivered to the Philippine Genome Center in the National Institute of Molecular Biology and Biotechnology in the University of the Philippines Diliman for the 16S ribosomal RNA Extraction to Sequencing.

When the results arrived, the FASTA-formatted sequence was checked using the BioEdit software and was then inputted in the NCBI-GenBank. Basic Local Alignment Search Tool (BLAST) was used to find matching sequences in the NCBI databank. The highest matching sequence identifies the species of the bacteria.

B. **Seaweed Species**
(Almarza, Gatila, Inosanto)

Small representative portions of each individual sample were preserved in 70% ethanol for verification purposes. The species were identified by comparing the morphological characteristics of the samples to existing photographs of the species found on Taklong Island, Guimaras. The identified species were *Dictyota dichotoma*, *Sargassum cristaefolium*, and *Padina minor*.

C. **Parasites**
(Constantino, Guillergan, Yabut, Navarro)

The gill filaments were examined under the Olympus BX51 compound light microscope and the Howell Binocular Compound Microscope at 40x and 100x magnification. Photographs of parasites present in the gills were then taken at 100x magnification. The intestines were teased and examined under the Howell Binocular Stereomicroscope. Parasites found in the gills and intestines were identified up to the genus level based on morphology, using the book Health Management in Aquaculture by Cruz-Lacierda et al. (2001) and further verified by Mrs. Gregoria Pagador of the Fish Health Section of SEAFDEC/AQD. After identification, parasites were counted based on their types.

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INCUBATION

OVERVIEW

This chapter covers methods involving incubation, particularly of chicken eggs. This category will specifically identify the equipment, temperature range, and relative humidity utilized and discuss the processes adapted such as egg candling and its corresponding criteria for exclusion of samples, and egg turning to ensure the integrity of the incubated eggs for research.

A. **Chicken Eggs**

(Aguirre, Sombiro, Valdestamon, Mediodia)

All chicken eggs purchased were put inside a manual industrial egg incubator with a temperature range of 38°C to 38.5°C and relative humidity of 57%. The eggs were candled on the third day of incubation to check their fertility, as measured by the visible growth of blood vessels in the egg when viewed under a candler. All infertile eggs were properly disposed of after candling. During the entire incubation period, the eggs were turned to a 45° angle from their vertical positions six times a day for seven days to ensure balanced temperature flow throughout the eggs and prevent the blood vessels from sticking to the shell.

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INFECTION

OVERVIEW

In determining the efficacy of treatments against infectious disease, experimental units can be initially challenged with the pathogen and then administered with the treatment. As in this chapter, for example, the method involving in vivo infection of a viral disease in chickens is described.

A. **Virus**

(Sira, Valzado, Larroder, Cabarles)

Preparation of Virus-concentrated Solution

The LaSota vaccine, bought from a local livestock supply store, was diluted with a vaccine solvent specifically for freeze-dried avian vaccines provided by CPU - CARES. The ratio of the vaccine to the solvent was modified by the Department of Agriculture (DA) so that the final solution would be more concentrated with live viruses.

Infection of Samples

The final solution was injected to either the thigh or the breast part of the chicken samples. Cotton was also soaked with the solution and was taped at the top of the cages to contaminate the air with the virus. This was done under the supervision of Dr. Jaime Cabarles Jr. of CPU - CARES and Dr. Jonic Natividad of DA.

MICROBIOLOGY

OVERVIEW

This chapter is broad and it covers methods encompassed by the field of microbiology. The field includes many sub-disciplines (e.g. bacteriology and virology), all defined by a common object of interest which is microscopic organisms. The chapter includes culture methods, staining, assays, and use of equipment.

A. Microplate Use
(Cabalfin, Gerona, Labrador, Remaneses, Mediodia)

A 96-well microtiter plate was used to grow the biofilms of *S. aureus*. 200 L of the 80 Biofilm Eradication previously diluted liquid media was transferred to columns 2, 4, 6, 8 and 10 of the plate using a micropipette. The microtiter plate was sealed and placed in the incubator for 24 hours at 30°C. (Yadav, 2015). This was conducted inside the laminar flow hood.

B. Isolation & Culture

Algae Isolation
(Yeban, Baranda, Antenor, Sinco)

Samples from respective CM and F/2 media were dispensed to 15 mL test tubes for serial dilution of 10^{-1} to 10^{-10}. An inoculum of 1 mL was added to 9 mL of the respective media, with CM for brown algae and F/2 media for green algae. The 10th and 9th inoculated samples were kept in capped test tubes to avoid contamination, and exposed to a 40-W fluorescent lamp, continuous lighting at room temperature, 25°C (± 2°C) for a minimum of one week.

Agar-selective Bacteria
(Suarez, Apdon, Balinas, Baldonado, Hernando)

To culture the bacteria, a triangular, glass hockey was used. Initially, the hockey has to be sterilized with the use of heat. After which, 0.1 mL of the diluted coral mucus was placed on the agar plate, and then spread throughout the plate using the sterilized triangular, glass hockey. The agar plate was then closed and sealed, and then incubated at room temperature for three days.

After three days, the cultured bacteria were isolated using the Four Quadrant Streaking Method. A sterilized inoculating loop was used to streak the bacteria onto three new agar plates, after which they were then incubated for another three days at room temperature. This method was done once again after three days in order to ensure the purity of the samples. Morphological assessment was then conducted visually with the use of a vernier caliper, a compound microscope and an ultraviolet lamp.

After the assessment, an agar plate, containing the cultured bacteria with isolated colonies was packed tightly then secured to be delivered to the Philippine Genome Center in the National Institute of Molecular Biology and Biotechnology in the University of the Philippines Diliman for the 16S ribosomal RNA Extraction to Sequencing.

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Staphylococcus aureus
(Cabalfin, Gerona, Labrador, Remaneses, Mediodia)

An inoculating loop was used to transfer an isolated colony from the agar plate to a test tube filled with 15 mL of Tryptic Soy Broth with 0.4 grams of glucose to create a subculture. The test tube containing the subculture was incubated for 18 to 24 hours at 30°C. A McFarland procedure was conducted to compare the optical densities of the cultures, using a bond paper with black and white stripes. A 96-well microtiter plate was used to grow the biofilms of S. aureus. 200 L of the previously diluted liquid media was transferred to columns 2, 4, 6, 8 and 10 of the plate using a micropipette. The microtiter plate was sealed and placed in the incubator for 24 hours at 30°C. (Yadav, 2015). This was conducted inside the laminar flow hood.

C. **Co-culture**
(Serra, Cabalfin, Lamzon, Mediodia, Catedral)

Separate cultures were prepared for the co-culturing of these organisms. The same method was used in order to acquire the needed population for each species. As soon as the dinoflagellate reaches cells/mL 1.00×10^4 and bacteria reaches 1.00×10^7 cells/mL. This was determined through counting the population of each sample dinoflagellate through hemocytometer (De la Peña and Franco 2013) and spread plate method for bacteria, and diluting the media to achieve the desired population density. Both samples were co-cultured to one another and were transferred into a 500 mL dextrose bottle with F - medium however the setup did not contain vitamin. Then will be placed in a well lit area equipped with aerators.

D. **Simple Staining**
(Triol, Dionela, Ecube, Mediodia)

The ethanolic extract was applied as a stain to 15 slides of *Staphylococcus aureus* and 15 slides of *Escherichia coli* bacterial smears using the simple staining method. Crystal violet, the positive control, was utilized as a stain to *S. aureus* and *E. coli*. Crystal violet and the ethanolic extract were used as the primary stain for the positive control and treatment, respectively. The primary stain was added to the bacterial smear and decolorizer was applied to remove the excess stain. Negative control without any stain was also used. The procedure for the simple staining was obtained from *Merck Microbiology Manual 12th Edition* (2010).

E. Gram Staining

Staphylococcus aureus, Escherichia coli, and mixture of the two bacteria
(Triol, Dionela, Ecube, Mediodia)

The ethanolic extract was applied as a stain to 15 slides of *Staphylococcus aureus*, 15 slides of *Escherichia coli*, and 15 slides of mixed bacterial smears using the Gram staining method. Crystal violet, the positive control, was utilized as a stain to *S. aureus*, *E. Coli*, and mixed bacteria. Crystal violet and the ethanolic extract were used as the primary stain for the positive control and treatment, respectively. Gram's iodine solution was utilized as a mordant which was added after the primary stain. A decolorizer was also used to remove the excess stain and safranin was added as counterstain. Negative control without any stain was also used. The procedure for the Gram staining was obtained from *Merck Microbiology Manual 12th Edition* (2010).

Using Bixa orellana Extract as Stain
(Venturina, Comuelo, Samaniego, Jolito)

Extracts from plants such as Bixa orellana, commonly known as Annatto, presents a potential to be an alternative to the commonly used synthetic stains. Thus, this study aimed to evaluate Bixa orellana methanolic extracts as a substitute for safranin in Gram Staining. Bixa orellana extracts were used to stain *S. aureus*, *E.coli* and mixed bacteria smears. A drop of normal saline solution was placed on the slide. Using an inoculating needle, the cultured bacteria was smeared on the slide and allowed to dry. The slide was then passed quickly over the flame of an alcohol lamp three times. The slide with the heat fixed smear was then flooded with crystal violet for one minute and was then rinsed with distilled water. The smear was gently flooded with Gram's iodine for one minute and was then rinsed with distilled water. The smear was then decolorized using a 50 v/v% mixture of acetone and alcohol.

Endophytic bacterial isolates
(Halaba, Molinos, Superficial, Bela-ong)

Endophytic bacterial isolates were isolated from the roots of *Zea mays L. var. rugosa* (sweet corn) Sugar King variety at vegetative stage. A total of eight different bacterial strains were isolated, characterized; through gram staining and colony and cell morphology; and screened for a positive reaction for nitrogen fixation, ammonia production and zinc solubilization through the use of selective media. Jensen's media was used to screen for nitrogen fixing bacteria, peptone water with nessler's reagent for ammonia producing bacteria, and zinc incorporated media for zinc solubilizing bacteria. Gram staining was conducted to differentiate bacteria based on their different cell wall constituents. From each pure culture colony grown, a bacterial smear was prepared. Smears were prepared by heat fixing bacteria onto a sterile glass slide. The smears were then saturated with the following reagents: the primary stain crystal violet for 1 minute; Gram's iodine solution for 1 minute; 95% ethanol for 5 seconds; and the counterstain safranin for 1 minute rinsing the glass slide with distilled water after the addition of each reagent. Microscopy was conducted to determine the results for the gram staining. Purple stained bacteria were considered Gram-positive while pink stained bacteria were considered Gram-negative.

F. Counting of Microalgal Cells Via Hemocytometer
(Derramas, Gonzalez, Villaflor, Mediodía)

Ten milliliters (10 mL) of the algae culture was pipetted from the source container into a test tube. It was agitated in a vortex mixer for five seconds. A micropipette was used to extract ten microliters (10 µL) of the culture and was dispensed into slot A of the hemocytometer. Algae colonies in the five smaller squares of the hemocytometer's central square were counted. The algae colonies whose bodies overlap between squares were not counted. The concentration of algae per milliliter was computed based on the number of microalgal cells per microliter (µL).

G. Antifeedant Assay
(Bendicion, Dael, Genterola, Aban, Seredrica)

Anethum graveolens has been known to have insecticidal activity on several insect pests. Studies state that components of the essential oil of *A. graveolens*, which are mainly composed of carvones, and limonenes, can be used as antifeedant against insect pests. This study used the essential oil of *A. graveolens* in order to determine whether it has potential antifeedant bioactivity against *Cochlochila bullita* (lace bugs) on *Ocimum kilimandscharicum*.

Three mature leaves (fourth or fifth leaves from the bottom of a branch) of one *O. kilimandscharicum* were placed inside an 11 cm diameter petri dish, their stalks covered in sections of moist paper towel. This constituted one replicate. There were three replicates in total (Nair et al. 2012).

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The basil leaves on which the lace bugs were placed were exposed to a concentration of essential oil from *A. graveolens* through dipping on the essential oil solution for ten seconds and then air-dried, whereas the negative control only included water (Shukla et al. 2012).

The dishes were arranged in a randomized complete block design and placed under conditions of 27 ± 1°C , the temperature range of 19°C-33°C is suitable for the development of lace bugs in laboratory conditions (Ju et al. 2011), and a photoperiod of 14:10 (L:D) hours (Nair et al 2012). Observations on the number of frass spots on the leaves per treatment were conducted every six (6) hour intervals for two (2) days (JeniLapinangga et al. 2018). The petri dishes were labeled as "Ag - (replicate number) (ppm concentration)" to denote the replicate number and concentration to be used. The control group were labeled as "(Control Group) - (replicate number)".

After every 6-hour interval, the leaves were assessed for damage by counting the number of frass spots. Frass spot numbers are highly correlated with leaf damage and serves as an index for the amount of *C. bullita* feeding on the basil (Nair et al. 2012). The number of frass spots per leaves per treatment was analyzed using One-Way ANOVA. The means were separated using Fisher's protected least significant difference (LSD) test.

MICROSCOPY

OVERVIEW

This chapter presents a detailed description of the methods for the proper handling and use of various types of microscopes. Additionally, it provides a rundown of the techniques involved in preparing different types of microscopic samples before processing, as well as the criteria for their identification and classification. Included among the wide range of specimens under this category are blood cells, phytoplanktons, algae, pollen, parasites, bacterial colonies, nanoparticles, and microplastics.

A. Haemocytometer

Counting Hemocytes
(Yap, Bungay, Alfonso, Libo-on, Cordero)

Hemolymph samples were withdrawn from one shrimp randomly selected from each tank 0, 1, 3, 4, 24, 72, and 120 hours after immersion has commenced. The hemolymph mixtures taken were of the following ratios: 100uL hemolymph: 200uL buffered formalin solution; 300uL hemolymph: 300uL anticoagulant solution; and 100uL hemolymph: 200uL anticoagulant solution for total hemocyte count, phenoloxidase activity, and respiratory burst activity, respectively.

For total hemocyte count, 10 uL of hemolymph-buffered formalin mixture was pipetted into the space formed between Neubauer hemocytometer and coverslip while avoiding bubble formation. This was viewed under a compound light microscope for counting. The calculation of the total hemocyte count was based on the formula:

$$Total\ Haemocyte\ Count\ =\ \frac{(N \times D \times 10^3)}{A \times 10} cells \cdot mL^{-1};$$

where N = total number of cells counted; D = dilution of haemolymph; A = total area counted (in mm^2, 10^3 = conversion factor from uL to mL, 10 = conversion factor from mm^2 to uL).

Respiratory burst activity was assessed using the extracted 300 uL of hemolymph mixture (200uL hemolymph: 100uL anticoagulant). The mixture was centrifuged at 1000g for 10 min at room temperature. The supernatant was removed and 100 uL of HBSS was added.

The resulting solution was incubated for 30 min at 37°C. After which the sample was washed three times with HBSS, stained with 100 uL of 0.3% NBT solution, and incubated for 30 min at 37°C. Then, the NBT was removed before adding 100 uL of absolute methanol. The methanol was removed, and the solution was washed three times with 70% methanol. The pellet was air-dried for 5 min before adding 120 uL of 2M KOH and 140 uL DMSO. The solution was transferred into microtiter plate wells and the optical density was read at 620 nm using a microplate reader. 120 uL of 2M KOH and 140 uL DMSO was used as the blank control reaction for subtraction from the absorbances of the samples. The formula used for the final absorbance was:

$$A_s\ =\ A_o\ -\ A_b;$$

where A_s = final absorbance of the sample; Ao = observed absorbance of the sample; and A_b = absorbance of the blank sample (solvent).

Counting Phytoplankton Cells
(Cordova, Occeña, Demandante, Bela-ong)

The procedure for counting phytoplankton cells using a hemocytometer was adopted from Delta Environmental (2015). Each phytoplankton sample from the screw cap bottles was transferred to a 250-mL graduated cylinder and then covered with cling wrap. The samples were left undisturbed at a shaded area at room temperature for 24 hours, allowing the phytoplankton cells to settle at the bottom of the graduated cylinder. The top layer of the phytoplankton samples was removed leaving 30mL of the samples.

The remaining sample was then transferred to another screw cap bottle and was homogenized by manual mixing. Two drops of the concentrated sample were transferred to the counting chambers of the hemocytometer.

A compound light microscope (LW Scientific) with a mechanically adjustable stage was used for counting phytoplankton cells. Counting was done for the two counting chambers of the hemocytometer.

Drawings and pictures of phytoplankton were used to count and identify the species. Reference literature is listed in Appendix B. Each phytoplankton was identified down to at least the genus level and, whenever possible, the species level. Those that remained unidentified were given accession numbers and their illustrations were appended.

B. Algae Identification
(Yeban, Baranda, Antenor, Sinco)

Pure algal isolates from the final serial dilution were examined under a compound microscope under the low power objective (10x magnification) and high-power objective (40x magnification). The morphology of the suspected microalgae species was considered using several manuals (Hyun & Serediak 2011; Hyun & Serediak 2006; Belcher & Swale 1976; Silva et. al. n.d.) for the identification of the microalgal isolates. The preliminary identification, based on the image, measurements, and parameters of the microalgal growth, was verified by an expert.

C. Parasite Identification
(Lopez, Aguirre, Dalisay)

When all of the samples have undergone processing, each sample was examined using a microscope. Using a pipette, about one (1) ml from each test tube was dropped on a glass slide and then covered using a glass slip. Under the low power objective, each slide was examined using the traditional S-direction scanning. All eggs found were counted and recorded.

To identify the genus of the parasite, the morphology of each suspected egg was compared to actual parasitic eggs. See Table 1:

Table 1. *Standard Morphology of Eggs*

Parasitic Egg	Morphology
Dipylidium spp.	Refer to Appendix B.1
Taenia spp.	Refer to Appendix B.2
Toxocara spp.	Refer to Appendix B.3
Trichuris spp.	Refer to Appendix B.4
Ancylostoma spp.	Refer to Appendix B.5

D. Cell/Colony Morphology
(Halaba, Molinos, Superficial, Bela-ong)

Endophytic bacterial isolates were isolated from the roots of *Zea mays L. var. rugosa* (sweet corn) Sugar King variety at a vegetative stage. A total of eight different bacterial strains were isolated, characterized; through gram staining and colony and cell morphology; and screened for a positive reaction for nitrogen fixation, ammonia production, and zinc solubilization through the use of selective media. Jensen's media was used to screen for nitrogen-fixing bacteria, peptone water with Nessler's reagent for ammonia-producing bacteria, and zinc incorporated media for zinc solubilizing bacteria.

Three sets of three test tubes: labeled 10-1,10-2, and 10-3 respectively, were filled with 9 mL of 0.9% saline solution. One gram of the sample was added to the test tubes labeled 10-1.

Before the sample settled, one mL of the suspension was transferred into the test tube labeled 10-2 using a micropipette. The procedure was repeated once again to achieve a concentration of 10-3.

Mixed culture colonies were cultured to determine the colony morphologies of individual isolates. One drop of the diluted samples from the test tubes labeled10-3 was pipetted onto one side of an agar plate. Using an inoculating loop, heat sterilized using an alcohol lamp, the sample was quadrant streaked on the agar plate. The agar plates were then incubated for 24 hours at 37° C. The plates were upside-down during the incubation period to avoid the interference of condensation in the growth of the microbes. After the incubation period, the colony morphology of the microbial plates was observed. Those with the same morphology were classified as the same colonies before the isolation of pure cultures to avoid multiple cultures of the same bacteria.

Colony morphology was done during the observation times before and after the isolation of pure culture bacterial colonies to ensure that the colonies isolated were correct. The shape, margin, elevation, size, color, and texture of the bacterial colony were identified. This determined the groupings, based on the similarities of their characteristics, for the isolation of pure culture colonies.
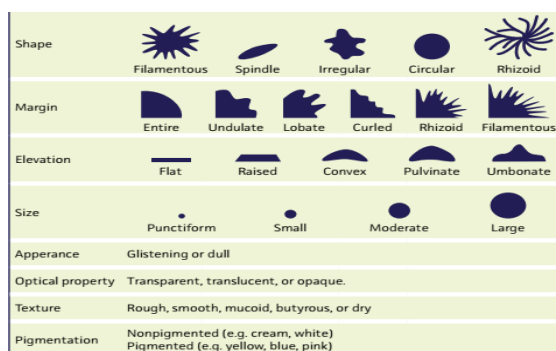
For the cell morphology, microscopy was conducted to determine the shape and arrangement of the bacterial cell.

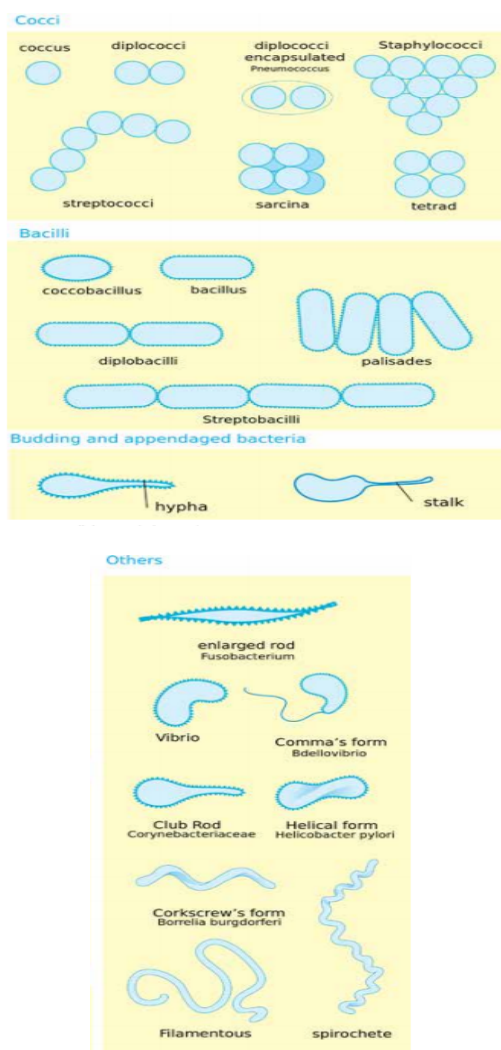**Figure 4.** *Cell morphology guide (ATTC 2006)*

Figure 3. *Colony morphology guide (ATTC 2006)*

E. **Atomic Force Microscopy**
(Socrates, Tang, Tionko, Bautista, Padernal)

Images of AgNPs deposited on a mica surface were taken to determine the size and observe the size distribution of the synthesized AgNPs at optimal conditions. For this purpose, a Shimadzu SPM-9700HT AFM was used in contact mode.

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F. **Microplastics Visual Inspection**
(Colacion, San Diego, Secondes, Oberio)

The microplastics of size ≤ 2 mm acquired after the extraction process were inspected using a compound inverted microscope at 40x magnification. Microplastics were identified based on the criteria provided by Norén (2007). They were also classified into four types according to their morphological type – fibers, films, fragments, and pellets based on the criteria provided by Free et al. (2014) and Frias et al. (2018).

