EFFECTIVENESS OF GOLDEN APPLE SNAIL SHELLS (Pomacea cana-liculata) AND YELLOWFIN TUNA BONES (Thunnus albacares) AS A POTENTIAL SOURCE OF BIOCERAMICS HYDROXYAPATITE APPLICATION

Mapatac, Levitah C.

Cahiles, Eloisa Marie G.

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Salise, Jo Dee B.

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EFFECTIVENESS OF GOLDEN APPLE SNAIL SHELLS (*Pomacea cana-liculata*) AND YELLOWFIN TUNA BONES (*Thunnus albacares*) AS A POTENTIAL SOURCE OF BIOCERAMICS HYDROXYAPATITE APPLICATION

[1]Levitah C. Mapatac, Ph.D., [2]Eloisa Marie G. Cahiles, [3]Apphia Kezia R. Trillo and [4]Jo Dee B. Salise
Professor 5, Caraga State University, Student Researchers- Phil. Science High School, Caraga Region
Corresponding author email: lcmapatac@carsu.edu.ph

Abstract: *Commercial dental filling, which has been the most used restorative material in dentistry, is highly susceptible to corrosion. Consequently, substances released from dental fillings during corrosion are toxic and can increase the risk of releasing toxic components within the oral cavity. This research aims to address the problems of commercial dental filling by using golden apple snail shells and yellowfin tuna bones as an alternative. Utilization of golden apple snail shells and yellowfin tuna bones created a practical use for biogenic material that had little to no practical use. Fourier Transform Infrared Spectroscopy and Energy Dispersive X-Ray Spectroscopy analyses proved the presence of hydroxyapatite, a biomaterial used to restore bone tissue defects, in the golden apple snail shells and yellowfin tuna bones. Scanning Electron Microscopy and X-Ray Powder Diffraction were used to analyze the physical structure of the samples to commercial dental filling. Results from Acidity and Basicity Tests and Brine shrimp Bioassay showed that the samples were less toxic and corrosive than commercial dental filling. With the presence of hydroxyapatite in the samples, the researchers were able to create an alternative to commercial dental filling from biogenic material that is less toxic and corrosive.*

Keywords: Golden apple snail shell, yellowfin tuna bones, hydroxyapatite, FTIR, X-Ray Spectroscopy and SEM

1. INTRODUCTION

The golden apple snail (*Pomacea canaliculata*) is native to South America. It was introduced to farmers in the Philippines in the 1980s from Argentina via Taiwan, and to other countries in Asia, to increase the income of farmers and enrich the protein in their diet. The Global Invasive Species Programmed lists golden apple snails as one of the world's 100 worst invasive alien species. It has brought about economic losses to aquatic crops in the Philippines. The prevalence of this invasive species particularly in rice fields has only been a problem and a burden to local farmers [18].

The yellowfin tuna (*Thunnus albacares*) is a large pelagic fish originating from the Scombridae family. This fish can be found almost in all tropical and subtropical waters [10]. The yellowfin tuna covers the majority of the tuna catch of the Philippines, which is one of the major fisheries commodities in the country [3]. However, the bones of yellowfin tuna are considered a waste and have little to no practical use.

Hydroxyapatite is one of the most attractive materials for bone implants due to its compositional and biological similarity to host materials. It also has a unique biocompatibility feature among phosphate groups [2]. Natural bones, such as bovine bones, fish bones, cuttlefish shells, oyster shells, and corals are identified as natural sources of hydroxyapatite [1].

Dental resin composite fillings have become an esthetic alternative to dental amalgam restorations and there has been a remarkable improvement of their mechanical properties to restore posterior teeth [4]. However, the researchers found commercial dental fillings to be susceptible to corrosion. In rare cases, the filling may shrink after it is placed, which can lead to more cavities if there is a gap between the filling and the tooth. The bonding agent must be placed with care, and the filling must be placed in such a way to minimize shrinkage [20]. In relation to this, substances released from dental fillings during corrosion are cytotoxic and can lead to the risk of releasing toxic components within the oral cavity [17]. With this information, the researchers decided to test whether golden apple snails and yellowfin tuna are a potential source of hydroxyapatite. Moreover, this study aims to produce an alternative to commercial dental filling using golden apple snail shells and yellowfin tuna bones. This alternative convert shell and bone waste into a sustainable biomaterial that is less corrosive and toxic.

