Biosorption Capability of Kambu-Ay (*Pila ampullacea*) Shell as Accumulation Indicator for Local Monitoring of Lead (Pb) and Mercury (Hg) Pollutions In Agusan Marsh

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ABSTRACT

Improper disposal of toxic metals such as mercury (Hg) and lead (Pb) from transport and industrial activities can cause harmful environmental and biological effects. Hg contamination can damage the organ-systems. Meanwhile, Pb causes behavioral and learning problems, seizures and death.

In Agusan Marsh, leaded gasoline is used by residents. In nearby areas, mining is present. Surveying and monitoring of toxic metal accumulation levels are to be taken seriously to ensure protection of wildlife in this ecologically significant wetland.

For monitoring toxic metal accumulation, Kambu-ay (*Pila Ampullacea*) shells were collected in Masagansang Creek, a part of Agusan Marsh where livelihood activities occur. The shells were pounded and then subjected to Direct-Air-Acetylene Flame AAS and Cold Vapor AAS to determine the inherited levels of Pb and Hg, respectively. The shells were further tested for accumulation and elimination of these heavy metals under 50 mg/L-set-up laboratory condition to determine if biosorption was still possible.

Result showed that Kambu-ay shell contained 0.22 mg/L of Hg and 220mg/L of Pb. Further examination revealed that the shells were able to remove an average of 49.43 mg/L of Pb and 43.88 mg/L of Hg in laboratory set-up. The project's contribution is two-fold. First, Kambu-ay shell was proven to remove heavy metals. Second, the strong accumulation of contaminants by the shell is well suited for local monitoring programs.

Caution should be taken if the meat of the shell is eaten. Continuous bioaccumulation of heavy metals in the food chain will threat Agusan Marsh's biodiversity. Thus, monitoring is necessary.

KEYWORDS: Direct-Air-Acetylene Flame AAS, Cold Vapor AAS, Biosorption, Bioaccumulation, Safety

Definition of Key Terms

**Atomic Absorption Spectrometer:** Atomic Absorption Spectrometer (AA) use light absorption to measure the concentration of gas-phase atoms. An analyte, usually a solid or liquid, is vaporized in a flame or in an graphite furnace with a temperature of 1000 degrees – 1200 degrees K. atoms absorb ultraviolet and achieve higher levels. Absorption amounts determine the analyte concentration.

**Adsorption:** Adsorption is the process by which molecules of a substance, such as gas or a liquid, collect on the surface of another substance, such as a solid. The molecules are attracted to the surface but do not enter the solid’s minute spaces as in absorption. Some drinking water filters consist of carbon cartridges that adsorb contaminants.

**Biosorption:** Biosorption is the removal of metal ions or organic compounds from solution by microorganisms. The goal of biosorption may be the removal of heavy metals such as gold or silver, or the removal of pollutants from soil.

**Calcium Carbonate:** Calcium Carbonate is a calcium salt CaCO₃ that is found in limestone, chalk, marble, plant ashes, bones, and many shells, that is obtained also as a white precipitate by passing carbon dioxide into a suspension of calcium hydroxide in water, and that is used in dentifrices and in pharmaceuticals as an antacid and to supplement bodily calcium stores.

**Lead:** Lead is one of the elements, a heavy, pliable, inelastic metal, having a bright, bluish color, but easily tarnished. It is both malleable and ductile, though with little tenacity, and is used for tubes, sheets, bullets, etc. It is easily fusible, forms alloys with other metals, and is an ingredient of solder and type metal. It is chiefly obtained from the mineral galena, lead sulphide.

**Mercury:** Mercury (Hg) is a heavy metal (liquid at normal temperature) which is asytemic poison that attacks brain, kidneys, and other body organs. It is most severely affects...
the central nervous system, and children and elderly are most susceptible to its poisoning. Like other heavy metals, mercury is a bioaccumulator that excretes very slowly from the body, collects in body tissues and climbs up the food chain over time. Its maximum allowable concentration is 0.05 parts per million (ppm).

Large Asiatic Apple Snail (Pila ampullacea): Large Asiatic Apple Snail (Pila ampullacea) is a species of freshwatersnail with an operculum, an aquatic gastropod mollusk in the family Ampullaridae, the apple snails.