Table 2. Criteria for the classification of microplastics based on definition (Free et al. 2014).

Definitions and potential sources of microplastic types.

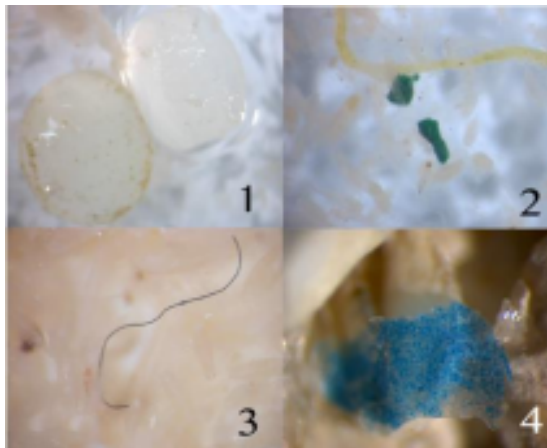| Microplastic type	Definition	Potential sources
Fragment	Hard, jagged plastic particle	Bottles; hard, sturdy plastics
Line/fiber	Thin or fibrous, straight plastic	Fishing line/nets; clothing or textiles
Pellet	Hard, rounded plastic particle	Virgin resin pellets; facial cleansers
Film	Thin plane of flimsy plastic	Plastics bags, wrappers, or sheeting

Figure 5. Criteria for the classification of microplastics based on photos of different microplastic types: (1) pellets, (2) fragments, (3) fiber, and (4) film under a stereomicroscope (Frias et al. 2018).

MOLECULAR BIOLOGY

OVERVIEW

This chapter involves methods from a descriptive study which includes the collection and preservation of samples, extraction of DNA, PCR amplification, gel electrophoresis, gene sequencing, and analysis of DNA samples using software and programs. The sample collection was done in Bagong Lipunan Market and Ivisan Market in Roxas City, Capiz, Philippines. DNA extraction, PCR amplification, and gel electrophoresis were conducted at Far Eastern University - Manila Molecular Laboratory.

A. DNA Extraction
(Areño, Cambel, Hilapad, Bela-ong, Castillo, Valdez)

DNA extraction of selected samples was performed using the Macherey-Nagel NucleoSpin Tissue kit (instructions included in the kit were followed).

B. Polymerase Chain Reaction
(Areño, Cambel, Hilapad, Bela-ong, Castillo, Valdez)

Extracted DNA was subjected to PCR amplification using the universal CO1 primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3').

Amplification was performed using the PCR mix consisting of 16.4 µL PCR grade water, 2.5 µL 10X PCR Buffer with 1.5 mM MgCl2, 2.5 µL 10mM DNTP, 0.5 µL Primer A, 0.5 µL Primer B, 0.10 µL Taq polymerase, 0.75 µL DMSO, 0.75 µL 3X BSA, and 1 µL DNA Template with a total reaction of 25 µL. PCRs were carried out by the following thermal regimen: 5 min at 95°C, then 35 cycles of 1 min at 94°C, 1 min at 43°C and 1:30 min at 72°C, followed by extension for 7 min at 72°C.

C. Agarose Gel Electrophoresis
(Areño, Cambel, Hilapad, Bela-ong, Castillo, Valdez)

Successfully amplified PCR products were subjected to gel electrophoresis to check the presence of DNA. The size and quality of PCR products were assessed in 1.5% agarose gel electrophoresis and stained with ethidium bromide.

D. DNA Sequencing (Areño, Cambel, Hilapad, Bela-ong, Castillo, Valdez)

Amplified PCR products were sent to the University of California, Berkeley for DNA sequencing.

E. Bioinformatics
(Areño, Cambel, Hilapad, Bela-ong, Castillo, Valdez)

All sequences were assembled in Geneious and aligned using MUSCLE in MEGA7. Each sequence was queried in BLAST for comparison of DNA sequences available in GenBank. Along with BLAST, BOLD was used to minimize the risk of using contaminated sequences. All identified species under BLAST search were checked on the IUCN red list of threatened species to identify endangered species.

For analysis of the base composition and visualization of the relationships among species included in the study, the software package MEGA7 was used. Phylogenetic analysis using the Neighbor-Joining (NJ) tree model was conducted. In addition, pairwise distances were also calculated along with the intraspecific and interspecific genetic divergences of the samples.

PLANT & ANIMAL CULTURE

OVERVIEW

This chapter includes the methods employed in culturing plant and animal specimens for the propagation of samples. Plant and animal cultures are grown in an artificial or natural environment and kept at favorable conditions for maintenance. In this process, it is important to consider growth factors (e.g. duration, temperature, pH), as well as the extraneous variables, that may affect the development and viability of the samples.

A. Cherry Tomato (*Solanum lycopersicum* var. *cerasiforme*)
(Pamati-an, Miraflores, Galino, Oberio)

Red Plum cherry tomato seeds, verified by the Department of Agriculture as true to the species that is named, as well as its hybrid and variety, were sown in an 8 in x 13 seedling tray using a soilless medium, Klasmann K TS1, which is mainly composed of white peat (FOREMOSTCO Inc. 2010). Two to three cherry tomato seeds were sown in each cell of the seedling tray, to a total of approximately 260 cherry tomato seeds sown in 104 cells. After 2 days, the seeds sprouted. The seedlings with the best growth attributes, indicated by the plant height, stem diameter, and the number of healthy true leaves were chosen for transplanting. Twenty-two (22) days after sowing the cherry tomato seeds, the seedlings produced true leaves. The seedlings were then transplanted to polyethylene pots with a diameter of 30 cm and height of 27.5 cm that were placed on pallets at the PSHS-WVC Academic Building rooftop.

B. *Allium* spp.
(Alogon, Horlador, Martinete, Mediodia)

Seeds were sterilized in 0.3% NaOCl solution for 5 minutes, washed thoroughly with sterilized distilled water three times, and transferred to Petri plates containing sterilized distilled water for germination.

After 8 days of germination in the dark at ambient temperature, plantlets were transferred to 9-cm-diameter petri plates with 5 mL 0.05M CaCl2 solution for further growth in the dark at ambient temperature, with each Petri plate having at most 100 plantlets.

C. Peanut Roots (*Arachis hypogaea*)
(Abellar, De Juan, Bela-ong)

Four peanut seeds were planted per pot, summing up to a total of 640 peanut seeds grown in 160 plastic pots. The pots were initially filled three-fourths of the way with rice hull compost soil, with the four peanuts seeds placed in a square orientation. An inch of soil was then added. These pots were situated in an area, wide enough for all pots to receive an equal amount of sunlight. These were grown in a controlled environment and given an equal amount of sunlight and water for 30 days before transferring half of the batch for UV exposure.

D. Onion Root Tips (*Allium cepa*)
(Abellar, De Juan, Bela-ong)

Fifteen commercial equal-sized *A. cepa* onion bulbs of 3 to 4 g were used. The bulbs were carefully unscaled, placed on top of beakers filled with tap water, and allowed to germinate at room temperature. After 48 hours, the onion bulbs were treated with 0.7% H_2O_2 for an hour. After the H_2O_2 treatment, onion bulbs with root tips which have grown up to 2-3 cm were washed for

an hour and were transferred to beakers containing the different treatments (mg/mL and 5 mg/mL crude ethanol extracts of both peanut plants exposed and unexposed to UV radiation, and tap water) for 72 h (Celik and Aslanturk, 2006). Assignation of bulbs to treatments was randomized.

E. Milkfish (*Chanos chanos*)

(Pasquin, Rodrigo, Villaluna, Aban)

Acquisition of *Chanos chanos* fingerlings was done with Southeast Asian Fisheries and Development Center (SEAFDEC) Region VI's affiliated institution, Dumangas Brackishwater Station (DBS). The fingerlings were placed in a 20-liter tank with dimensions of 43.18 x 22.86 x 27.94 (HxWxH) centimeters. They were then set to acclimatize for 48 hours under laboratory conditions. Water quality was maintained at a pH between 6 to 9, change in dissolved oxygen at 1 ppt to 3 ppt, and salinity at 0 ppt to 35 ppt. The *Chanos chanos* fingerlings were fed with commercial fish pellets daily and starved for 24 hours prior to and during the experiment. The water quality parameters (dissolved oxygen, pH, and temperature) were assessed using a PASCO PS-2230 water quality sensor, with an accuracy of ±0.6 mg/L right out of the box, provided by Philippine Science High School-Western Visayas Campus. Salinity was measured using an Original Equipment Manufacturer (OEM) brackish water salinity refractometer. The tests were done before and after the addition of extracts. Water quality was checked at 0, 1, 3, 6, 12, 24, 48, and 96 hours after the application of extracts.

F. Whiteleg Shrimp (*Litopenaeus vannamei*)

(Yap, Bungay, Alfonso, Libo-on, Cordero)

For the maintenance of the shrimp culture for the experiment, the seawater in the tanks was monitored with the following conditions: pH of 7.7 to 8.2, salinity of 33±1 ppt, temperature of 30±1°C, and constant aeration.

Commercial feeds were used and administered twice a day. Ten (10) shrimps were placed into separate 50 L tanks filled with 30 L of filtered seawater. Tanks used for each treatment (0, 100, and 200 mg/L hot-water extracts) were in triplicate.

G. Corn (*Zea mays L.*)

(Castañeda, Hallado, Lujan, Mediodia, Aguaras)

20-watt fluorescent bulbs were used and positioned 6 ft and 9 inches above the pots. Fluorescent light is an ideal source of artificial light for plants. A daily photoperiod of 14 hours was used as most facilities growing corn in a greenhouse use this duration of artificial light. Room temperature was kept at 25 C.

One corn seed was planted 2 inches deep into 2.5 kg of loamy soil in a 6 x 6 x 8 pot. The plants were watered every day with varying volumes depending on their growth stage requirements based on data. A 20% PEG-4000 solution was used to simulate drought conditions and was added at four-day intervals, while 0.23 g/ft^2 of potassium polyacrylate was added 15cm below the topsoil for water retention. Potassium Polyacrylate was not mixed with the soil to avoid breaking the polymers.

H. Corals (*Acropora spp.*)

(Dalabajan, Hilay, Velasco, Navarro, Olvido)

The branches were glued individually into cement cones with a diameter of 8 cm using an underwater epoxy. Marine epoxy was used because there are no recorded negative effects as coral adhesives and it is effective in attachment and transplant survival (Dizon et al. 2008). It is essential to plant the corals in order to simulate the natural conditions of the macroalgal-coral competition.

The treatments and control replicates (n=15 coral branches each) have separate metal racks. The metal racks were covered with a 1x1 cm grid metal screen to avoid other herbivores from entering. The racks were placed in designated areas around the island. Each of the coral branches within the

cement cones were interspersed 15-cm apart across the metal rack. As a means to minimize the extraneous variables and for easier measurement, there is a divider between each of the coral branches. The measurement of the whole metal rack is 115x46x50 cm (LxWxH). Three metal racks were used so that there would be 15 organisms for each experimental group for the statistical analysis.

I. Algae (*Chlorella sorokiniana*)
(Derramas, Gonzalez, Villaflor, Mediodia)

A culture of *Chlorella sorokiniana* was purchased from SEAFDEC, Tigbauan, Iloilo. The culture was sealed in a plastic bottle for transport to PSHS-WVC through an air-conditioned mode of transportation. It was then stored in a refrigerator at $3°C$ prior to usage to stabilize the algal cell concentration, which is accelerated by heat exposure. Twenty milliliters (20 mL) of *Chlorella sorokiniana* culture in exponential phase cultured in Conway medium was inoculated into each Erlenmeyer flask.

J. Lace Bugs (*Cochlochila bullita*)
(Bendicion, Dael, Genterola, Presno-Aban, Seredrica)

Anethum graveolens has been known to have insecticidal activity on several insect pests. Studies state that components of the essential oil of *Anethum graveolens*, which are mainly composed of carvones, and limonenes, can be used as antifeedants against insect pests. This study used the essential oil of *Anethum graveolens* in order to determine whether it has potential antifeedant bioactivity against *Cochlochila bullita* (lace bugs) on *Ocimum kilimandscharicum*.

Basil plants were procured from 3 Sunshine Garden, Tagbak, Jaro, Iloilo City. The basil plants were then transported to and raised in an improvised greenhouse created at Philippine Science High School -

Western Visayas Campus (10°45'10.1"N 122°35'15.9"E). For four days, the basil plants were watered every morning and afternoon. *Cochlochila bullita* colonies were collected from basil plants located at Orchard Valley, Pavia, Iloilo (10°46'09.7"N 122°33'03.8"E). Adult lace bugs were collected by cutting the entire basil leaves on which the lace bugs were situated using scissors and placed inside mega box containers which were covered with perforated cling wrap. The lace bugs were then transported to the greenhouse and transferred onto the basil leaves. The basil plants were transferred inside the laboratory in the Student Learning Resource Center Building at Philippine Science High School - Western Visayas Campus. The lace bugs were acclimatized inside the laboratory for two to three days (Rojht et al. 2009; Sathe 2014). Male and female adult lace bugs were collected from the basil plants for the antifeedant assay. As described by Sajap and Peng (2010), adult lace bugs were classified according to sex using a hand microscope.

K. Mosquitoes (*Aedes aegypti*)
(Carigaba, Leonida, Masculino, Mediodia, Garbo)

The *Aedes aegypti* larvae used in the study were cultured in the Department of Science and Technology - Industrial Technology Development Institute (DOST-ITDI) Entomology Section Insectary and were reared according to their standard procedures following the guidelines provided by the World Health Organization (WHO). The larvae were reared at a laboratory condition of $25 ± 2°C$ and relative humidity of $70\% ± 10\%$.

L. Bacteria from Plastic Waste
(Canja, Hilis, Galan, Jolito)

Plastics are known for being durable materials while still maintaining a low cost of production. It is a very important material for commercial use all over the world. However, due to the lack of a reliable method of disposal, the risk of plastic pollution is steadily increasing throughout the years.

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This study aims to isolate and extract bacteria from the Iloilo City Engineered Sanitary Landfill, in Mandurriao, Iloilo City, and to assess their biodegradation potential on LDPE (Low-density polyethylene), HDPE (High-density polyethylene), and PET (Polyethylene terephthalate).

Bacteria were sampled directly from swabbing the plastic as well as from the soil sample obtained from the site. The bacterial sample in the swabs was plated on nutrient agar (NA) medium using the streak method of inoculation. Three NA mediums were utilized for the growth of the bacteria. The plates were incubated at 30°C for 24-48 hours. Colonies with different morphological appearances were then sub-cultured onto fresh NA.

Four grams of the soil sample were suspended in 96 mL of sterile distilled water and shaken vigorously for 2 minutes. It was heated at 60°C for 60 minutes in a water bath. The mixture was put to rest to allow the soil particles to settle. It was plated on a nutrient agar using the streak method. Incubation was done at 30°C for 24 - 48 hours. Identification was done using morphological observation (Al-Humam, 2016).

Samples were then plated in selective media, eosin methylene blue agar (EMB) and mannitol salt agar (MSA), to further categorize the bacteria. Out of the ten plates, two were selected for incubation based on the most distinct colonies and were again cultivated in these selective media. Colonies were selected based on their morphological characteristics: form, elevation, and margin.

Plates 1-2. *Bacterial strains EMBP2A and MSAP2A for incubation.*

Figures 6-15. *The bacterial culture inside Petri dishes.*

PREPARATION

OVERVIEW

This chapter includes methods for preparing different types of solutions and organic materials before they are subject to further processing. This broad category covers methods in different levels of molecular complexity. Some methods employ stoichiometry in preparing solutions and some involve mechanically processing raw plant products.

A. **Substrates**
(Faciolan, Leonora, Majaducon, Sinco)

For the preparation of substrate mixture, the total mass of each fruiting bag was 750 grams, comprising 98 percent of the alternative substrate with 1 percent molasses and 1 percent lime. Each treatment had five replicates to account for mushroom mortality.

There are 7 treatments: (i) the control, (ii) 100% rice stalk, (iii) 100% rice bran, (iv) 100% rice husk, (v) 50% rice stalk and 50% sawdust, (vi) 50% rice bran and 50% sawdust, and (vii) 50% rice husk and 50% sawdust. The control was based on the substrate mixture ratio used by WESVIARC.

B. **Culture Media**

Mueller Hinton Broth
(Barrientos, Miraflores, Serisola)

The Mueller-Hinton broth was prepared by suspending 10.5 grams of the medium in 500 mL of distilled water. The mixture was then mixed and boiled over a hot plate then autoclaved for 15 minutes with a setting of 121°C and 15 psi.

The Mueller-Hinton agar was prepared in a media bottle by suspending 20 grams of the medium in 500 mL of distilled water. The mixture was then boiled over a hot plate, autoclaved for 15 minutes with a setting of 121°C and 15 psi. The cooled agar was poured into sterile petri dishes on a level, horizontal surface to give uniform depth.

Tryptic Soy Broth and Nutrient Broth
(Elizalde, Herida, Jaudian)

In a 500 mL media bottle, 9.9 grams of Tryptic Soy Broth powder was suspended in 330 mL distilled water. The concentration may vary based on manufacturer specifications.

The suspension was stirred using a stirring rod until the media powder had fully dissolved. The prepared broth was then evenly distributed to their respective test tubes using a glass pipette. Each test tube was capped using cotton balls, covered with aluminum foil, and gathered for sterilization. Lastly, the prepared media was autoclaved at an optimal temperature of 121°C and pressure of 15 psi for 15 mins, cooled, and stored in the refrigerator prior to usage.

Tryptic Soy Broth and Nutrient Broth
(Cabalfin, Gerona, Labrador, Remaneses, Mediodia)

The glasswares (petri plates, test tubes, micropipette tips, stirring rods) were autoclaved (Delixi LS-B35L) for 15 minutes at the optimal pressure of 15 psi. Using a 500 mL media bottle, 20 grams of Tryptic Soy Agar was mixed with 500 mL distilled water. The mixture was cooked in the hot plate (LMS HTS-1003) and continually stirred until the mixture cleared up. Using a separate 500 mL media bottle 2.4 grams of Nutrient Broth was suspended in 500 mL of distilled water. The solution was mixed using a sterile stirring rod until the solid was dissolved. Peptone water was prepared with 0.5 grams of Bacteriological Peptone, and 4.8 grams of Sodium Chloride. It was then suspended in 500 mL of distilled water in a 500 mL media bottle. The media was autoclaved for 15 minutes at 15 psi and was cooled down afterward. In four sterile test tubes, 15 mL of Nutrient Broth mixture was pipetted. Peptone water of 10 mL was pipetted into two test tubes. Nutrient agar (20-30 mL) was poured into each agar plate. After the agar has solidified it was stored in the refrigerator at 20C.

TCBS Agar
(Suarez, Apdon, Balinas, Baldonado, Hernando)

Thiosulfate-Citrate-Bile Salt-Sucrose (TCBS) Agar was prepared with the use of distilled water amounting to 400.00 mL to suspend 35.64 grams of the dehydrated agar in a media bottle. The mixture was then stirred on a hot plate until it was boiling and the dehydrated agar completely dissolved in the solvent. Once completely mixed, the agar solution did not undergo autoclaving as instructed but was cooled to 50°C and poured into sterilized Petri dishes. The agar plates were then left to cool overnight in the refrigerator to be preserved until they are used.

C. Stock Solution
Lemongrass
(Sira, Valzado, Larroder, Cabarles)

One kilogram of *Cymbopogon citratus* (lemongrass) leaves was washed with distilled water. The *C. citratus* leaves were then loaded into the juicer with 1 L of distilled water gradually being added at the same time. The produced aqueous solution was collected in a 1 L plastic container and then sealed and stored away from the sunlight until use in the preparation of serial dilutions.

Calamansi Essential Oil
(Carigaba, Leonida, Masculino, Mediodia, Garbo)

Two stock solutions of 10,000 ppm each (0.1 mL extract in 10 mL acetone and 0.1 mL in 10 mL 95% ethanol) were prepared and diluted in dechlorinated water as per the concentration used in the assay. Ethanol and acetone were used as solvents because the essential oil is not miscible in water, if applied directly.

Anethum graveolens essential oil
(Bendicion, Dael, Genterola, Presno-Aban, Seredrica)

Anethum graveolens has been known to have insecticidal activity on several insect pests. Studies state that components of the essential oil of *Anethum graveolens*, which are mainly composed of carvones, and limonenes, can be used as antifeedants against insect pests. This study used the essential oil of *Anethum graveolens* in order to determine whether it has potential antifeedant bioactivity against *Cochlochila bullita* (lace bugs) on *Ocimum kilimandscharicum*.

The essential oil of *Anethum graveolens* was acquired from Plant Therapy Essentials Oils Corporate, 510 2nd Ave S, Twin Falls, ID 83301, the United States of America through an online shopping site, Lazada PH, as a medium. The essential oil concentrations were diluted according to a previous study (Khosravi and Sendi 2013) in which 0.01, 0.02, 0.04, and 0.08 mL of essential oil were mixed in 0.4 milliliters of dimethyl sulfoxide (DMSO) inside a 100mL volumetric flask. Distilled water was added into the volumetric flask until the final volume of 100 mL was reached. This provided a 100 mL solution of 100, 200, 400, and 800 ppm concentrations of essential oil. A negative control group of distilled water was also prepared.

Chromium (VI) Stock Solution
(Faciolan, Leonora, Majaducon, Sinco)

Chromium in its hexavalent state, Cr^{6+}, is one of the prevalent heavy metals in aquatic ecosystems with its occurrence primarily attributed to industrial activities such as dye manufacturing and construction run-off. This paper presents the removal efficiency of organo-mineral composites from the shells of three mollusks abundant in the Philippines: *Crassostrea iredalei* (Slipper Cupped Oyster), *Perna viridis* (Green Shell), and *Telescopium telescopium* (Horned Snail).

A stock solution of 1000ppm Cr^{6+} solution was prepared by dissolving potassium dichromate ($K_2Cr_2O_7$) in distilled water (Baijnath et al. 2014; Onchoke and Sasu 2016). The dichromate ($Cr_2O_7^{2-}$) ion present in $K_2Cr_2O_7$ contains chromium in the hexavalent oxidation state (Cr^{6+}). The dichromate is being used instead of its trivalent counterpart, chromate (CrO_4^{2-}), in compliance with Method 7196A of the American Environmental Protection Agency, and the Standard Method for the Examination of Water and Wastewater followed by DOST-VI. The stock solution was subsequently diluted to the 100ppm standard solution from which 1, 5, 10, and 50ppm solutions were prepared.