2. METHODOLOGY

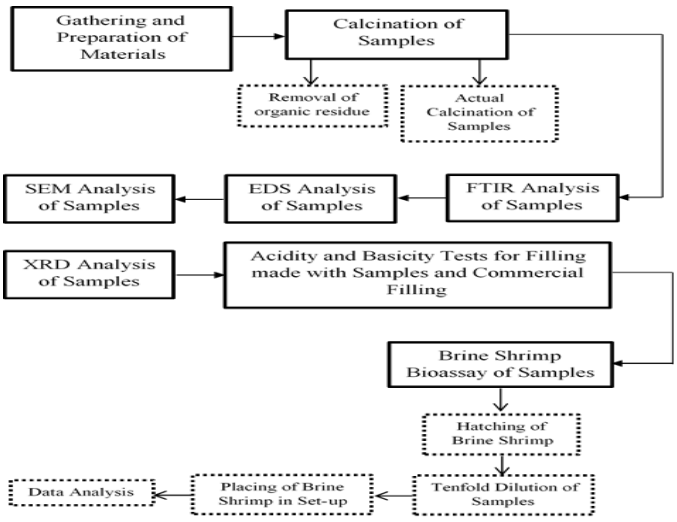

Figure 1. Schematic Diagram of the Study

2.1. Gathering and Preparation of Materials

The researchers gathered one kilogram of yellow fin tuna bones from the market of Langihan, Butuan City. One kilogram of golden apple snail shells was collected from local farms in Antongalon, Butuan City. The snail shells and tuna bones underwent a species characterization at the Caraga State University and Bureau of Fisheries and Aquatic Resources, respectively, to ensure that the samples collected were the accurate species.

The tuna bones were cleaned using distilled water. They were boiled for three hours to remove any remaining meat and impurities on the bones. Afterwards, the bones were dried under the sun for one hour to remove excess moisture. The bones were then chopped into smaller pieces for calcination.

The snail shells, after being washed and cleansed thoroughly, were boiled to aid in the removal of the meat from the shell. Similar to the fish bones, the shells were dried in the sun to remove excess moisture. The shells were crushed into smaller pieces optimal for calcination

2.1.1. Calcination of Samples

The samples were calcined through a two-stage thermal treatment at the Department of Agriculture Laboratory in Taguibo, Butuan City.

Removal of Organic Residue

The first stage consisted of heating the samples in an oven added with phosphoric acid to 200°C for two hours at the heating rate of 5°C/min to destroy any organic residue. This was to prevent any impurities that may interfere with the synthesis of hydroxyapatite.

Actual Calcination of Samples

The second stage consisted of heating one batch of samples in a furnace to 700°C for 6 hours, but with the heating rate of 0.5°C/min. Another batch of samples was heated at a temperature of 900°C, with the same heating rate. At this temperature, the calcined snail shells and tuna bones will have been transformed into calcium oxide in the form of ash by freeing carbon dioxide.

2.3. Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Samples

The researchers planned to submit the powder obtained from the calcined samples for the Fourier Transfer Infrared Analysis to a laboratory but were unable to do so as most laboratories in Mindanao were unavailable due to maintenance work.

Energy Dispersive X-Ray Spectroscopy (EDS) Analysis of Samples

The powders obtained from the calcined samples were submitted to the Ateneo de Davao University Laboratory for the Energy Dispersive X-Ray Spectroscopy.

Scanning Electron Microscopy (SEM) Analysis of Samples

The powders obtained from the calcined samples were submitted to the Ateneo de Davao University Laboratory for the Scanning Electron Microscopy to determine the morphological structure of the samples.

X-Ray Powder Diffraction (XRD) Analysis of Samples

The powders obtained from the calcined samples were submitted to the Ateneo de Davao University Laboratory for the X-Ray Powder Diffraction.

2.1.2 Acidity and Basicity Tests of Samples

The researchers compared the resistance of dental filling made with the powder from the samples to corrosion to the resistance of commercially made filling using acidity and basicity tests. The tests served as a rough simulation of the substances the fillings will encounter in an oral environment. The researchers created fillings using different ratios of the calcined sample mixed with the commercial ingredients and separated 5 grams of each filling for each of the tests. An analytical balance was used to weigh 5 grams of each filling. The ratios used were 50% commercial filling/50% calcined snail shells, 50% commercial filling/50% calcined tuna bones, 75% commercial filling/25% calcined snail shells, and 75% commercial filling/25% calcined tuna bones. Pure dental fillings were used in comparison to the filling made with the samples. 200 ml of 2.0M acetic acid was used for the acidity test while 200 ml 2.0M sodium hydroxide was used for the basicity test.

The acetic acid was placed in 250 ml beakers assigned for the acidity test while the Sodium hydroxide was placed in 250 ml beakers assigned to the basicity test. Once all of the beakers were filled, each of the fillings was submerged in one beaker for 30 minutes. After the allotted time has passed, the researchers weighed each of the fillings and recorded their weight loss due to corrosion.

In determining the percentage weight loss, the following formula was used:

$$\text{percentage weight loss} = \frac{\text{final weight}}{\text{initial weight}} \times 100$$

A one-way ANOVA was conducted to determine whether there was a significant difference among the recorded weight losses.

