Nano-sized Hydroxyapatite Adsorbent Synthesis from Calcined Green Mussel *(Perna viridis)* Shells (nHAP-cGMS) for Phosphate Sequestration

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Abstract

Anthropogenic activities, particularly urbanization, results in the excessive presence of phosphate ions in aquatic ecosystems which contribute to eutrophication. This phenomenon poses significant environmental and health risks that necessitates novel ways in nutrient sequestration. This study explores the synthesis of a nano-sized hydroxyapatite adsorbent from calcined green mussel (Perna viridis) shells (nHAp-cGMS) through chemical precipitation and its characterization in terms of surface morphology, elemental composition, and its constituent functional groups through testing of Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Spectroscopy (EDX), and Fourier Transform Infrared Spectroscopy (FTIR), respectively. Batch kinetic study and UV-Visible Spectroscopy (UV-Vis) were executed to test efficiency of nHAp-cGMS in adsorbing phosphate ions. As assessed from the results, the favorable structural and chemical characteristics of the absorbent, combined with the statistical analyses indicating significant differences in varying concentrations of phosphate before and after treatment, emphasizes the capability of the synthesized adsorbent in sequestering phosphate ions present in phosphate-contaminated water samples. This study highlights the potential of a green mussel shell-based adsorbent in nutrient adsorption for environmental remediation.

Keywords*: eutrophication, nutrient sequestration, nHAp-cGMS, adsorbent*

CHAPTER I: THE PROBLEM AND ITS BACKGROUND

a. Introduction, Background of the Study

Unsustainable anthropogenic activities result in the discharge of untreated effluents into the bodies of water, which contributes to the persistent water pollution and eutrophication (Suhani et al., 2021). Nutrient enrichment, such as that of cadmium (Cd), lead (Pb), nitrogen (N), phosphorus (P), and other elements and ions that pose significant threats to biodiversity through eutrophication and oxygen loss, health, and mainly water quality (Neijnens et al., 2024). One main eutrophication agent, phosphate, acts as a limiting nutrient, triggering algae overgrowth and eventually depleting oxygen supply in the water, resulting in an uninhabitable ecosystem for marine organisms (UN Environment Programme, 2024). As a result, various preventive efforts had been invented and implemented such as nutrient sequestration, particularly the primary, secondary, and tertiary stage, which includes adsorption to mitigate the aforementioned environmental impacts (Usman et al., 2022).

Adsorption, considered as one of the most effective techniques in water treatment, particularly for high volumes of samples is preferred due to its design and operation's simplicity and the process' high efficiency and selectivity (Usman et al., 2022) This surface process, where molecules from a fluid (gas or liquid) accumulate on a solid surface, forming a thin layer (Hussain et al., 2023). Still, creating solutions and assessing

adsorbent materials is still a global challenge. One promising strategy is the valorization of waste and biomass through recycling and conversion into new materials (Santhasivam et al., 2024). In recent years, studies have explored various natural sources such as waste materials to synthesize environmentally-friendly adsorbents which included egg shells, groundnut shells, coconut husk, and particularly calcium carbonate derived materials such as apatite (Agbeboh et al., 2020). Among these, a natural form of calcium apatite, hydroxyapatite (HAp, $Ca_5(PO_4)_3(OH)$), has high affinity to phosphorus (Shaker & Deftos, 2023). Due to its enhanced surface area and reactivity, it has been considered as a great source of material for nano-sized hydroxyapatite (nHAp) for phosphate absorbance. However, conventional nHAp synthesis often relies on expensive and non-renewable precursors, limiting its large-scale application. In addition to this, synthesizing nHAp requires high temperatures, especially in the precursor's calcination, which greatly influences the process' energy footprint (Oluremi, 2025).

This study explored the valorization of green mussel *(Perna viridis)* shells (GMS) as a calcium source for the synthesis of a nano-sized hydroxyapatite from calcined green mussel shells (nHAp-cGMS) (Rahman et al., 2024). Green mussels, referred locally as *"tahong,"* are a common biowaste in Philippine coastal areas as a result of improper disposal (Arellano et al., 2022). Furthermore, GMs are known for their affordability, adaptability, and nutritional value. However, their high demand generates significant volumes of shell waste, which, if not managed properly, might pose environmental risks (Ramoran, 2023). This study highlights their potential to be converted into a valuable adsorbent for water treatment.

The primary objective of this study is to synthesize nHAp-cGMS adsorbent to reduce the amount of phosphate ions present in water, alleviating eutrophication. Ergo, this study aims to answer the following research questions: (1) What are the characteristics of the synthesized nHAp-cGMS adsorbent in terms of (a) topography (surface features) and morphological properties and (b) elemental composition present in the material?; (2) How efficient is the nHAp-cGMS adsorbent from GMS in sequestrating phosphate ions in phosphate-concentrated water in terms of kinetic study?; (3) Is there a significant statistical difference in the phosphate ions concentration in the aqueous solution before (represented as the blank solution) and after treatment?

b. Statement of the Problem

Despite the availability of various water treatment methods, the persistent discharge of untreated effluents into bodies of water continues to cause severe pollution and eutrophication, largely driven by excessive nutrient loads such as phosphate ions. Addressing this challenge requires effective and sustainable adsorbent materials. However, green mussel shells—one of the most abundantly produced biowastes in the Philippines—remain improperly managed, even though they contain calcium compounds suitable for conversion into hydroxyapatite. Conventional hydroxyapatite synthesis relies on costly and non-renewable precursors, limiting its practical application in large-scale water treatment. Therefore, this study aims to identify a sustainable, low-cost, and efficient material for phosphate removal by evaluating green mussel shells as an alternative calcium source for hydroxyapatite synthesis.

c. Significance of the Study

By utilizing green mussel shell waste, this study not only supports cleaner water systems but also helps reduce pollutant accumulation along riverbanks and underscores the importance of waste valorization. It aligns with the United Nations' 6th and 14th Sustainable Development Goals, promoting access to clean water, proper sanitation, and the protection of marine ecosystems. Through converting discarded shells into a functional material for water treatment, the research encourages improved waste management practices and reinforces environmental responsibility. Furthermore, this study is beneficial to:

1) *Environmental sectors and policymakers* – The findings provide a sustainable and cost-effective strategy for addressing nutrient pollution and eutrophication. This offers policymakers evidence-based options for integrating waste-derived adsorbents into existing water treatment programs, potentially improving regulatory frameworks and environmental interventions.
2) *Local communities and aquaculture industries* – Communities involved in green mussel production and consumption benefit from reduced shell waste accumulation in coastal areas. By transforming biowaste into a valuable product, the study supports cleaner surroundings, promotes circular economy practices, and may create opportunities for small-scale livelihood initiatives tied to waste collection and processing.
3) *Researchers, academic institutions, and future innovators* – The study contributes additional knowledge on alternative calcium sources for hydroxyapatite synthesis, supporting ongoing efforts to develop eco-friendly adsorbents. It can serve as a reference for future research on biowaste valorization, nanomaterial synthesis, and sustainable water treatment technologies.

d. Scopes and Delimitation of the Study

This study focuses exclusively on the sequestration of phosphate ions from phosphate-concentrated water and does not evaluate the adsorbent's effectiveness against other contaminants. The adsorbent is synthesized solely from green mussel shells (GMS), and the study is limited to assessing its potential for phosphate removal without exploring other possible applications or material properties. Furthermore, the research is confined to small-scale production due to the high-temperature and energy-intensive requirements involved in synthesizing nano-sized hydroxyapatite from calcined GMS (nHAp-cGMS). The use of chemicals, such as disodium hydrogen phosphate (Na_2HPO_4), is also restricted to minimal quantities due to financial constraints associated with reagent costs.

CHAPTER II: REVIEW OF RELATED LITERATURE

a. Review of Related Literature

Excess presence of nitrogen and phosphate in waterworks acts as fertilizers for aquatic plants, making them grow faster than the capacity marine ecosystems can handle, causing harmful algal blooms (HABs) (US EPA, 2023). This mechanism causes eutrophication, and increases harm to water quality, food resources and natural habitats, and a depletion in the oxygen provided to fish and other aquatic organisms. Furthermore, HABs produce toxins harmful to humans and animals which can contaminate sources of drinking water, and cause gastrointestinal, neurological, and respiratory problems (Vantarakis, 2021).

In addition to this, phosphorus exists in the form of phosphate ions in environmental water systems due to it being the most stable, soluble form released from rocks through weathering and available for uptake by aquatic organisms. Phosphate promotes eutrophication which then blocks sunlight for marine organisms development. Fertilizers, manure, and other agricultural runoff, as well as wastewater (e.g. sewage and septic systems), and natural sources like weathering rocks, decomposing organic matter, and urban runoff are the primary sources of phosphorus content in water (UN Environment Programme, 2023).

Consequently, conservative efforts had been developed to combat the persistent problem of aquatic nutrient enrichment. There are three main stages in nutrient sequestration, mainly: primary, secondary, and tertiary (Usman et al., 2022). The primary stage focuses on letting the solid particles float or settle, integrating sedimentation, for it to become physically separated. The secondary treatment then employs aeration, filtration,

activated sludge, and biofilm formation to remove biological oxygen demand (BOD) and chemical oxygen demand (COD). However, while this eliminates residual biodegradable organic matter and suspended solids, it is not as efficient in removing nitrogen and phosphorus, resulting in high concentrations of phosphorus in aquatic environments. Secondary treatment produces excess sludge, increasing wastewater management costs to be higher (Cepan et. al, 2021). Lastly, the tertiary treatment is an advanced treatment that targets nitrogen and phosphorus, trace organics, and other pollutants by removing low concentrations of contaminants the previous stages cannot effectively eliminate.

Adsorption, part of the tertiary stage, is an effective alternative for water treatment which had been in recent development as previous strategies, especially for phosphate sequestration, had their set of drawbacks. This technology offers several advantages, such as low cost, high selectivity, effective removal of phosphate ions even at low concentrations, ease of operations, simple design, high adsorption capacity, and minimal by-products production (Mekonnen et. al, 2021). It is a sorption process where molecules, atoms, or ions from a gas, liquid, or dissolved solid create a film-like structure to adhere to a surface (Artioli, 2023). The efficiency of adsorption largely depends on the properties of the adsorbent, including the surface's charge, area, and functionality. The primary advantage of this process is that adsorbents may be renewed by regeneration through desorption (Kumar et al., 2024). The recovered phosphates can then be recycled and reused in various applications, most notably as raw material for fertilizer production to enhance agricultural productivity (Almanassra, 2021).

Various materials had been proposed for phosphorus sorption in water, but sorbents, particularly nano-sorbents, are recyclable, allowing for easy desorption and recovery of adsorbed pollutants (Oghyanous, 2022). Hydroxyapatites (HAps), a naturally-occurring mineral mainly found in tooth enamel and bone mineral, is inexpensive, has high adsorption capacity, is biodegradable, and is chemically stable, is being developed recently as an efficient adsorbent (He, 2024). HAp adsorbents, specifically nano-sized HAps, have exceptional capacity to clean wastewater by eliminating fluoride ions, organic contaminants, radioactive elements, and heavy metal ions. The ionic exchange reaction, surface complexation, co-precipitation of new partly soluble phases, and physical adsorption processes including electrostatic contact and hydrogen bonding are all supported by the hexagonal crystal structure of HAp (Balasooriya et. al, 2022). Natural sources include eggshells, seashells, fish bones, coral reefs, and bovine bones (Mohd Pu'ad, 2019). These sources are abundant and eco-friendly, providing the necessary calcium and phosphate for HAp production.

The Philippines, being a tropical coastal country, has fishery as one of its main sources of livelihood, containing the sub-category of the mussel industry. Requiring little capital investment, mussel farming provides additional income and livelihood for fisherfolk in the country (Site Suitability Assessment for Mussel, n.d.). The most common and commercially farmed mussel species is the green mussel *(Perna viridis)*. Being a common food ingredient, its shell is also a major unmanaged waste as it is considered as a significant environmental issue, but is also considered valuable for valorization reasons. These calcium carbonate rich shells can be converted into useful products such as construction materials (e.g. concrete aggregate), soil conditioners and fertilizers, fire retardants for paints, adsorbent for waste water treatment, and even nanomaterials (Ramoran, 2023). Due to its calcium carbonate content, which can act as an immobilizer, and its capability as a nanomaterial, GMS has a high potential as a nHAp phosphate sorbent.