D. **Treatment Solution**
(Derramas, Gonzalez, Villaflor, Mediodia)

Using a graduated cylinder, four liters of distilled water were measured and poured into a clean plastic container. After this, 57.3 mg of potassium dihydrogen phosphate (KH_2PO_4) was measured and mixed into the water via agitation to create the control (base) solution. One liter (1 L) of the KH_2PO_4 solution was then measured for each of the four treatments using a graduated cylinder and transferred into respectively labeled beakers. From the separated solution, 180 mL was measured using a graduated cylinder and was transferred into each of the five Erlenmeyer flasks designated to hold the replicates of the control setup with the treatment containing 0 mg of nitrogen. The remaining 350 mL of the solution was stored inside a beaker. This process was then repeated for the three other treatments wherein 5 mg, 10 mg, and 15 mg of nitrogen as potassium nitrate (KNO_3) was added respectively to the one liter of base solution for each treatment.

E. **Cadmium and Lead Metal Solution**
(Bandiola, Galotera, Sampiano, Mediodia)

Synthetic solutions with a concentration of 10 mg/L and 30 mg/L for lead and cadmium ions, respectively, were prepared from the following salts: $3CdSO_4 \cdot 8H_2O$ (cadmium sulfate octahydrate, Scharlau) and $Pb(NO_3)_2$ (lead nitrate, Farco) (Vera et al. 2019). Using the Shimadzu AUX220 (Min 0.1 g; Max 220 g) analytical balance, 60.44 mg of $3CdSO_4 \cdot 8H_2O$ and 47.95 mg of $Pb(NO_3)_2$ were weighed. The salts were then poured into three one-liter volumetric flasks. Deionized water was poured to mark into each of the volumetric flasks. The flasks were agitated until there were no visible solid salt particles in the solution. The three-liter heavy metal solution was transferred and stored in a four-liter HDPE bottle. A pH of 6.0 was obtained using 0.1 M of hydrochloric acid. A strip of pH paper with an accuracy of 1 pH was used to determine the pH of the solution prior to adsorption.

The salts (NaCl, KCl, and $CaCl_2$) were weighed using the Shimadzu Analytical Balance. For the different concentrations, the respective amount of salt needed was measured and dissolved in 250 mL of distilled water in separate beakers. The solution was then tested with the probe to test if the solution has achieved the desired salinity level. When the desired level was not obtained, the solution was manually diluted with distilled water or increased in salt concentration.

The salts were weighed using Shimadzu Analytical Balance (AUX220), located at the Biology Stockroom of PSHS-WVC. For the 4 dS/m treatment, 0.64 grams of each salt was weighed. For the 6 dS/m treatment, 0.512 grams of each salt was weighed. For the 8 dS/m treatment, 0.1024 grams of each salt was weighed. These values were scaled down according to the Electrical Conductivity formula. A spare plastic container acted as a tray for the weighing of the salt samples. A plastic spoon was used to transfer salt from the original container to the acting tray. The containers for the salt were closed immediately upon opening to prevent moisture from accumulating inside the container, which may have led to the contamination of the salt samples.

$$EC = TDS / 640 \text{ (at } 25°C); \text{ wherein}$$
$$EC = Electrical\ Conductivity$$
$$TDS = Total\ Dissolved\ Salts\ in\ mg/L$$

The salts were dissolved in 250 mL of distilled water in separate one-liter beakers using separate stirring rods. This adjustment in proportions was performed in order to avoid wastage of salt samples and distilled water. The solutions were then tested with the conductivity probe to check if the desired level of salinity was obtained. When the solution was not equal to the desired level, the solution was manually diluted with distilled water or increased in concentration.

Virus-Concentrated Solution
(Sira, Valzado, Larroder, Cabarles)

Freeze-dried live LaSota strain Newcastle disease virus vaccine was diluted using a vaccine solvent. The volume ratio of vaccine to solvent was modified by authorized personnel from the Department of Agriculture to assure a more virus-concentrated final solution.

F. Flavonoid Treatments
(Sombiro, Valdestamon, Aguirre Mediodia)

For the treatment with anthocyanin only and quercetin only, 0.0151g each of anthocyanin and quercetin was weighed on an analytical balance. The two flavonoid powders were then separately transferred to 50-mL beakers and mixed with 50 mL of dimethyl sulfoxide (DMSO). The solutions were stirred until the powders dissolved. Each solution was transferred to a one-liter beaker where it was diluted with distilled water until the 1-liter line was reached. The mixture was stirred.

For anthocyanin and quercetin, 0.0076 g each of anthocyanin and quercetin were combined and the same process was followed. All treatments were then transferred to reagent bottles and stored in a refrigerator for 24 hours at a normal refrigeration temperature,1.6 C.

G. Calcium Chloride Solution
(Florentino, Santos, Templonuevo)

Three varying calcium chloride concentrations were made at 1, 2, and 3 percent w/v concentrations. Calcium chloride weighing 2.5, 5.0, and 7.5 grams were separately mixed in distilled water in three 250mL volumetric flasks.

H. Sodium Alginate Solution
(Florentino, Santos, Templonuevo)

Three percent of sodium alginate solution was made by putting 1.5 grams of sodium alginate in a 50 mL conical tube. It was then initially added with a small amount of distilled water and was mixed using the vortex mixer and was diluted to mark. Continuous mixing was done after every addition of distilled water until it was fully mixed. The mixture was then left to settle overnight to allow it to dissolve completely.

I. Corn Stalks
(Rentoy, Angot, Mabaquiao, Larroder)

The corn stalks were removed from its dirt and leaves, and the outer sheaths were peeled to reduce impurities and to produce a smoother surface. The stalks were chopped, blended, and used immediately after. Using a top-loading balance set to 0.1 milligrams accuracy, 200 g of the blended corn stalks were weighed in preparation for the next procedure. The outer sheath remained intact in the first repetition, but since it resulted in a rough bioplastic sheet, in the end, the outer sheath was peeled off in the succeeding repetitions.

J. Viscose
(Rentoy, Angot, Mabaquiao, Larroder)

A sodium hydroxide solution was used in order to convert cellulose pulp into alkali cellulose. The solution was made by preparing a 500 mL solution of 18% hydroxide. Afterward, ninety grams of NaOH was added into a 500 mL volumetric flask filled to form the solution. The pulp was then poured into the beaker containing the solution then covered with aluminum foil and left to stand for 60 minutes. The solution was then transferred to a Buchner funnel which afterward the alkali cellulose was then hand pressed to remove as much solution as possible.

The alkali cellulose underwent xanthation which was done by adding 7.70g of carbon disulfide to the alkali cellulose in a one-liter media bottle and left to soak at $30°C$ for two hours. The bottle was rotated periodically to ensure uniform xanthation. The yellow pulp had turned a deep orange color afterward 240.28mL of cold water ($5°C$) and 48.56 g of 18% sodium hydroxide were added. The result was a viscous, thick, orange-colored liquid which was then stirred for two hours using an electric mixer. The "viscose" was then transferred into a clean media bottle for storage which was loosely sealed and stored at $5°C$ in a refrigerator for 24 hours.

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K. Bacterial Smears
(Triol, Dionela, Ecube, Mediodia)

Staphylococcus aureus, Escherichia coli, and a mixture of the two bacterial smears were prepared by placing a drop of normal saline solution on a glass slide and mixing it with a small colony of bacteria obtained from an agar slant tube using an inoculating loop which was obtained from the Biology Science Research Assistant (SRA) of Philippine Science High School - Western Visayas Campus (PSHS-WVC). The bacteria were then heat-fixed by having the glass slide pass over a flame. Seventy-five slides of *Staphylococcus aureus* bacterial smear, 75 slides of *Escherichia coli* bacterial smear, and 45 slides of mixed bacterial smear were prepared in a biosafety cabinet.

L. Pyrolysis
(Diaz, Golo, Villaluna, Aban)

Research regarding biochar remediation efficiency of excess nutrients such as phosphates is limited due to its low adsorption capacity. The study aimed to determine the potential of pineapple peel-derived biochar in adsorbing phosphates. Pineapple peel biochar was produced via pyrolysis at 300°C, 400°C, and 500°C, and then characterized using a Fourier Transform Infrared Radiation (FTIR) spectrometer.

The procedure for ash and biochar separation was acquired from the study of Wang et al. (2016). The resulting products from pyrolysis were immersed in 0.1 M HCl solution for 12 hours, washed with distilled water repeatedly, then oven-dried at 60°C for 12 hours. After cooling down to room temperature in a desiccator, the solids passed through a 100-mesh sieve to obtain the final biochar sample. The resulting biochar produced from each temperature were referred to as PP300, PP400, and PP500.

Pineapples were obtained from Leganes Public Market, Iloilo. The peels were thoroughly separated from the flesh by careful scraping using a knife. The peels were air-dried for 24 hours, then oven-dried at 70°C for 12 hours. The oven-dried peels were ground using a blender and passed through a 1 mm sieve. Five grams of pineapple peels were weighed using an analytical balance and were placed into ten constant-weighed ceramic crucibles for each pyrolysis temperature.

All crucibles used for pyrolysis were previously oven-dried at 110°C for 1 hour, placed inside a desiccator for 15 minutes, then weighed. Afterward, the crucibles were oven-dried again for 30 minutes at the same temperature, and then placed again inside the desiccator for 15 minutes, and finally weighed again. After weighing, the oven drying and the 15-minute cooling process were repeated until successive weighing was agreed to within ±0.0005 g.

The dried pineapple peels underwent slow pyrolysis and were incubated at the peak temperature (300°C, 400°C, 500°C) for 2 hours using a muffle furnace at the DOST- Region VI laboratory. Ten crucibles with a total of 50 grams of the sample were pyrolyzed for each temperature. After pyrolysis, the crucibles were then cooled to room temperature in a desiccator, then weighed using an analytical balance.

The procedure for ash and biochar separation was acquired from the study of Wang et al. (2016). The resulting products from pyrolysis were immersed in 0.1 M HCl solution for 12 hours, washed with distilled water repeatedly, then oven-dried at 60°C for 12 hours. After cooling down to room temperature in a desiccator, the solids passed through a 100-mesh sieve to obtain the final biochar sample. The resulting biochar produced from each temperature were referred to as PP300, PP400, and PP500.

M. Rice Husks and Mango Peels Absorbents
(Bandiola, Galotera, Sampiano, Mediodia)

One kilogram husks of *Oryza sativa* (rice) from Iloilo Central Market was initially washed with tap water in a colander and then with distilled water thrice by batches. After washing, the rice husks were evenly placed on a glass baking tray and oven-dried using the Binder ED53 laboratory oven for eight hours at 70°C to remove any moisture. It was then ground using the Oster household blender. Ten kilograms of ripe Philippine mangoes, *Mangifera indica,* ranging from class four to five on the ripeness chart (Nambi et al. 2015), was obtained from Iloilo Central Market.

The excess flesh on the mango peels was removed and the peels were sliced into small squares using a kitchen knife. The mango peels were initially washed with tap water in a colander by batch and then with distilled water thrice. The mango peels were then evenly placed on a glass baking tray with a skin-side down orientation to prevent curling of the mango peels when drying. They were then oven-dried for 72 hours at 70°C (Iqbal et al. 2009b). After drying, the dried mango peel adsorbents were placed inside a plastic container and were crushed manually. Both the adsorbents were sieved using the Hubbard Scientific Sieve Mesh Size 10 at a particle size larger than 2 mm. After sieving, the adsorbents were stored in separate air-tight containers at constant room temperature until the implementation of the column adsorption.

N. Seed Priming Agents
(Legurpa Mi, Legurpa Ma, Oberio)

Seed priming is a technique used to improve the overall germination behavior of rice through the imbibition of solutions. This study employed three different priming methods (mannitol, glycerol, and sorbitol) on two local Philippine varieties of *Oryza sativa* (rice) and compared germination behaviors between primed and unprimed seeds.

This study is an experimental study that investigated and compared the effect of three different priming agents (sorbitol, mannitol, and glycerol) with specific concentrations on two different local varieties of rice (red and black). Several germination parameters were used to compare the different setups in order to test the seed priming effect on seed germination.

The methods were provided by Mr. Stephen Timple from International Rice Research Institute (IRRI) taken from De Guzman 2018. This study was conducted at the second-floor laboratory of Philippine Science High School - Western Visayas Campus' SLRC Building. Three different Priming Agents were used in the study and one unprimed control for each variety. These concentrations below were taken from previous studies on seed priming (Theerakulpisut et al. 2017) (Debnath et al. 2017).

The table below shows the priming agent, what concentration was used, and their treatment numbers.

Table 3. Priming agents.

Priming Agent	Concentration	Treatment Number
mannitol	2% (Theerakulpisut et al. 2017)	1
glycerol	5% (Debnath et al. 2017)	2
sorbitol	0.25M (Theerakulpisut et al. 2017)	3

Twenty-five grams of mannitol was measured using an analytical balance (Shimadzu aux-220) and then dissolved in 500mL of water in a 2L beaker and stirred using a stirring rod in order to achieve the desired 2% concentration. A 100mL graduated cylinder was used to measure 25mL of glycerol and 475mL of water to create a 5% glycerol solution, in a 2L beaker, mixed using a stirring rod. The same process was repeated to achieve a 0.25M sorbitol solution with 15mL of sorbitol and 485mL of water.

During the priming process, the seed weight to solution volume ratio of 1:5 was used. 100g of seeds from each variety were soaked in 5% solution of glycerol for 24 hours, the same was done for mannitol and sorbitol. This was done in 2L beakers. Soaked seeds were then recovered from the solution, spread on a metal tray with paper towels and allowed to dry for 24 hours.

O. Oryza sativa (Rice)
Seed Priming
(Legurpa Mi, Legurpa Ma, Oberio)

Seed priming is a technique used to improve the overall germination behavior of rice through the imbibition of solutions. This study employed three different priming methods (mannitol, glycerol, and sorbitol) on two local Philippine varieties of *Oryza sativa* (rice) and compared germination behaviors between primed and unprimed seeds.

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250g of seeds of both Black and Red rice were obtained from a local farmer. The priming agents; mannitol, glycerol and sorbitol, were all obtained from Patagonian Enterprises. The beakers, stirring rods, Petri dishes, flasks and graduated cylinders were all from PSHS WVC's Chemistry Supply.

Seeds were tested for their moisture content at Western Visayas Agricultural Research Center. A grain moisture tester (Riceter f511) was used to measure the moisture content. A small number of seeds were placed into the test chamber and then the handle was turned to crush the grain and the moisture content was instantly provided.

Salt Treatment Setups
(Geroche, Sombiro, Villegas, Olvido, Patricio)

Salinity is one of the leading causes of crop yield loss worldwide. Harmful cations and anions are present in the soil as seawater intrusion is the main cause of soil salinization in the Philippines. With this, the study focused on the effect of three types of salts: NaCl, KCl, and $CaCl_2$ on the germination stage of rice.

The NSIC Rc 442 rice variety seeds that were used for the study had a moisture content (MC) of 13.4%, which fell within the desired MC range of 8% to 14%. The seeds and their certification were obtained from WESVIARC.

Pre-germinated seeds were separated between pure and impure seeds. Fifty seeds of rice were randomLy placed in 9cm-diameter Petri dishes. Twenty milliliters of each solution were added to the Petri dishes. Distilled water was used for the control set-up.

P. *Bixa orellana*
(Venturina, Comuelo, Samaniego)

Extracts from plants such as Bixa orellana, commonly known as Annatto, present the potential to be an alternative to the commonly used synthetic stains. Thus, this study aimed to evaluate Bixa orellana methanolic extracts as a substitute for safranin in Gram Staining. Bixa orellana extracts were used to stain *S. aureus, E.coli,* and mixed bacteria smears.

The seeds were separated by hand then washed with distilled water and sterilized in a hot air oven at 60°C for 24 hours (Braide et al. 2010). The seeds were then powdered using a grinder.

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SAFETY PROTOCOLS

OVERVIEW

When handling biological material, researchers must be cautious of the possible presence of parasite species of varying infectious stages. To avoid ingestion, proper personal protective equipment (PPE) must be used throughout all stages. These typically include laboratory gowns, surgical gloves, and surgical masks, but may vary depending on the type of material being handled. Finally, young researchers must always remember to properly disinfect articles of clothing, and thoroughly wash their hands after each handling of these samples.

A. **Fecal Samples**
(Lopez, Aguirre, Dalisay)

Segregation of waste materials was followed thoroughly. All materials that were used were labeled. Ideal proper laboratory attire was worn. Laboratory gown, pair of gloves, and masks were used during the entire duration of the study. All glassware was handled with care. All glassware were rinsed three times into the waste container before they were cleaned. Extreme caution was followed when handling the chemicals. Glasswares were stored in a safe place to avoid scratches and breakage. After every experiment, the glasswares were cleaned and dried as soon as possible prior to storing. All the glassware used were placed on a pot full of water and were left for boiling after using them. The glassware were then rinsed with tap water twice, they were first rinsed with liquid detergent and then rinsed for the second time with distilled water. Glasswares were then placed upside down in a tray for drying. Uncontaminated materials and contaminated materials were labeled. Then when dry, the materials were ready for storage or for disposal. Glasswares contaminated with biological material were emptied and decontaminated by boiling. They were rinsed thoroughly with tap water, drained, and air-dried.

SCREENING

OVERVIEW

Screening is one of the most reliable methods for gathering data from phenomena occurring beyond the microscopic scale. A variety of reagents, procedures, and samples can be utilized to cater to different scientific investigations. This chapter provides a brief description of the different methods of screening for chemical and biochemical studies.

A. Antibacterial
(Elizalde, Herida, Jaudian)

A 10-fold serial dilution was performed in order to dilute the extract in the prepared broths into 8 different concentrations using a micropipette. The bacteria samples were then inoculated into the treated test tubes using a micropipette with 100 µm each. One test-tube for every trial was reserved for normal growth without any extract present as a negative control. The test tubes were then incubated for 18-24 hours under an optimal temperature of 30°C. The turbidity of each test tube was measured using a UV-2100 spectrophotometer with a wavelength of 600 µm.

B. Phytochemical
(Andonaque, Dorado, Ledesma)

The antioxidant activity of Lansium domesticum seed extracts was determined by phytochemical screening, adopting and modifying methods from Tiwali, et. al. (2011). After drying, crushing, and sifting, Lansium domesticum seeds were added to amber reagent bottles containing hexane, methanol, and ethyl acetate, respectively, with a solvent to dry weight ratio of 5:1. The presence of phenols was tested by adding 3-4 drops of 5% ferric chloride solution to 1 mL of the stock solution, with a change in color to brownish-black indicating a positive result. Flavonoids were tested by adding 10% sodium hydroxide solution dropwise into 1 mL of the stock solution in a test tube. At the appearance of an intense yellow color, 5N hydrochloric acid was then added dropwise until the solution turned colorless, indicating the presence of flavonoids. For saponin testing, one mL of the stock solution was placed in a graduated cylinder, diluted with 20 mL of distilled water, and manually agitated for 15 minutes. The formation of a foam layer roughly 1 cm thick at the top indicated the presence of saponins. Alkaloid testing was done by adding 5-15 drops of Wagner's reagent into 1 mL of stock solution in a test tube. The formation of a brown precipitate indicated the presence of alkaloids.

C. Anthocyanin (Triol, Dionela, Ecube, Mediodia)

The ethanolic extract of *Clitoria ternatea* was tested for the presence of anthocyanin following the procedure from the book entitled "A Guidebook To Plant Screening: Phytochemical and Biological" edited by Guevara (2005). Three milliliters of the ethanolic extract were added with five drops of 1% (v/v) hydrochloric acid (HCl) and was subjected to boiling. A change from the original color of the extract to an orange-red to blue-red color upon addition of HCl was used as an indicator for the presence of anthocyanin in the extract.

D. Nitrogen Fixation (Halaba, Molinos, Superficial, Bela-ong)

Endophytic bacterial isolates were isolated from the roots of Zea mays L. var. rugosa (sweet corn) Sugar King variety at the vegetative stage. A total of eight different bacterial strains were isolated, characterized; through gram staining and colony and cell morphology; and screened for a positive reaction for nitrogen fixation, ammonia production, and zinc solubilization through the use of selective media. Jensen's media was used to screen for nitrogen-fixing bacteria, peptone water with Nessler's reagent for ammonia-producing bacteria, and zinc-incorporated media for zinc solubilizing bacteria.

Bromothymol blue was used as an indicator for the screening of nitrogen-fixing bacteria. The reagent was provided by the Chemistry SRA of PSHS-WVC.

Following the procedures for the making of Jensen's media by Richard et al., 20 grams of sucrose, one gram of dipotassium phosphate, 0.500g of magnesium sulfate, 0.500g of sodium chloride, 0.100g of ferrous sulfate, two grams

of calcium carbonate and 15g of agar were suspended with 1 L of distilled water in a culture bottle. The mixture was then boiled using a hot plate and continuously stirred using a sterile glass stirring rod. After boiling, the solution was sterilized in an autoclave at 121° C for 15 minutes. The solution was then allowed to cool down for 3-4 minutes before being distributed into sterile agar plates. The plates were filled until the bottoms were fully covered and allowed to cool and solidify inside the biosafety cabinet. The plates were then labeled and stored at 2-8° C.

Nitrogen-free malate agar (Jensen's media) was used in the screening for nitrogen fixation with bromothymol blue (BTB) acting as an indicator (Gothwal et al. 2007). The isolates were then incubated at 37°C for 24 hours. Isolates that exhibit nitrogen fixation will provide nitrogen that the media lacks and will change its color from creamy white to blue.

    E.   Ammonia Production
        (Halaba, Molinos, Superficial,
        Bela-ong)

Endophytic bacterial isolates were isolated from the roots of Zea mays L. var. rugosa (sweet corn) Sugar King variety at the vegetative stage. A total of eight different bacterial strains were isolated, characterized; through gram staining and colony and cell morphology; and screened for a positive reaction for nitrogen fixation, ammonia production, and zinc solubilization through the use of selective media. Jensen's media was used to screen for nitrogen-fixing bacteria, peptone water with Nessler's reagent for ammonia-producing bacteria, and zinc-incorporated media for zinc solubilizing bacteria.

The guidelines reported by HiMedia were followed in the preparation of peptone water. In a culture bottle, 10g of peptone and 5g of sodium chloride were suspended with 1 L of distilled water. The mixture was then boiled using a hot plate and continuously stirred using a sterile glass stirring rod. After boiling, the solution was autoclaved for sterilization at 121° C for 15 minutes. Then, 16 test tubes were each filled with 10 mL of peptone water solution while the remaining were left inside the culture bottle. Both were then labeled and stored at 2-8° C.

The guidelines reported by HiMedia were followed in the preparation of peptone water. Inside a culture bottle, 10g of mercuric chloride, 7g of potassium iodide,

16g of sodium hydroxide were suspended and mixed with 100 mL of distilled water. The mixture was then stirred continuously using a sterile glass stirring rod. The solution was then allowed to cool down before being stored in the refrigerator at 2-8° C.