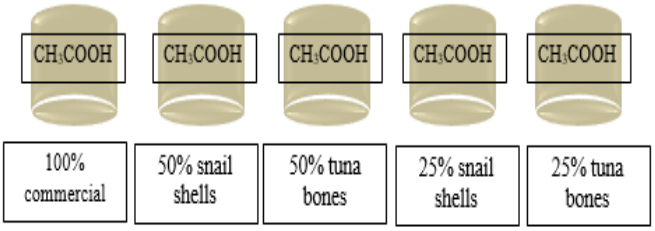

Figure 1. Experimental Set-up for Acidity Test

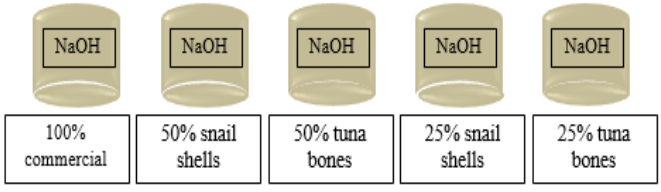

Figure 2. Experimental Set-up for Basicity Test

2.1.3. Brine Shrimp Bioassay of Samples

The preparation was done in a 1L plastic container filled with 1000 ml of artificial seawater by dissolving 35 grams of NaCl or rock salt water for the hatching of the brine shrimp.

Hatching Brine Shrimp

Brine shrimp eggs were placed in the plastic container prepared earlier. An aerator was used to provide sufficient oxygen. The container was placed under a lamp for illumination and was covered with perforated parchment paper. An aerator was used to provide sufficient oxygen. The brine shrimp eggs were left in the set-up after 48 hours to hatch.

Tenfold Dilution of Samples

Twelve 10 ml test tubes were used for each sample. One test tube was filled with 10 ml of artificial seawater, while five test tubes were filled with 9 ml. One gram of sample was added to the test tube with 10 ml of artificial seawater. One ml of solution from the test tube with 10 ml of artificial seawater was then added to each of the five test tubes. To create the set-up for the second trial, the solution in the six test tubes were halved and placed in six other test tubes. The procedure was repeated four more times for the four remaining samples.

Placing Brine Shrimp in Set-up

After being hatched for 48 hours, 15 brine shrimp nauplii were delivered into each of the test tubes using a syringe. The test tubes were kept illuminated during the treatment period.

Data Analysis

The number of dead and alive shrimps was counted after 24 hours. The researchers determined the percent mortality rate of brine shrimp in each of the test tubes by using the formula:

$$\% \text{ Mortality} = \frac{\text{number of dead brine shrimp}}{\text{initial number of brine shrimp}} \times 100$$

Probit analysis was used to find the LC_{50}, or the lethal concentration required to kill 50% of the brine shrimp.

3.RESULTS

3.1. Fourier Transform Infrared Spectroscopy (FTIR)

The researchers were unable to pass the samples as most laboratories in Mindanao were unavailable due to maintenance work. However, the researchers' previous iteration of the study showed the FTIR analysis of the samples.

Figures 4 and 5 show the spectrum of the golden apple snail shells calcined at temperatures of 700°C and 900°C, respectively. The bands in figure 4 at 3642 cm^{-1} with an absorbance abundance of 96.26%T represent the hydroxyl compounds and at 1403.88 cm^{-1} with an absorbance abundance of 89.97%T derived from stretching and vibrational modes of OH groups in $Ca_{10}(PO_4)_6(OH)_2$. While figure 5 shows bands at 3642 cm^{-1} with absorbance abundance of 93.95%T which represents the hydroxy compounds. However, the phosphate bond cannot be seen as the result, indicating an absence of the compound.