1. INTRODUCTION
1.1 Background of the Study
Heavy metals are natural components of the Earth’s crust. They occur in ecosystem. To a small extent, they enter our bodies via food, drinking water and air. As trace elements, some heavy metals are essential to maintain the metabolism of the human body. Yet at higher concentrations, heavy metals can lead to poisoning.

The top two most pollutant heavy metals are lead (Pb) and mercury (Hg). It negatively affects people’s health. With scarce public recognition for the harm they cause, these toxic metals do untold damage to people’s lives.

In humans, exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. It causes kidney and gastrointestinal damage and mental retardation in children. It is a dangerous element. It is harmful even in small amounts. It can be inhaled in dust from lead paints, or waste gases from leaded gasoline. It is found in trace amounts in various foods, notably fish, which are heavily subject to industrial pollution. Some old homes may have lead water pipes, which can then contaminate drinking water.

Meanwhile, mercury is a global pollutant with complex and unusual chemical and physical properties. It can damage to nervous system and can cause protoplasm poisoning. High exposures to this metal could eventually result to death. In its pure form, it is generally not harmful to humans, but if it is allowed to combine with other elements (as the oxygen and hydrogen in water or air), it can form organic compounds which is very dangerous. In aquatic environments, eating too much fish from streams and rivers contaminated with mercury is a dangerous way of life.

Heavy metal contamination have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi, et al., 2007; Vosyliene and Jankaite, 2006; Ashraj, 2005). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants, (Olafia et al., 2004; Clarkson, 1998; Dickman and Leung, 1998).

Mining is one of the leading sources of the toxic wastes. In Agusan and Compostela Valley, it has played an important role in their economy. Improper disposal of its toxic wastes is becoming a concern, however, in the Agusan Marsh, an ecologically significant wetland ecosystem in the Philippines. It is the catch basin of the Agusan-Davao, draining from mining’s of Mt. Diwata Ranges, and the Mt. Bukidnon Ranges.

The water from Agusan marsh is used as drinking water by the residents living there. Moreover, fishing is the most common livelihood of the people in Agusan Marsh. Toxic metals are dangerous for them to tend to bioaccumulate. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted.

Heavy metals like lead and mercury bioaccumulated and flowed around the creeks of Diwalwal into the Manunga and Naboc Rivers that in turn about 24 km away from the mining site. Hence, the impact of possible mercury pollution due to mining in Diwalwal extends far beyond the site. The provinces of Agusan del Sur and Agusan del Norte that are crossed by the Agusan River. Butuan Bay is also affected. Thus, the water in Agusan Marsh needs to be monitored.

Large Asiatic Apple Snail (Pila ampullacea) known as Kambu-ay are presently abundant in the site. It serves as one of the residents’ food. The shells are thrown away by the residents. A study of G. Crinib (2004) proved that shells are a good adsorptive material because it contains modified biopolymers in waste water treatment. It contains calcium carbonate (CaCO₃) that adsorbs great amount of heavy metals locking them into solid form. The shells can be used as indicator for the level of heavy metals in the water.

Using shells as an adsorptive material would be a great advantage because of its low cost and good performance.

1.2 Statement of Problem
The main problem that this research sought to answer is: Can the Large Asiatic Apple Snail’s (Pila ampullacea) shell which is presently abundant in Agusan Marsh be used as an accumulation indicator for local monitoring of Hg and Pb levels?

Specifically, the study sought answers to the following problems:
1. Is there an inherited level of lead (Pb) and mercury (Hg) adsorbed by powdered Large Asiatic Apple Snail’s (Pila ampullacea) shell in Agusan Marsh?
2. Can Large Asiatic Apple Snail’s shell accumulate and remove Hg?
3. Can Large Asiatic Apple Snail’s shell accumulate and remove Pb?
1. To determine the inherited level of lead (Pb) and mercury (Hg) adsorbed by powdered Large Asiatic Apple Snail’s (Pila ampullacea) shell in Agusan Marsh.
2. To check the accumulation and removal capability of Large Asiatic apple snail to Hg.
3. To check the accumulation and removal capability of Large Asiatic apple snail to Pb.