Isolates were inoculated into 10 mL peptone water in separate test tubes then incubated for 2-3 days at 28±2 degrees Celsius. After the addition of 0.5 mL of Nessler's reagent, isolates that exhibit ammonia production will cause the water to discolor due to the sudden addition of ammonia to the solution.

    F.   Zinc Solubilization
        (Halaba, Molinos, Superficial,
        Bela-ong)

Endophytic bacterial isolates were isolated from the roots of Zea mays L. var. rugosa (sweet corn) Sugar King variety at the vegetative stage. A total of eight different bacterial strains were isolated, characterized; through gram staining and colony and cell morphology; and screened for a positive reaction for nitrogen fixation, ammonia production, and zinc solubilization through the use of selective media. Jensen's media was used to screen for nitrogen-fixing bacteria, peptone water with Nessler's reagent for ammonia-producing bacteria, and zinc incorporated media for zinc solubilizing bacteria.

Following the procedures for the making of zinc chloride medium by Kamran et al., 0.5g of zinc chloride was suspended with 500 mL of TSA. The mixture was then boiled using a hot plate and continuously stirred using a sterile glass stirring rod. After boiling, the solution was autoclaved for sterilization at 121°C for 15 minutes. The solution was then allowed to cool down for 3-4 minutes before being distributed into sterile agar plates. The plates were filled until the bottoms were fully covered and allowed to cool and solidify inside the biosafety cabinet. The plates were then labeled and stored at 2-8° C.

Zinc chloride (ZnCl2) medium plates were used in the screening for zinc solubilization. The isolates were aseptically inoculated as spot on the respective medium plates and covered with aluminum foil. They were then incubated in the dark at 28° C for 1 day. Isolates exhibiting zinc solubilization formed clear zones around the bacterial colonies due to the bacteria breaking down the zinc present in the media into simpler forms.

SELECTION

OVERVIEW

This chapter discusses the selection criteria for different aspects of research studies. It includes the selection of study sites and of animal subjects.

A. Study Site

Site for Dog Feces Collection
(Lopez, Aguirre, Dalisay)

Iloilo is one of the four (4) provinces in Panay Island located in Region VI Western Visayas of the Philippines to which its capital is Iloilo City. Currently, there are seven (7) districts in Iloilo City which are Arevalo, Iloilo City Proper, Jaro, La Paz, La Puz, Mandurriao, and Molo. Based on the 2010 Census on Population and Housing (CPH), the current population of Iloilo City is 424,169. According to a study conducted by Robinson et al. 1996, the human to dog ratio in the Philippines is one to three (1:3). This means that for every dog, there are three (3) humans accompanying it. Based on this data, there should be about 142,000 dogs in Iloilo City. Among these districts only one (1) district was selected. The number of barangays was considered due to the limited time frame given for the study. Added with safety reasons, the district of Arevalo was selected as the district to be the study site. It is located 10.6859 N, 122.5118 E having 13 barangays which are Bonifacio, Calaparan, Dulonan, Mohon, Quezon, San Jose, Santa Cruz, Santa Filomina, Santo Domingo, Santo Nio Norte, Santo Nio Sur, Sooc, and Yulo Drive.

Site for Collection of Microplastics
(Colacion, San Diego, Secondes, Oberio)

Anilao, Iloilo was selected as the coastal community to investigate due to its large impact on the province's seafood industry, as well as its accessibility to the researchers. Three (3) coastal areas in the municipality, specifically Barangays Dangula-an (10°58'58.2" N 122°46'44.2" E), Pantalan (10°57'5" N 122°45'52" E), and San Carlos (10°58'48" N 122°46'42" E) were selected as sampling sites based on the fish-ing and mariculture activities in the areas. The sediment sample collection was conducted during low tide in the coastal areas. A transect of length 100 m was placed along the high tide line of each coastal area. Three quadrats of dimension 0.25 m x 0.25 m were then randomly placed along the 100 m stretch. A metal trowel was used to collect sand to a depth of approximately 2 cm from the surface. The obtained samples were placed inside a glass container with a metallic lid, stored inside an ice chest, and transferred to the PSHS-WVC Research Laboratory. A total of three (3) samples per site were collected.

B. Domestic Dogs
(Lopez, Aguirre, Dalisay)

Selection criteria for the dogs included the presence of leashed dogs in households and consent from the owners to collect samples. The fecal samples needed to be approximately one (1) to three (3) days old. Information such as the age, breed, diet, and medical treatment such as vaccination and deworming of the dogs were also taken. After one household was selected we selected the next household approximately 10 households away from the first one to avoid the auto infections of parasites in dogs.

STORAGE

OVERVIEW

Storage is the action of preserving samples for future use. This section deals with the storage and preservation of human platelets. Though some samples take a while to degrade, always ensure that the length of storage does not compromise the quality of the sample. It is always recommended to work on the sample upon harvest/preparation unless otherwise required for the study.

A. Platelet Storage and Preservation
(Alvarez, Oberio)

CPDA-1 has been added during blood extraction (contained in the blood bag) during blood extraction at around 63 mL per blood unit to preserve the platelets and prevent coagulation. The samples were stored at a volume of 20 mL at 20-24oC with constant gentle agitation in their respective satellite bags. Three replicates were prepared, and each replicate consisted of the three setups: l-carnitine (positive control), saline (negative control), and ALCAR.

The chosen concentration for the preservatives used in the study was 15 mM based on a pilot study of Deyhim et al. (2014) in determining the best concentration and volume of l-carnitine in preserving PCs.

L-carnitine solution was prepared by dissolving the l-carnitine powder in sterile, normal saline at a concentration of 15 mM.

A volume of 1 mL l-carnitine solution was added to the positive control setup (Deyhim et al. 2014). ALCAR was prepared and added to the variable setup the same way as l-carnitine. The preservatives were added to the PCs one day after blood extraction.

One mL of l-carnitine and ALCAR each were introduced into the platelet bag via aseptic infusion one day after the extraction. Insulin syringes were used after filtering the preservative solution through a 0.22 µm filter. Sterile normal saline was used to dissolve the l-carnitine and ALCAR powders. As a control, an equal volume of 1 mL of saline was also added to the third setup. The site of puncture was sealed and a stripper was used to mix the preservative with the blood bag contents. A biosafety cabinet level II located in a nearby hospital from the site of storage was used. Transportation of samples was done using an approved Styrofoam box and was returned after the application of preservatives.

TREATMENT

OVERVIEW

In experimental studies, specific treatments are administered to experimental units to determine their effects, usually to establish whether the treatment is effective or is at par with current standard treatments. This category covers methods in different forms of treatment administered to various experimental units. Some methods employ manipulation of main treatment components, while some manipulate only the concentration of the same treatment. Other methods focus on the manipulation of storage conditions.

A. Virus-infected Samples
(Sira, Valzado, Larroder, Cabarles)

Infected samples were drenched using 1 mL syringes with aqueous extracts from the *C. citratus* plant every afternoon. Groups from vaccinated samples vaccinated against Newcastle's Disease (ND) were labeled as Group VA (vaccinated, group A), Group VB (vaccinated, group B), Group VC (vaccinated, group C), and Group VD (vaccinated, group D). Groups from the unvaccinated samples were labeled as Group UA (unvaccinated, group A), Group UB (unvaccinated, group B), Group UC (unvaccinated, group C), and Group UD (unvaccinated, group D). Samples from groups VA and UA were administered with aqueous extract dosed at 1 mL extract, groups VB and UB with 1 mL extract/5 mL, groups VC and UC with 1 mL extract/10 mL, and groups VD and UD with 1 mL extract/20 mL. Survival rate and live weight gain were measured 14 days after the start of treatment. Survival rate was computed by treatment group using the equation (total number of chickens - number of deaths)total number of chickens) x 100. With regards to the side effects of the phytochemicals on chickens, the study of Raza et al. (2015) showed that plants containing alkaloids, flavonoids, saponins, and tannins that were tested against the ND virus in vitro and in vivo exhibited positive results and did not show any adverse reactions on chickens.

B. Light Cycle for Algal Cultures
(Derramas, Gonzalez, Villaflor, Mediodia)

Twenty milliliters (20 mL) of Chlorella sorokiniana culture in exponential phase cultured in Conway medium was inoculated into each Erlenmeyer flask. The flasks were linearly arranged in a ventilated, isolated room. The cultures were subjected to light exposure with a 20-watt fluorescent lights distance of 40.64 cm (16 in) away from the flasks on a light/dark regimen of 14/10 h simulation of the day and night light exposure cycle (Patel et al. 2012). After submerging the connected tubing into the filled flasks, the water aerator, set on low, was switched on to induce agitation of the medium by continuously bubbling the air in the flasks. These conditions were maintained for the entire duration of the observation period.

The setup was monitored for nine days. The lights were switched on and off at 6:26 AM and 8:26 PM, respectively. This simulated a 14/10-hour day and night cycle.

C. Seed Priming
(Legurpa Mi, Legurpa Ma, Oberio)

Seed priming is a technique used to improve the overall germination behavior of rice through the imbibition of solutions. This study employed three different priming methods (mannitol, glycerol, and sorbitol) on two local Philippine varieties of Oryza sativa (rice) and compared germination behaviors between primed and unprimed seeds.

The priming agents mannitol, glycerol, and sorbitol were prepared. During the priming process, the seed weight to solution volume ratio of 1:5 was used. 100g of seeds from each variety were soaked in a 5% solution of glycerol for 24 hours, the same was done for mannitol and sorbitol. This was done in 2L beakers. Soaked seeds were then recovered from the solution, spread on a metal tray with paper towels, and allowed to dry for 24 hours. After that seeds were brought to WESVIARC to have their moisture contents measured following the same methods as the first test.

D. Germination
   (Geroche, Sombiro, Villegas, Olvido, Patricio)

Salinity is one of the leading causes of crop yield loss worldwide. Harmful cations and anions are present in the soil as seawater intrusion is the main cause of soil salinization in the Philippines. With this, the study focused on the effect of three types of salts: sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride (CaCl2) on the germination stage of rice.

Each researcher was assigned to a table, in which they were responsible for counting the number of germinated seeds daily, performing maintenance, and properly managing the set-up when it was needed.

The germination rate was recorded for every petri dish daily for ten consecutive days. Counting began at 4:30 PM daily.

Ambient temperature and relative humidity were measured using the LabdiscEnviro daily for 10 consecutive days. Ambient factors were gathered at 8:00 AM, 12:00 PM, and 4:00 PM daily.

The leaf color of the seeds was measured by matching the leaves of the best seeds on a Leaf Color Chart produced by the Philippine Rice Research Institute (PhilRice) acquired from the Department of Agriculture- LGU Oton.

Ten of the best seedlings per petri dish were chosen to represent the petri dish. Shoot and root length were measured using a plastic Vernier caliper with a precision of 1mm. The germinated seed's shoot was laid flat beside the Vernier caliper, and the measurement was taken. The same process was repeated for the roots. In the case of the roots, the longest root was chosen as the main root for measurement.

The 10 best seedlings used for the length of the roots and shoots were also utilized for the weight of the roots and shoots. The roots and shoots were separated from the seedlings using tweezers and laid on tissue paper. The roots and shoots were then patted dry and stored for measurement. The roots and shoots were weighed using Shimadzu Analytical Balance (AUX220), and a plastic petri dish cover was used as a tray for the samples. The roots and shoots were weighed within two hours upon being separated from the seedling.

The germination rate (or periodic germination percentage) provides a measure of the time course of seed germination. It was measured daily, and its unit of measurement is in percent (%). Its formula is as given below:

$$Germination\ Rate = \frac{GR_0 - GR_1}{50} \times 100$$

Equation 1. Formula for calculating Germination Rate

Where GR_0 = Total number of germinated seeds today and GR_1 = Total number of germinated seeds yesterday

The germination percentage was recorded after the 10th day. Germination percentage expresses the proportion of the total number of seeds that are alive. A seed is considered germinated if its main radicle has reached a length of 2 mm (Vibhuti et al. 2015). Good seeds have more than an 80% germination rate. The rate of germination is an indicator of vigor. The formula for germination percentage is as given below (Vibhuti et al. 2015).

$$Germination\ Percentage = \frac{No.\ of\ total\ germinated\ sees}{Total\ no.\ of\ tested\ seeds} \times$$

Equation 2. Formula for Germination Percentage

A one-way Analysis of Variance (ANOVA) at a 95% confidence interval (α = 0.05) was used to evaluate whether a significant difference exists between the germination percentage, lengths, and weights of both shoots and roots, in relation to the type of salt and the level of concentration utilized. This test was performed using Statistical Packages for the Social Sciences (SPSS) version 23.

CHEMISTRY

DEGRADATION

OVERVIEW

Degradation reduces a product into its components through physical and/or chemical means. The methods outlined in this section are from studies interested in the degradation of plastic. Provided are the methods for organic enzymatic degradation and photodegradation by irradiation. Both induce a chemical response from the object of interest. Substances have varied responses to different degrading agents; hence, ensuring compatibility between the agent and the substance of interest is vital in drawing a favorable result.

A. Photodegradation

M. oleifera sp.

(Bastareche, Catolico, Secular, Larroder)

Forty mL of 0.03 molar concentration (M) silver nitrate was measured. The silver nitrate was stirred at 400 rotations per minute (rpm). Then, ten mL of *M. oleifera sp.* seed extract were slowly dropped into the silver nitrate using a pipette. The same procedure was also done with the synthesis of the 10-g and 15-g *M. oleifera sp.* seed extract.

LDPE films

(Agno, Gilongos, Jalandoni, Sinco)

Before light exposure, each of the 12 LDPE films was labeled with the following: TNP-UV (treated with undoped titanium dioxide nanoparticles under UV light), TNP-VL (treated with undoped titanium dioxide nanoparticles under visible light), N-TNP-UV (treated with nitrogen-doped titanium dioxide nanoparticles under UV light), and N-TNP-VL (treated with nitrogen-doped titanium dioxide nanoparticles under visible light), for a total of four experimental set-ups with three replicates each. The LDPE films were initially placed in their respective 250 mL beakers containing 150 mL of 20mM TNP and N-TNP aqueous suspensions (Kim et al. 2003). It was ensured that the tested surface of LDPE films was exposed to the suspension at all times.

Two sets of beakers containing the samples were enclosed in a 24" x 8" x 6" wooden box. The first set, which contained three replicates of TNP or N-TNP treated set-ups, was constantly irradiated using a UV- A light (18W, <320nm primary wavelength), and the second set, also containing three replicates of TNP or N-TNP treated set-ups, with a light-emitting diode (LED) bulb (9W that is equivalent to 18W-fluorescent lamp, 400-700 nm wavelength) at a 10 cm distance from the base of the beaker (Ali et al. 2016). The photodegradation process was carried out for 336 hours. During this period, the set-ups were stored in ambient air and room temperature (Tofa et al. 2018). After irradiation, LDPE films were thoroughly rinsed with distilled water.

EVALUATION

OVERVIEW

Products, whether resulting from or used for research, undergo an evaluation to ensure stability, effectivity, and safety. This section provides the evaluating methods from various studies as it relates to products such as hydrogel, bioplastic, antibacterial soap, and biodiesel. Further, parameters for oil adsorption, antioxidant property, and a makeshift reaction chamber are included. Aside from the final product, the proprietary of some parameters also requires evaluation to ensure a controlled environment throughout the study.

A. **Bioplastic Sheets**
(Rentoy, Angot, Mabaquiao, Larroder)

The first and the fourth repetitions were successful in producing solid sheets that could be tested. The first batch was tested on its mechanical properties, particularly the density, percent elongation, and tensile strength as these were the only available tests in Central Philippine University - Packaging Engineering. The fourth batch was tested of its chemical composition using the FT-IR Spectroscopy in Philippine Science High School Western Visayas Campus. All the tests were compared with commercial cellophane.

B. **Oil Adsorption**
(Janiya, Lopez, Magtoles)

The initial weight of the samples was recorded with the use of an analytical balance. The samples were submerged in crude oil for 30 minutes. Then after the adsorption process, the samples were removed with a strainer and then weighed. The amount of oil adsorbed was calculated using the formula by Tolba et al (2011):

$$qe = (\frac{Wo - We}{M})$$

Where:

q_e = amount of oil adsorbed per unit weight of adsorbent
w_o = initial weight of oil (g)
w_e = weight of oil sample after the adsorption process (g)
M = mass of adsorbent (g)

C. **Device**
(Gurrea, Peregrino, Regalado, Salvador)

For added information, the fluid dynamics of the bubbling CO_2 may also be calculated based on the data. Utilizing 20L of CO_2 over 14.56stranslates to 1.37L/s of CO_2 being bubbled into the solution. Using the Bernoulli equation one can also derive its velocity which is equal to 4.84m/s and its pressure which is 23.24Pa or 0.003 PSI. This means that only as much as 0.003 PSI back pressure from cars is necessary to facilitate the mineral carbonation process. This shows that 1.37L/s of gas is very doable in practical as well as experimental situations since it generates only minimal backpressure.

Assuming a 1:1 ratio (i.e. a 100percent efficiency) approximately 39.32g of CO_2 will necessitate 68.87g of $Ca(OH)_2$. $Ca(OH)_2$ was set as the excess reactant and spread evenly amongst the 3 chambers and 24g of CO_2 was added per chamber for a total of 72g. Furthermore, based on previous studies, an optimal concentration of 30 ppm or 0.09g of Nickel nanoparticles were also added into the solution, evenly spread amongst the 3 chambers.

As a result of the reaction of $Ca(OH)_2$ and CO_2, $CaCO_3$ was expected to precipitate at the bottom of the chambers. The contents of each chamber were then drained, filtered and washed, oven-dried, and weighed. The precipitates were washed with distilled water while being filtered to ensure the purity of the $CaCO_3$. This was then oven-dried at 60ºC overnight. These were done to determine the amount of $CaCO_3$ precipitate present in the solution.

D. **Physiochemical Properties**

(Hembra, Henderin, Pareñas, Sinco)

Leaves of Mangifera indica (mango) contain phytochemicals that promote antibacterial activity. This study aimed to determine whether *M. indica* leaves extract can be an alternative antibacterial agent for triclosan.

The formulated soap was subjected to multiple tests for its physicochemical properties as described below.

Measuring the pH of soap is significant in determining whether the formulated solution is corrosive to the skin or not (Vivian et al. 2014). The pH test was done in triplicates and was based on the standard protocol recommended by American Oil Chemists' Society (AOCS) (1997). Using buffer solutions of pH 4.00, 7.00, and 10.00, the EUTECH pH700 pH meter was calibrated. After calibration, the probe was dipped into 10% solutions of each of the formulated soaps. The obtained pH value was then recorded.

The density of liquid soap is influenced by the amount and molecular weight of its components (Handrayani et al. 2015). The density of each of the soap solutions was determined by first preparing 10mL of each soap solution and measuring each of the masses of the prepared solutions using the Sartorius top loading balance. The obtained mass was divided to 10mL to calculate the density.

Foam stability is dependent on the properties of the surfactant (Osei-Bonsu et al. 2015). For the assessment of foam stability, 10mL of each soap solution was measured and then mixed in a vortex for five (5) minutes. The foam height produced was measured using a ruler with +0.5mm precision. After allowing the solution to stand for 5 minutes, the foam height was again measured. Foam Stability was measured using the formula (Handrayani et al. 2015):

$$\text{Foam Stability} = \frac{\text{Final Foam Height}}{\text{Initial Foam Height}} \times 100$$

Equation 3. The equation was used to calculate the foam height.

Similar to pH, free caustic alkali is also responsible for the abrasiveness of the soap and is used to determine if the product is hypoallergenic or not. For the determination of free caustic alkali, 5g of each soap solution was measured and then dissolved in 30mL ethanol. After dissolution, three (3) drops of phenolphthalein indicator and 10mL of 20% barium chloride (BaCl2) were added into each of the solutions. After thorough mixing, each solution was then titrated with 0.05M H2SO4. Free caustic alkali was determined using the formula by Vivian et al. (2014):

$$FCA = \frac{0.31}{W} \times VA$$

Where W is the weight of the soap, and VA is the volume of H_2SO_4.

E. **Biodiesel Properties**

(Almarza, Gatila, Inosanto)

Sixteen biodiesel properties were calculated using the FAME composition of each species. Fuel properties derived from FAME profiles are the following: degree of unsaturation (DU), long-chain saturation factor (LCSF), cold filter plugging point (CFPP), iodine value (IV), saponification value (SV), cetane numbers 1 and 2 (CN1 and CN2), saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), kinematic viscosity, density, higher heating value (HHV), amount of C18:3, number of double bonds (Db), and oxidation stability.

The FAME profile of the seaweed species involved is the direct result of the analysis conducted by the GC-MS equipment. These data are used to calculate and predict the values of the parameters using their respective formula under the biodiesel standards EN 14214 and ASTM D6751-02, which will determine the biodiesel fuel quality of the organisms.

FABRICATION

OVERVIEW

Fabrication involves the design and manufacturing of a product or object. The methods in this section provide the proposed process of production and specifications for sugarcane bagasse-based mesh and bioplastic sheets. Written here are experimental methods aimed at the production of the aforementioned products. Fabrication is a rigorous process requiring multiple trials - time is an essential factor in identifying points for improvement.

A. Mesh
(Bayer, Farinas, Navarra, Yturralde)

The mesh was created by using sterile gauze as the base. The base was used because of the bagasse fibers' short length. Then, the epoxy was beforehand diluted with 100 percent denatured alcohol to effectively spread the epoxy between the fibers. The solution was then added to the mesh to hold the fibers. The mesh is then left to dry overnight. The bagasse meshes were treated with concentrated sulfuric acid for 30 minutes and then were kept in an oven at 150C for 24 hrs to activate the adsorbent.

B. Bioplastic Sheets
(Rentoy, Angot, Mabaquiao, Larroder)

The cellophane casting stage is when the viscose is poured onto the glass slides and bathed into the chemical baths of 40% ammonium sulfate and 12% sulfuric acid-18% sodium sulfate. In a 250-mL beaker, 200 g of ammonium sulfate was weighed to create 500 mL of 40% solution of ammonium sulfate. Subsequently, the sulfuric acid-sodium sulfate bath was made by adding 60 mL of sulfuric acid into a one-liter graduated cylinder. After sulfuric acid, 90 g of sodium sulfate was added. The solution produced was a 12% sulfuric acid-18% sodium sulfate solution. The two baths, ammonium sulfate, and sulfuric acid-sodium sulfate baths were heated over a hot plate up to 45◦C.