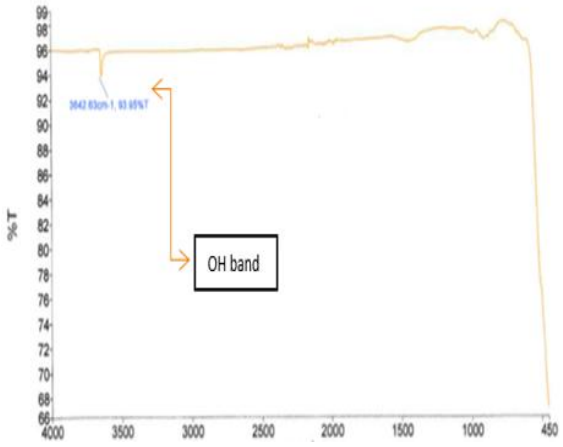

Figure 4. FTIR Graphical Presentation of Snail Shell Ash at 700°C calcination

Figures 6 and 7 show the spectrum of the yellowfin tuna bones calcined at temperatures of 700°C and 900°C, respectively. Both the powders have indicated the formation of an apatite phase. The tuna bones calcined at 700°C show bands for the apatite group at 1023.65cm^{-1} with an absorbance abundance of 63.39%T. While the powder of yellowfin tuna calcined at 900°C shows bands for the apatite group at 1024.16 cm^{-1} with an absorbance abundance of 85.11%T. However, the band of yellowfin tuna calcined at 900°C is more prominent than the band spectrum of yellowfin tuna calcined at 700°C with difference in the absorbance of 63.39%T for 700°C and 85.11%T for 900°C. The hydroxyl group is not present in the calcined yellowfin tuna at both temperatures.

The FTIR results showed that hydroxyapatite is completely present in the calcined golden apple snail shells at 700°C. Apatite is absent in golden apple snail shells calcined at 900°C. Moreover, it is also present in the calcined yellow fin tuna bones at 700°C and 900°C, but the hydroxyl group is absent in both samples.

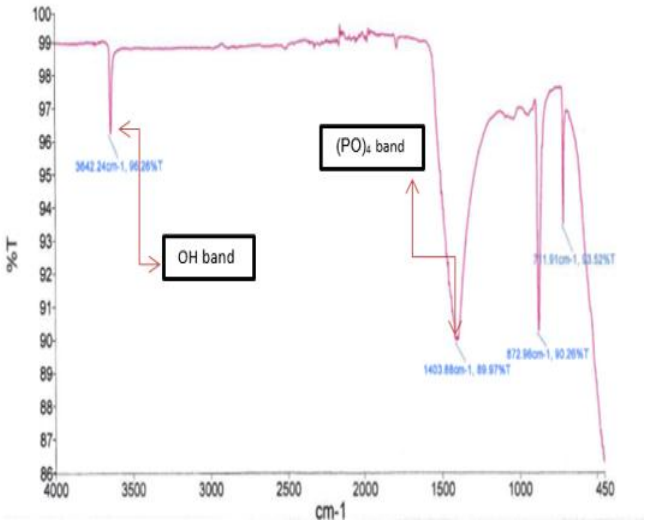

Figure 5. FTIR Graphical Presentation of Snail Shell Ash at 900°C calcination

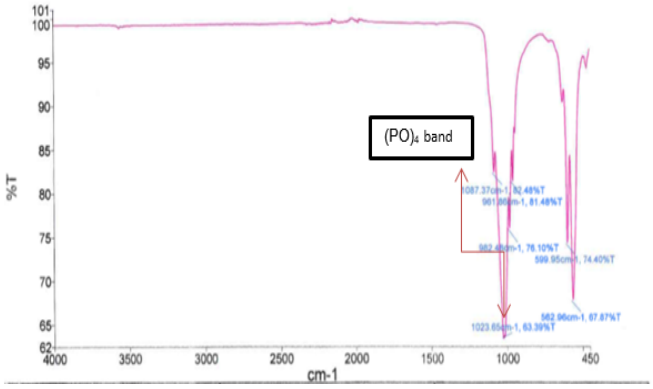

Figure 3. FTIR Graphical Presentation of Fish Bone Ash at 700°C calcination

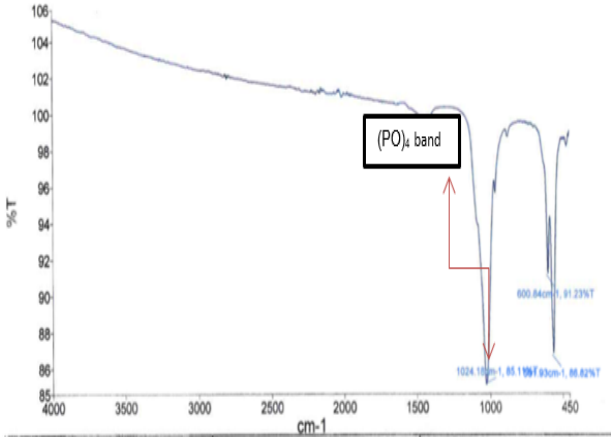

Figure 4. FTIR Graphical Presentation of Fish Bone Ash at 900°C calcination

3.2. Electron Dispersive X-Ray Spectroscopy

The chemical compositions of fish bone and snail shell powder were analyzed through Electron Dispersive X-Ray Spectroscopy. The snail shell powder calcined at 700°C had the highest Ca/P ratio with a ratio of 12.40. The least Ca/P ratio belonged to the snail shell powder calcined at 900°C with a Ca/P ratio of 1.45 [7].