Synthesis

Aquatic eutrophication, driven by the excessive input of nitrogen and phosphorus, has been widely recognized as a major environmental problem affecting water quality, ecosystem services, and human health. Excess nutrient loading into aquatic systems stimulates rapid algal growth, leading to harmful algal blooms

(HABs), oxygen depletion, and the disruption of aquatic food webs, which ultimately degrade water quality and biodiversity (US EPA, 2023; Wang 2024). HABs not only reduce dissolved oxygen, causing hypoxic "dead zones" that cannot support healthy fish populations, but also produce toxins that may pose health risks to humans and animals via contaminated drinking water and seafood (US EPA, 2023; Wang 2024). These ecological and public health impacts underscore the importance of effective nutrient management strategies in aquatic environments.

Phosphorus, particularly in the form of soluble phosphate ions, is a key nutrient that exacerbates eutrophication once it enters water bodies from agricultural runoff, wastewater discharges, and natural weathering. Phosphate stimulates algal proliferation, which in turn blocks sunlight necessary for submerged aquatic vegetation and ultimately alters ecosystem structure (UN EPA, 2023; Wang 2024). Due to its high solubility and biological availability, phosphorus is more challenging to remove with conventional wastewater treatment processes alone, necessitating advanced removal techniques to mitigate ecosystem degradation.

Conventional wastewater treatment typically involves primary, secondary, and tertiary stages. Primary and secondary treatments focus on removing solids and reducing organic loads but are limited in their ability to remove dissolved nutrients such as nitrogen and phosphorus, often resulting in elevated nutrient levels in treated effluents (Usman et al., 2022; Cepan et al., 2021). These limitations highlight the need for more effective tertiary processes that specifically target nutrient removal.

Among tertiary treatment approaches, adsorption has emerged as an effective and versatile method for removing nutrients at low concentrations. Adsorption relies on surface-based interactions where dissolved ions adhere to the surfaces of solid adsorbents, and it offers advantages such as high selectivity, simplicity of design, and low secondary pollution (Artioli, 2023; Mekonnen et al., 2021). Crucially, adsorption can be reversible, allowing for regeneration of the adsorbent and recovery of nutrients for reuse, which contributes to circular resource management (Kumar et al., 2024; Almanassra, 2021).

The kinetics of phosphate adsorption are critical to understanding and optimizing treatment performance. Studies consistently show that adsorption capacity often increases rapidly at initial contact times, which reflects abundant available adsorption sites, but slows as equilibrium is approached due to site saturation and diffusion limitations. Pseudo-second-order kinetic models frequently provide the best fit to experimental data, indicating that chemisorption processes largely control adsorption rates (Brahmi, 2025; Yu et al., 2024). Such kinetic behavior underscores the importance of optimizing contact time and adsorbent characteristics for efficient phosphate removal.

Hydroxyapatite (HAp)-based materials have attracted significant research interest as phosphate adsorbents due to their highly porous structure, large surface area, and abundance of reactive functional groups, which enhance adsorption capacity and selectivity (Brahmi, 2025). Hydroxyapatite's surface structure supports mechanisms such as ion exchange, surface complexation, and co-precipitation, contributing to its effectiveness in sequestering orthophosphate ions from aqueous environments (Balasooriya et al., 2022). In addition, HAp exhibits chemical stability and biodegradability, making it a promising candidate for sustainable water treatment applications.

The synthesis and application of HAp from low-cost and waste-derived materials have further expanded its potential utility. Natural calcium-rich wastes such as eggshells, seashells, fish bones, and coral can serve as precursors for HAp production, providing eco-friendly alternatives to synthetic reagents (Mohd Pu'ad, 2019). This approach aligns with circular economy principles by valorizing waste streams and producing functional adsorbents with minimal environmental impact.

In the Philippine context, green mussel *(Perna viridis)* shells represent an abundant and underutilized waste resource with high calcium carbonate content. The valorization of green mussel shell waste not only mitigates shell disposal issues but also offers a locally relevant source for synthesizing nano-hydroxyapatite (nHAp) adsorbents with high phosphate removal potential. Nano-sized HAp derived from such biogenic sources may exhibit enhanced surface area and reactivity, further improving adsorption performance for nutrient sequestration.

Despite the recognized effectiveness of HAp-based sorbents, research directly examining nHAp synthesized from green mussel shells for phosphate adsorption remains limited, particularly within the Philippine setting. Addressing this gap would contribute to both improved wastewater treatment strategies and sustainable waste utilization, further advancing efforts to manage eutrophication and protect coastal water quality.

c. Theoretical Framework/Conceptual Framework

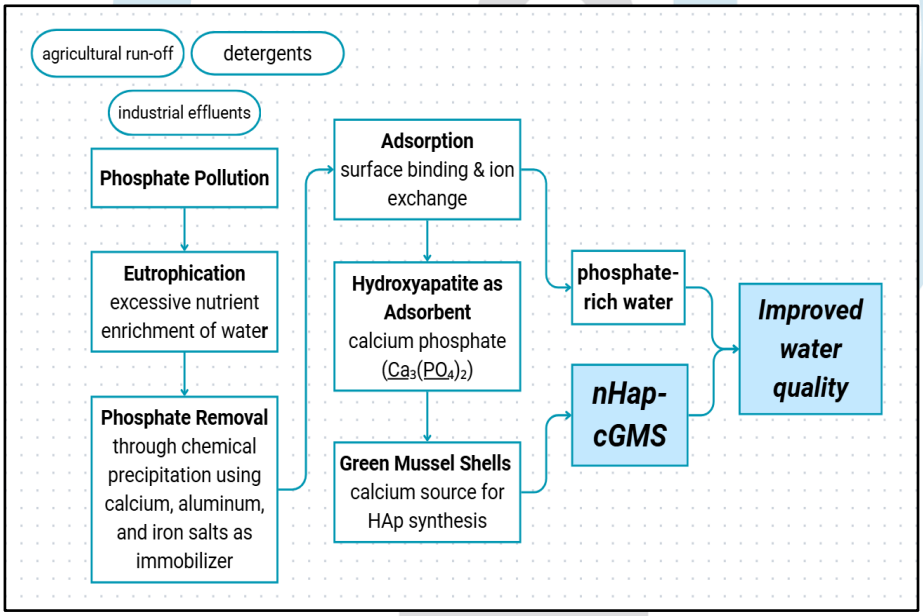

Figure 1. Conceptual Framework

d. Definition of Terms

Chemisorption - involves the formation of a stable chemical bond between a modifier molecule (the adsorptive) and a surface (the adsorbent) (Atif et al., 2022).

Desorption - essentially the opposite of adsorption; the release of substances that was previously bound to a substance's surface (WetlandInfo, 2023).

Eutrophication - high nutrient richness in water, often brought on by runoff from the land, which reduces the amount of oxygen available in the ecosystem, resulting in dense plant growth and animal mortality (NOAA, 2024).

Green mussel shells (GMS) - referred locally as "tahong," are a common biowaste in Philippine coastal areas as a result of improper disposal (Arellano et al., 2022).

Hydroxyapatite (HAp) - stable naturally-occurring mineral form of calcium carbonate and is mostly found in tooth enamel and bone structures (He, 2024).

Nanosized hydroxyapatite (nHAp) - hydroxyapatite particles that are extremely small, usually between 50 and 1000 nanometers. For a variety of uses, their nanoscale size enables them to pass through pores and mimic non-organic bone structure (Bordea et al., 2020).

Nanosized hydroxyapatite from calcined green mussel shells (nHAp-cGMS) - HAp synthesized using calcined green mussel shells powder as its precursor.

Precipitated Calcium Carbonate (PCC) - synthetic form of calcium carbonate ($CaCO_3$) produced through a controlled precipitation process (Mining, 2024).

Precipitation - the process of converting metal ions into hydroxide, sulfide, carbonates, or other compounds with lower solubility (Rahimpour and Haghighatjoo, 2024).

Waste valorization - a process of turning wastes into a more valuable and useful material.

CHAPTER III: METHODOLOGY

a. Research Design

This study adopted an experimental quantitative research design, integrating both chemical characterization and performance evaluation to assess the efficiency of nHAp-cGMs for phosphate ions sequestration in phosphate-contaminated waters. The experimental phase involved the synthesis of the nHAp-cGMS through wet precipitation method followed by batch adsorption experiments, which aimed to assess how rapid the synthesized material works.

b. Sample and sampling procedure/Sample Preparation

GMS were acquired from a local wet market in Bacoor, Cavite. The shells were thoroughly washed with distilled water and sun-dried for 24 hours. Once dried, the shells were grounded into a fine powder using a marble mortar and pestle and electric blender. The fine powder was then sieved to ensure consistency and remove contaminants such as gritty bits of shells and leftover meats.

The disodium hydroxide phosphate (Na_2HPO_4), which was utilized for the preparation phase was purchased from DKL Laboratory Supplies. The solution was prepared using distilled water.

c. Data Gathering Procedure

C.1. Preparation of the nHAp-CGMS

C.1.1. Shell Calcination

The calcination process, an endothermic reaction, was conducted to convert calcium carbonate ($CaCO_3$) present in the mussel shells into calcium oxide (CaO) (Neisan et al., 2025) as shown in the following reaction:

$$CaCO_3 \rightarrow CaO + CO_2 \Delta H = 182.1 \text{ kJ mol}^{-1}$$

The powdered shells was calcined in a muffle furnace at 900 °C for five hours at Measure Engineering Services in San Pedro, Laguna. Subduing the material in exorbitant temperature yielded 76 grams of calcium oxide from the initial 125 grams of calcium carbonate.

C.1.2. Hydroxyapatite Synthesis

The calcined powdered shells were used to synthesize Precipitated Calcium Carbonate (PCC) through wet precipitation. 5 grams of powdered shells were treated with 40 mL of 1.33 M disodium hydrogen phosphate (Na_2HPO_4) solution to achieve a 1.67 Ca/P molar ratio (Cescon et al., 2025). The mixture is stirred under room temperature (25 ± 0.5 °C) for 15 minutes at 700 revolutions per minute (rpm) to allow crystal formation. Subsequently, a filtration system was set up using a qualitative filter paper with pore size 4-12 μm.

The heterogeneous solution was carefully poured through the filter paper to separate the precipitate HAp from the liquid, allowing the gradual flow of carbon dioxide gas. The resulting milky white precipitate was then washed and filtered with distilled water to a pH of 7 and then dried in the oven at 100°C for 2 hours to remove residual moisture at Magayon Ventures OPC in Silang, Cavite. The obtained material was referred to as: nHap-cGMS.

C.2. Characterization

C.2.1. Scanning Electron Microscopy (SEM)

The morphology of the synthesized material was observed through Scanning Electron Microscopy using Hitachi Tabletop Microscope TM4000II in Nanotech Analytical Services and Training Corporation (NASAT) Failure Analysis Laboratory in C. Arellano, Muntinlupa. Sample preparation involved attaching an aluminum stub to the nHAp-cGMS using a conductive carbon table, allowing electron flow. The images were acquired using Backscattered electron (BSE) with an accelerating voltage of 15 kV at a working distance of 8.1 mm and low vacuum level. Magnification levels were fixed at 100x, 500x, 1500x, and 5000x, and were analyzed in different scale bars ranging from 10 μm to 500 μm. The images acquired were presented into five different spectra.

C.2.2. Energy Dispersive X-ray Analysis (EDX)

The Energy Dispersive X-Ray Analysis (EDX) was performed using Oxford Xplore Compact 30 (NASAT Failure Analysis Laboratory, C. Arellano, Muntinlupa). This test was conducted to identify the elemental composition of the nHAp-cGMS.

C.3. Formulation of Phosphate-concentrated Water

A mother simulated phosphate-contaminated solution yielded 1000 parts per million (ppm) solution. 0.906 g of hydrated monopotassium phosphate (KH_2PO_4) was diluted in 200 mL of distilled water.

Following this method, the mother solution yielded a 100 ppm stock solution and a 10 ppm working solution. Seven standard phosphate solutions were then prepared from the 10 ppm working solution, with concentrations of 0 ppm (distilled water as blank), 1 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm.

C.4. Batch Experiment

C.4.1. Kinetic Study

A phosphate ion solution with an initial concentration of 10 mg L^{-1} was mixed with the adsorbent at a solid-to-liquid ratio of 5:1 (mg mL^{-1}) in a 20 mL glass vial to hold the solution during the experimentation process. Polypropylene (PP) screw caps with polytetrafluoroethylene (PTFE) septa — a type of cap incapable of generating a chemical reaction — was used to seal the bottles tightly and prevent phosphorus contamination. The mixture was stirred at room temperature (25 ± 0.5 °C) using an orbital shaker which ran at 120 rpm. The experiment was carried out at pH 7 to prevent phosphorus precipitation and simulate natural water conditions.