The sheet of bioplastic was prepared by spreading a thin layer of viscose on the 1x3x8 inch glass plate using a rubber spatula. The viscose was allowed to coagulate by immersing the sheets in an ammonium sulfate bath (45◦C) for 60 seconds. After the immersion, the coagulated sheet that was still on the plate was immersed in a sulfuric acid-sodium sulfate bath (45◦C) for two minutes.

Upon contact, the yellow sheets gradually turned white and a bubbling effect was observed. The cellulose-based bioplastic films were then soaked in hot distilled water (80◦C) for 10 minutes. To obtain a more flexible bioplastic, the bioplastic sheets were treated with a 5% glycerin solution for 15 minutes. The bioplastic sheets were flipped during the glycerin treatment to allow even plasticizing. The sheets were air-dried for three days at room temperature.

In the first repetition, the viscose was spread onto the inside of the glass slides, which surrounds the viscose with walls. Sheets were successfully made. In the fourth repetition, the chemical used for the first bath was ferrous ammonium sulfate instead of ammonium sulfate. Moreover, the underside of the glass plates which had no walls surrounding was used instead of casting the sheets inside the glass plates with walls. The sheets were then immersed into pans filled with coagulating and regenerating reagents. Sheets were successfully made. In repetitions three and five, the sheets would tear at every attempt to remove them from the glass since they adhered to the surface of the glass plate. The films were not appropriate for testing since they would tear easily and the sizes of the film were too small.

METHODS OF ANALYSIS

OVERVIEW

Chemical analysis is the process of quantifying and/or identifying a chemical species. This section provides broad-scope methods in analyzing Chromium (VI) and phosphates. Each species may require different methods to be accurately measured. There are various considerations in this type of analysis such as the oxidation state of the target species, phase of the substance, and the target data, e.g. quantity, concentration, etc.

A. Chromium (VI)
(Faciolan, Leonora, Majaducon, Sinco)

Chromium in its hexavalent state, Cr^{6+}, is one of the prevalent heavy metals in aquatic ecosystems with its occurrence primarily attributed to industrial activities such as dye manufacturing and construction run-off. This paper presents the removal efficiency of organo-mineral composites from the shells of three mollusks abundant in the Philippines: *Crassostrea iredalei* (Slipper Cupped Oyster), *Perna viridis* (Green Shell), and *Telescopium telescopium* (Horned Snail).

A fresh 10ppm Cr^{6+} solution was prepared for treatment. Prior to the start of treatment, the initial absorbance and pH of the stock solution were measured using Shimadzu UV-vis Spectrophotometry and a pH meter, respectively. The pH of the solution was acidified with 1M HCl to a pH level of at most 3. This was to prevent the reduction of Cr^{6+} ions to Cr^{3+} which occurs upon the addition of bases—which in this case are the mollusks' shells themselves (Sanchez-Hacchair and Hofmaan 2018). Blanks were prepared using distilled water with the same mass of shells as the treatments.

Three set-ups—composed of triplicates for each shell powder treatment, and a blank— were used for the duration of the treatment phase. Mollusk shell samples were introduced into the Cr^{6+} stock solution by adding 1.0 mg of the crushed powder to 100 mL of the solution per replicate (Abeynaike et al. 2011; Baijnath et al. 2014).

The same amount of shell powder was added to 100 mL of each blank. Scilogex SK-L180-Pro laboratory shaker was used to agitate the mixtures at 100rpm for 20 minutes (Weerasooriyagedra and Kumar 2018; Zhou et al. 2019), after which, the final absorbance and pH of each replicate were measured.

The calibration curve was used to convert absorbance data to concentration, expressed in parts per million (ppm) Cr^{6+}. The concentration of Cr^{6+} removed was obtained by subtracting the final

concentrations of the stock solution and the blank replicate to the initial concentration of the 10ppm Cr^{6+} stock solution.

Adsorption efficiency (Q) for the three species was calculated using Equation 4:

$$Q = \frac{C_0 - C_1}{C_0} \times 100$$

Equation 4. Formula for adsorption efficiency (Q)

where C_0 is the initial concentration of Cr^{6+} and C_1 is the resulting concentration following treatment.

B. Ascorbic Acid Method (For Phosphates)
(Derramas, Gonzales, Villaflor, Mediodia)

Fifteen milliliters (15 mL) of 36 N concentrated sulfuric acid (H_2SO_4) was diluted to 108 mL to form 5 N sulfuric acid. A solution of potassium antimonyl tartrate ($K_2Sb_2(C_4H_2O_6)_2$) was made through dissolving 1.3716 g of the compound in 400 mL of distilled water in a beaker. Twenty

grams (20 g) of ammonium molybdate $((NH_4)_9MoO_4)$ was also dissolved in 500 mL of distilled water. A solution of ascorbic acid was prepared through mixing 1.76 g of ascorbic acid to 100 mL of distilled water in another beaker. A combined reagent was made through mixing 50 mL of 5 N sulfuric acid, 5 mL of potassium antimonyl tartrate solution, 15 mL of the ammonium molybdate solution, and 30 mL of the ascorbic acid solution for a total of 100 mL of combined reagent that was used for the chemical analysis of the samples (Clesceri et al. 1992).

The ascorbic acid method was utilized to dye the samples a blue color proportional to the phosphorus concentration, making the samples analyzable via UV-visual spectrophotometry, a colorimetric method of analysis. Potassium antimonyl tartrate and ammonium molybdate, under the acidic conditions set by sulfuric acid, creates a chemical complex, which when reduced by the addition of ascorbic acid, dyes the samples. For each sample, 50 mL of the solution was pipetted into an Erlenmeyer flask. One drop of phenolphthalein indicator was added. If a red color developed, 5 N H_2SO_4 was added drop by drop to discharge the color. Eight milliliters (8 mL) of the combined reagent was added to the samples and mixed thoroughly. This process was repeated for each replicate for all treatments.

C. [Phosphate Adsorption of Biochar (Stannous Chloride Method)](#)

(Diaz, Golo, Villaluna, Presno-Aban)

Research regarding biochar remediation efficiency of excess nutrients such as phosphates is limited due to its low adsorption capacity. The study aimed to determine the potential of pineapple peel-derived biochar in adsorbing phosphates. Pineapple peel biochar was produced via pyrolysis at 300 °C, 400 °C, and 500 °C, and then characterized using a Fourier Transform Infrared Radiation (FTIR) spectrometer.

then oven-dried at 60° C for 12 hours. After cooling down to room temperature in a desiccator, the solids passed through a 100-mesh sieve to obtain the final biochar sample. The resulting biochar produced from each temperature were referred to as PP300, PP400, and PP500.

Three concentrations (5 ppm, 15 ppm, 25 ppm) of monopotassium dihydrogen phosphate (KH_2PO_4) solutions were prepared by dissolving respective amounts (5 mg, 15 mg, 25mg) of KH_2PO_4 in 1 liter distilled water. All glassware used was washed using 1:1 HCl and rinsed with distilled water to prevent contamination of other chemicals (APHA 1992).

In each 250 mL Erlenmeyer flask, 150 mL of phosphate solutions were placed with a total of 45 flasks with 15 flasks for each phosphate concentration (5 ppm, 15 ppm, 25 ppm). Out of the 15 flasks, 5 flasks were each added with 0.3 g of biochar pyrolyzed at the same temperature (300° C, 400° C, 500° C), and each flask was agitated for 24 hours at 200 rpm using a mechanical shaker (Yao et al. 2011). This process was repeated for all three types of biochar obtained from different pyrolysis temperatures, and for all three concentrations.

The agitated phosphate solutions were first filtered with Whattman 150 mm filter paper. Afterward, 0.45 μm nylon membrane filters which were soaked in 2 liters of distilled water for 24 hours, were used to further filtrate the remaining contaminants (APHA 1992).

The phosphate content of the treated solutions was determined using the stannous chloride acid method (APHA 1992). In a volumetric flask, 25 g of ammonium molybdate $((NH_4)_6Mo_7O_{24}\text{-}4H_2O)$ was dissolved in 175 mL distilled water. In another volumetric flask, 280 mL of concentrated H_2SO_4 was added to 400 mL distilled water. After the solution was cooled to room temperature, the H2SO4 was added to the ammonium molybdate solution and diluted to 1 L and was then labeled "Ammonium Molybdate Solution".

In a 100 mL beaker, 2.5 g fresh stannous chloride ($SnCl_2$ - $2H_2$) was mixed with 100 mL glycerol. It was heated using a hot plate and stirred using a glass rod to hasten dissolution. The reagent is stable and requires neither preservatives nor special storage. It was labeled "$SnCl_2$ Solution".

OPTIMIZATION

OVERVIEW

Optimization is the process of maximizing or minimizing a study parameter to make the best or most effective use out of a particular resource. This section includes methods that involve separating mixtures using a separatory funnel and a rotary evaporator. These may be used by young researchers aiming to optimize the methanolic mixtures of aloe gel and aloe latex with rind.

A. **Aloe Gel**
(Alcalde, Tajo, Valencia)
The aloe gel was mixed with 100 mL hexane and 100 mL methanol and placed in the separatory funnel. The mixture was shaken to hasten the distribution of solutes. The layered mixture was separated using the separatory funnel. The methanolic extract was placed in a rotary evaporator. A more concentrated aloe gel and the methanolic mixture were then retrieved from the rotary evaporator.

B. **Aloe Latex with Rind**
(Alcalde, Tajo, Valencia)
The aloe latex powder was dissolved in 100 mL hexane. The solution was mixed with 100 mL methanol and placed in the separatory funnel. The mixture was shaken. The mixture of two immiscible solutions was separated using a separatory funnel. The methanolic solution containing the aloe latex and rind was placed in a rotary evaporator. A more concentrated aloe latex, rind, and methanolic mixture were then retrieved from the rotary evaporator.

bit.ly/PamaagiChem

PRODUCTION

OVERVIEW

Production is the process of making something from raw materials. This section provides a method for the production of materials required for the implementation of the study. Specifically, this method may be used in producing carbon dioxide to trigger a reaction that would be necessary to evaluate a new device.

A. **Carbon Dioxide**
(Gurrea, Peregrino, Regalado)

To evaluate the device, CO_2 was necessary for the reaction to take place and hence the purchasing of a CO_2 tank. A makeshift regulator was made by using adapters, tees, hose, and hose clamps. An -in brass adapter for the CO_2 tank was fitted. This was then connected to an -in stainless steel tee. The other two junctions of the tee were connected to a pressure gauge and a customized hose nozzle. The customized hose nozzle was made by welding a to -in adapter and -in to -in hose nozzle. Thus, the resulting customized hose nozzle had -in socket and -in hose. A -in hose was then connected in the hose nozzle and was fastened by the hose clamp. Teflon was also wrapped between the adapters to ensure that no gas would leak out.

SPECTROPHOTOMETRY

OVERVIEW

Spectrophotometry is an experimental technique used in several quantitative analyses in various fields. It measures the amount of chemicals in a solution or the amount of absorbed light based on the principle of compounds absorbing or transmitting light over a particular range of wavelengths. This section provides spectrophotometric methods using various instruments namely Ultraviolet-Visible (UV-Vis) spectrophotometer, Fourier-Transform Infrared (FTIR) Spectroscopy, Microwave Plasma - Atomic Emission, and Has Chromatography - Mass Spectrometry for various studies.

A. UV-Visible

Absorbance of Chromium(VI) Solutions
(Faciolan, Leonora, Majaducon, Sinco)

Chromium in its hexavalent state, Cr^{6+}, is one of the prevalent heavy metals in aquatic ecosystems with its occurrence primarily attributed to industrial activities such as dye manufacturing and construction run-off. This paper presents the removal efficiency of organo-mineral composites from the shells of three mollusks abundant in the Philippines: Crassostrea iredalei (Slipper Cupped Oyster), Perna viridis (Green Shell), and Telescopium telescopium (Horned Snail).

The stock solution was subsequently diluted to the 100 ppm standard solution from which 1, 5, 10, and 50 ppm solutions were prepared from. This is observing the validity range for the linearity of the Beer-Lambert Law (Sanchez-Hacchair and Hofmaan 2018). The spectrum absorptions of the standard solutions were measured via the Shimadzu UV-visible Spectrophotometer from 600 to 200 nm, as prescribed by Onchoke and Sasu (2016). A line of best fit relating the absorption to concentration in parts per million (ppm) was then modeled in Microsoft Excel.

Phosphate Analysis
(Diaz, Golo, Villaluna, Presno-Aban)

Research regarding biochar remediation efficiency of excess nutrients such as phosphates is limited due to its low adsorption capacity.

The study aimed to determine the potential of pineapple peel derived biochar in adsorbing phosphates. Pineapple peel biochar was produced via pyrolysis at 300 °C, 400 °C, and 500 °C, and then characterized using a Fourier Transform Infrared Radiation (FTIR) spectrometer.

The procedure for ash and biochar separation was acquired from the study of Wang et al. (2016). The resulting products from pyrolysis were immersed in 0.1 M HCl solution for 12 hours, washed with distilled water repeatedly, then oven-dried at 60° C for 12 hours. After cooling down to room temperature in a desiccator, the solids passed through a 100-mesh sieve to obtain the final biochar sample. The resulting biochar produced from each temperature were referred to as PP300, PP400, and PP500.

The phosphate analysis was conducted by batches of five to maximize the capacity of the Shimadzu Ultraviolet–visible (UV-Vis) spectrophotometer. A volume of 25 mL of the phosphate solution samples were placed in 50-mL Erlenmeyer flasks. One (1) mL of the ammonium molybdate solution was added and mixed. Then, two drops of the stannous chloride solution was added and swirled. The flask was manually shaken several times to mix the solution. After waiting between 5 to 15 minutes, samples were transferred to cuvettes, and absorbance of samples were measured using the UV-Vis spectrophotometer at a wavelength of 650 nm (APHA 1992).

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A calibration curve (y = 0.0658571x+ 0.174952) was prepared using six phosphate solutions of known concentrations ranging from a blank sample to 25 ppm, with an increment of 5 ppm. The absorbance value for each concentration was measured at a wavelength of 650 nm. The values were then recorded and plotted. An absorbance versus concentration graph was constructed by making the absorbance values as the y-axis and the concentrations as the x-axis. The absorbance of the standard concentrations were plotted on the graph. A "best fit" line was drawn through the points using the free online application Desmos Graphing Calculator™. The concentration of the phosphate solution samples were interpreted using the graph.
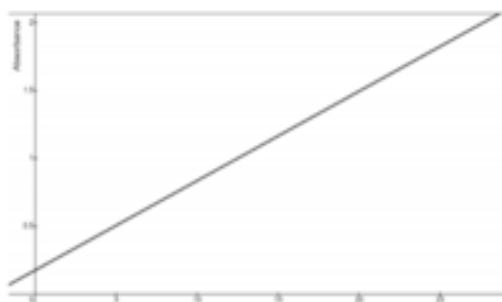
Figure 16. *Concentration versus absorbance calibration curve.*

Absorbance of *Chlorella sorokiniana* culture samples

(Derramas, Gonzalez, Villaflor, Mediodia)

After 20 minutes of exposure of the treatment solutions to the ascorbic acid method reagents, the absorbance of each sample was measured at 880 nm, using distilled water as a blank solution for reference. For every round of analysis, the cuvette was first rinsed with distilled water and then with the analyte solution. After this, it was filled to mark with the analyte solution. After properly inserting the cuvette to be analyzed into the UV-visual spectrophotometer, the absorbance of the sample was measured. This was repeated for every replicate. After the individual analysis of the samples per replicate, the absorbance

of the Conway medium content added to the samples was analyzed and then subtracted from the sample absorbances, providing the values for the phosphorus remaining in the samples, disregarding the phosphorus content of that of the Conway medium.

$A_{actual} = A_{sample} - A_{Conway}$

Where: A_{sample} = *absorbance of sample*

A_{Conway} = *absorbance of Conway medium*

A_{actual} = *absorbance of sample disregarding Conway*

Characterization of Copper-Chitosan Complexes
(Loquias, Placido, Mediodia)

The synthesized copper-chitosan complexes were characterized in terms of Ultraviolet-Visible Spectra (Surface Plasmon Resonance) using a UV-1800 Shimadzu UV Spectrophotometer (Ultraviolet-Visible Spectrophotometry).

Determination of AgNP Absorbance/Yield
(Socrates, Tang, Tionko, Bautista, Padernal)

The reaction mixture samples were studied at a wavelength of 300 to 500 nm using a Shimadzu UV-1800 Ultraviolet-visible spectrophotometer. The absorbance at λ_{max} (~400-420 nm) for each sample was obtained from the spectra. The yield or concentration of NPs in the NP solution is proportional to the absorbance at λ_{max} following Beer-Lambert's Law (Paramelle et al. 2014):

Yield (in M) = A/Lε (1)

where A is the absorbance at λ_{max}, L is the path length = 1 cm and ε is the extinction coefficient expressed in $M^{-1} cm^{-1}$.

This yield can also be expressed in number of particles per unit volume by multiplying the yield (in M) to the Avogadro's number (N_A):

Yield (in particles/L) = Yield (in M) x N_A (2)

B. Fourier-transform infrared spectroscopy (FTIR)

Characterization of Pineapple Peel Biochar
(Diaz, Golo, Villaluna, Presno-Aban)

Research regarding biochar remediation efficiency of excess nutrients such as phosphates is limited due to its low adsorption capacity. The study aimed to determine the potential of pineapple peel derived biochar in adsorbing phosphates. Pineapple peel biochar was produced via pyrolysis at 300 °C, 400 °C, and 500 °C, and then characterized using a Fourier Transform Infrared Radiation (FTIR) spectrometer.

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The Attenuated Total Reflection (ATR) method for solids was used in order to identify functional groups using the Fourier Transform Infrared (FTIR) spectroscopy. Two grams of each biochar sample with different pyrolysis temperatures (300, 400, 500) were prepared for the analysis through powderizing the biochar using mortar and pestle. The pre-installed computer program for the test was set to identify functional groups within the 4000 to 500 cm−1 spectrum, but other preset parameters for the analysis were not changed. After the prior preparations, the samples were placed under the ZnSe crystal. After the samples were fixed for the analysis, the program was run to collect 256 scans to obtain the FTIR graph from the sample using transmittance (%). The results for each sorbent were saved and were named according to their pyrolysis level (PP300, PP400, PP500) (Wang et al. 2016).

Characterization of Copper-Chitosan Complexes
(Loquias, Placido, Mediodia)

The synthesized copper-chitosan complexes were characterized in terms of Absorbance Spectra using an IRAffinity-1S Shimadzu Fourier Transform Infrared (FTIR) Spectrophotometer.

Characterization of LDPE Films
(Agno, Gilongos, Jalandoni, Sinco)

Before the start of the exposure under ultraviolet and visible light, LDPE films were characterized using Fourier Transform Infrared (FTIR) Spectroscopy. After irradiation to ultraviolet and visible light, the LDPE films were subjected again to FTIR spectroscopy (Shimadzu IRAffinity-1). A wave range of 4000-400 cm^{-1} was used. The vibration peaks were recorded and analyzed for the chemical transformation of the films and to determine the functional groups present in the film (Ashraf 2014). The degree of chemical changes in the LDPE films in terms of its carbonyl and vinyl indices was then determined.

Microplastics Analysis
(Colacion, San Diego, Secondes, Oberio)

Petri dishes containing the obtained microplastics of size ≤2 mm from each sampling location were sent to Advanced Device and Materials Testing Laboratory (ADMATEL) in Taguig City, Metro Manila for analysis. The Perkin Elmer FTIR Spectrometer Frontier ATR FTIR model was used to determine the chemical composition of the identified microplastics. One representative microplastic piece from each sample was selected and subjected to the analysis. The microplastic particles to be analyzed were selected based on whether they can be manually handled for the procedure. The instrument was set to reflection mode with a 4000-600 cm-1 range with 20 scans at 8 cm-1 resolution. A spectra library linked to the ATR-FTIR was used to determine the identity of the acquired sample spectra.

C. Gas Chromatography- Mass Spectrometry

Analysis of Fatty Acid Composition
(Almarza, Gatila, Inosanto)

The samples for lipid analysis were stored in an icebox and transported to the laboratory of the College of Fisheries and Ocean Sciences department of University of the Philippines - Visayas (UPV) in Miag-ao, Iloilo where it was subjected to pretreatment for Chromatographic analysis. The lipids underwent derivation using 14% BF3 as reagent in order to produce Fatty Acid Methyl Esters, afterwards these lipids were to be injected in the Gas Chromatography with Mass Spectrometry (GC-MS). The process was done with the aid of the laboratory technician of UPV.

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The pretreated samples were injected in the Gas Chromatography with Mass Spectrometry (GC-MS) equipment for analysis of FAMEs. The complete procedure was done using GC: Clarus 600 Gas Chromatograph; MS: Clarus 600 T-Mass Spectrometer of UPV.

Calamansi Peel Essential Oil
(Carigaba, Leonida, Masculino, Mediodia, Garbo)

Twenty (20) mL of the calamansi peel essential oil was submitted to the Department of Science and Technology - Industrial Technology Development Institute (DOST-ITDI) Standards and Testing Division Organic Chemistry Section and was subjected to Gas Chromatography Test for Limonene in order to determine the limonene content present in the product.

SYNTHESIS

OVERVIEW

To synthesize is to produce a compound by letting simpler materials react with each other. This section focuses on the synthesis of various compounds. Specifically, the following methods will help young researchers to form nanoparticles, calcium alginate-based beads, hydrogels, chitosan-hydroxyapatite composites, copper-chitosan complexes, and liquid antibacterial soaps.

A.   Nanoparticles

Nickel
(Gurrea, Peregrino, Regalado, Salvador)

The chemical reduction method will be applied in the synthesis of Nickel Nanoparticles. First, 7.132 g of nickel chloride hexahydrate ($NiCl_2 \cdot 6H2O$) was dissolved in 30 mL distilled water in a 250mL beaker. After which, 16 g of sodium citrate dihydrate ($C_6H_5NaO_7 \cdot 2H_2O$) was added to the solution to act as a capping agent. The prepared solution was then placed in a water bath where it was heated (at 40°C) and magnetically stirred (at 400 rpm) for one hour. During the one-hour stirring time, a separate solution of 2.27 g sodium borohydride ($NaBH_4$) was prepared to act as a reducing agent.

This concentration corresponds to a 2:1 molar ratio of $NaBH_4$ to $NiCl_2 \cdot 6H_2O$ (Nayak et al.). The $NaBH_4$ solution was kept for temporary storage in a sealed 50 mL volumetric flask at room temperature while waiting for the $C_6H_5NaO_7$ and $NiCl_2 \cdot 6H_2O$. After an hour of stirring, the $NaBH_2$ solution was then added dropwise to the $NiCl_2 \cdot 6H_2O$ and sodium citrate solution for 10 minutes while continuously stirring at 400 rpm (at 180°C). The solution turned black, which indicated the reduction of the nickel ions. After the addition of $NaBH_4$, the temperature of the hot plate was decreased to 80°C and left constant for two hours to allow the reaction to complete.