Table 1. Stoichiometric Ratio of Ca/P in Samples

Sample	Weight of Calcium	Weight of Phosphorous	Ca/P Ratio
Fish bone Powder (700°C)	1.87	1.26	1.48
Fish bone Powder (900°C)	21.66	-	-
Snail Shell Powder (700°C)	20.71	1.67	12.40
Snail Shell Powder (900°C)	19.57	13.51	1.45

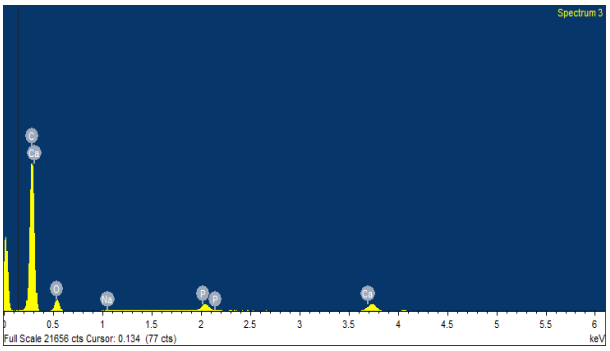

Figure 8. Spectrum of Snail Shell Ash Calcined at 700°C

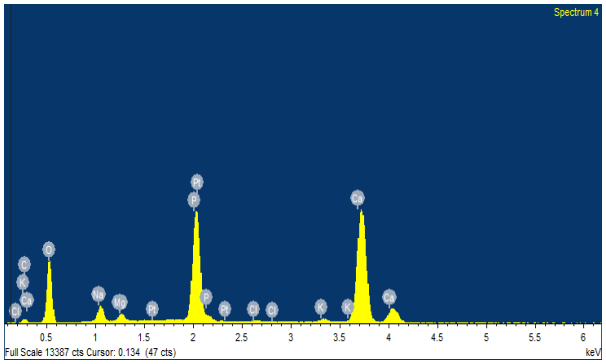

Figure 6. Spectrum of Snail Shell Ash Calcined at 900°C

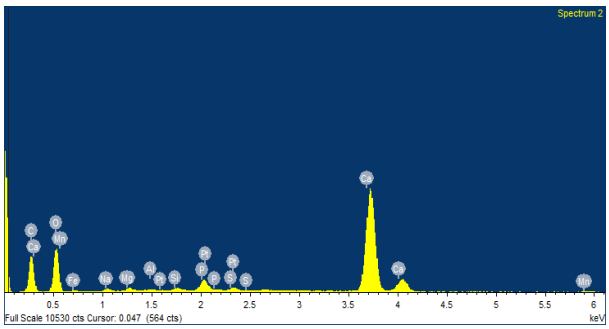

Figure 7. Spectrum of Fish Bone Ash Calcined at 700°C

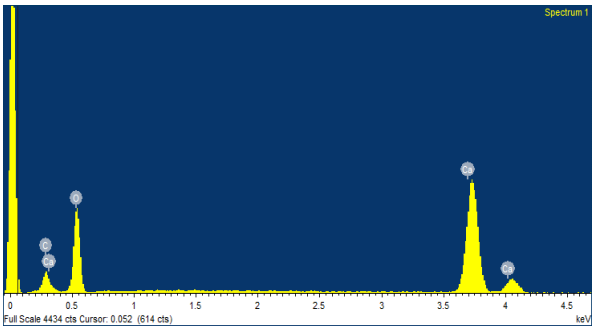

Figure 8. Spectrum of Fish Bone Ash Calcined at 900°C

3.3. Scanning Electron Microscopy (SEM) Results

Figures 12(a) to 12(p**).** represents the morphology of the surface of the fish bone powder and snail shell powder after calcination at temperatures of 700°C and 900°C, respectively. As compared to the fish bone powder, the microstructure of snail shell powder was found to be smaller in size, with a closer netted molecular structure. The snail shell powder was also found to be more compact and spherical in form. Consequently, the

particle size of the snail shell powder is greater and more crystalline in structure than that of the fish bone powder, which is proportional to the increment of the hydroxyapatite content in the structure of the powder [5].