Afterwards, the time-dependent samples — with each having time intervals equal to 20, 40, and 60 mins) were transferred in a 10 mL glass vial. After each interval, the solution was filtered using a qualitative filter paper.

d. Research Instrument

Ultraviolet-Visible (UV-VIS) Spectroscopy was used to quantify the concentrations of phosphate ions in solutions before (0 ppm) and after contact with nHAp-cGMS during kinetic adsorption experiments. To prepare the molybdenum blue reagent for phosphate determination, four stock solutions were prepared: (1) 2.5 M sulfuric acid (H_2SO_4); (2) 4.0 g of ammonium molybdate dissolved in 100 mL of distilled water; (3) 0.28 g of potassium antimonyltartrate (KAT) dissolved in 100 mL of distilled water; and (4) 1.76 g of ascorbic acid ($C_6H_8O_6$) dissolved in 100 mL of distilled water. A mixed reagent was then formulated by combining 10 mL of the sulfuric acid solution, 3 mL of the ammonium molybdate solution, 1 mL of the KAT solution, and 6 mL of the ascorbic acid solution (Blackburn, 2020). This mixture served as the color-developing agent in the subsequent phosphate analysis.

For the colorimetric analysis, seven 10 mL phosphate standard solutions (0 ppm, 1 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm) were intermixed with 3 mL of the molybdenum blue reagent in separate 10 mL vials. The resulting solutions were used for the spectrophotometric determination of phosphate ions concentration. All procedures were completed in JBL Scientific Lab in Sta. Ana, Manila with the supervision of Mr. Juan Benigno Luarca.

e. Statistical Treatment

To analyze the amount of visible light transmitted by the nHAp-cGMS across different wavelengths, the Independent-Samples Kruskal-Wallis Test ($\alpha = 0.05$) compared the mean ranks of four sample groups: 10 ppm standardized phosphate solution, and samples collected at 20, 40, and 60 mins adsorption time. This analysis aimed to determine whether significance differences existed among the varying concentrations. The Dunn-Bonferroni test was then applied to all 6 possible pairwise combinations among the four groups to determine which specific pairs exhibited statistically significant differences.

f. Ethical Consideration

This study was conducted with careful attention to ethical and environmental responsibility. All materials used in the synthesis of nHAp-cGMs were handled in accordance with standard laboratory safety and waste disposal protocols to prevent harm to researchers and the environment. Green mussel shells utilized in the study were collected as discarded biowaste, ensuring that no harm was caused to living organisms or natural ecosystems. The phosphate solutions used were prepared in controlled, small-scale quantities to minimize chemical waste and environmental risk. All experimental procedures were performed under proper supervision, and no human or animal subjects were involved in the study. The researchers ensured accurate data recording, honest reporting of results, and proper acknowledgment of all referenced studies to uphold academic integrity.

CHAPTER IV: PRESENTATION, ANALYSIS AND INTERPRETATION OF DATA

a. Characterization

A.1. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to examine the surface morphology of the nHAp-CGMS. The images collected for the sample are reported in Figures 2 to 6. The sample exhibited agglomerated particles of a heterogenous and irregular morphology and varying size. Larger, blockline crystalline structures are intermixed with clusters of fine, granular particles. This mechanism explained the rough and porous appearance of the surface, which aligned with the represented imperceptible structure of the sample having a non-uniform distribution of particles, with several void-like spaces due to the dense packings of some regions. This structure implies that the synthesized nHAp-cGMS demonstrates a large surface area, allowing enhanced adsorption capacity (Söğüt and Gülcan, 2023).

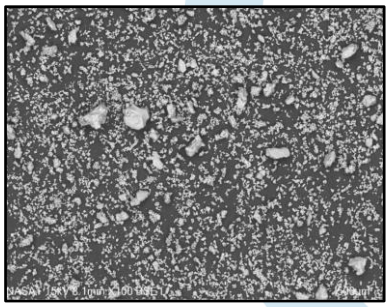

Figure 2. *SEM image of nHap-cGMS under 100x magnification*

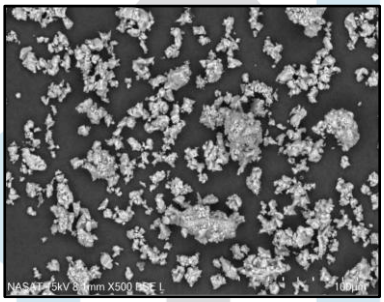

Figure 3. *SEM image of nHap-cGMS under 500x magnification*

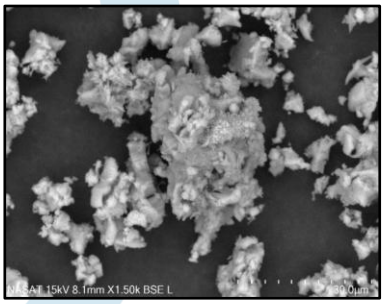

Figure 4. *SEM image of nHap-cGMS under 1500x magnification*

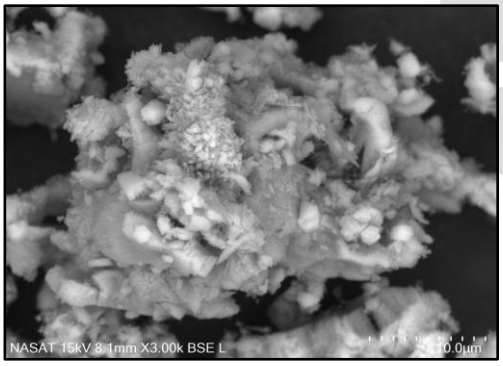

Figure 5. *SEM image of nHap-cGMS under 3000x magnification*

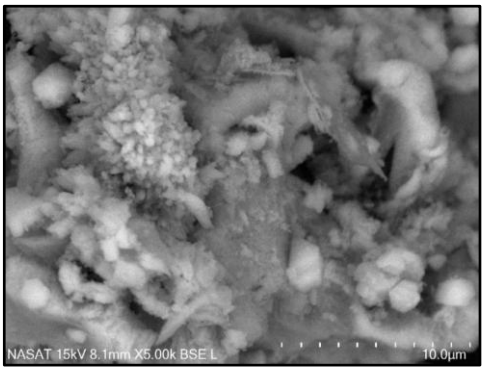

Figure 6. *SEM image of nHap-cGMS under 5000x magnification*

A.2. Energy Dispersive X-Ray Analysis (EDX)

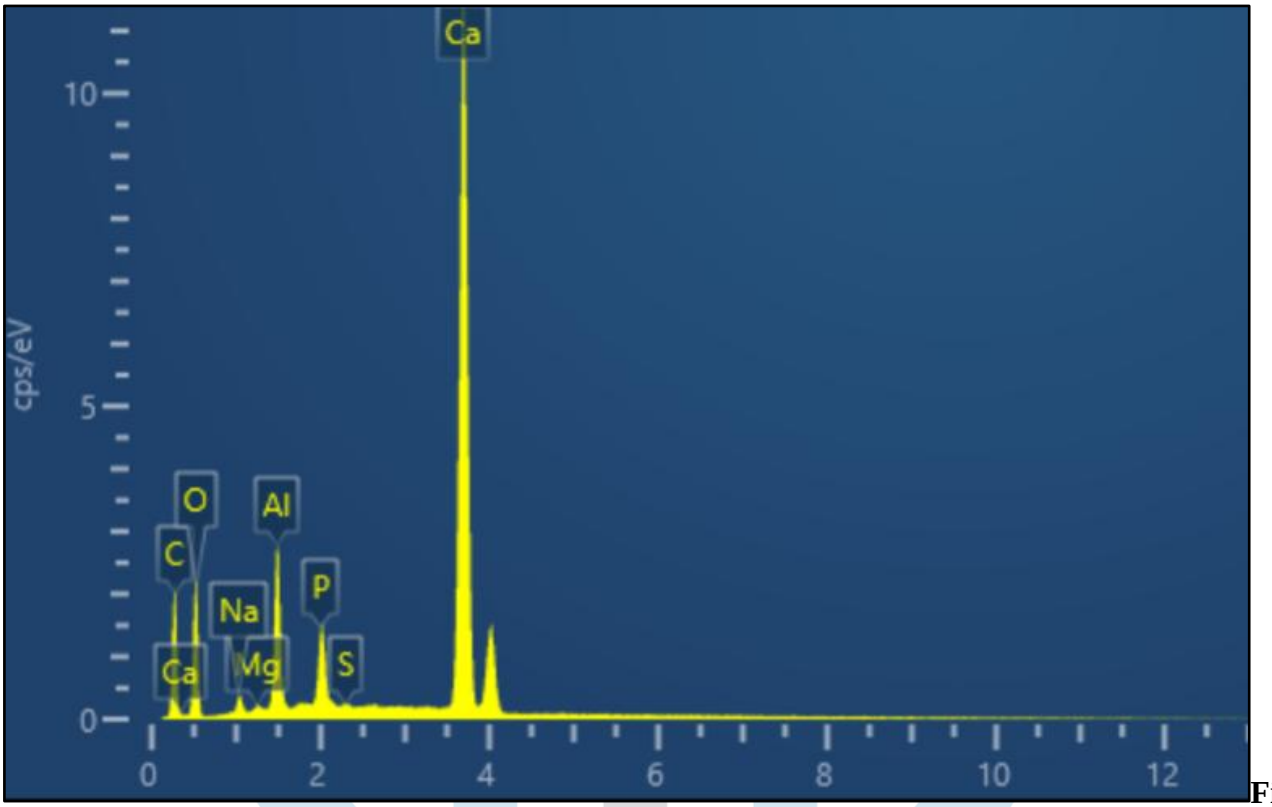

Figure 7. *EDX Analysis of nHap-cGMS for Spectrum 1*

Table 1.
EDX Analysis of nHap-cGMS for Spectrum 1

Spectrum 1			
Element	**Weight %**	**Weight % Sigma**	**Atomic %**
C	22.98	0.33	38.39
O	28.17	0.31	35.32
Na	0.77	0.04	0.68
Mg	0.17	0.03	0.14
Al	4.62	0.06	3.43
P	2.48	0.05	1.61
S	0.13	0.03	0.08
Ca	40.67	0.26	20.35
Total	100.00		100.00

In the first spectrum of the analyzed nHAP-cGMS, by percentage, the three main constituents of the sample are carbon (C), oxygen (O), and calcium (Ca), having a weight percentage of 22.98, 28.17, and 40.67, respectively. Aside from this, other minor elemental compositions of the formulated nHAP-cGMS are aluminum (Al), phosphorus (P), sodium (Na), magnesium (Mg), and sulfur (S), with all the constituting elements' weight percentage and atomic percentage summing 100.00.

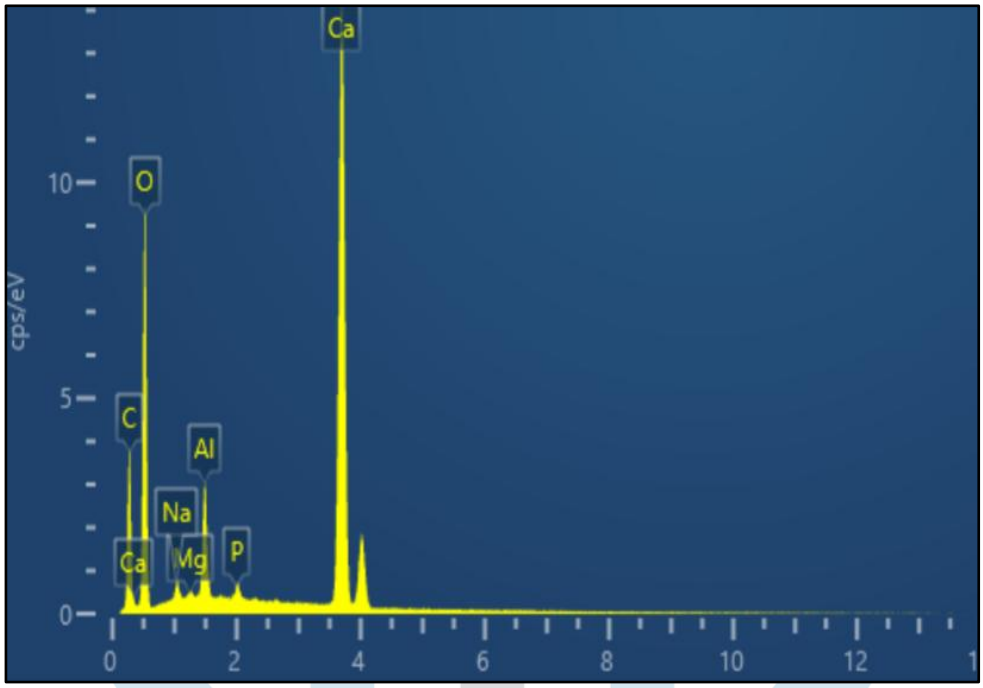

Figure 8. *EDX Analysis of nHap-cGMS for Spectrum 2*

Table 2.
EDX Analysis of nHap-cGMS for Spectrum 2

Spectrum 2			
Element	**Weight %**	**Weight % Sigma**	**Atomic %**
C	19.35	0.27	29.39
O	48.13	0.27	54.89
Na	0.66	0.05	0.52
Mg	0.15	0.03	0.11
Al	2.73	0.05	1.84
P	0.36	0.03	0.21
Ca	28.62	0.18	13.03
Total	100.00		100.00

For the second spectrum of the nHAP-cGMS, it was found that it was also composed of carbon (C), calcium (Ca), and oxygen (O), with the difference with spectrum 1 as oxygen became the major constituent,

comprising 48.13% of the weight of the sample, while carbon and calcium comprised 19.35% and 28.62%, respectively. Additionally, it was also composed of the following elements: aluminum (Al), sodium (Na), magnesium (Mg), with sulfur (S) not being present in the spectrum.