The resulting solution was filtered using filter paper and alternately washed thrice with distilled water and ethanol. To prevent excess moisture that would contaminate the nickel nanoparticles, it was dried using a hot air oven at 80°C for one hour.

The weight of the produced nickel nanoparticles was recorded. Characterization techniques were not done for the synthesis of the NiNPs since this method has already been confirmed to work via transmission electron microscopy from the researchers' previous studies.

Silver
(Dogeno, Gamboa, Pefianco, Aban, Larroder)

40 mL of 0.03 molar concentration (M) silver nitrate was measured. The silver nitrate was stirred at 400 rotations per minute (rpm). Then, ten mL of *M. oleifera* sp. seed extract was slowly dropped into the silver nitrate using a pipette. The same procedure was also done with the synthesis of the 10-g and 15-g *M. oleifera* sp. seed extract.

Silver
(Socrates, Tang, Tiongko, Bautista, Padernal)

An 80-mL solution of 6.0×10^{-4} M ascorbic acid and 3.0×10^{-3} M trisodium citrate was stirred for five minutes at 100°C. Then, 15 mL of the silver nitrate ($AgNO_3$) solution of desired concentration was poured into the previous solution. The resulting solution was kept heated and stirred at 900 rpm using a magnetic stirrer. All experiments utilized the same process; however, variables were varied according to the values presented in Table 4.

The pH, time, and concentration values were converted to their coded factors (-1, 0, +1). Coding reduces the range of each factor to a common scale, -1 to +1, regardless of its relative magnitude. Coded factors could then be used for the design description and analyses in Response Surface Methodology (RSM) (Farahi et al. 2012).

Table 4. *Coded factors and the corresponding values for each variable.*

Variables	-1	0	1
pH	6	7	8
Digestion Time (m)	2.5	3.5	4.5
AgNO$_3$ concentration	0.005	0.010	0.015

Nanoparticle Suspension and LDPE films
(Agno, Gilongos, Jalandoni, Sinco)

TiO2 nanoparticles (TNPs and N-TNPs) aqueous suspensions at 20mM concentration were prepared by mixing 1.599g of TNPs and 1.878g of N-TNPs each with 1 L of distilled water in separate beakers. The mixtures were then ultrasonicated for 30 minutes. A total of 12 pieces of LDPE films were cut into 4 cm by 26 cm strips prior to the exposure to light (Tofa et al. 2018). Only the 4 cm by 4 cm at the center of the LDPE films were subjected to analysis leaving the remaining area touchable.

B. **Hydrogel**
(Gerona, Remaneses, Sorongon)

This copolymerization method was taken from the Microwave Initiated Synthesis and Application of Polyacrylic Acid Grafted Carboxymethyl Cellulose of Mishra et al. Five (5) grams of CMC was dissolved in 100 mL distilled water. Fifteen grams of Chitosan dissolved in a 100 mL solution before it was added to the CMC solution. Constituents were mixed in the reaction vessel (500 mL beaker). The reaction vessel was subsequently placed on the turntable of a microwave oven.

Microwave irradiation using American Home AMW-6510W with an operation frequency of 2450 MHz at a power of 700 W was performed for 3, 6, and 9 minutes. Periodically, the microwave irradiation was paused (as the reaction mixture started to boil, i.e. at 65 C) and was cooled by placing the reaction vessel in cold water. This was to avoid competing homopolymer formation reactions or the reaction between CMC-CMC molecules and chitosan-chitosan to the minimum and also to prevent any thermal damage to the backbone polymer chain. The reaction vessel and its contents were cooled and kept undisturbed for 12 h to complete the polymerization.

C. **Chitosan-hydroxyapatite Composite**
(Janiya, Lopez, Magtoles)

A ratio of 1:1 chitosan and hydroxyapatite was followed. Twelve (12) grams of chitosan was dissolved with the use of a magnetic stirrer at 600 rev/min in 250 mL distilled water with 1% v/v acetic acid. The same amount of hydroxyapatite was added slowly to the mixture while stirring. Followed by the addition of 12.5 mL of 2.5% glutaraldehyde solution to the mixture. After 90 minutes of stirring, 15 g of gelatin powder was added to the mixture while continuously stirred at 40°C. While warm, the mixture was poured into eight (8) molds which were divided into two (2) batches, batch A and B. Both batches of the composite were air-dried to form completely for 48 hours.

D. **Copper-chitosan Complex**
(Loquias, Placido, Mediodia)

The chitosan-copper complexes were prepared using a method adapted from Usman et al. (2012, 2013), wherein 10 mL of CuSO$_4$ • 5H$_2$O (0.05 M) was added to 40 mL of acetic acid solution (0.1 M) containing chitosan (0.1, 0.2, and 0.5 wt%). After constant stirring and refluxing at around 100°C-140°C for 20 minutes, a lighter blue-colored solution was obtained, and 0.5 mL of ascorbic acid (0.05 M) was added and stirred for twenty (20) minutes at room temperature.

Two (2) mL of NaOH (0.6 M) was then added, obtaining a darker blue-green solution after stirring for another twenty (20) minutes. Then, 0.5 mL of N$_2$H$_4$ (0.05M) was added, and the solution was stirred for five (5) minutes. The pH was kept at an average of 8.0 throughout the process utilizing NaOH and HCl solutions. The synthesis solution was centrifuged at 10 000 G for 10 minutes and washed with acetone (90%, v/v). The precipitate was dissolved in distilled water and vacuum dried at 50°C for 18 hours at the Regional Research Center, University of the Philippines-Visayas.

E. Liquid Antibacterial Soap

(Hembra, Henderin, Parenas, Sinco)

Leaves of *Mangifera indica* (mango) contain phytochemicals that promote antibacterial activity. This study aimed to determine whether *M. indica* leaves extract can be an alternative antibacterial agent for triclosan.

The soap formulation using hot process was based on Widyaningsih et al. (2018) with revisions. The liquid soap base was formulated by heating 21.4% (w/w) of palm oil to 80°C. The potassium hydroxide (KOH) solution was separately prepared by dissolving 4.29% (w/w) of KOH to 10.0% (w/w)of distilled water. The KOH solution was then poured into the heated palm oil. The process was exothermic so the solution was allowed to cool down to 80°C. After which, 1.07% (w/w) of sodium lauryl sulfate (SLS) and paraben were added into the solution. The soap solution was then stirred at 600rpm - 700rpm until the stir bar was no longer able to rotate. Upon reaching this state, the solution was manually stirred using a stirring rod until a semi-solid consistency was achieved. The weight of the semi-solid solution was then measured using a top-loading balance. To dilute the soap, distilled water was added to the solution while being heated.

The amount of the added distilled water was triple the recorded weight of the solution to ensure dissolution. The weight of the resulting liquid soap was measured using the Sartorius top loading balance. For the negative control, 21.4% (w/w) of the prepared soap base was set aside and stored in a properly labeled Erlenmeyer flask.

For the positive control, $4.29 \times 10^{-5}\%$ (w/w) of Triclosan was added to 21.4% (w/w) of liquid soap base. For the preparation of the soap with the crude extract, the temperature of the soap base was first lowered to 40°C. After which, 0.536% (w/w) of M. indica extract was incorporated into the 21.4% (w/w) of liquid soap base and was mixed using a magnetic stirrer until the solution is homogeneous.

PHYSICS

ANALYSIS

OVERVIEW

Analysis is crucial to scientific research as it is a means for researchers to show the accuracy and reliability of their obtained data. This chapter discusses numerical analysis, percent deviation, non-parametric test, and thermal analysis. The following methods describe how different analyses can be used to compare results and to screen relevant from irrelevant data. Analysis can also be done with equipment such as the thermogravimetric analyzer in this chapter.

A. **Numerical**
(Clement, Josue, Ledesma, Murga, Madriñan)

One of the two techniques for observing the Golden ratio in structures is numerical analysis, where the ratio of the different measurements of the structure under study is taken. In this study, a spreadsheet was used for the automation of calculations.

The analysis is carried out by dividing a, the greater length, to b, the lesser length given by the formula; ratio = $a/b \approx$ 1.6.1812297. Subsequent calculations follow using the same basic idea of dividing the greater by the lesser. This is done on all lengths, widths, and areas with priority on locations where the Golden ratio is typically found eg. areas of facades or floor plans, lengths or distances of columns and arches.

B. **Percent Deviation**
(Clement, Josue, Ledesma, Murga, Madriñan)

Following the Golden ratio deliberately may lead to inconveniences in applying certain designs and adjustments should be made. Therefore in comparing the ratio to the golden ratio it was decided that the relevant ratios are those within 5% deviation from the golden ratio.

The percent deviation was calculated by getting the absolute value of:

$$\left(\frac{Dervied\ Ratio}{Golden\ Ratio} \times 100 \right) - 100.$$

D. **Non-Parametric Test**
(Barrios, Cortez, Herman, Larroder, Yu Jeco, Watanabe, Okada)

The Wilcoxon Signed Ranks Test was used to compare the daily output power of both the convex and Fresnel setups. It is a non-parametric test used to compare matched samples to determine if a significant difference exists for their population mean ranks. This non-parametric test was used since the data is not entirely random due to the assigned time for gathering the values, and thus cannot be assumed to be normally distributed.

Average values for voltage, current, output power, irradiance, and temperature were obtained by averaging the values at each hour for the 3-day testing period.

E. **Thermal Analysis**
(Tukasim, Beñosa, Pe, Jolito)

The simultaneous Thermal Analyzer 8000 (STA 8000) is an equipment used in measuring the mass time change while controlling the temperature at 15°C-1600°C. The weight loss history and exothermic phenomenon of the individual blends in a linearly heated environment were studied using a thermal analyzer (PerkinElmer STA 8000) which is coupled with a differential scanning calorimetry (DSC) to measure the heat flow into or out of the sample over time. The thermal analysis was done at the University of the Philippines Visayas-Miag-ao Campus.

The Thermogravimetric analyzer (model: PerkinElmer Simultaneous Thermal Analyzer 8000) was used in the gasification process.

For each experiment, a sample weighing 20 mg was heated in a small furnace in the thermal analyzer to study its thermal degradation. The samples were heated from 20°C to 900°C at a heating rate of 50°C·min⁻¹. The samples were gasified at a temperature of 900°C, this was decided by basing on a study done by Patra and Sheth (2015) which states that the gasification of biomass is usually conducted between 800°C- 1000°C, to promote the different endothermic reactions that are occurring during gasification. In summary, a sample run typically contains five steps namely, isothermal step for stabilization, temperature ramp step to the drying temperature of 110°C, another isothermal step to fully remove the moisture content, another temperature ramp step to the desired gasification temperature, and Finally, another isothermal step where the gasification would take place. Table 5 shows the summary of the strides.

Table 5. Running program inputted in STA 8000.

Seg No.	Method	Description	Purpose
1	Isothermal	Heat for 2.0 min at 20°C	Mass stabilization
2	Temperature Ramp	Heat from 20°C to 110°C at 50°C/min	Increase temperature to the drying temperature
3	Isothermal	Hold for 4.0 min at 110°C	Removal of moisture
4	Temperature-Ramp	Heat from 110°C to 900°C at 50°C/min	Increase to the devolatilization, gasification, and combustion temperature
5	Isothermal	Hold for 10.0 min at 900°C	Devolatilization, gasification, and combustion

CALCULATION

OVERVIEW

Calculations often involve using standard equations in quantifying values of interest. This chapter discusses the quantification of gasification (conversion of biomass to fuel) reaction and the conversion efficiency of photovoltaic cells. The first study shows the use of previously established models to determine the most appropriate that can describe their experimental data. While the latter shows the calculations for the efficiency of photovoltaic cells and the measure of quality of the solar cell.

A. **Gasification Reaction**
(Tukasim, Beñosa, Pe, Jolito)

Co-gasification of biomass and coal is emerging as a potential clean fuel technology as it reduces greenhouse gas emissions, lowers gross power output, and increases thermal efficiency. The present study aimed to determine the potential of Theobroma cacao (UIT Variety) pod husks as an alternative to coal by investigating the gasification kinetics. After obtaining the mass time curve data from the thermal analysis it was then analyzed in order to determine the proximate composition. The proximate composition includes the percentage mass of moisture, volatile matter, fixed carbon, and ash content. As seen in Figure 17, the graph is subdivided into four different parts namely the removal of moisture and volatile matter, carbon dioxide gasification of char, and the combustion of the remaining char. The data were analyzed using a series of steps by determining the amount of moisture, volatile matter content, fixed carbon content, and the ash content of the sample that was being tested.
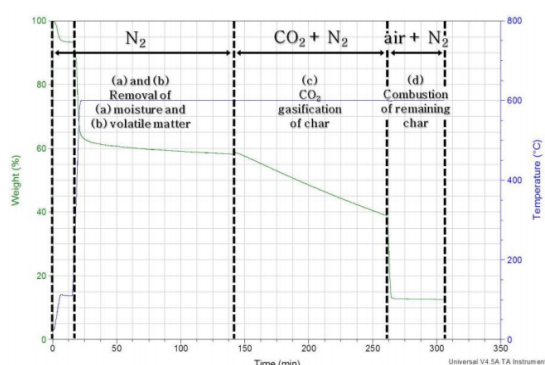
Figure 17. *Graph of char combustion.*

The evaluation was carried out by determining the fractional conversion using the equation below. This is done in order to determine the best model. After which, it was fitted into the gas solid reaction model.

$$X = \frac{W_0 - W}{W_0 - W_{ash}}$$

Where W_0 is the initial mass of the pre-gasified char, W_{ash} is the mass of ash in the primary char sample, and W is the mass of the char at any time t. (Massoudi Farid *et al*, 2016) The reaction rate of the gas solid models which are expressed as the carbon mass balance, Volumetric model and Shrinking Core model, were determined using the respective equations. The volumetric model is considered to be the simplest and straightforward model, while the Shrinking Core model is described to be solid molecule used or being consumed by either dissolution or through the chemical reactions happening.

$$X = 1 - e^{-k_{VM}t}$$

where k_{VM} is the first-order reaction rate constant and X is the fractional conversion of carbon.

$$X = 1 - (1 - k_{SCM}t)^3$$

where k_{scm} is the average reaction rate constant.

As for the Random Pore model, there is another step to go through before determining the rate constant. The surface parameter will be first determined, it was used to determine the rate constant. The Random Pore model considers the growth of pores and the coalescence of these pores which causes a reduction of area through a combination of overlapping of pore surfaces in the process of gasification, which can determine the peak reactivity of the reaction (Bhatia et al. 1980).

$$X = 1 - e^{-k_{RPM}t\left(1 + \frac{\psi k_{RPM}t}{4}\right)}$$

Where ψ is a structural parameter using a reduced quantity that describes the particle's internal structure.

$$\psi = \frac{4\pi L_0(1 - \epsilon_0)}{S_0^2}$$

Where S_0^2 is the pore's surface area per volume, S_0^2 is the pore length per volume and ϵ_0 is the solid porosity parameter. However, these values weren't available hence an alternate formula is used.

$$\psi = \frac{2}{2\ln(1 - X_{max}) + 1}$$

Once Parameter is obtained, the reaction constant can be estimated similarly to the previous models. In determining the best fit model, the values will be fitted into the equation . The equation will help find the coefficient of determination (R) which will be used in determining the best fit model. Using the standard deviation formula , the overall goodness of the model will be determined.

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}} = 1 - \frac{\sum_1^N (X_{exp} - X_{model})^2}{\sum_1^N (X_{exp} - \overline{X})^2}$$

Where SS_{res} is the sum of squares of residuals from the experimental and empirical values while the SS_{tot} is the sum of squares from the experimental and the average values of char conversion.

$$SD = \sqrt{\frac{\sum (X_{exp} - X_{model})^2}{N - p}}$$

where X_{exp} and X_{model} are the conversion data from the experiment and each individual model and N is the number of data while p is the number of parameters fitted. After determining the best model for the sample, the activation energy was then determined using Arrhenius equation. The best kinetic model and parameters obtained were then compared to the other samples. This was all done using Microsoft Excel.

$$\ln\left(\frac{k_2}{k_1}\right) = \frac{E_a}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

B. **Conversion Efficiency**
(Barrera, Umadhay, Canson, Larroder)

Photovoltaic (PV) panels are subject to extreme heat and radiation while exposed to sunlight. For sustainability reasons, organic bio-based Phase Change Materials (PCMs) are used to cool the temperature of the PV panel since they have a desired thermodynamic and kinetic criteria for low-temperature latent heat storage. This study aims to check the potential of bio-based phase change material which is the 1:7 mixture of rice bran wax to rice bran oil (RBW/RBO) in determining the conversion efficiency of the photovoltaic cells.

The wattages of the PV/PCM systems were computed using the equation of power which is the current multiplied by the voltage that was obtained using a multimeter. Efficiency was calculated and the gathered data was compared to the PV cell without PCM attached, and to the PV cell with paraffin wax.

Conversion Efficiency of the PV cell was determined using the following equation:

$$\eta = \frac{FF \times I_{sc} \times V_{oc}}{P_{in}}$$

Where V_{OC} was the open-circuit voltage, I_{SC} was the short-circuit current, FF was the fill factor, P_{in} was the power input and η was the efficiency. The Fill Factor (FF) was essentially a measure of the quality of the solar cell. It was calculated by comparing the maximum power (P_{MAX}) to the theoretical power (PT) that would be output at both the open-circuit voltage and short circuit current together.

$$FF = \frac{P_{max}}{P_T} = \frac{I_{MP} \cdot V_{MP}}{I_{SC} \cdot V_{OC}}$$

CONSTRUCTION

OVERVIEW

Geometric construction is the process of drawing a geometrical figure using geometrical instruments. This chapter discusses one of the techniques on how to construct a figure to determine whether the Golden ratio exists in the structures under study. The methods included in this chapter may be used as a reference to replicate similar studies.

A. **Geometric**
(Clement, Josue, Ledesma, Murga, Madriñan)

One of the two techniques for observing the Golden ratio in structures is geometric construction, where the ratio of the different measurements of the structure under study is taken. In this study, a spreadsheet was used for the automation of calculations.
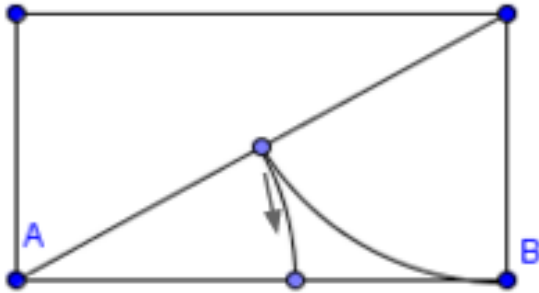
Figure 18. *Geometric construction.*

A line was partitioned to locate its golden section by first drawing a line with the length of AB. Next, the rectangle with the length AB and width AB/2 is drawn. A diagonal was drawn from A to the opposite corner of A.

Then, the width (AB/2) was then subtracted from the diagonal by drawing an arc with the width as the radius. The diagonal was then divided into two segments as a result of the intersection of the arc with the diagonal. Finally, the longer segment of diagonal was rotated onto the adjacent side, AB. The intersection point, C subdivides AB into the golden ratio. C is called the golden section of AB.

The figure was then scaled and overlaid on the scaled illustrations or blueprints of the structures under study to visually locate golden ratios.

DESIGN

OVERVIEW

Equipment design, in the absence of standardized equipment, is crucial in research methodology as it directly affects the results of the study and how data is interpreted. This chapter is a catalog of methodologies involving equipment and apparatus design. The following designs have been modeled from previous works of the same field but have been modified for feasibility and to better suit the purpose of their respective studies. Design heavily involves the kind of material used, the dimensions of the material, and in some cases, the chemical compositions of a particular layer.

A. Device
(Gurrea, Peregrino, Regalado, Salvador)

The core aspects of the design involve the utilization of 3 bubbling chambers. As CO_2 is bubbled through the first solution, it reacts in the first chamber with $Ca(OH)_2$ to initiate the carbon mineralization process and is then converted to $CaCO_3$. However, since not all CO_2 is expected to react, additional chambers have been added to allow the escaping CO_2 to be sequestered and react again with the $Ca(OH)_2$ filled chambers.

The design was also conceived through the aid of a professional mechanical engineer, Edgar Allan Vargas. The engineer was able to contribute mostly to the practicality of the device design rather than the chemical design behind the device. The extent of his contribution was mostly centered with regards to its feasibility to manufacture, its practicality, and changes in dimensions to facilitate the welding of stainless steel tubes.

A rectangular chamber was chosen for the sake of fabrication. Stainless steel is a relatively hard metal to bend and weld. Also, allowing for other geometrical shapes would have also meant an irregularly shaped device and would have made it bulkier. Rectangular shapes decrease the total space the device occupies. Hence, for our device, a rectangular chamber was chosen. To empty the $Ca(OH)_2$ solution in the device, a

The design also includes input and output tubes to facilitate the flow of air. Some padding was placed between the roof of the device and the chamber so that it may be sealed airtight. The roof of the device is removable with nuts and bolts holding it together.

B. Assembly of Lens
(Ebojo, Magan, Dumalag, Larroder, Aban)

The concentrator photovoltaics requires a light source to be within the system's acceptance angle to achieve the highest output. A concentrator lens configuration was designed for a tandem III-V multi-junction solar cell in order to address this problem, improve the power output of the setup, and thereby reduce the need for a solar tracker.

The modified setup, Double Convex-Hemispherical lens (DCX-HSL), is composed of three lenses: the convex, Fresnel, and the hemispherical lens as shown in Figure 19. The convex lens was situated 10 cm above the multi-junction solar cell of the setup which is the topmost lens. The convex lens acts as the collector lens that collimates the beam of light from the sun to the next lens of the setup, the Fresnel lens. The concentrated light was distributed by the hemispherical lens on the surface of the solar cell for uniform irradiance distribution.
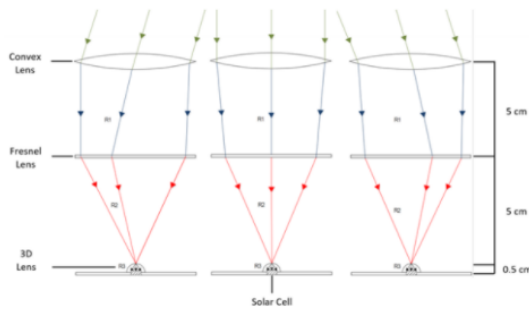
Figure 19. Diagram of the compound convex-hemispherical setup

A convex lens and hemispherical lenses were added to the optical system of the Fresnel lens setup. The convex lens was situated 10 cm above the multi-junction solar cell of the setup. The Fresnel lens was situated 5 cm on top of the multi-junction solar cell and concentrates the light on the solar cell. A hemispherical lens was situated on the solar cell for the distribution of light on the solar cell. The placements and specs of the lenses were optimized based on Köhler illumination where light from a certain range of angles is still redirected onto the surface of the solar cell. Köhler illumination is a method for generating an even illumination of the source on the object which in this case is the solar cell.