Null Hypotheses (H₀):
1. There is no inherited level of lead (Pb) and mercury (Hg) adsorbed by powdered Large Asiatic Apple Snail’s (Pila ampullacea) shell in Agusan Marsh.
2. Large Asiatic apple snail shell cannot accumulate and remove mercury (Hg).
3. Large Asiatic apple snail shell cannot accumulate and remove Lead (Pb).
Alternative Hypotheses (H₃):
1. There is an inherited level of lead (Pb) and mercury (Hg) adsorbed by powdered Large Asiatic Apple Snail’s (Pila ampullacea) shell in Agusan Marsh.
2. Large Asiatic apple snail shell can accumulate and remove mercury (Hg).
3. Large Asiatic apple snail shell can accumulate and remove lead (Pb).

1.3 Significance of the Study
The prevalence of people getting sick of some diseases has increased year by year due to the widespread contamination of heavy metals like mercury and lead. Due to this issue, the search for removal process and detection instruments is a problem in the field of environmental science and management.

This study is found to be timely and relevant to test the potential use of commonly available animals like the Large Asiatic Apple Snail. The feasibility of using the shell of the Large Asiatic Apple Snail as an indicator of Pb and Hg accumulation in Agusan Marsh and as a biosorptive agent of heavy metals could be of great importance to the awareness of the people in Agusan Marsh.

To the Community
This study significantly warns the community to be very careful on eating kambu-ay meat. It is a common knowledge that the shells are thrown away and become garbage/waste. Tellingly, the price and abundance of the shells is lower when compared to oysters, clams and other edible bivalves. That’s why it is preferably to use it than others. This yields significant to people to focus on the local materials provided by nature as alternative sources of products.

To Department of Health (DOH)
The use of Kambu-ay shells as biosorption is significant since pollution is a big problem and one of the causes of poor health and even death of the people and the living organisms in the society.

To the mining operators and pump boat users
The shells can also be used as a filter of mercury and lead at the mining sites.

To Department of Environment and Natural Resources
By the series of research, the researchers found out that the shells have the tendency to attract chemical pollutants because it contains a non-metal calcium carbonate which is considerably a hint in chelation process.

To Local Government Units (LGU)
The shell-based techniques presented here provided useful means of local monitoring of Hg and Pb accumulation. Relevant strategies demonstrated here could be of particular importance in areas with pollution histories and may affect biological necessities of the environment and local ecosystems affected by some classified and unclassified pollutants including the heavy metals.

Moreover, the availability of the said shell in the area is ultimately high thus, a good factor in making this research possible.

This research will give further information in the local monitoring sectors of pollution and to other researchers in the field of environmental science as well.

1.4 Scope and Limitation of the Study
This study was experimentally conducted last July 21-22, 2011 at FAST laboratories, Cagayan de Oro. The researchers were under the supervision of a laboratory chemist, Mr. R.L. Borres and the FAST Laboratory manager, Mrs. Ronalyn V. Soliva.

This study is delimited on the use of large Asiatic apple snail known as kambuay shell as an indicator of water pollutants in Agusan Marsh Wildlife Sanctuary (AMWS) and as a removal of heavy metals such as Mercury (Hg) and Lead (Pb).

Intervening variable such as water source and the location of the Large Asiatic Apple Snails were facilitated thoroughly in order to ensure unbiased interaction within solutions.

1.5 Review of Related Literature
Large Asiatic Apple Snail
Large Asiatic apple snail (Pila ampullacea) in Bunawan, Agusan del Sur is locally known as Kambuay. It is a freshwater animal in the Ampullariidae family which is found to be abundant in Agusan Marsh Wildlife Sanctuary (AMWS) (Travis, 2007).

Kambu-ay is amphibious. They can adapt in both land and water. They borrow in mud to a depth of 1 m. They feed on all kinds of vegetation and algae and hibernate during dry season. It has an exoskeleton that supports and protects an animal’s body known as shells. The shell varies from 90-100 mm high and 85-90 mm wide, bright green to orange-brown with reddish spiral bonds. The shell of kambuay contains calcium carbonate that makes the shell hard to break. These snails are not selective and eat almost everything available in their environment (Komalamisra, C., Nuamtanong, S & Dekumyoy, P. 2009).

These aquatic animals are abundant throughout South Asia such as Philippines, Singapore, Borneo, Sumatra, and many more. In the Agusan Marsh, Philippines, kambuay is abundant and is usually sold in Bunawan public market. The meat is edible; however the shells become waste and are just thrown away.