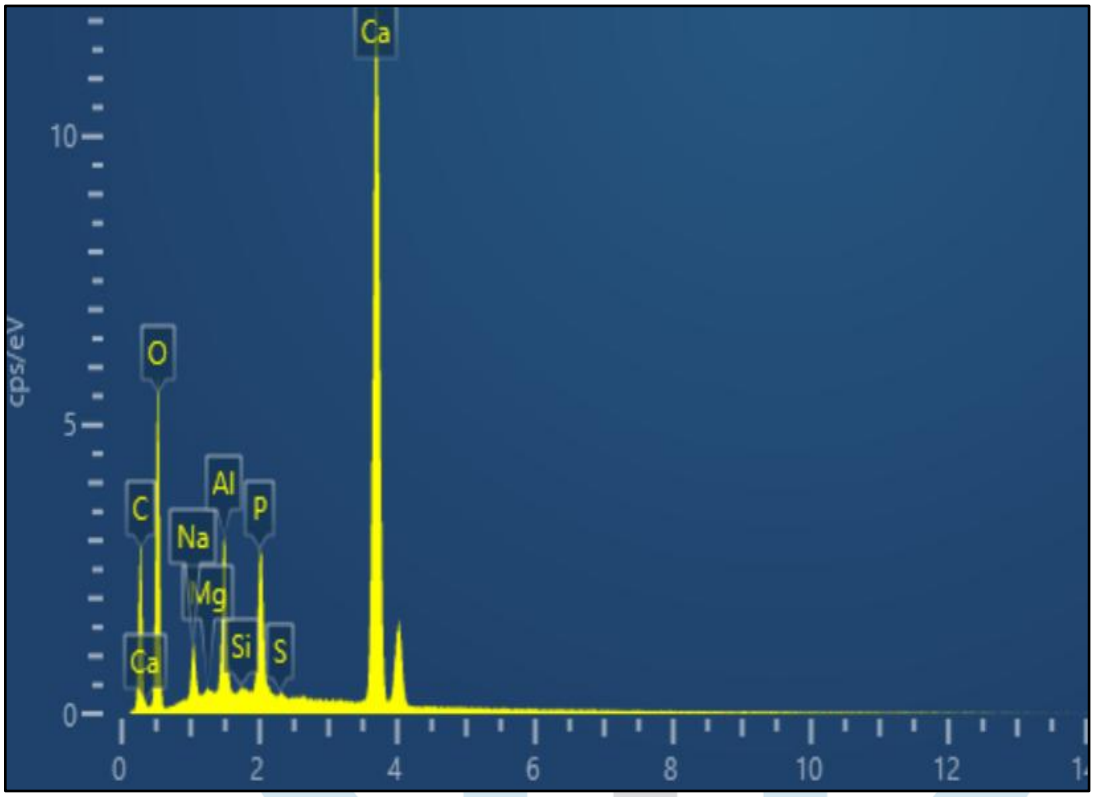

Figure 9. *EDX Analysis of nHap-cGMS for Spectrum 3*

Table 3.
EDX Analysis of nHap-cGMS for Spectrum 3

Spectrum 3			
Element	**Weight %**	**Weight % Sigma**	**Atomic %**
C	22.31	0.32	34.63
O	39.07	0.29	45.53
Na	1.74	0.06	1.41
Mg	0.12	0.03	0.09
Al	3.32	0.05	2.29
Si	0.09	0.03	0.06
P	3.35	0.05	2.02
S	0.12	0.03	0.07
Ca	29.87	0.20	13.90
Total	100.00		100.00

Spectrum 3 had the most elements comprising it, being composed mainly of carbon (C), calcium (Ca), and oxygen (O), making up 22.31%, 29.87%, and 39.07%, respectively. Oxygen became the major constituent, similar to spectrum 2. Moreover, trace amounts of phosphorus (P), aluminum (Al), sodium (Na), magnesium (Mg) and sulfur (S), and minimal traces of silicon (Si) were detected.

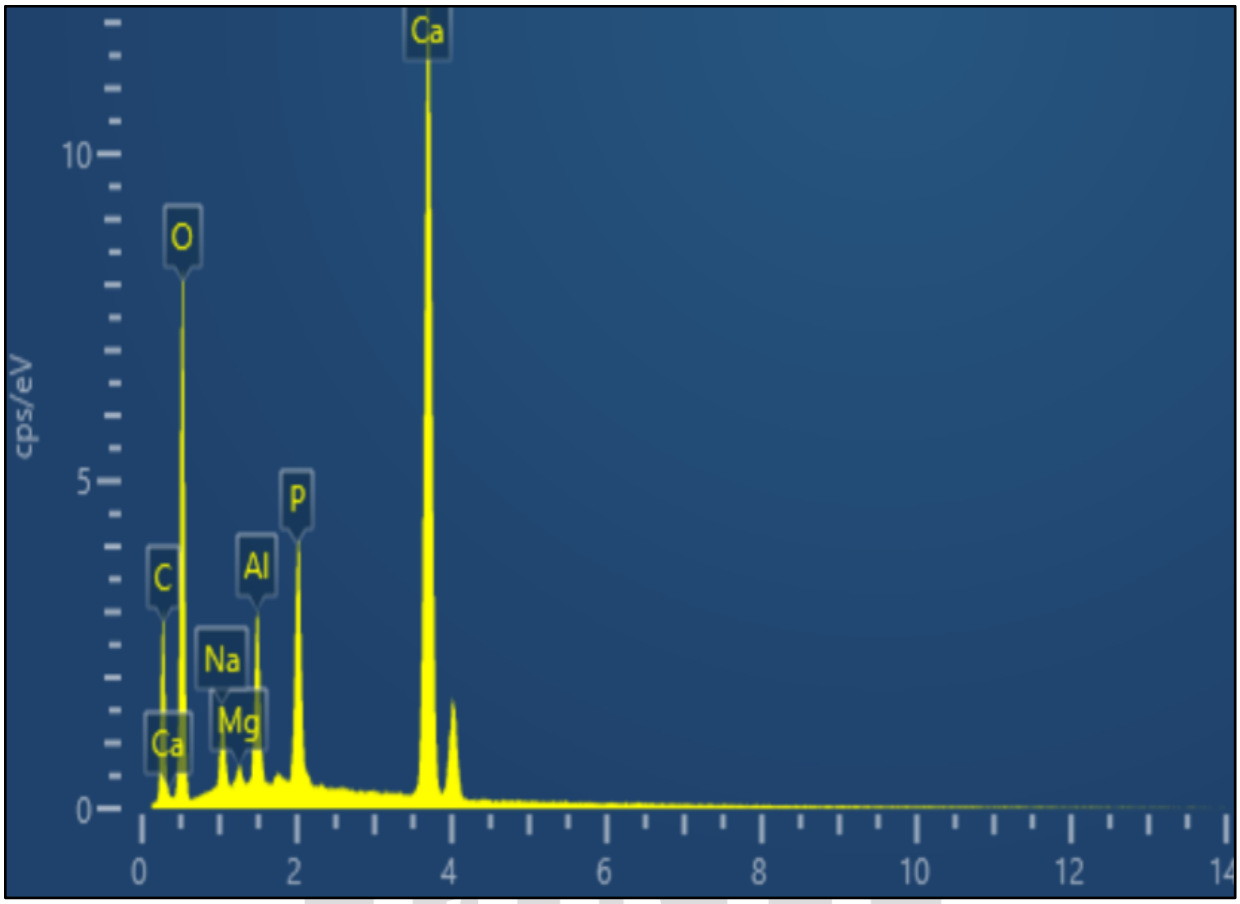

Figure 10. *EDX Analysis of nHap-cGMS for Spectrum 4*

Table 4.

EDX Analysis of nHap-cGMS for Spectrum 4

Spectrum 4			
Element	**Weight %**	**Weight % Sigma**	**Atomic %**
C	20.38	0.32	31.21
O	43.75	0.28	50.30
Na	2.11	0.06	1.69
Mg	0.35	0.03	0.26
Al	2.83	0.05	1.93
P	4.29	0.06	2.55
Ca	26.29	0.18	12.07
Total	100.00		100.00

In Spectrum 4, most of the weight is still made up of carbon (C), calcium (Ca), and oxygen (O). Each made up the following percentages in the total weight of the sample: carbon (20.38%), calcium (26.29%), and oxygen (43.75%). It was also composed of small quantities of phosphorus (P), aluminum (Al), sodium (Na), and magnesium (Mg).

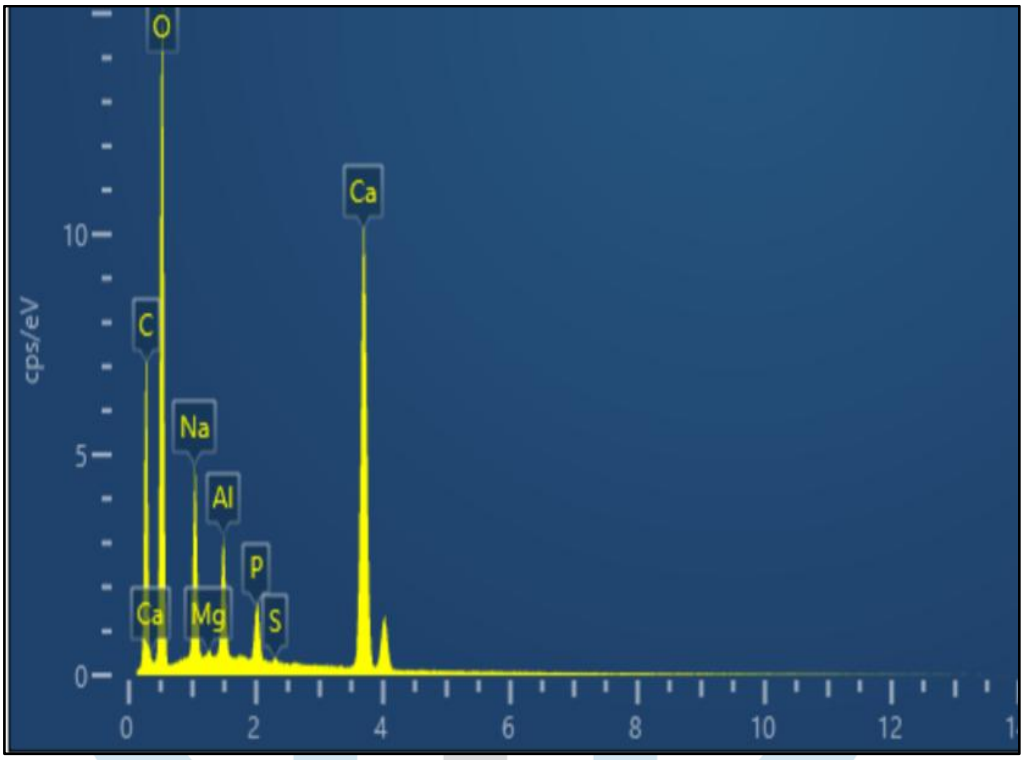

Figure 11. *EDX Analysis of nHap-cGMS for Spectrum 5*

Table 5.

EDX Analysis of nHap-cGMS for Spectrum 5

Spectrum 5			
Element	**Weight %**	**Weight % Sigma**	**Atomic %**
C	29.26	0.27	39.92
O	47.33	0.24	48.47
Na	4.79	0.07	3.41
Mg	0.13	0.03	0.09
Al	2.06	0.04	1.25
P	1.12	0.03	0.59
S	0.09	0.02	0.05
Ca	15.24	0.11	6.23
Total	100.00		100.00

For Spectrum 5, carbon (C), calcium (Ca), and oxygen (O), are the major elemental components, comprising 29.26%, Gülcan15.24%, and 47.33%, respectively. It also had the same elemental composition

as that of the first spectrum: sodium (Na), aluminum (Al), phosphorus (P), and sulfur (S) comprising the rest of the weight of the sample.