C. Column Design
(Bandiola, Galotera, Sampiano, Mediodia)

Before the columns were packed, they were washed with distilled water thrice and 10% nitric acid and were left to air-dry. They were stored in a 10% nitric acid wash bath overnight. Using a wooden rod, glass wool was packed inside the column up to a height of 2 cm. After making sure that that layer was flat, the adsorbent layer (rice husks for setup A; mango peels for setup B. An equal combination of rice husk and mango peels for setup C) was then poured inside the column until a 15 cm layer was formed. For combining the adsorbents in setup C, a 50:50 adsorbent ratio based on bed height was implemented, and the mixing process was

performed by pouring the adsorbent inside a beaker and performing manual agitation. The adsorbent inside each column was weighed and was controlled for all replicates. Figure 20 shows the schematic diagram of the column setup. The sides of the column were tapped for an even distribution of the adsorbents. A final layer of glass wool with a height of 1 cm was placed on top of the setup in order to secure the adsorbent in place. As a primer, 100 mL of deionized water was passed through the column.
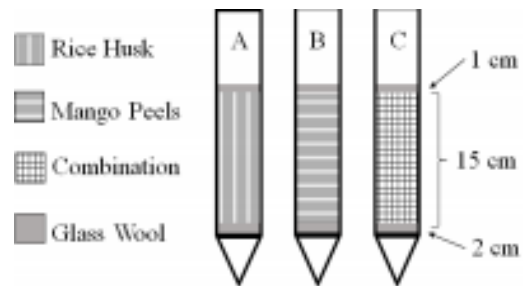
Figure 20. The schematic diagram of the column setups is composed of a) rice husks only, b) mango peels only, and c) a combination of rice husks and mango peels.

ELECTRONICS

OVERVIEW

      Electronics deals with the design of circuits using transistors and microchips, and with the behavior and movement of electrons in any medium. This chapter discusses the different designs of Fresnel Lens, and Two Lens Setup. By varying measurements of length (spacing) and angles, these experimental setups were able to determine which of their proposed designs produces the highest power output.

A. Fresnel Lens Setup

Method A

(Barrios, Cortez, Herman, Larroder, Yu Jeco, Watanabe, Okada)

      The Fresnel lens setup is comprised of a Fresnel lens mounted 5 cm above the solar cell, as shown in Figure 21. The solar cell is placed 5 cm under a Fresnel lens measuring 5 cm x 5 cm with a measured optical efficiency of 96.2% as measured using an irradiance meter and sourcemeter. Additionally, the lens has an estimated acceptance angle of 84.7 to 101.6°relative to the lens surface as measured with a laser pointer and a protractor.
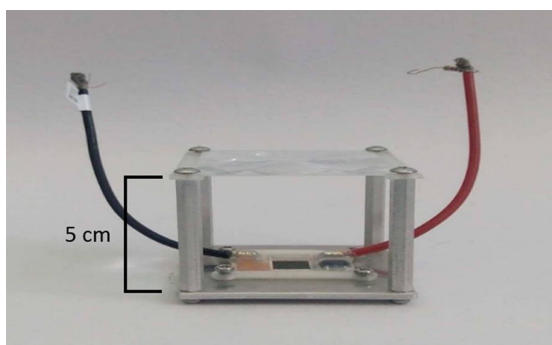
Figure 21. *The Fresnel lens setup with a Fresnel lens concentrator.*

Method B

(Ebojo, Magan, Dumalag, Laroder, Aban)

      The concentrator photovoltaics requires a light source to be within the system's acceptance angle to achieve the highest output. A concentrator lens configuration was designed for a tandem III-V multi-junction solar cell in order to address this problem, improve the power output of the setup, and thereby reduce the need for a solar tracker.

      For the theoretical modeling of the DCX-HSL setup, the software Edraw Max™ was utilized to create a light ray diagram. A diagram was created to have an expected result and view of how the setup will function when assembled. For the controlled setup, the Fresnel lens setup was used. It consisted of a solar cell and the Fresnel lens which served as the primary concentrator. In this setup, the Fresnel lens was situated 5cm above the solar cell as shown in Figure 22.
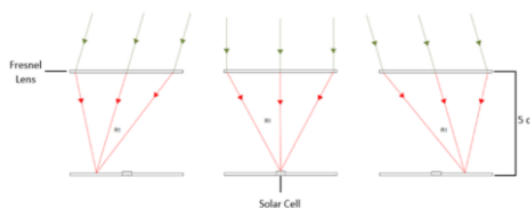
Figure 22. *Diagram of the Fresnel lens setup.*

      The Fresnel lens setup is composed of one Fresnel lens and a multi-junction solar cell. The solar cell was secured on the aluminum backplate with the solar cell holder and screws. The spacers were then screwed to the aluminum backplate with the washers between the aluminum backplate and screws. The lens holders were screwed to the spacer, then the Fresnel lens was lodged into place and was secured using screws. The Fresnel lens in this setup was placed 5 cm above the multi-junction solar cell as shown in Figure 21. It served as the primary concentrator for the setup.

B. Two-lens setup
(Barrios, Cortez, Herman, Larroder, Yu Jeco, Watanabe, Okada)

A convex lens with a diameter of 5 cm, a focal length of 10 cm, and an estimated acceptance angle of 83.7 to 97.5° relative to the lens surface was selected. It was mounted at a fixed height of 5 cm above the Fresnel lens using the iron stand and iron clamp, as shown in Figure 23.
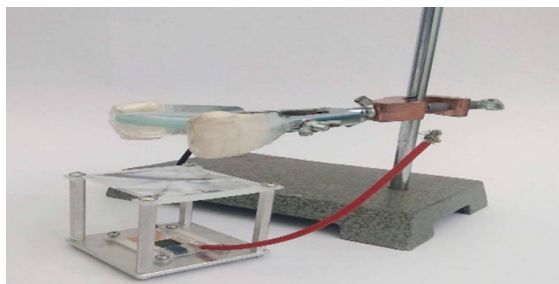
Figure 23*. The two-lens system with a convex lens as primary concentrator located 5 cm above the Fresnel lens secondary concentrator.*

The specific measurements for the lens installation were calculated using the Lens-Maker's equation (1), where the dimensions were selectively aimed at having the focal point of the two-lens system placed exactly on the solar cell's surface. f1 and f2 represent the focal lengths of the convex and Fresnel lenses, respectively, d represents the distance between the two lenses, and f represents the composite focal length of the entire lens system.

$$\frac{1}{f} = \frac{1}{f_1} + \frac{1}{f_2} - \frac{d}{f_1 f_2}.$$

EVALUATION

OVERVIEW

Evaluation provides a systematic method to determine the efficacy of a certain quality or process. This chapter discusses experimental methods for evaluating the plastic degradation of three types of plastics and the absorbency of a hydrogel, a superabsorbent polymer. Evaluating the qualities of different materials may support future studies on product design, and later on manufacturing.

A. Plastic Degradation (% Weight Loss)
(Canja, Hilis, Galan, Jolito)

Plastics are known for being durable materials while still maintaining a low cost of production. It is a very important material for commercial use all over the world. However, due to the lack of a reliable method of disposal, the risk of plastic pollution is steadily increasing throughout the years. This study aims to isolate and extract bacteria from the Iloilo City Engineered Sanitary Landfill, in Mandurriao, Iloilo City, and to assess their biodegradation potential on LDPE (Low-density polyethylene), HDPE (High-density polyethylene), and PET (Polyethylene terephthalate).

The three different types of plastics (PET, LDPE, HDPE) were cut into strips having dimensions of 2cm x 2cm with 3 times replication for each plastic in each set-up. They were sterilized in 70% ethanol for 30 minutes, washed with distilled water, and subsequently dried in an incubator at 60° C for 24 hours. Afterward, plastics were put into a silica gel containing desiccator for 24 hours for total water evaporation. The initial dry weight of plastic was measured with an analytical balance (Kyaw et al., 2012).

After bacterial culture, three set-ups were made. Colonies were selected based on their morphological characteristics: form, elevation, and margin. The first being the medium inside the Petri dish where Bacteria 1 was cultivated with the three types of plastic strips (HDPE, LDPE, and PET) having each type of test plastics in triplicates.

The second setup was the medium with Bacteria 2, and the same was done for this setup. The two colonies were distinguished using the nutrient agar eosin methylene blue agar (EMB) and mannitol salt agar (MSA) as shown in Plate 1. The last set-up is the control, which was maintained with polyethylene strips in the microbe-free medium. Pre-weighed strips of sterilized plastics of each type were aseptically transferred to the Petri dish containing mineral salt medium (MSM) and inoculated with the bacteria to be tested.

Plate 3-4. *Bacterial strains EMBP2A and MSAP2A for incubation.*

Triplicates were maintained for each type of plastic and were left on the incubator. After 10 days, the plastic discs were collected and washed thoroughly using distilled water. They were then dried in a hot air oven at 50°C overnight for at least 10 hours and were weighed for final dry weight. The percentage weight loss was calculated using the formula below (Usha et al., 2011).

$$\text{Weight loss } (\%) = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} x100$$

B. **Superabsorbent Polymer Absorbency**

(Gerona, Sorongon, Remaneses)

The absorbency of the hydrogel or the volume of water the polymer can absorb was determined as follows: 1 g of hydrogel was immersed in 200 mL distilled water at room temperature (30°C) and the weight was measured every hour for five hours.

The tea bag was allowed to drain for 10 min or until the excess water stopped dripping. The equation for the swelling capacity or absorbency is as follows: Absorbency = $(W2 - W1)/W1$ where $W1$ and $W2$ represent the weight of the dry polymer and the swollen gel, respectively. The rate of absorption was obtained by determining the absorbance at consecutive time intervals.

FIELD TESTING

OVERVIEW

Field testing is an important procedure in product research and development as it is pertinent in gauging whether the design of the product is effective in its target environment. This then refines the scope of the research and the specifications of the product. In this chapter, two modified and controlled setups of field testing involving lens and PVC with bio-based PCM are cataloged; both of which required sunlight and had taken place at Philippine Science High School - Western Visayas.

A. **Lens**
(Ebojo, Magan, Dumalag, Larroder, Aban)

The concentrator photovoltaics requires a light source to be within the system's acceptance angle to achieve the highest output. A concentrator lens configuration was designed for a tandem III-V multi-junction solar cell in order to address this problem, improve the power output of the setup, and thereby reduce the need for a solar tracker.

The Fresnel lens (controlled) setup and Double Convex-Hemispherical lens (modified) setups were tested on the rooftop of the Student Learning Resource Center (SLRC) building located at Philippine Science High School - Western Visayas. The modified and controlled setups were placed on a table and the angle of the controlled and modified setups was adjusted to 0 degrees in reference to the ground using the built-in compass app of Apple to make sure it was flat as shown in Figure 24. Voltage, current, solar irradiance, and the time of data gathering, were recorded during the outdoor data gathering.
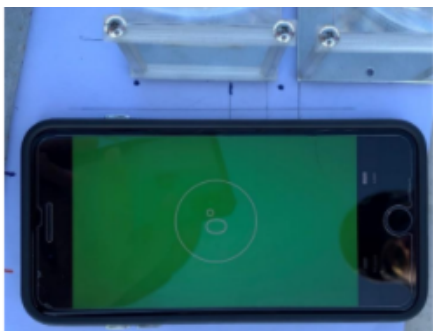
Figure 24. *Angle of the modified and controlled setup in reference to the ground*.

Two multi-testers were used for measuring the voltage and the current while a solar irradiance meter was used to measure the solar irradiance for two setups. The multimeters were calibrated by setting the two to the highest resistance range option and the red and black probes that were connected to the multimeter were touched together until the reading on the multimeters displayed "0 ohms". After calibration, the measuring devices were used to measure the parameters. The measurements were repeated until three of the values have a difference equal to or less than one on the rightmost significant digit for the reliability of the data. This process was repeated every hour starting from 6:15 am until 5:15 pm. Weather conditions such as the cloud cover were recorded and were taken into consideration during the data gathering because it may affect the data gathered.

B. **PVC with Bio-based PCM**
(Barrera, Umadhay, Canson, Larroder)

Photovoltaic (PV) panels are subject to extreme heat and radiation while exposed to sunlight. For sustainability reasons, organic bio-based Phase Change Materials (PCMs) are used to cool the temperature of the PV panel since they have a desired thermodynamic and kinetic criteria for low-temperature latent heat storage. This study aims to check the potential of bio-based phase change material which is the 1:7 mixture of rice bran wax to rice bran oil (RBW/RBO) in determining the conversion efficiency of the photovoltaic cells.

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From the simulation result of Indartano et al. (2015), the optimum thickness of the PCM was at 80 mm. The thicker the PCM mounted on the solar module is, the greater the drop in temperature experienced by the solar modules, especially at the backside of the solar module. This was due to the thickness of the PCM being proportional to the total volume of PCM. The larger the volume of PCM, the more time it needs to melt all the PCM. The aluminum tube was used for the purpose of heat collection, removal, and possible recovery. Two fabricated rectangular containers made of aluminum held the PCMs; one for the rice bran wax and rice bran oil mixture and one for the paraffin wax. The more contact of the material to a metal, the greater the thermal conductivity, thus, spreading the heat more evenly throughout the PCM (Hasan et al. 2010).
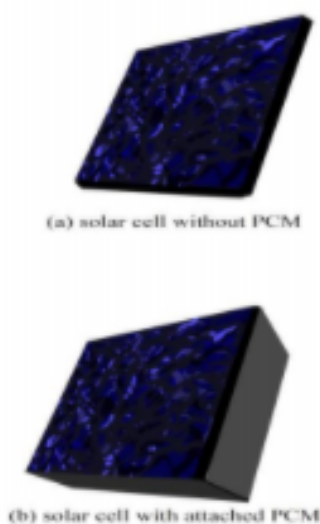
Figure 25. *PV cell Schematics (not drawn to scale)*
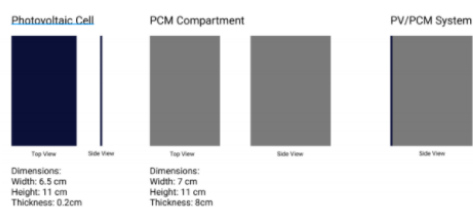
Figure 26. *The dimensions and components of the PV cell and schematics of PV with PCM attached to it (not drawn to scale).*

The containers were placed under the PV module to create the PV/PCM system. K-type thermocouples were attached to the back of the PV cell and to the PCM to monitor temperature changes.

The actual testing of the PV cell with and without attached phase change materials was performed in the Smart Classroom 2 of Philippine Science High School- Western Visayas Campus. A box made of illustration board 21 cm high, 9 cm wide, and 15 cm long was constructed to act as a dark room for the PV cell and to hold in place the xenon lamp. The temperature, current, and voltage were measured every 15 minutes using thermocouples and a multimeter. The open-circuit voltage (VOC) and short circuit current (ISC) were first measured by connecting the PV cell to an equivalent circuit with a single diode and series resistance mode, under the illuminated light of a xenon lamp. The VOC was measured when the current in the circuit is equal to zero while the short circuit current was measured when the voltage in the circuit is equal to zero. Only parameters VOC and ISC were measured due to the unavailability of specialized equipment for voltage sweep analysis used in determining the actual measurement of maximum power output (PMAX) in the PV cell. Instead, the actual values of the two were used and derived to calculate the efficiency, together with its underlying parameters-- fill factor (FF) and (PMAX). The voltage and current of the PV cells were simultaneously measured using a multimeter. The temperature of the PCM and the back surface of the PV cell were also measured simultaneously. The value of the solar irradiance was also measured every 15 minutes using the solar irradiance meter.
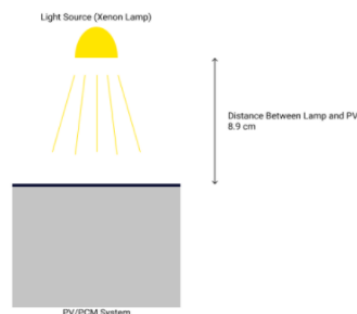
Figure 27. *Set-up with the distance between Xenon lamp and PV/PCM*

MATERIAL TESTING

OVERVIEW

This chapter provides different methods of determining quantitative characteristics of a raw material and a commercial product. This category discusses three mechanical properties, i.e. tensile strength, tear resistance, and stiffness, which are important for textiles and bag-making, and an optical property, reflectivity, which may be used for urban planning and design. These research investigations promote studying locally-available materials for their potential uses and possible commercial value.

A. Tensile Strength
(Aguilar, Lignig, Togonon, Brana, Tanoy)

The tensile test was performed using the Universal Testing Machine(UTM) Instron Model 1000 by pulling the samples under tension until breakage. The data for the results were then calculated and derived using the maximum force and extension at break displayed by the machine. The standard used was ASTM D828-97(2002): Test Method for Tensile Properties of Paper and Paper Board Using Constant-Rate-of-Elongation Apparatus, using Instron Model 1000 Universal Testing Machine(UTM). This standard was used as procedure reference only for the test and not a specific test standard for leaf sheaths or canvas.

B. Tear Resistance
(Aguilar, Lignig, Togonon, Brana, Tanoy)

For the tear test, the machine used was Elmendorf Model 60-100 Tear Tester, and the standards used were ASTM D689-"Test Method of Internal Tearing Resistance of Paper" and TAPPI T414-"Internal Tearing Resistance of Paper (Elmendorf Type)". The standards were used as procedure reference only and not a specific standard test for leaf sheaths or canvas. The test conditions were consistent throughout the test, with 26.7℃ at 44% relative humidity. The average tearing force in grams was calculated by multiplying 16 the scale reading of the machine, and then dividing by the number of plies(from the ASTM and TAPPI standards used).

C. Tear Resistance
(Aguilar, Lignig, Togonon, Brana, Tanoy)

For the tear test, the machine used was Elmendorf Model 60-100 Tear Tester, and the standards used were ASTM D689-"Test Method of Internal Tearing Resistance of Paper" and TAPPI T414-"Internal Tearing Resistance of Paper (Elmendorf Type). The standards were used as procedure reference only and not a specific standard test for leaf sheaths or canvas. The test conditions were consistent throughout the test, with 26.7℃ at 44% relative humidity. The average tearing force in grams was calculated by multiplying 16 the scale reading of the machine, and then dividing by the number of plies(from the ASTM and TAPPI standards used).

D. Reflectivity
(Apdon, Frange, Salistre, Larroder)

A Samsung J7 Prime with a 13-megapixel camera that can run the Albedo: Reflectance App™ was used for the study. The application works by comparing the image of the sample with an image of a photographer's gray card as a calibrator (which has a known albedo of 0.18). Since the objective of the study is to determine if there is an increase in albedo due to the addition of *Placuna placenta*, the absolute albedo readings are not necessary but rather, albedo testing of each slab must be at identical background conditions to have the same basis for the application to determine the relative albedo values.

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MEASUREMENT

OVERVIEW

Measurement is the quantification of the various attributes of an object. This section will deal with the measurement of thermophysical properties, particularly that of rice bran wax and rice bran oil mixture. The method outlined in this section may be adapted to substances of similar properties.

A. Thermophysical Properties

(Barrera, Umadhay, Candon, Larroder)

Photovoltaic (PV) panels are subject to extreme heat and radiation while exposed to sunlight. For sustainability reasons, organic bio-based Phase Change Materials (PCMs) are used to cool the temperature of the PV panel since they have a desired thermodynamic and kinetic criteria for low-temperature latent heat storage. This study aims to check the potential of bio-based phase change material which is the 1:7 mixture of rice bran wax to rice bran oil (RBW/RBO) in determining the conversion efficiency of the photovoltaic cells.

The thermophysical properties of the rice bran wax and rice bran oil mixture which are the melting point, the heat of fusion and supercooling/subcooling, latent heat of fusion, and heat capacity were measured using differential scanning calorimetry (DSC) analysis. The samples were taken to the University of the Philippines-Visayas Miag-ao for the DSC analysis. One gram of the rice bran mixture sample was heated and the changes in its heat capacity were tracked as changes in the heat flow. This allowed the detection of transitions such as melting point and phase changes. In DSC, the 1:7 ratio of rice bran wax to oil mixture was heated at a constant rate. The latent heat of fusion of the material was measured by using the area below the peak and melting temperature was measured by the tangent at the point of maximum slope on the face part of the peak.

The samples were held for 1 minute at 15 °C. It was heated at a linear rate of 10°C/min from 15°C to 36°C in a nitrogen atmosphere. The differential scanning calorimetry (DSC) is to determine if the sample was starting to melt. It was held again for 2 minutes at 36°C and was heated from 36°C to 40°C at 1°C/min before it was held for 2 minutes to check if the samples were completely melted. It was heated again at 40°C to 500°C with a heating rate of 10°C/min before it was cooled down from 500°C to 40°C at 20°C/min. The percentage of the thermophysical properties was determined by extrapolating the slopes of the thermographic curves above and below the inflection temperature. The intersection of these slopes allowed the determination of the weight loss and thus the percentage of the components and the reactions that were present.

Data on the thermophysical properties of rice bran wax and rice bran oil mixture were compared to the established thermophysical properties of paraffin wax as it is the most common type of PCM that is being attached to PV cells for cooling. The basis of the thermophysical properties of paraffin wax was acquired from the research of Kavitha and Arumugam (2013). Paraffin wax is an organic PCM that has been tested to improve PV panel efficiency and it was the most common PCM that can be used in lowering the PV cell temperature (Hasan et al. 2010; Wei et al. 2017; Cellura et al. 2008).

PROCUREMENT

OVERVIEW

Procurement is the act of acquiring materials or values needed to proceed with the research design. This chapter discusses how measurements of a structure were obtained through various methods, and how Phase Change Materials (PCM) were selected according to established standards. It is important to be critical in the procurement stage as it would affect the analysis in the later part of the study.

A. **Measurements**
(Clement, Josue, Ledesma, Murga, Madriñan)

In using the measurement of structures as a subject of the study, acquiring blueprints or plans of the structure is the most common method. Should there be no available blueprints or measurements of the whole structure or certain parts of the structure needed, manual measurement of the structure with the aid of a laser distance measuring device or tape measure can be done.

The relatively longer lengths in this study were measured with the laser device while the lengths that were hard to get using this device were measured with tape measure. The measurements in meters were taken at least three times and then averaged. Since the laser measurement device has an area for error of 1.5 mm, then each measurement obtained should be placed in a range of ± 1.5 mm. However, the value 0.0015 was too small to be significant in collecting measurements because the measurements obtained have been rounded off to two decimal places.