Bioaccumulation
Heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of these heavy metals are mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (TI) and lead (Pb). They occur naturally in the Earth’s crust. They cannot be degraded nor destroyed. Even to a small extent, they enter our bodies by way of food cycles, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are vital to maintain the proper metabolism of the human body (Lenntech, 2004). However, at higher concentrations they are toxic to living organisms. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain (Duruihe, Ogwuegbu & Egwurugwu, 2007).
According to Gopinathan, K.M. & R. Sobhana Amma,R., the presence of heavy metals causes hazardous impact on the flora and fauna of earth. They are threatening because they have the tendency to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical’s concentration in the environment. The worse thing is these compounds build up in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater (Lenntech, 2004).

A study by Kesavan, Babu and Ravi (2011) in India about mercury concentration in mollusks and sediments in Uppanar Estuary, Southeast Coast of India revealed that "concentration of the mercury in the dry weight sample of sediment ranged from (1.2 to 1.8 ppm), tissue ranged from (0.4 to 2.4 ppm); while in the shell samples it was (0.6 to 3.4 ppm)."

The study concluded that Hg level is in the acceptable range of concentration compared with the previous studies. Moreover, they demonstrated that the mollusks are safe for human consumption and that the ecosystems where they were harvested do not pose any hazard to man in terms of health risk. However, caution should be taken if the bivalves are continuously used; especially children have about five times more gastrointestinal absorption rate of adults.

In addition, Huanxin, Lejun, & Presley (1999) investigated sample of shells collected at the major US Gulf of Mexico bays and estuaries. Presence of heavy metals such as Cd, Cr, Cu, Fe, Mn, Pb, and Zn were analyzed. Results showed that oyster shell is rich with cadmium. Accordingly, accumulated heavy metals are results of both nature and human activity.

In Ijaw area of the Niger Delta, bioaccumulation of heavy metals in fauna and flora were investigated. Selected faunal-mollusks and crustacean, and flora Hibiscus esculentus and Vernonia amygdalina were used for this study. Results showed an accumulation of considerable amounts of heavy metals on Mollusks and crustacean. Furthermore, higher levels of all the metals determined such as Hg, Pb, Cr, Cu, Ni, and Zn were observed in the shells. The roots of Hibiscus esulentus and Vernonia amygdalina accumulated mercury levels is 0.010 µg/g ± 0.00, while the stem 0.17 ± 0.03 µg/g and leaves 0.25 ± 0.02µg/g was recorded for the V. amygdalina. The H. esculent fruit has lead levels of 0.22 ± 0.03 µg/g, Cu, Ni, and Zn levels are generally higher than those of Hg, Pb and Cr in all the samples analyzed (Gbaruko & Friday, 2007).

One of the biggest mining operations in the Philippines is located in Diwalwal, one of the Barangays in the municipality of Monkayo, in Compostela Valley. The creeks around Diwalwal flow into the Manguna and Naboc rivers that in turn drain into the Agusan River, which is about 24 kilometers away from the mining site. Hence, the impact of mercury pollution due to mining in Diwalwal extends far beyond the site. The provinces of Agusan del Sur and Agusan del Norte that are crossed by the Agusan River to Butuan Bay are also affected. According to Roa (2007), these heavy metals threatened life in Agusan Marsh Wildlife Sanctuary (AMWS), a catchment area which is highly environmentally sensitive because of its location.

AMWS is one of the most significant wetland and biodiversity sites in the Philippines and is considered as a Key Biodiversity Area (site no. 180). Unfortunately, the area is being threatened by some problems and issues. One is the pollution from mining upstream. This makes AMWS potentially in danger that hinders ecological functions and services. Behind this problem is the low awareness about the importance of the AMWS among the stakeholders. Participation in planning, management and decision-making to help conserve the environment is greatly encouraged (Society of Philippine Wetland Conservation, 2011).

Biosorption of Heavy Metals
Serious health risks to humans and aquatic organisms pose by large quantities of toxic metals such as mercury lead, cadmium, zinc or others made the scientific community under pressure to upgrade new methods for the detection and elimination of toxic contaminants from wastewaters in efficient and economically feasible ways.