A.3. Summary of the EDX Analysis of nHap-cGMS

Carbon (C), oxygen (O), and calcium (Ca) emerged as the major constituents present in nHAp-cGMS in all the five spectra. In addition to the major constituents, minor amounts of sodium (Na), magnesium (Mg), aluminum (Al), and phosphorus (P) were observed. Minuscule amounts of sulfur (S) were detected in Spectra 1, 3, and 5, and silicon (Si) was observed exclusively in Spectrum 3. These amounts were treated as merely contaminants and did not entirely affect the adsorption capabilities of the nHap-cGMS.

b. Ultraviolet-Visible (UV-Vis) Spectroscopy

Spectrophotometric analysis was conducted on seven standardized phosphate solutions ranging from 0 to 10 ppm alongside three time-dependent simulated phosphate-contaminated water samples stirred for 20, 40, and 60 minutes. The absorbance spectra were recorded across the wavelength range of 400–1000 □m.

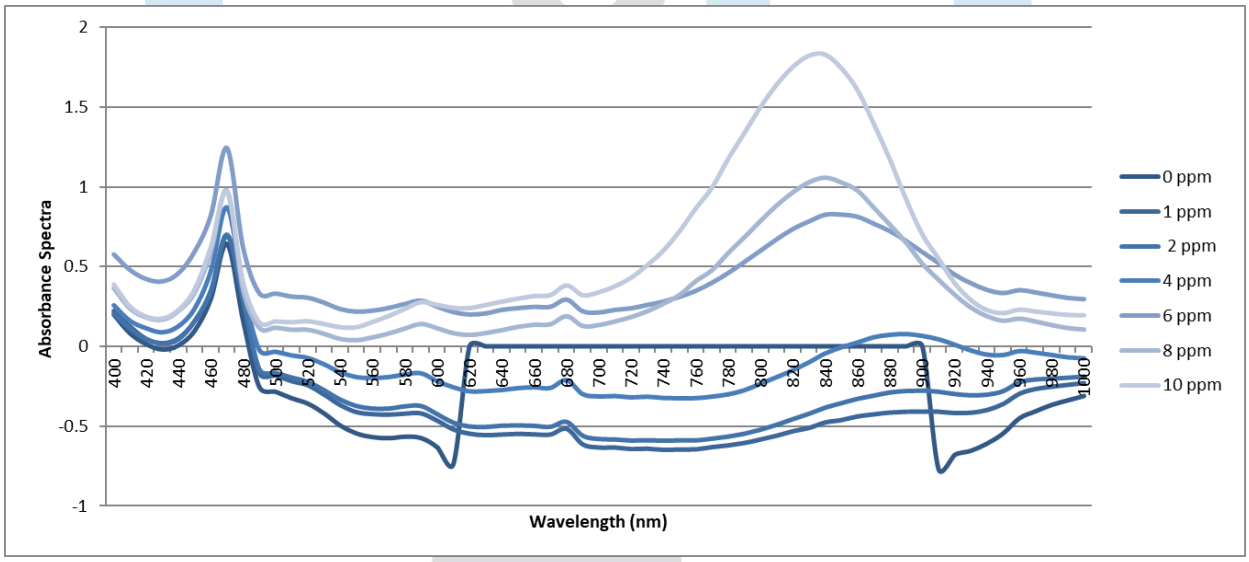

Figure 12. *Absorbance spectra of seven standard phosphate solutions*

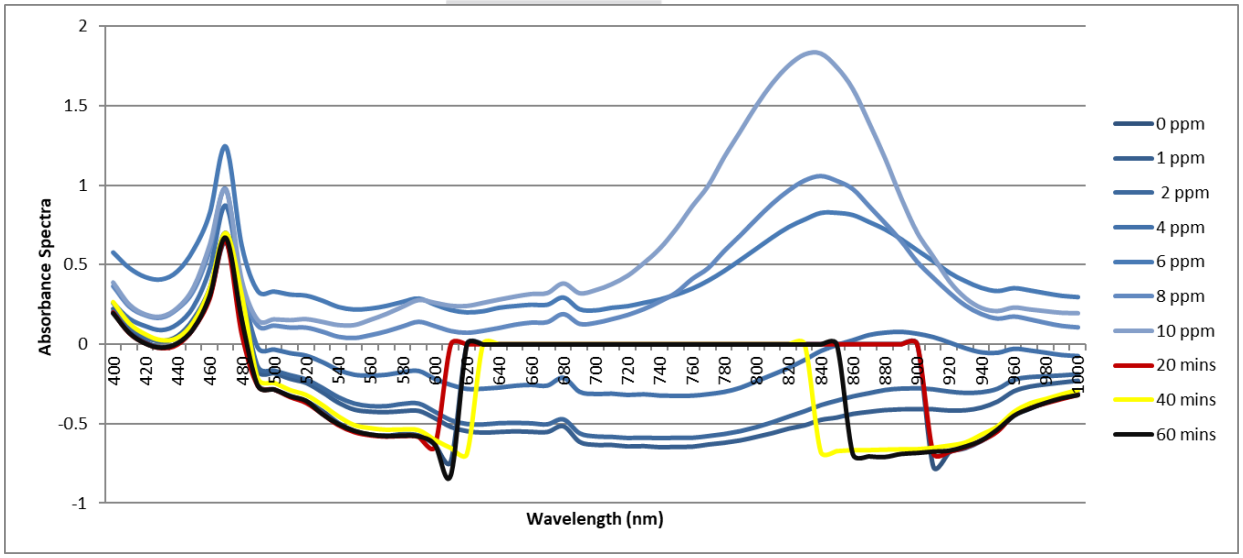

Figure 13. *Absorbance spectra of seven standard phosphate solutions and three time-dependent phosphate-concentrated water*

A prominent absorbance peak was consistently identified at approximately 470 □m, exhibiting a direct, proportional relationship with phosphate concentration. This finding indicates that the highest sensitivity for phosphate detection occurs at this wavelength. Therefore, 470 □m is optimal for quantitative phosphate analysis based on the Beer-Lambert Law, which states that the absorbance intensity increases with the concentration of the sample at a specific wavelength (Bolic, 2023). Another prominent broad peak was observed between 820-880 □m; however, these peaks were less defined and did not demonstrate a clear linear correlation with concentration, indicating that they are less reliable for analytical purposes.

Furthermore, the time-dependent samples emphasized the role of incubation duration in phosphate detection. At 20 minutes, absorbance at 470 □m remained low, mimicking that of the 0 ppm standards due to the incomplete formation of the molybdenum blue complex (Heidari-Bafroui et al., 2021). By 40 minutes, a moderate increase in absorbance was observed, indicating partial complex formation and improved sensitivity. While some color development is visible, it remains insufficient to distinguish from lower concentrations because the phosphate concentration is low. In other words, there was a slight increase in its observed absorbance behavior; however, it remains close to the standardized blank solution. Lastly, the 60-minute sample demonstrated the highest absorbance intensity, implying that the colorimetric reaction had established a near-completion to full color development, which is a pre-requisite for accurate phosphate detection.

The data demonstrated that both phosphate concentration and incubation period are key factors affecting spectrophotometric response in this system. Although longer incubation resulted in complete color development reaction, phosphate ions which were initially absorbed by the nHap-cGMS reappeared at regions 840-860 □m, suggesting detachment due to weakened binding over time. The initial interaction between the two materials is caused by a phenomenon called chemisorption, which allows the formation of a stable chemical bond between a modifier molecule (the adsorptive) and a surface (the adsorbent) (Atif et al., 2022). Such absorption systems with a heterogenous material, however, reverses when the equilibrium is shifted; ergo, the phosphate ions gradually lose bond with the nHap-cGMS.

Therefore, the use of 470 nm as the primary analytical wavelength, coupled with a minimum reaction time of 20 minutes, is the most accurate experimental condition for phosphate detection before and after adsorption tests.

c. Analysis and Discussion

C.1. Independent Samples Kruskal-Wallis Test

Table 6.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The synthesized nHAp-cGMS adsorbent is ineffective in adsorbing significant amounts of phosphate ions in phosphate-concentrated water.	Independent-Samples Kruskal-Wallis Test	<.001	Reject the null hypothesis.

a. The significance level is .050.

b. Asymptomatic significance is displayed.

Table 6 summarized the results of the Independent Samples Kruskal-Wallis Test. The null hypothesis is rejected because the p-value corresponding to the H-statistic is lower than 0.50.

Table 7.

Descriptive Statistics

Group	n	Mean	Mean Rank	Median	Std. Dev.
10 ppm	61	0.5665	208.9344	0.3220	0.5141
20 mins	61	-0.1537	99.9344	0.000	0.2812
40 mins	61	-0.2266	90.6639	-0.2160	0.3283
1 hr	61	-0.2198	90.4672	0.000	0.3227
Total	244				

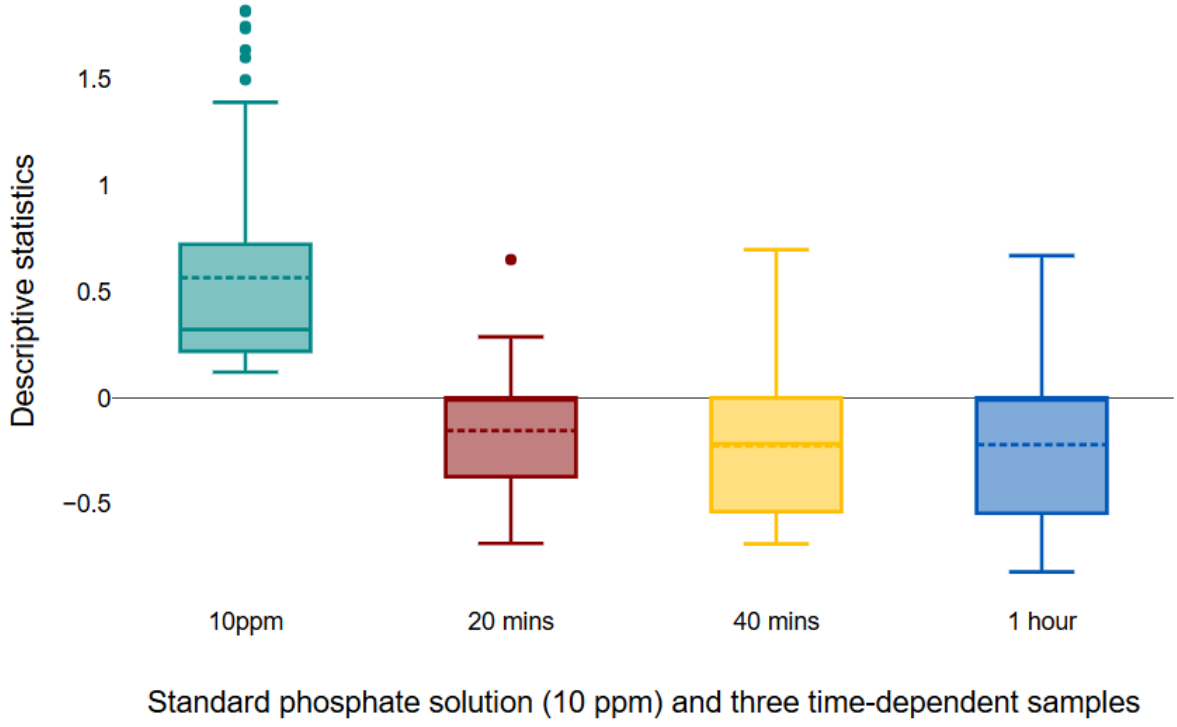

Figure 14. *Visual diagram (box-plot) of the descriptive statistics*

Descriptive statistics were calculated for four independent groups — standard phosphate solution (10 ppm) and three time-dependent samples (20 mins, 40 mins, 1 hr) — each with a sample of 61 (n = 244). According to the findings, the phosphate content of the 10 ppm group ($\bar{x}_{10\ ppm}$ = 0.5665; Mean Rank$_{10\ ppm}$ = 208.9344; Median$_{10\ ppm}$ = 0.3220 : Standard Deviation$_{10\ ppm}$ = 0.5142) is higher compared to all three time-dependent groups — 20 mins ($\bar{x}_{20\ mins}$ = -0.1537; Mean Rank$_{20\ mins}$ = 99.9344; Median$_{20\ mins}$ = 0.000: Standard Deviation$_{20\ mins}$ = 0.2812), 40 mins ($\bar{x}_{40\ mins}$ = -0.2266; Mean Rank$_{40\ mins}$ = 90.6639; Median$_{40\ mins}$ = -0.2160; Standard Deviation$_{40\ mins}$ = 0.5142), 1 hr ($\bar{x}_{1\ hr}$ = -0.2198; Mean Rank$_{1\ hr}$ = 90.4672; Median$_{1\ hr}$ = 0.000; Standard Deviation$_{1\ hr}$ = 0.3227). The 20 mins, 40 mins, and 1 hr groups demonstrated negative mean values and comparable variability, with the 20 mins group obtaining the lowest standard deviation (0.2812). Lower standard deviation implies less variability and consistent values in the dataset. In other

words, the similarity in values among the time-dependent groups suggested that any temporal effect beyond 20 minutes is minimal or absent.