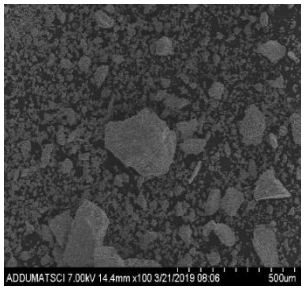

Figure 12(a). Snail Shell Ash (700°C) at 100x magnification

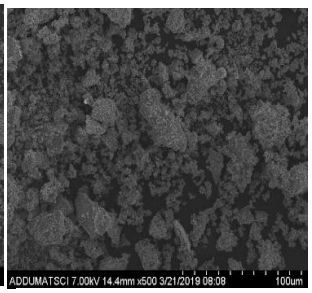

Figure 12(b). Snail Shell Ash (700°C) at 500x magnification

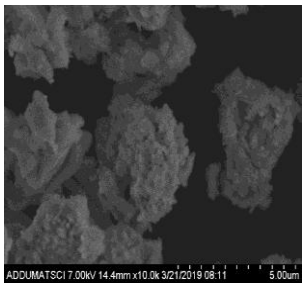

Figure 12(c). Snail Shell Ash (700°C) at 10000x magnification

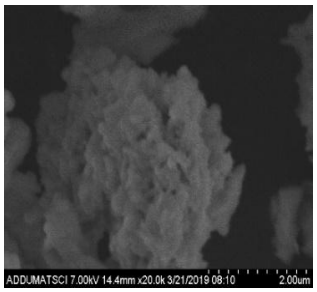

Figure 12(d). Snail Shell Ash (700°C) at 20000x magnification

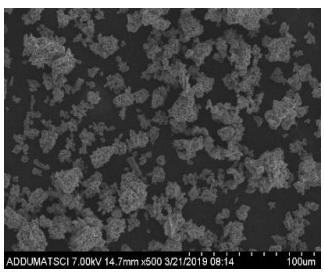

Figure 12(e). Snail Shell Ash (900°C) at 500x magnification

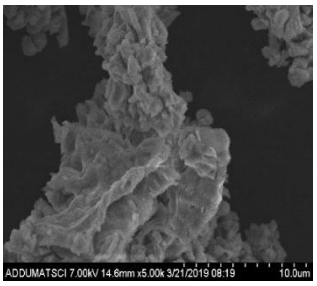

Figure 12(f). Snail Shell Ash (900°C) at 5000x magnification

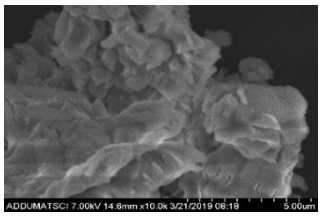

Figure 12(g). Snail Shell Ash (900°C) at 10000x magnification

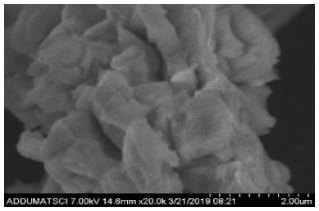

Figure 12(h). Snail Shell Ash (900°C) at 20000x magnification

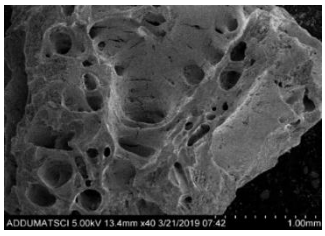

Figure 12(i). Fish Bone Ash (700°C) at 40x magnification

Figure 12(j). . Fish Bone Ash (700°C) at 200x magnification

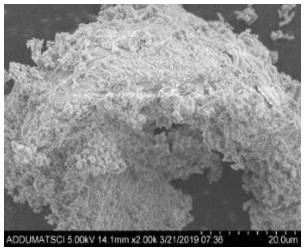

Figure 12(k).Fish Bone Ash (700°C) at 2000x magnification

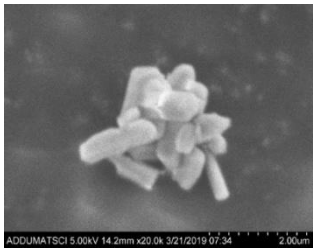

Figure 12(l). Fish Bone Ash (700°C) at 20000x magnification

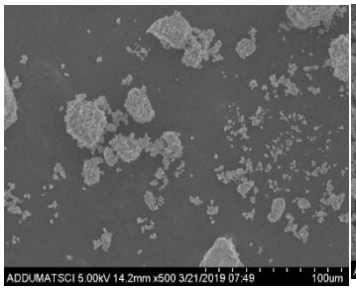

Figure 12(m). Fish Bone Ash (900°C) at 500x magnification

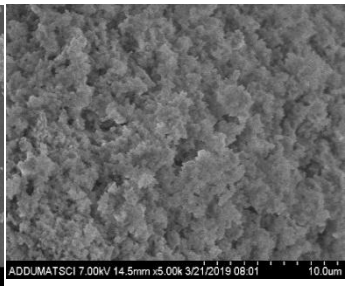

Figure 12(n). Fish Bone Ash (900°C) at 5000x magnification

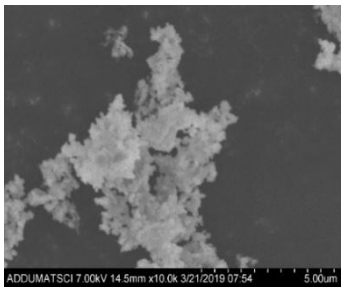

Figure 12(o). Fish Bone Ash (900°C) at 10000x magnification

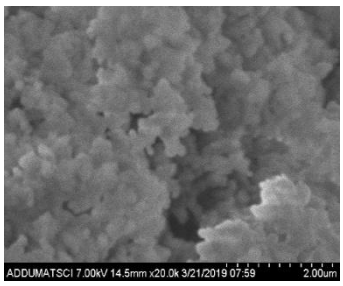

Figure 12(p). Fish Bone Ash (900°C) at 20000x magnification

3.4. X-Ray Diffraction (XRD) Results

XRD analysis was used to confirm the purity and stability of the powder after the calcination process. Figures 13(a) to figure 13(d) exhibit the XRD patterns for yellow fin tuna bones and golden apple snail shells that had been treated at different temperatures. The hydroxyapatite crystalline composition of the yellowfin tuna bones calcined at 900°C which had an absence of