B. **Bio-Based Phase Change Materials (PCM)**
(Barrera, Umadhay, Candon)

Photovoltaic (PV) panels are subject to extreme heat and radiation while exposed to sunlight. For sustainability reasons, organic bio-based Phase Change Materials (PCMs) are used to cool the temperature of the PV panel since they have a desired thermodynamic and kinetic criteria for low-temperature latent heat storage.

This study aims to check the potential of bio-based phase change material which is the 1:7 mixture of rice bran wax to rice bran oil (RBW/RBO) in determining the conversion efficiency of the photovoltaic cells.

The criteria for PCM selection is broadly based on the following physical, thermal and chemical properties. According to Xu et al. (2017) first, the phase change temperature of this material should satisfy the operating temperature range of the latent heat storage energy system. A PCM with an easily adjustable melting point would be a necessity as the melting point is the most important criterion for selecting a PCM for passive solar applications (Farid et al. 2004). Second, the PCM should possess high latent heat of fusion and large specific heat, to ensure the high storage density of the system. Lastly, the material is required to have high thermal conductivity in order to achieve high discharge power.

According to the Certificate of Analysis, the melting point of the rice bran oil was only 15°C. The phase change temperatures of rice bran oil, which is a fatty acid, could be adjusted by mixing fatty acids in a suitable proportion. A more appropriate solution was to utilize another type of PCM with a more reasonable melting point that was suitable for the application.

One of the ways on how the melting point of the PCM could increase was to adjust its characteristics, or by mixing it with other types of PCM with a lower melting point until the desirable melting point was achieved. Rice bran wax has a melting temperature of 79°C (Kramer 2016). It was

then mixed with rice bran oil to create a mixture that will have a melting point higher than the rice bran oil but lower than that of the rice bran wax. There were seven mixtures having different ratios of rice bran wax to rice bran oil in terms of volume, which were 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, and 1:7 respectively. The rice bran wax was melted first using the hot plate and rice bran oil was added to it while it was being heated and was mixed and stirred using a stirring rod. Different ratios created mixtures with differences in the melting point. It was found out that the mixture with the 1:7 ratio was the most suitable for the study since its melting temperature is in line with the operating temperature of the PV cell.

COMPUTER SCIENCE

APP DEVELOPMENT

OVERVIEW

Application development is responsible for the creation of various softwares relevant in an increasingly technologically demanding world. This section deals with a method in the use of Thunkable and Firebase in developing a dormitory pass application. Application development is a tedious process often requiring keen insight not only on the method of addressing an issue, but also the issue being addressed. Identify and focus on stakeholders prior to beginning the project.

A. **Thunkable and Firebase**
(Roxas, Subong, Fuentes, Salazar, Briones)

The flowchart shows the steps in developing Fygo. These steps include the gathering of information from the manual leave pass system for developing Fygo, developing flowcharts that would determine the logic of Fygo, developing the subsystems, debugging each subsystem, integrating each subsystem, testing and gathering to the interns, dorm managers and security guards for the feedback.
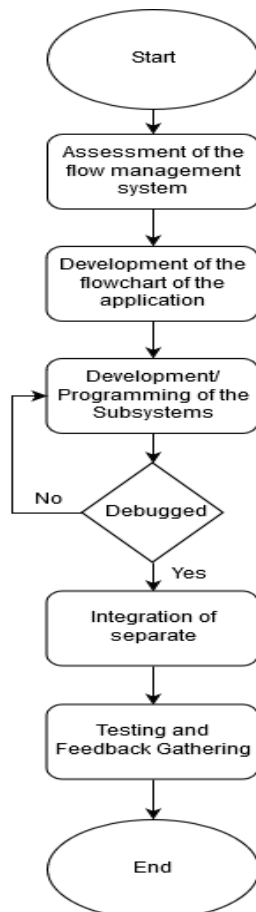
Figure 28. *Flowchart of the timeline of the developmental phases of the application.*

User interface (UI) is a vital concept when developing an application. The first impression of users is usually based on the UI of the application. If its UI is less attractive, users would tend to be bored in using the application. As stated by Nam (2011), the manager of a developed mobile application which is the interface of the system, UI is implemented for smartphone applications in the Android platform to support easy installation and usability with Graphic User Interface (GUI). Disregarding an application's function, its UI must follow a set of standards in order to be accessible. While doing work on Fygo proper, a user interface was designed so as to streamline development of what the front-end of the application would look like.

B. **Fygo**
(Roxas, Subong, Fuentes, Salazar, Briones)

The development phase of Fygo is composed of 6 phases: assessment of the conventional method, development of application operation flowchart of Fygo, development of the Fygo subsystems, debugging, integration of subsystems, and testing and feedback gathering.

The assessment of the conventional method identified the different persons involved and their respective tasks for the current dormitory leave pass system. The application operation flowchart for Fygo was then modeled from this system. This flowchart served as a guide to develop the application. Another flowchart was designed to help visualize the final product of Fygo.

Separate subsystems were created for each type of user (interns, dorm managers, and security guards) to perform different, specific tasks. There are a total of 5 subsystems: logging in, registration, for interns, for dorm managers, and for security guards.

The login subsystem is the primary screen in which the users first encounter upon opening Fygo. On this subsystem, a user logs in using their username and password. The application will then identify if the user is registered and determine whether the user is an intern, a dorm manager,or a security guard then redirects the user to his/her respective subsystem. The login subsystem is an additional feature added to the system of Fygo.

New users who want to create a new account can proceed to the registration screen. The registration is only applicable to interns only. There is no need for security guards and the dorm manager to create a new account. Premade accounts have been created for the dorm manager and the security guards. The registration subsystem is also an additional feature added to the system of Fygo.

After the intern logs in, the application would redirect to the screen where he/she could file a leave pass. The subsystem designed for the interns are based on the paper-form leave pass that have been used in the current dormitory leave pass system.

When the dorm managers log in, Fygo would redirect to the screen consisting of three options: the list of requests, log records, and pending registrations. The list of requests screen contains a list of interns who filed for a leave pass. Log records contain the dates in which interns logged in and logged out. Lastly, the pending registrations screen contains a list of new interns registering for a new account.

When the security guards log in to Fygo, they would be able to see approved leave passes. They could also log in or log out the interns who have approved leave passes.

Each of the subsystems was debugged separately for easier spotting of any minor errors. After successfully debugging, the subsystems were integrated to become a functioning application. The screens for the different subsystems were also made and programmed. The application included a registration screen and a main menu or login screen.

The application was tested by 40 interns who were tasked to file a leave pass. The number of testers were determined by the Minimum Sample Size Needed for Interval Estimate of a Population Proportion. The average number of leave passes filed each weekend was used as the population size, and the confidence level and confidence interval was set to be 95% and 5, respectively. The application was also tested by a dorm manager who was tasked to approve the leave passes filed by the interns. Lastly, the application was also tested by a security guard who was tasked to log in and log out the approved leave passes. The time spent for each user to accomplish a task was recorded.

The ISO 9241-11 standard measure of usability has been used to evaluate Fygo. The application was evaluated using the effectiveness (completion rate), time-based efficiency, overall relative efficiency, and satisfaction of each user.

DESIGN

OVERVIEW

A proper workflow breaks down the key process/es an application undergoes to serve its function. This provides the programmer with workable goals that makes the application easier to code. This section provides the fundamental design for an assistive medical application. Program design guides the programming process eventually manifesting in the final product - allot sufficient time in designing the flowchart.

A. **Application Structure**
(Montero, Baldonado, Cruz)
Before the application could be programmed, its structure should be designed. The application structure should be designed based on the flow of events when using the application, as depicted in Figure 29.
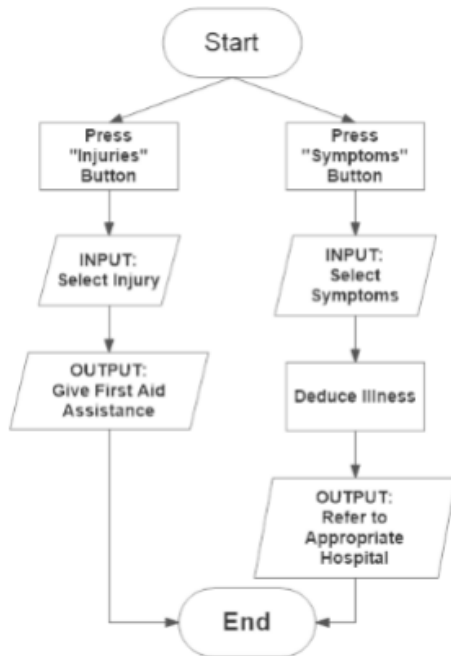
Figure 29. *Flow of events in the mobile application.*

IMAGE ACQUISITION AND ANALYSIS

OVERVIEW

Various functions such as acquisition, analysis, and comparison of data can be automated through the use of modern technology. This section provides the parameters in the acquisition of fish-eye images as well as introduces the software, tools, and processes to analyze them, particularly as they relate to color, value, and saturation. This may be applicable in similar studies involving image acquisition and analysis. A controlled environment, i.e. lighting, angle, etc., is crucial for research studies using images as it establishes the validity of the analysis and/or comparison. In image analyses, each software has specifications that make them ideal for certain uses, i.e. in the conduct of research; hence, the pros and cons of each software must be considered prior to its inclusion in the method.

A. Fish Images Acquisition
(Cadorna, Chan, Salmon, Salazar)

A color camera and an illumination chamber or lightbox as described by Dowlati et al. (2003) were used in an image acquisition system to capture the images of fish. Two LED lamps (Natural Daylight, 240 V / 4W) with a color temperature of 6500 K were used to capture high quality images under reproducible lighting conditions. The lamps with lengths of 30 cm were installed opposite of each other on the left and right sides of a wooden lightbox of dimensions of 50 x 50 x 30 cm. The interior walls were painted matte black to minimize background light and light reflectance. The sample tray was green in color for adequate contrast with the fish sample (Dowlati et al. 2013). The images of fish eyes were captured using a digital color camera (Nikon AF-S DX NIKKOR 18-55mm 1:3.5-5.6G VR II). Top-view images were taken at a vertical distance of 30 cm (Lehnert et al. 2011). All images were taken using constant camera settings (see Table 6). The LED lamps were turned on an hour before image capturing to ensure stable lighting (Dowlati et al. 2013).

Table 6. Camera control settings.

Variable	Settings
Image size	6000 x 4000 pixels
Zoom	No zoom
Flash mode	No flash
Sensitivity	ISO-200
Operation mode	Manual
Aperture Av.	f/6.3
Exposure time Av.	1/15 s
Image type	JPEG
Macro	On
Focal Length	18mm

B. Fish Image Analysis
(Cadorna, Chan, Salmon, Salazar)

Segmentation is the process of partitioning an image into regions that correlate strongly to features of interest in an image. The regions of interest, the eye and the pupil, were manually segmented using GNU Image Manipulation Program 2.10.8 to isolate them for color analysis (Lehnert et al. 2011). The elliptical marquee tool was used to select and segment images of the eye which includes the pupil and iris, and the pupil which appears as a black circle in the middle of the eye (Kanamori et al. 2017). The RGB values of the processed images were converted to their respective HSV equivalents. From the HSV matrices, only the saturation and value layers were used for analysis. All empty pixels of the image were removed before calculating for the mean saturation and value of the respective layers. Image analysis was done using Scilab 6.0.2 (Sengar et al. 2017).

IMAGE PROCESSING

OVERVIEW

Analyzing images can be made easier through the use of softwares and applications. The methods in this section provide a means of automating tasks using the software ImageJ. Each software has specifications that make them ideal for certain uses, i.e. in the conduct of research; hence, the pros and cons of each software must be considered prior to its inclusion in the method.

A. **Fractal Dimension**
(Aguirre, Sombiro, Valdestamon)

All 300 x 210 px photographs of the chorioallantoic membrane were analyzed using the software ImageJ. The blood vessels on the image were first reinforced by being manually overlaid with black using any image-editing software to have a strong contrast against the background.

These reinforced images were then converted to 8-bit grayscale in ImageJ and the range of dark colors that represent the blood vessels were isolated from the background. The blood vessels were skeletonized and the fractal dimensions were retrieved using the box-counting method set to a maximum of 64 boxes on ImageJ.

PROCUREMENT AND PROGRAM IMPLEMENTATION

OVERVIEW

Each hardware and database has different functions and efficiency in terms of their performance; thus, before any implementation is done, it is necessary to have the appropriate hardware and database for the conduct of the study. Making or modifying a program is also needed to determine a particular thing depending on the objective of the study. This section focuses on the procurement of hardware and database and the use of an Apriori algorithm that has been modified to provide the necessary details needed for the study. In this case, it aimed to output the execution time of the algorithm and the frequency of the most frequent itemsets.

A. **Hardware and Database Procurement**
(Celestial, Ibarreta, Tiron, Nulla, Oberio)
An ASUS Vivobook X442U laptop which had an Intel Core i5 3.4GHz Processor with 4GB RAM was used in the testing process. A grocery database having 9,835 transaction records in total was acquired from Salem Marafi (Salem 2014). It contained a collection of receipts with each line representing one (1) receipt and the items purchased. The database was then converted from a comma-separated values (.csv) file to a text (.txt) file by replacing the commas with spaces, since the source code used required plain text for its input.

B. **Algorithm Implementation**
(Celestial, Ibarreta, Tiron, Nulla, Oberio)
The Java source code of Apriori was acquired from Github (Umanghome 2016) and was modified to output the execution time of the algorithm and the frequency of the most frequent itemsets. The modified source code was then checked by two (2) consultants, Mr. Christian Chiu, the owner of SoftArtifact Incorporated, a product development company based in Koronadal City, South Cotabato, and Mr. Marc San Pedro, an engineer at Samsung Electronics.

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GENERAL

MAPPING

OVERVIEW

Maps represent the world on a smaller scale. To do mapping, methods such as study area determination, satellite image referencing, digitization, and image classification are done. This section provides the methods aforementioned to map the mangrove forest cover change in Jaro Floodway, Brgy. Bito-on, Jaro, Iloilo City.

A. Study Area Determination
(Flores, Larroder)

The study area included the mangrove cover area at the mouth of the Jaro Floodway in Brgy. Bito-on, Jaro, Iloilo City (Figure 30). A grid with a 200 m spacing bounded by geographic coordinates 122.5836°E, 10.7487°N and 122.5982°E, 10.7361°N was created in Quantum Geographic Information System (QGIS) software, version 3.4.6. The grid served as a guide in downloading high resolution images from Google Earth for the years 2005, 2009, 2012, 2014, 2016 and 2018.
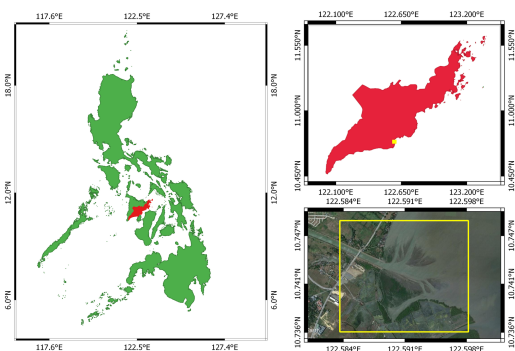
Figure 30. *Study area.*

B. Satellite Image Georeferencing
(Flores, Larroder)

Satellite images available from Google Earth as of December 2018 were georeferenced using Google Earth Pro, version 7.3.2 and Smart Geographic Information System (SmartGIS) 2019, version 19.11. Images were available for the years of 2005, 2009, 2012, 2014, 2016, and 2018. The georeferenced images were saved as a Geospatial Tagged Image File Format (GeoTIFF), a format that incorporates the latitude and longitude data into an image, then imported as a raster layer to QGIS for digitization.

C. Digitization and Image Classification
(Flores, Larroder)

The georeferenced images for each year were then classified into four (4) thematic classes using visual interpretation namely water, fishpond, mangrove, and non-mangrove areas. Water was identifiable as a dark blue color in the satellite image. Fishpond areas were rectangular shaped with a well-planned distribution, as seen through the satellite image. Mangroves were identifiable in the satellite images as green regions along the coast. These are the only plant species which could thrive on the salinity of the brackish water. Other areas which do not fit the criteria for the other three thematic classes were classified into a non-mangrove area. Every thematic class was then manually traced over using QGIS and saved as a shapefile layer. After the digitization process, the area of each thematic class, which was measured in hectares, was automatically determined by the software. An ocular inspection was also conducted in order to verify the classified areas.

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RESEARCH PROGRAM

The research program is a three-year undertaking that aims to inculcate the scholars with the quality and skills becoming of a world-class Filipino scientist. Each year starting from Grade 10 focuses on an aspect of research paramount to the growth of scholars in becoming leading figures in their respective fields.

RESEARCH 1

Research as a subject is introduced in Grade 10. It is the formative year for the budding researcher with in-depth discussions on the Scientific Method, and the Research Process. Particularly, it introduces scholars in topic selection, literature search, research design, and paper presentation. Scholars were also taught the basics of writing a proposal, and a research paper – both in instruction, and in practice. Each group or work unit is composed of at most five members with general topics provided by the Research 1 teachers, and further developed by the students. Research 1 culminates with the paper presentation of the Grade 10 scholars in *Pagsuguidadon*.

RESEARCH 2

Research 2 is the reinforcement year in Grade 11. The writing of the proposal, and the research paper are further scrutinized in this year. Each section of a standard research paper: abstract, introduction, method, results, discussion, and conclusion are more pronounced in the syllabus. Research 2 is performed in work units of, at most, three. Scholars are allowed to select any topic in alignment with their science options and/or elective under the supervision of their advisers, propose and defend their proposal to a panel, develop and/or enhance skills related to the study, perform the method outlined in their proposal, and report their findings in a full-fledged research paper. Research 2 concludes with the presentation of the results in the final defense.

RESEARCH 3

Research 3 is the culminating year of the research curriculum. It is reserved for Grade 12 scholars who organize activities and events that share their respective study's findings, including but not limited to: research presentation (*Pagbantala*), poster presentation (*Pagbalandra*), seminar-workshop *(Pahisayod)*, and community science conference (*Pagwaragwag*) with more events described at the end of this journal. This very journal is a fruit of the previous two batches' research experience. Research 3 focuses on finalizing the study and networking the results. It focuses on community-oriented approach in making science more accessible. This is evident in the oral and poster presentations for students and professionals, workshops and seminars for high school students, and gamification of each study for elementary students during the aforementioned events. Each study is given the opportunity to be published in this journal, provided that they have accomplished the review process outlined at the left.

RESEARCH EVENTS

The Research Events circuit is the series of research-related activities prepared by the Research Unit and/or the scholars with the aim of bringing their studies to the community. The logo to the right is the overarching symbol of the events circuit. The multicolored flame representing the multitude of activities the scholars undertake as embodied in each of the event logos. The interwoven circles represent the infinite possibilities in research. The logo itself is reminiscent of the PSHS logo, representing the values of truth, excellence, and service.

START OF SCHOOL YEAR

PAGBANTALA
SCHOOL-BASED RESEARCH CONGRESS

BANTALA
Hiligaynon
To inform

G12

PAGBANTALA is the first event in the circuit. It is a local research congress where scholars present their studies to a panel of experts. The logo therefore features a caricature of the main scene in Pagbantala: a panel of three, sitting behind the table facing the scholars and their presentation – collectively represented by the incomplete frame. As the first event, Pagbantala is colored red, symbolizing the flames igniting the Research Circuit.

PAGBALANDRA
SCHOOL-BASED POSTER PRESENTATION

BALANDRA
Hiligaynon
To show

G12

PAGBALANDRA is where scholars present their posters to an audience. The logo therefore features a caricature of an individual presenting his/her poster. Here, scholars prepare and showcase three materials: a technical poster for professionals, a community poster for elementary school students, and a headline poster to capture their audience's attention.

PAGHINUN-ANON
GRADE 11 RESEARCH SEMINAR

hinunan-anon
Hiligaynon
To inquire

G11

PAGHINUN-ANON is an event for Grade 11 scholars. As in Pagbantala, the logo features the main scene in Paghinun-anon: the work unit, a group of scholars, and a three man panel sit face to face to discuss the research proposal, the panel guiding the scholars in their proposed study. The logo is yellow – a joyful color – as this is a time of celebration, the first step towards realizing a study in the research curriculum.

PAHISAYOD
RESEARCH WORKSHOPS & GIMICKS

HISAYOD
Akeanon
To announce

G12

PAHISAYOD is among the largest events in the circuit. Scholars teach skills they've acquired while performing their studies to a group of students from provincial high school, as well as present their study to elementary students through an interactive game. The logo therefore shows the caricature of two individuals: one representing the scholars, the other the students they're presenting to, the white matter being the knowledge shared in this event. The green marks a transition in energy, from school-based events we now move on towards the larger community.

Cont'd

Cont'd

PAGWARAGWAG
COMMUNITY-BASED RESEARCH CONGRESS

WARAGWAG
Hiligaynon

To broadcast

G12

PAGWARAGWAG is Pagbantala, Pagbalandra and Pahisayod combined, this time brought to a larger audience outside the school. Every year, Pagwaragwag is brought to a different province. This is represented in the logo where the caricature shows a group of individuals facing a group of three: the scholars. The incomplete frame that represented the scholars in Pagbantala are now complete researchers in Pagwaragwag with the ability to communicate science in an elementary and secondary level.

PAINDIS-INDIS
JOURNAL COVER PAGE COMPETITION

INDIS-INDIS
Hiligaynon

To compete

G12

PAINDIS-INDIS is an on-campus cover page competition. This is how the cover of Publiscience is decided. Hence, the logo shows multiple panels that showcase each submission, two individuals are shown holding similar panels, as if they were issuing a vote. Each of Batch 2020's work units submitted an entry, the number of which is reduced in every stage of voting with teachers issuing the final vote.

PAGPABALHAG
RESEARCH JOURNAL PUBLICATION

PABALHAG
Hiligaynon

To publish

G12

PAGPABALHAG is the event that formally launches this year's Publiscience issue. Through the journal, the audience of the scholar's studies are expanded, networking through various individuals or groups that possess a copy. This is represented in the logo by caricatures of individuals surrounding the journal, giving it the collective shape of an atom. This is reminiscent of the PSHS logo, a symbol of the institution which nurtured the scholars up to this point. The same atom which can diffuse through borders and catalyze the exchange of knowledge.

PAGSUGUIDADON
GRADE 10 RESEARCH CONGRESS

SUGUID
Hiligaynon

To report

G10

PAGSUGUIDADON is the culminating event for Grade 10 scholars. Work units composed of five scholars present their study to a panel. In the logo, the five scholars are shown, the descending white matter representing their initiation into the research process. The white-grey color represents a blank slate, scholars that are ready to be molded, brimming with unknown potential.

END OF SCHOOL YEAR

INDEX

Page numbers followed by f, t, and p indicate figures, tables, and plates, respectively.

ACKNOWLEDGMENT

https://bit.ly/Pamaagi