Table 8.
Independent-Samples Kruskal-Wallis Test Summary

Total N	244
Chi^2	126.36
Degree of Freedom	3
p	<.001

Table 8 illustrates the results of the Independent-Samples Kruskal-Wallis Test, which was conducted to examine the phosphate content of four groups. According to the findings, there was a significant difference in outcome measures between four experimental groups ($Chi^2 = 126.36$; $p < .001$) since the p-value corresponding to the H-statistic is lower than .050. The results implied that one or more treatments are significantly different.

Table 9.
Dunn's Pairwise Comparisons of Standard Phosphate Solution and Three Time-dependent Phosphate-Concentrated Water

	Z-Statistic	p	Adj. p
10 ppm vs 20 mins	8.5288	<.001	<.001
10 ppm vs 40 mins	9.2542	<.001	<.001
10 ppm vs 1 hr	9.2696	<.001	<.001
20 mins vs 40 mins	0.7254	0.4682	1.0000
20 mins vs 1 hr	-0.7408	0.4588	1.0000
40 mins vs 1 hr	-0.0154	0.9877	1.0000

Post-hoc analysis using Dunn-Bonferroni Test identified significant differences between the following groups: 10 ppm vs 20 mins, 10 ppm vs 40 mins, 10ppm vs 1 hour at $\alpha = 0.05$.

The result of the Dunn-Bonferroni Test aligned with the analysis of the UV-Vis spectrometry, which demonstrates a significant difference in phosphate concentration before and after treatment of the simulated phosphate-contaminated water. This statistically significant result confirmed the effectiveness of nHAp-cGMS as an adsorbent in reducing the levels of phosphate ions. Moreover, the analysis revealed distinct differences in removal efficiency across the five different treatment conditions, highlighting the influence of time-dependent variables on the standardized 10 ppm phosphate solution based on adsorption performance. Overall, these findings suggest that nHAp-cGMS has strong potential in phosphate remediation, offering solutions for water purification efforts and allowing eutrophication control.

CHAPTER V: SUMMARY, CONCLUSIONS AND RECOMMENDATION

a. Summary of Findings

This study evaluated the structural properties and phosphate adsorption effectiveness of the synthesized nHAp-cGMS through the utilization of the following instruments: SEM, EDX, and UV-Vis spectroscopy. To further evaluate adsorption capacity, the researchers conducted Independent Samples Kruskal-Wallis Test to compare the mean ranks of four sample groups: 10 ppm standardized phosphate solution, and samples collected at 20, 40, and 60 mins adsorption time.

The nHAp-cGMS exhibits a heterogenous and agglomerated morphology composed of block-like crystalline structures interspersed with fine granular particles, with carbon (C), oxygen (O), and calcium (CA) identified as its major constituents. The rough and porous surface, characterized by non-uniform particle distribution and void-like spaces, indicates a large surface area allowing adsorption capacity of hydroxyapatite-based materials. Moreover, UV-Vis analysis demonstrated a prominent absorbance peak at 470 □m, establishing this wavelength as optimal for quantitative phosphate demonstration. Results also demonstrated that absorbance increased with incubation time; however, reappearance of phosphate signals at higher wavelengths suggested partial desorption over extended contact time. Therefore, a minimum reaction time of 20 minutes was identified as sufficient for reliable phosphate analysis.

Lastly, the Independent Samples Kruskal-Wallis Test revealed a statistically significant difference ($Chi^2 = 126.36$; $p < .001$) in phosphate concentration among the standard 10 ppm solution and the three time-dependent samples (20 mins, 40 mins, and 1 hr) at 5% significance level. Post-hoc Dunn–Bonferroni comparisons confirmed that all treated groups differed significantly from the untreated 10 ppm standard, while no significant differences were observed among the time-dependent treatment groups themselves.

b. Conclusion

This study successfully achieved its primary objective of synthesizing a nano-hydroxyapatite–carbonized green mussel shell (nHAp-cGMS) adsorbent and evaluating its potential to reduce phosphate ion concentration in water as a strategy for mitigating eutrophication. Overall, the results indicate that nHAp-cGMS possesses both suitable structural characteristics and functional effectiveness for phosphate sequestration.

In terms of material characterization, the heterogeneous and agglomerated morphology, characterized by block-like crystalline structures and fine granular particles on a rough and porous surface, suggests the presence of numerous active sites available for adsorption. This structural feature supports previous findings that hydroxyapatite-based materials exhibit high adsorption efficiency due to their porous structure and large surface area (Oluremi, 2025). Moreover, the identification of calcium (Ca), oxygen (O), and carbon (C) as the major elemental constituents confirms the successful formation of a calcium phosphate–based material derived from biogenic waste, which is consistent with earlier studies on mussel shell–based hydroxyapatite adsorbents (Shaker & Deftos, 2023).

With respect to adsorption efficiency and kinetics, the selection of 470 nm as the optimal wavelength for phosphate quantification allowed for reliable monitoring of adsorption behavior over time. The observed trend indicates that phosphate uptake occurs rapidly during the initial stages of contact, after which the rate of adsorption stabilizes. Similar kinetic behavior has been reported in previous studies on

hydroxyapatite adsorbents, where optimal contact times were achieved within short durations due to early attainment of adsorption equilibrium (Manna et al., 2022). This suggests that prolonged exposure offers limited additional benefit for phosphate removal.

The significant difference between untreated and treated samples indicates that nHAp-cGMS effectively reduces phosphate ions in water. The absence of significant variation among treatment times suggests that adsorption occurs rapidly and reaches stability at an early stage, with most phosphate removal achieved within the first 20 minutes. Extending contact time therefore provides little additional benefit, reflecting fast adsorption kinetics where surface sites are quickly occupied. This behavior highlights the practical advantage of nHAp-cGMS, as efficient phosphate removal can be attained without prolonged treatment durations, consistent with previous findings on hydroxyapatite-based adsorbents (Manna et al., 2022).

In conclusion, the synthesized nHAp-cGMS demonstrates strong potential as a sustainable, low-cost, and efficient phosphate adsorbent derived from green mussel shell waste. Its favorable structural features, rapid adsorption behavior, and demonstrated effectiveness in phosphate reduction underscore its applicability for water treatment approaches aimed at controlling eutrophication. Future research may focus on adsorption isotherm modeling, regeneration and reuse potential, and application in real wastewater systems to further assess its long-term environmental and practical viability.

c. Recommendation

The researchers recommend conducting further studies using higher phosphate concentrations ranging from 100 ppm to 1000 ppm, as well as testing different concentrations of nHAp-cGMS in relation to the phosphate solution. This will help in identifying nHAp-cGMS' equilibrium point and maximal adsorption capacity. To gain a better understanding of the adsorption process over longer time periods, it is also recommended to increase the contact time in batch adsorption tests from 0 to 180 minutes while keeping the pH level constant.

In addition, the researchers suggest subjecting the nHAp-cGMS to advanced characterization analysis such as X-Ray Powder Diffraction (XRPD) and thermal analysis. These methods will provide insights into the structural stability of the material under heat and reveal the crystalline phases that are relative to the synthesized nano-hydroxyapatite material that could affect its performance in water treatment applications.

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Appendix A

ISEF Forms

Checklist for Adult Sponsor (1)

This completed form is required for ALL projects.

To be completed by the Adult Sponsor in collaboration with the student researcher(s):

Student's Name(s): Bai Zandra Q. Orao, Samantha A. De Mesa, Mikaela D. Teodosio

Project Title: Nano-sized Hydroxyapatite Adsorbent Synthesis from Calcined Green Mussel (Perna viridis) Shells (nHAP-cGMS) for Phosphate Sequestration

1. ☑ I have reviewed the ISEF Rules and Guidelines, including the science fair ethics statement.
2. ☑ I have reviewed the student's completed Student Checklist (1A) and Research Plan/Project Summary.
3. ☑ I have worked with the student and we have discussed the possible risks involved in the project.
4. ☐ The project involves one or more of the following and requires prior approval by an SRC, IRB, IACUC or IBC:
   - ☐ Humans
   - ☐ Vertebrate Animals
   - ☐ Potentially Hazardous Biological Agents
     - ☐ Microorganisms ☐ rDNA ☐ Tissues
5. ☑ Items to be completed for **ALL PROJECTS**
   - ☑ Adult Sponsor Checklist (1)
   - ☑ Student Checklist (1A)
   - ☑ Research Plan/Project Summary
   - ☑ Approval Form (1B)
   - ☑ Regulated Research Institutional/Industrial Setting Form (1C) (when applicable; after completed experiment)
   - ☐ Continuation/Research Progression Form (7) (when applicable)

Additional forms required if the project includes the use of one or more of the following (check all that apply):

- ☐ **Humans,** including student designed inventions/prototypes. (Requires prior approval by an Institutional Review Board (IRB); see full text of the rules.)
  - ☐ Human Participants Form (4) or appropriate Institutional IRB documentation
  - ☐ Sample of Informed Consent Form (when applicable and/or required by the IRB)
  - ☐ Qualified Scientist Form (2) (when applicable and/or required by the IRB)
- ☐ **Vertebrate Animals** (Requires prior approval, see full text of the rules.)
  - ☐ Vertebrate Animal Form (5A)-for projects conducted in a school/home/field research site (SRC prior approval required
  - ☐ Vertebrate Animal Form (5B)-for projects conducted at a Regulated Research Institution. (Institutional Animal Care and Use Committee (IACUC) approval required prior experimentation.)
  - ☐ Qualified Scientist Form (2) (Required for all vertebrate animal projects at a regulated research site or when applicable)
- ☐ **Potentially Hazardous Biological Agents** (Requires prior approval by SRC, IACUC or IBC, see full text of the rules.)
  - ☐ Potentially Hazardous Biological Agents Risk Assessment Form (6A)
  - ☐ Human and Vertebrate Animal Tissue Form (6B)-to be completed in addition to Form 6A when project involves the use of fresh or frozen tissue, primary cell cultures, blood, blood products and body fluids.
  - ☐ Qualified Scientist Form (2) (when applicable)
  - ☐ The following are exempt from prior review but require a Risk Assessment Form 3: projects involving protists, archae and similar microorganisms, for projects using manure for composting, fuel production or other non-culturing experiments, projects using color change coliform water test kits, microbial fuel cells, and projects involving decomposing vertebrate organisms.
- ☐ **Hazardous Chemicals, Activities and Devices** (No SRC prior approval required, see full text of the rules.)
  - ☑ Risk Assessment Form (3)
  - ☐ Qualified Scientist Form (2) (required for projects involving DEA-controlled substances or when applicable)
- ☐ **Other**
  - ☑ Risk Assessment Form (3)

☑ **I attest to the information checked above and that I have read and agree to abide by the science fair ethics statement.**

Jerrel Ann M. Surabia		08/08/25
Adult Sponsor's Printed Name	Signature	Date of Review (mm/dd/yy)
09562645395	Jerrelann.surabia@deped.gov.ph	
Phone	Email	

Appendix A.1. *ISEF Form 1 (Checklist for Adult Sponsor)*

Student Checklist (1A)

This form is required for ALL projects.

1. a. Student/Team Leader: Bai Zandra Q. Orao Grade: 12

   Email: baizandrao@gmail.com Phone: 09154262320

   b. Team Member: Mikaela D. Teodosio c. Team Member: Samantha Ysabelle A. De Mesa

2. Title of Project: Nano-sized Hydroxyapatite Adsorbent Synthesis from Calcined Green Mussel (Perna viridis) Shells (nHAP-cGMS) for Phosphate Sequestration

3. School: Manila Science High School School Phone: (02) 8525 6197
   (if multiple schools, list of the team leader or list all schools).