phosphorus had a bigger and distinct microporous structure compared with the yellowfin tuna bones calcined at 700°C which had a presence of both calcium and phosphorus[9-10]. On the other hand, the golden apple snail shells calcined at 700°C showed higher purity with its transparent molecular patterns compared to the golden apple snail shells calcined at 900°C.

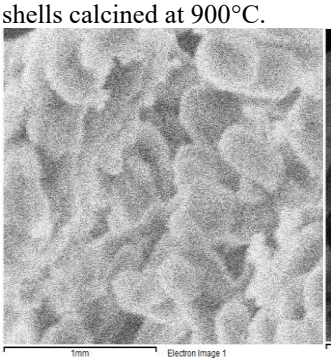

Figure 13(a). XRD Pattern of Snail Shell Ash (700°C) crystalline composition

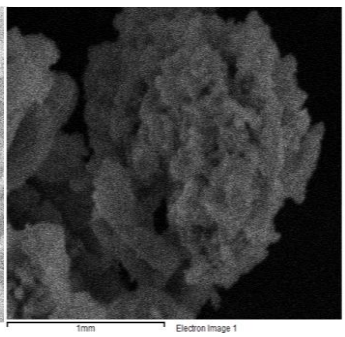

Figure 13(b). XRD Pattern of Snail Shell Ash (900°C) crystalline composition

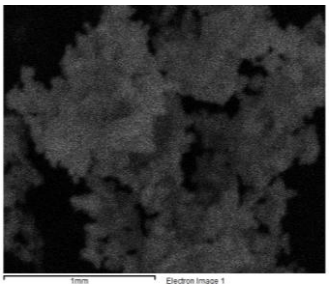

Figure 13(c). XRD Pattern of Fish Bone Ash (700°C) crystalline composition

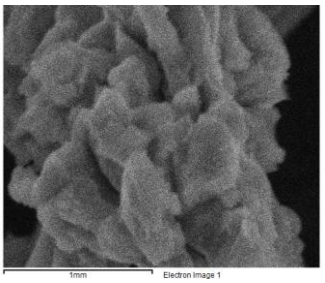

Figure 13(d). XRD Pattern of Fish Bone Ash (900°C) crystalline composition

3.5. Acidity and Basicity Test Results

As shown in (fig.14), the 100% commercial filling had the highest percentage of weight loss at 12.50%. The filling comprised of 50% commercial filling and 50% calcined snail shells at 900°C had the lowest amount of weight loss, with a weight loss percentage of 1.44%. A one-way ANOVA conducted on the samples yielded a P-value of 0.001. With the critical value of 0.05, the researchers concluded that there is a significant statistical difference among the recorded weight losses of the fillings made with filling and samples [14-17].

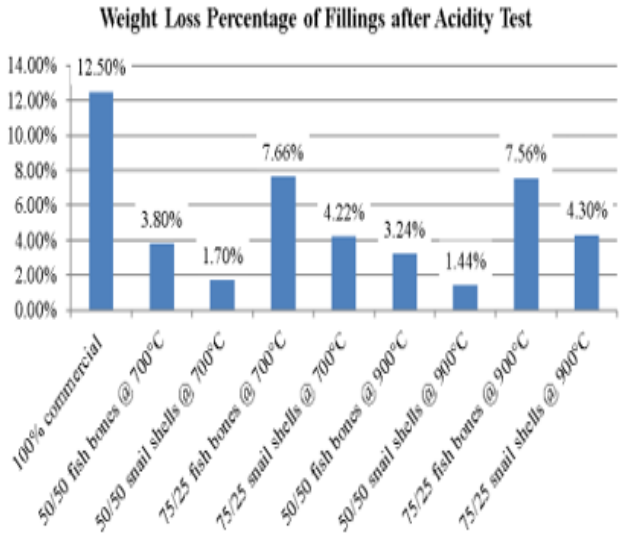

Figure 14. Acidity Test Rest

The graph above(fig.15) shows that the 100% commercial filling had the highest percentage of weight loss at 25.10%. The filling comprised of 50% commercial filling and 50% calcined snail shells at 900°C had the lowest amount of weight loss, with a weight loss percentage of 4.02%. A one-way ANOVA conducted on the samples yielded a P-value of 0.001. With the critical value of 0.05, the researchers concluded that there is a significant statistical difference among the recorded weight losses of the fillings made with filling and samples.