New technologies used shells for heavy metals clean-up. Shells contain calcium carbonate (Lechtanski, 2000,) that adsorbs heavy metals and locks them into a solid form. Pouring metal and acid-laden water over a bed of crushed clam or mussel shells provides an easy fix. The shells are made of aragonite, a form of calcium carbonate that readily swaps out its calcium atoms in favor of heavy metals, locking them into a solid form. The pH of shells is 8.3 when dissolved in water.

The properties of waste shells which cause pollution problems are investigated in Southern beach, Korea. The absorbent surface area was affected by the contaminant gas removal efficiency of the waste shell absorbents, pore structures, absorbent deliquesence and hygroscopicity during the contaminant gas reaction (Jung, Yoo, & Oh, 1999).

Calcium Carbonate helps in the biosorption of heavy metals in water, it is described as cardiac stimulant, digestive observe any possible change in heavy metal and calcium stimulant Aphoric. Pouring metal and acid laden water over a bed of crushed clam or mussel shells provides an easy fix. The shells are made of Aragonite, a form of calcium carbonate that readily swaps out its calcium atoms in favor of heavy metals, locking them into a solid form. The shells are naturally basic too—when dissolved they have a pH of 8.3.

According to Horne (2006), the exoskeletons of snails and atoms, or their shells in common parlance differ from the exoskeletons of turtles in several ways. Seashells are the exoskeletons of mollusks such as snails, clams, oysters, and many more. Such shells have three distinct layer's and are composed mostly of calcium carbonate with only a small quantity of protein –no more than 2%.

Simmons (1989) introduced CaCO3 into the Whetstone Brook in Massachusetts. His hope was that the calcium carbonate would counter the acid in the stream from acid rain and save the trout that had ceased to spawn. Although his experiment was a success, it did increase the amounts of aluminum ions in the area of the brook that was not treated with the limestone. This shows that CaCO3 can be added to
neutralize the effects of acid rain in river ecosystems. Currently calcium carbonate is used to neutralize acidic conditions in both soil and water. Since the 1970s, such liming has been practiced on a large scale in Sweden to mitigate acidification and several thousand lakes and streams are limed repeatedly.

The study investigated by Khosravi, Ganji & Rakhsheae (2005) on the biosorption of Pb, Cd, Cu and Zn from the wastewater by treated Azolla filiculoides with H2O2/MgCl2 revealed that the maximum uptake capacities of the collected Azolla from rice field at the optimal conditions for Pb, Cd, Cu and Zn ions were approximately 228, 86, 62 and 48 mg/g (dry Azolla), respectively while the maximum uptake capacities of the collected Azolla from the Anzali International Wetland in the north part of Iran at the same conditions for these heavy metals were about 124, 58, 33 and 34 mg/g (dry Azolla), respectively.

**Mercury (Hg)**

Mercury is one of the heavy metals because it is very dense. A cup of mercury for example weighs more than three (3) kilograms, that’s nearly fourteen times heavier than a cup of water. Mercury is generated naturally in the environment from the degassing Earth’s crust, from volcanic eruptions. Mining operations, chloralkali, plants and paper industries are significant procedures of mercury (Goyer, 1996). According to the environmental engineers at Duke University that harmful form of mercury which is formed is called Methyl mercury which is toxin for living beings nerve cells.

Mercury pollution can be a serious threat. Inhalation is the most frequent cause of exposure to mercury. The organic form is readily absorbed in the gastrointestinal tract (90-100%); lesser but still significant amounts of inorganic mercury are absorbed in the gastrointestinal tract (7-15%). Target organs are the brain and kidneys (Roberts 1999).

Although the metallic mercury in these products rarely poses a direct health hazard, industrial mercury pollution becomes a serious threat when it is released into the air by power plants, certain chemical manufacturers and other industrial facilities, and then settles into the oceans and waterways, where it builds up in fish that we eat. Once in the human body, mercury acts as a neurotoxin, interfering with the brain and nervous system. Prenatal and infant mercury exposure can cause mental retardation, cerebral palsy, deafness and blindness. Mercury may affect a child’s development, delaying walking and talking, shortening attention span and causing learning disabilities. In adults, mercury poisoning affects fertility and blood pressure regulation and can cause memory loss, tremors, vision loss and numbness of the fingers and toes. It also leads to heart disease. High exposures have also resulted in death.