   School Address: Taft Avenue, corner Padre Faura St, Ermita, Manila, 1000 Metro Manila

4. Adult Sponsor: Jerrel Ann M. Surabia Phone/Email: 09562645395

5. Does this project need SRC/IRB/IACUC or other pre-approval? ☐ Yes ☒ No Tentative start date: _____

6. Is this a continuation/progression from a previous year? ☐ Yes ☒ No
   a. If yes, attach the previous year's ☐ Abstract **and** ☐ Research Plan/Project Summary
   b. Explain how this project is new and different from previous years on
   ☐ Continuation/Research Progression Form (7); include forms for all previous years

7. This year's experimentation/data collection (include forms for all previous years):

   08/09/25 (Actual Start Date: (mm/dd/yy)) 09/01/25 (End Date: (mm/dd/yy))

8. Where will you conduct your experimentation? (check all that apply)
   ☑ Research Institution ☐ School ☐ Field ☑ Home ☐ Other: _____

9. Source of Data:
   ☑ Collected self/mentor ☐ Other List all URL(s) in Research Plan: _____

10. List the name and address of all non-home and non-school work site(s), whether you worked there virtually or on-site:

    Name: Measure Engineering Services
    Address: San Pedro, Laguna
    Phone/email:

    JBL Scientific
    2270 Aragon, San Andres Bukid
    Metro Manila
    jblscientific@gmail.com

11. **Complete a Research Plan/Project Summary following the Research Plan/Project Summary instructions and attach to this form.**

12. **An abstract is required for all projects after experimentation.**

Appendix A.2. *ISEF Form 1A (Student Checklist)*

Approval Form (1B)

A completed form is required for each student, including all team members.

1. To Be Completed by Student and Parent

a. **Student Acknowledgment:**

- I understand the risks and possible dangers to me of the proposed research plan.
- I have read the ISEF Rules and Guidelines and will adhere to all International Rules when conducting this research.
- I have read and agree to uphold all aspects of the student researcher ethics statement.

Student researchers are expected to maintain the highest standards of honesty and integrity. Scientific fraud and misconduct are not condoned at any level of research or competition. Such practices include but are not limited to plagiarism, forgery, use or presentation of other researcher's work as one's own, and fabrication of data. Fraudulent projects will fail to qualify for competition in affiliated fairs and ISEF.

Bai Zandra Q. Orao		07/17/25
Student's Printed Name	Signature	Date Acknowledged (mm/dd/yy) (Must be prior to experimentation.)

b. **Parent/Guardian Approval:** I have read and understand the risks and possible dangers involved in the **Research Plan/Project Summary**. I consent to my child participating in this research.

Olivia V. Quirabu		07/17/25
Parent/Guardian's Printed Name	Signature	Date Acknowledged (mm/dd/yy) (Must be prior to experimentation.)

2. To be completed by the local or affiliated Fair SRC

(Required for projects requiring prior SRC/IRB APPROVAL. Sign 2a or 2b as appropriate.)

a. **Required for projects that need prior SRC/IRB approval BEFORE experimentation** (humans, vertebrates or potentially hazardous biological agents).

The SRC/IRB has carefully studied this project's **Research Plan/ Project Summary** and all the required forms are included. My signature indicates approval of the **Research Plan/Project Summary** before the student begins experimentation.

SRC/IRB Chair's Printed Name

Signature | Date of Approval (mm/dd/yy) (Must be prior to experimentation.)

OR

b. **Required for research conducted at all Regulated Research Institutions with no prior fair SRC/IRB approval.**

This project was conducted at a regulated research institution **(not home or high school, etc.)**, was reviewed and approved by the proper institutional board before experimentation and complies with the ISEF Rules. **Attach (1C) and any required institutional approvals (e.g. IACUC, IRB).**

SRC Chair's Printed Name

Signature | Date of Signature (mm/dd/yy) (May be after experimentation)

3. Final ISEF Affiliated Fair SRC Approval (Required for ALL Projects)

SRC Approval After Experimentation and Before Competition at Regional/State/National Fair

I certify that this project adheres to the approved **Research Plan/Project Summary** and complies with all ISEF Rules.

Regional SRC Chair's Printed Name	Signature	Date of Approval (mm/dd/yy)
State/National SRC Chair's Printed Name (where applicable)	Signature	Date of Approval (mm/dd/yy)

Appendix A.3. *ISEF Form 1B*

Approval Form (1B)

A completed form is required for each student, including all team members.

1. To Be Completed by Student and Parent

a. **Student Acknowledgment:**

- I understand the risks and possible dangers to me of the proposed research plan.
- I have read the ISEF Rules and Guidelines and will adhere to all International Rules when conducting this research.
- I have read and agree to uphold all aspects of the student researcher ethics statement.

Student researchers are expected to maintain the highest standards of honesty and integrity. Scientific fraud and misconduct are not condoned at any level of research or competition. Such practices include but are not limited to plagiarism, forgery, use or presentation of other researcher's work as one's own, and fabrication of data. Fraudulent projects will fail to qualify for competition in affiliated fairs and ISEF.

Samantha Ysabelle A. De Mesa		07/17/25
Student's Printed Name	Signature	Date Acknowledged (mm/dd/yy) (Must be prior to experimentation.)

b. **Parent/Guardian Approval:** I have read and understand the risks and possible dangers involved in the **Research Plan/Project Summary**. I consent to my child participating in this research.

Catalina De Mesa	Catalina R. De Mesa	07/17/25
Parent/Guardian's Printed Name	Signature	Date Acknowledged (mm/dd/yy) (Must be prior to experimentation.)

2. To be completed by the local or affiliated Fair SRC

(Required for projects requiring prior SRC/IRB APPROVAL. Sign 2a or 2b as appropriate.)

a. **Required for projects that need prior SRC/IRB approval BEFORE experimentation** (humans, vertebrates or potentially hazardous biological agents).

The SRC/IRB has carefully studied this project's **Research Plan/ Project Summary** and all the required forms are included. My signature indicates approval of the **Research Plan/Project Summary** before the student begins experimentation.

SRC/IRB Chair's Printed Name

Signature | Date of Approval (mm/dd/yy) (Must be prior to experimentation.)

OR

b. **Required for research conducted at all Regulated Research Institutions with no prior fair SRC/IRB approval.**

This project was conducted at a regulated research institution **(not home or high school, etc.)**, was reviewed and approved by the proper institutional board before experimentation and complies with the ISEF Rules. **Attach (1C) and any required institutional approvals (e.g. IACUC, IRB).**

SRC Chair's Printed Name

Signature | Date of Signature (mm/dd/yy) (May be after experimentation)

3. Final ISEF Affiliated Fair SRC Approval (Required for ALL Projects)

SRC Approval After Experimentation and Before Competition at Regional/State/National Fair

I certify that this project adheres to the approved **Research Plan/Project Summary** and complies with all ISEF Rules.

Regional SRC Chair's Printed Name | Signature | Date of Approval (mm/dd/yy)

State/National SRC Chair's Printed Name *(where applicable)* | Signature | Date of Approval (mm/dd/yy)

Appendix A.4. *ISEF Form 1B*

Approval Form (1B)

A completed form is required for each student, including all team members.

1. To Be Completed by Student and Parent

a. Student Acknowledgment:

- I understand the risks and possible dangers to me of the proposed research plan.
- I have read the ISEF Rules and Guidelines and will adhere to all International Rules when conducting this research.
- I have read and agree to uphold all aspects of the student researcher ethics statement.

Student researchers are expected to maintain the highest standards of honesty and integrity. Scientific fraud and misconduct are not condoned at any level of research or competition. Such practices include but are not limited to plagiarism, forgery, use or presentation of other researcher's work as one's own, and fabrication of data. Fraudulent projects will fail to qualify for competition in affiliated fairs and ISEF.

Mikaela D. Teodosio		07/17/25
Student's Printed Name	Signature	Date Acknowledged (mm/dd/yy) (Must be prior to experimentation.)

b. Parent/Guardian Approval: I have read and understand the risks and possible dangers involved in the **Research Plan/Project Summary**. I consent to my child participating in this research.

Arleen D. Teodosio		07/17/25
Parent/Guardian's Printed Name	Signature	Date Acknowledged (mm/dd/yy) (Must be prior to experimentation.)

2. To be completed by the local or affiliated Fair SRC

(Required for projects requiring prior SRC/IRB APPROVAL. Sign 2a or 2b as appropriate.)

a. Required for projects that need prior SRC/IRB approval BEFORE experimentation (humans, vertebrates or potentially hazardous biological agents).

The SRC/IRB has carefully studied this project's **Research Plan/Project Summary** and all the required forms are included. My signature indicates approval of the **Research Plan/Project Summary** before the student begins experimentation.

SRC/IRB Chair's Printed Name

Signature

Date of Approval (mm/dd/yy) (Must be prior to experimentation.)

OR

b. Required for research conducted at all Regulated Research Institutions with no prior fair SRC/IRB approval.

This project was conducted at a regulated research institution **(not home or high school, etc.)**, was reviewed and approved by the proper institutional board before experimentation and complies with the ISEF Rules. **Attach (1C) and any required institutional approvals (e.g. IACUC, IRB).**

SRC Chair's Printed Name

Signature

Date of Signature (mm/dd/yy) (May be after experimentation)

3. Final ISEF Affiliated Fair SRC Approval (Required for ALL Projects)

SRC Approval After Experimentation and Before Competition at Regional/State/National Fair

I certify that this project adheres to the approved **Research Plan/Project Summary** and complies with all ISEF Rules.

Regional SRC Chair's Printed Name	Signature	Date of Approval (mm/dd/yy)
State/National SRC Chair's Printed Name *(where applicable)*	Signature	Date of Approval (mm/dd/yy)

Appendix A.5. *ISEF Form 1B*

Risk Assessment Form (3)

Must be completed before experimentation; recommended for all projects. May be required for projects involving Human Participants, Hazardous Chemicals, Materials or Devices or Potentially Hazardous Biological Agents.

Student's Name(s) Bai Zandra Q. Orao, Samantha Ysabelle A. De Mesa, Mikaela D. Teodosio

Title of Project Nano-sized Hydroxyapatite Adsorbent Synthesis from Calcined Green Mussel (Perna viridis) Shells (nHAP-cGMS) for Phosphate Sequestration

To be completed by the Student Researcher(s) in collaboration with Direct Supervisor/Qualified Scientist: (All questions must be answered; additional page(s) may be attached.)

1. Identify and assess the risks and hazards involved in this project.
   This study involves handling corrosive acids (sulfuric acid), fine powdered materials that may cause respiratory irritation, CO_2 gas, hot equipment, and fragile glassware all of which present potential hazards such as chemical burns, inhalation risk, thermal burns, and glass cuts.
2. a) List all hazardous chemicals, activities or devices to be used; b) identify and list all microorganisms to be used that are exempt from pre-approval (see Potentially Hazardous Biological Agent rules).
   - sulfuric acid
   - ammonium molybdate
   - potassium amonyltartrate
   - ascorbic acid
   - disodium hydrogen phosphate
   - hotplate

   B. N/A
3. Describe the safety precautions and procedures that will be used to reduce the risks. If you conducted field work, include permits received and safety plans, as applicable.
   All procedures will be conducted in a well-ventilated laboratory with students wearing full PPE (lab coat, gloves, goggles, and face and hair mask). Corrosive acids will be handled carefully with acid-resistant glassware, fine powders will be weighed carefully to avoid inhalation. CO_2 gas will only be used in well-ventilated areas and hot glassware will be handled with heat-resistant gloves.
4. Describe the specific disposal procedures that will be used (when applicable).
   All liquid wastes of residual acids or phosphate will be collected in a designated acidic aqueous waste container and neutralized with sodium bicarbonate before disposal. Solid residues will be placed separately in a solid chemical waste container for proper disposal by the laboratory personnel. Glassware will be rinsed with distilled water and broken glasses will be disposed in a designated broken glass box.
5. List the source(s) of safety information.
   Oklahoma State University. (2022, July 13). Laboratory Safety Rules - Oklahoma State University. Ehokstateu.edu.https://ehs.okstate.edu/laboratory-safety/lab_safety_rules.html

To be completed and signed by the Direct Supervisor (or Qualified Scientist, when applicable):
I agree with the risk assessment and safety precautions and procedures described above. I certify that I have reviewed the Research Plan/Project Summary and the International Rules, including the science fair ethics statement and will provide direct supervision.