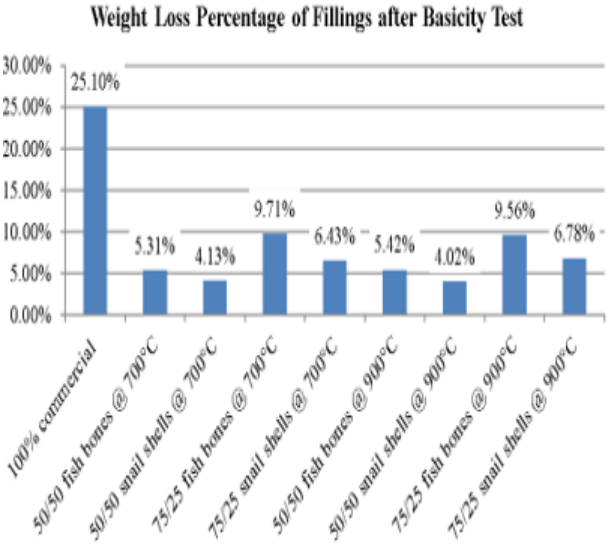

Figure 15. Basicity Test Results

3.6. Brine Shrimp Bioassay Results

Figure 16, the commercial dental filling had the smallest value of LC_{50} at 2.69 mg/L. Thus, it possesses the least value of lethal concentration required to kill 50% of a population of brine shrimp. On the other hand, the calcined yellowfin tuna bones at 700°C had the greatest value of LC_{50} at 213.71 mg/L. This implies that the mentioned sample has the greatest value of lethal concentration required to kill 50% of a population of brine shrimp. These two implications prove that the commercial dental filling is more toxic than the four other samples.

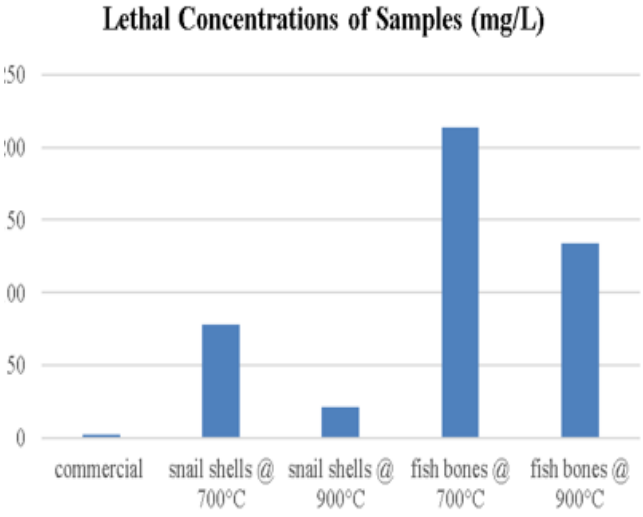

Figure 16. Lethality Result of Sample

4.CONCLUSION

From the results of the FTIR analysis in the previous iteration of the research, golden apple snail shells and yellowfin tuna bones are a source of hydroxyapatite. However, the samples that were calcined at the temperature of 900°C, are shown to have less of the compound mentioned, which suggests that calcination at the temperature of 700°C produces a more optimal sample. Results of the EDS analysis determined that the snail shell ash calcined at 700°C had the highest Ca/P ratio. Analysis of the morphological structure of the samples through SEM showed that the calcined snail shells were more crystalline in molecular structure than the calcined tuna bones. Another analysis done through XRD implied that the snail shells calcined at 700°C showed the most purity after calcination [13].

Consequently, acidity and basicity tests show that the commercial dental filling was more prone to corrosion, having the highest weight loss percentage in both tests. Lastly, Brine Shrimp Bioassay results determined that the commercial dental filling was more toxic than the samples based on lethal concentration. These results imply that the calcined golden apple snail shells and yellowfin tuna bones may be used as an additive to commercial dental filling. The most optimal sample was the calcined snail shell ash at 700°C after showing desirable results in all the tests [8-12].

Some applications of HAP such as sciatic nerve implants of HAP and Al_2O_3 were inserted into rat tibias wherein at 24 weeks after insertion. After sciatic nerve resection, the bone was demonstrated to have a superior affinity to HAP, compared with Al_2O_3.[23].

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