**Lead (Pb)**

Lead is a bright and silvery when freshly cut. It is a dense, ductile, very soft, highly malleable, bluish-white metal that has poor electrical conductivity when compared to most other metals. Lead can be used as a pure metal, alloyed with other metal or as a chemical compound. It is estimated that more than 1 million tons of Lead is retrieved annually by recycling batteries.

Lead released into the environment makes its way into the air, soil and water that can contribute pollution especially in urban areas. Lead can enter the environment through mining practices, steel industry, crop enhancers, improperly disposed of batteries or improperly stored metal part such as machinery that can break and leave pieces or shavings which may leech into the environment.

Lead toxicity at high levels as well as at low levels may pose adverse health effects especially if there is a continual exposure to low levels of heavy metals through the consumption of these fauna and flora. Physiological and behavioral effects of these trace metals in human population are a growing concern. (Gbaruko & Friday, 2007).

## 2. METHODOLOGY

### 2.1 Materials, Reagent and Equipment

<table>
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<tr>
<th>Item</th>
<th>Description</th>
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<tr>
<td>Mortar and Pestle</td>
<td>Stirring Rod</td>
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<td>Graduated Cylinder</td>
<td>Erlenmeyer flask</td>
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<td>Analytical Balance</td>
<td>Volumetric flask</td>
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<td>Watch</td>
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<td>Beaker</td>
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<tr>
<td>Watch Glass</td>
<td>potassium per sulfate</td>
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<tr>
<td>Map</td>
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### 2.2 Area Selection

The area for the collection of shells was carefully selected. The criteria for such selection include (but not limited to): 1. Area must be part of Agusan Marsh; 2. Kambu-ay shells were present; 3. And livelihood activities occur.

The area that will be used for the study must be part of the marsh. The study will assess the level of accumulated mercury and lead in the area.

It must warrant an economic or livelihood activity. Surveying an area for toxic metal contamination where there are people and where there are economic and livelihood activities will double the impact and will motivate researchers, residents and policymakers to act if evidence of contamination will be proven.

Kambu-ay must also be present. This mollusk will be used in the study since the shells are thrown after the meat is eaten.

### 2.3 Collection and Preparation of Sample

#### 2.3.1 Collection of Kambu-ay

The researchers focused only to *Pila ampullacea*, a variety of Large Asiatic Apple Snail, commonly known as Kambu-ay. It was collected at Masagsang Creek, Nueva Era, Bunawan, Agusan del Sur with the help of a Kambu-ay hunter.

#### 2.3.2 Preparation of Kambu-ay shell

A long knife was inserted into its small opening to sweep it along the inside of the shell which cuts the muscle. The shell was opened and the meat was separated. Then, the shells were washed with clean water and were sun dried. The dried samples were pounded and weighed using a platform balance.
Obtained 100 grams of Kambu-ay shell powder were placed on a silver foil to avoid contamination and were brought to F.A.S.T. Laboratory at Cagayan de Oro City for actual experimentation.

2.4 Inherited Level of Pb and Hg of Kambu-ay Shells

2.4.1 Digestion of Kambu-ay Shells (Baseline Survey)
One (1) gram powdered Kambu-ay shell was weighed and added with 10mL concentration of nitric acid and digested over a hotplate till clear solution was obtained. Then, it was diluted to 100 mL.

The digested sample for mercury was further added with 15 mL of KMnO₄ (5%). After 15 minutes, 8 ml potassium persulfate (5%) was added.

2.4.2 Chemical Analysis
The digested reading using Direct-Air-Acetylene Flame AAS for Pb and Cold Vapor AAS for Hg were done after 24 hours of standing.

Biosorption Capability of Kambu-ay Shells
For safety purposes, the researchers read the Material Safety Data Sheet (MSDS) for lead and mercury. Such action will help prevent any improper actions and in case of accident, will help in carrying out necessary steps.

2.5 Preparation of Hg and Pb Solutions
Three replicates were made for every Hg and Pb solution set-up. Set-up replicates were labeled accordingly.

To obtain 50 ppm lead solution, 0.005g of Pb was emerged with 100mL distilled water making up 50 ppm solution. To obtain 0.005g of mercury, 0.005g of Hg was also emerged with 100mL distilled water making up 50 ppm solution.