Jerrel Ann M. Surabia — Direct Supervisor's Printed Name

Signature

08/08/25 — Date of Review (mm/dd/yy)

Special Science Teacher I / Manila Science High School — Experience/Training as relates to the student's area of research

Research Adviser — Position/Institution

Jerrelan.surabia@deped.gov.ph /09562645395 — Phone or email contact information

Appendix A.6. *ISEF Form 1B*

Appendix B

Laboratory Results

Scanning Electron Microscope (SEM)

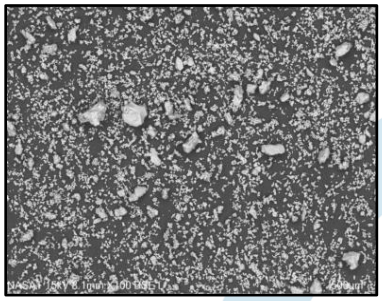


Figure 2. *SEM image of nHap-cGMS under 100x magnification*

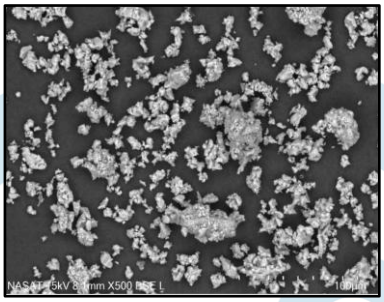


Figure 3. *SEM image of nHap-cGMS under 500x magnification*

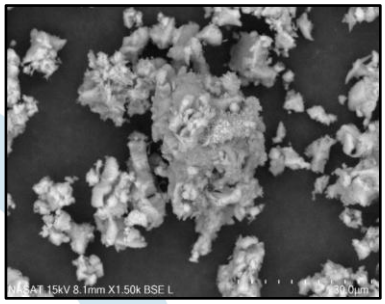


Figure 4. *SEM image of nHap-cGMS under 1500x magnification*

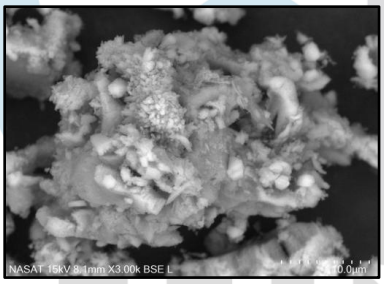


Figure 5. *SEM image of nHap-cGMS under 3000x magnification*

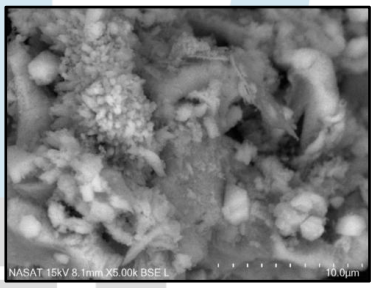


Figure 6. *SEM image of nHap-cGMS under 5000x magnification*

Energy Dispersive X-Ray Analysis (EDX)

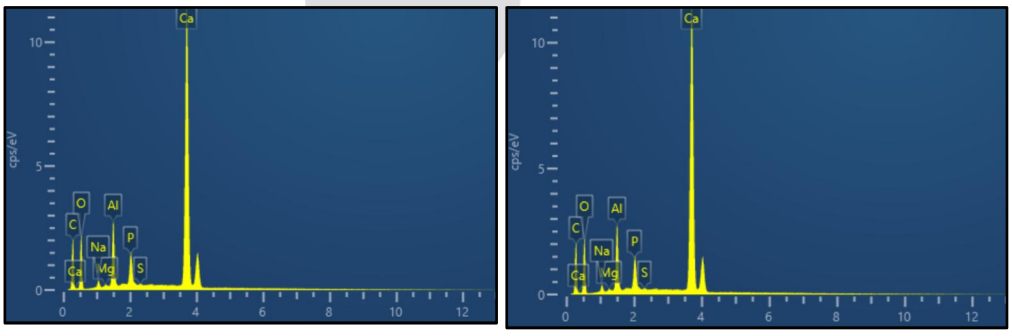


Figure 7. *EDX Analysis of nHap-cGMS for Spectrum 1*

Appendix C

Computations and Statistical Analysis

Table 7.
Descriptive Statistics

Group	n	Mean	Mean Rank	Median	Std. Dev.
10 ppm	61	0.5665	208.9344	0.3220	0.5141
20 mins	61	-0.1537	99.9344	0.000	0.2812
40 mins	61	-0.2266	90.6639	-0.2160	0.3283
1 hr	61	-0.2198	90.4672	0.000	0.3227
Total	244				

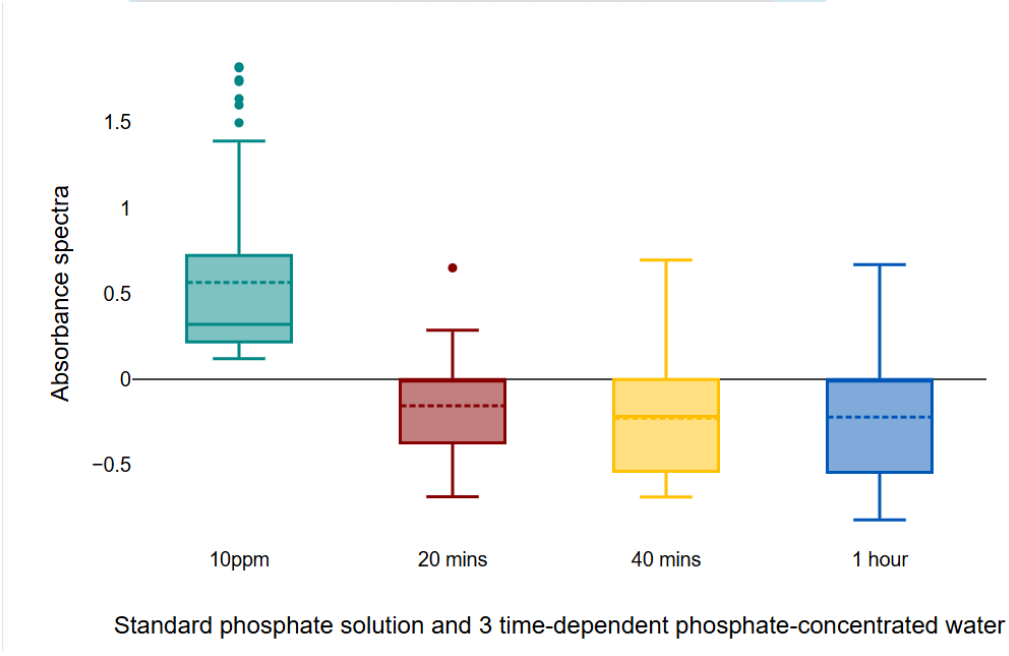


Figure 14. *Visual diagram (box-plot) of the descriptive statistics*

Table 8.

Independent-Samples Kruskal-Wallis Test Summary

Total N	244
Chi^2	126.36
Degree of Freedom	3
p	<.001

Table 9.

Dunn's Pairwise Comparisons of Standard Phosphate Solution and Three Time-dependent Phosphate-Concentrated Water

	Z-Statistic	p	Adj. p
10 ppm vs 20 mins	8.5288	<.001	<.001
10 ppm vs 40 mins	9.2542	<.001	<.001
10 ppm vs 1 hr	9.2696	<.001	<.001
20 mins vs 40 mins	0.7254	0.4682	1.0000
20 mins vs 1 hr	-0.7408	0.4588	1.0000
40 mins vs 1 hr	-0.0154	0.9877	1.0000

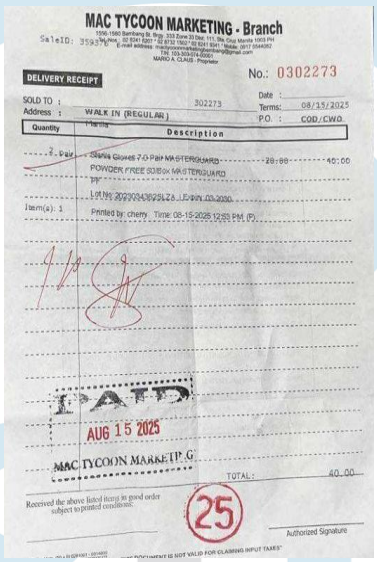
Appendix D

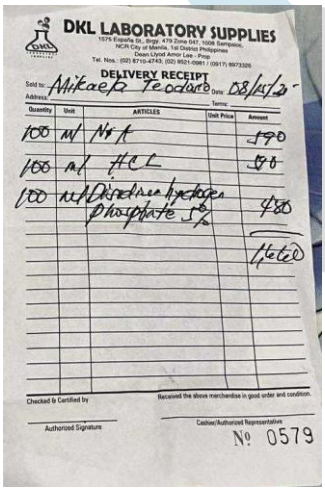
Output

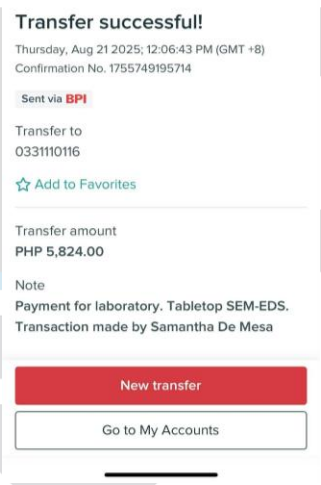
Appendix D. nHAp-cGMS sample as received by NASAT Labs.

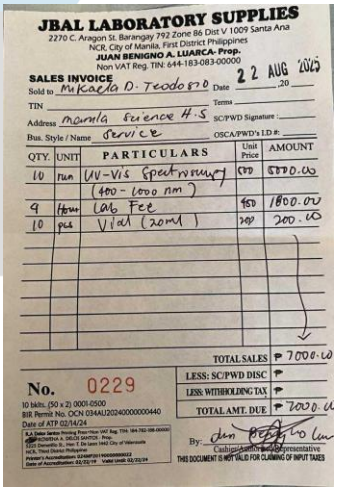
Appendix E

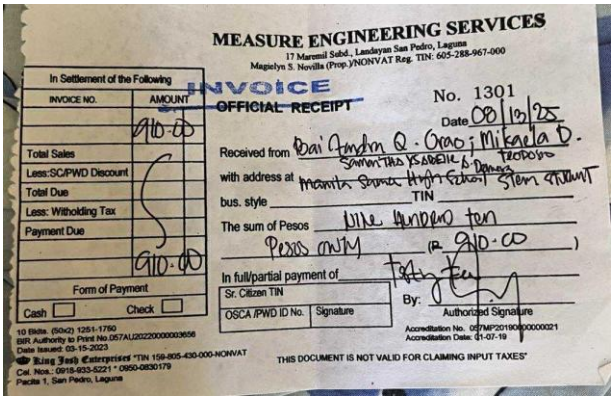
Receipts

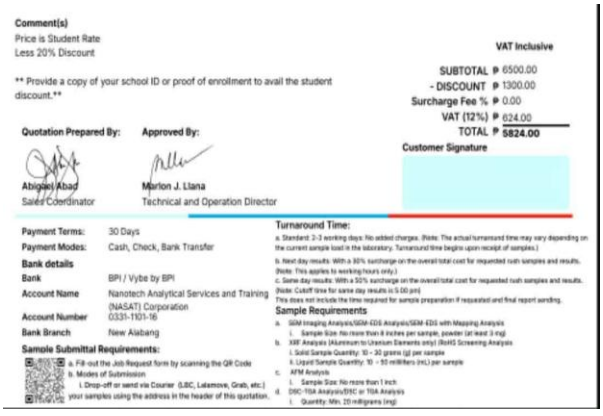








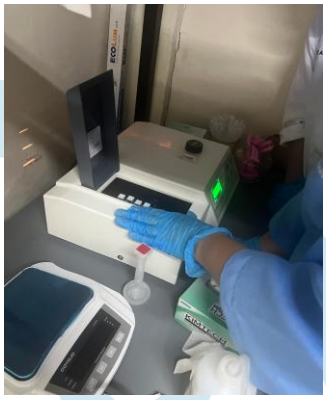




Appendix F

Documentation

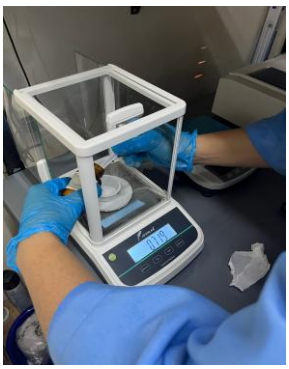






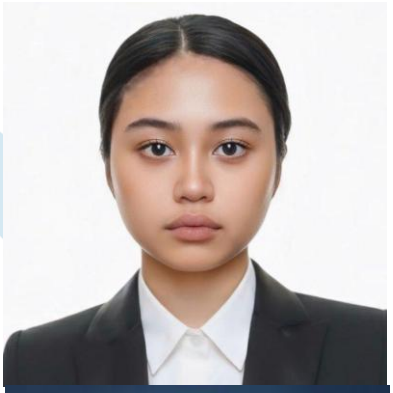





Curriculum Vitae

Name: Samantha Ysabelle A. De Mesa
Contact Number: (+63) 927 551 1864
Email: demesasamanthay@gmail.com



Name: Bai Zandra Q. Orao
Contact Number: (+63) 915 426 2320
Email: baizandrao@gmail.com

Name: Mikaela D. Teodosio
Contact Number: (+63) 991 014 2879
Email: mikaela.dionisio.teodosio@gmail.com