A total care was applied in the preparation of the solutions to avoid explosion of heavy metals and destruction of laboratory apparatus being used.

2.6 Addition of Kambu-ay shells to Hg and Pb Solutions
Fifteen grams of Kambu-ay shells were carefully poured to each 50 ppm-solution set-up manually considering a minute for each mixture. Shaking/stirring was done.

2.7 Separation from Solution
Only three hours was the considered time interval. Three hours was chosen to allow enough time for the shells to act/bind/adsorb with the Hg and/or Pb in the solution.

Note that the purpose of laboratory set-up was to prove that the shells can still adsorb despite the inherited level of Hg and/or Pb in the shells. Thus, three hours was enough. In the actual setting, there are other factors that are essential in the accumulation of toxic metals in to the shells as discussed by previous studies (Kesevan et. al, 2011; Boyden, 1977).

After three hours, the mixture was separated. With the use of Whatman papers no.3 as suggested by the F.A.S.T. Laboratory which is very porous that will allow the passage of heavy metals to pass through, separately, the mixtures were filtered simultaneously and carefully so as to avoid spilling. Slowly, the filtered solutions were then set aside, ready for analysis.

2.8 Chemical Analysis
The filtrates were analyzed at F.A.S.T. Laboratory. Using Direct-Air-Acetylene Flame AAS and Cold Vapor AAS, the amount of Pb and Hg was determined, respectively.

2.9 Disposal of Experimental Variables (mixtures of heavy metal solutions and shells)
After the heedful course of mixture filtration and chemical analyzation, the remaining residues were left behind the chemical laboratory in-charge for they have the eminent skills in handling such hazardous substances.

Summary of Biosorption Capability of Kambu-ay Shells

2.10 Data Interpretation and Analyses
2.10.1 Computation for the mean
The data was analyzed and interpreted utilizing the mean/average values. The average value was calculated using the equation:

\[
\text{Ave. value} = \frac{\sum x_1 + x_2 + x_3 + \cdots + x_n}{N}
\]

2.10.2 Computation for the Removed Toxic Metal
The results of the chemical analyses revealed the toxic metals that were not removed from the 50 mg/L-solution. In order to get the removed Hg or Pb, this formula was used:

\[
\frac{\text{result of AAS}}{50} = \frac{\text{metal to toxic removed}}{50}\text{mg/L} = 50 - \text{AAS result}.
\]

2.10.3 Computation for the Removed Toxic Metal in Percentage (%)
In order to get the removed Hg or Pb in percentage, this formula was used:

\[
\frac{50 - \text{AAS result}}{50} \times 100 = \text{removed toxic metal (mg/L)}
\]

2.10.4 Units of Measurement
The units used in this study were mg/L, µg/g and ppm. A part per million or ppm is equal to mg/L and µg/g. Thus:

1 mg/L = 1 ppm = 1 µg/g.
3. RESULTS AND DISCUSSION

3.1 Area Selection

The area selected was Nueva Era, Bunawan, Agusan del Sur. Nueva Era is a barangay in the Municipality of Bunawan in the province of Agusan del Sur, Caraga Region.

Nueva Era is located in an area of Agusan Marsh where residents have difficulty travelling to the public market. The fare going to this place is 40 pesos. As a result, residents have no choice but to eat foods like Kambu-ay which are present in the barangay.
In Campo 6 of Nueva Era, Masagansang Creek can be found. It is connected to Lake Mihaba. Both Masagansang Creek and Lake Mihaba are part of Agusan Marsh.

Masagansang Creek is surrounded by different kinds of grasses, trees and water lilies. It is also inhabited by different species of animals like crocodiles. In this creek, residents get their food if they cannot go to the market. Fishing and farming is their main livelihood since most of the people live near the lake.

Kambu-ay, a snail abundantly found in the creek is one of the easily found foods. Adobong Kambu-ay is a popular dish. This serves as one of their native delicacies. While the meat is eaten, the shells are thrown away.

Since the Masagansang Creek is part of Agusan Marsh and there are livelihood activities occurring, it was selected for the collection of Kambu-ay shells.

### 3.2 Inherited Levels of Mercury (Hg) and Lead (Pb)

#### Table 3.1

<table>
<thead>
<tr>
<th>Detected toxic metal in shells</th>
<th>Inherited Level (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead (Pb)</td>
<td>220</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Table 3.1 presented the inherited level of Lead (Pb) adsorbed by the shells of Large Asiatic Apple Snail commonly known as Kambu-ay (Pila ampullacea) in Lake Masagansang.

Pb was present at 220 mg per liter. The possible reason for this high level of Pb was the use of leaded gasoline by the residents in their boat and other activities in the lake. The detected amount of Pb was high and warrants a further investigation.

Meanwhile, Hg was present at 0.22 mg per liter. This concentration was lower than the level of Pb. Nevertheless, bioaccumulation of Hg will be dangerous to the wildlife in the area and to the health of the residents. Thus, monitoring is essential.

#### 3.3 Pb and Hg in Shells around the World

This was the first study of Kambu-ay shell and its biosorption capability as indicator of Hg and Pb accumulation in Agusan Marsh. Given this, the level of toxic metals will be compared to the detected amount of toxic metals in shells around the world.

#### 3.3.1 Detected Amount of Pb

A study by Notten, Walraven, Beets, Vroon, Rozema and Aerts in 2008 used the snail Cepaea nemoralis to determine the amount of Pb in three areas of Netherlands: a forestry area (REF: 52˚28' N, 5˚26' E) and two locations in Biesbosch National Park (BB1: 51˚45' N, 4˚46' E and BB2: 51˚48’ N, 4˚45’ E).

Result of the study revealed that Pb concentration in BB1 was 0.19 µg/g, in BB2 was 0.17 µg/g and REF was 0.44 µg/g. The shells from the creek had a considerably higher Pb content than from any of the three areas in Netherlands. Table 3.2 tabulated this.

#### Table 3.2. Detected Pb in Agusan Marsh and Netherlands

<table>
<thead>
<tr>
<th>Location</th>
<th>Detected amount of Pb (mg/L=µg/g)</th>
<th>Used snail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masagansang Creek, Agusan Marsh</td>
<td>220</td>
<td>Pila ampullacea</td>
</tr>
<tr>
<td>BB1</td>
<td>0.19</td>
<td>Cepaea nemoralis</td>
</tr>
<tr>
<td>BB2</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>REF</td>
<td>0.44</td>
<td></td>
</tr>
</tbody>
</table>

The amount of Pb inherited by the shells in Lake Masagansang must be studied further. The high amounts of this toxic metal in the shell suggested that the lake had high amount of Pb. The source of such should be known. Meanwhile, monitoring must be taken for the safety of the flora and fauna of the area.

#### 3.3.2 Detected Amount of Hg

Kesavan, Babu and Ravi (2011) studied the mercury concentration in mollusks and sediments from Uppanar Estuary, Southeast Coast of India. The shells of Crassostrea madrasensis, Meretrix meretrix and Cerithidea cingulate contained 3.4 mg/L, 0.6 mg/L and 0.6 mg/L of Hg, respectively.

The shells from the creek had a lower Hg content than those from Uppanar Estuary. Table 3.3 summarized the Hg content.
Table 3.3. Detected Hg in Agusan Marsh and Uppanar Estuary

<table>
<thead>
<tr>
<th>Shell of..</th>
<th>Detected amount of Hg (mg/L)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pila ampullacea</em></td>
<td>0.22</td>
<td>Agusan Marsh, Philippines</td>
</tr>
<tr>
<td><em>Crassostrea madrasensis</em></td>
<td>3.4</td>
<td>Uppanar Estuary, Southeast Coast of India</td>
</tr>
<tr>
<td><em>Meretrix meretrix</em></td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td><em>Cerithidea cingulata</em></td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

The level of Hg in the shells of *Pila ampullacea* suggested that the area had lower Hg. This low level of Hg may be attributed to chemical control order for mercury and mercury compounds of Department of Environment and Natural Resources (DENR Administrative Order No. 38, Series of 1997). Other factors may also be the reason for a low level of Hg.

Range of the mercury level of the shell in Agusan Marsh was close to the shellfish in Japan (0.050-0.152 mg/kg) than that of Canada (0.024-0.051 mg/kg) as reported by Dabeka et al. in 2011.

3.4 Biosorption Capability of Kambu-ay

3.4.1 Pb Removal Capability of Kambu-ay

Table 3.4 shows the mean and standard deviation of the remaining amount of 50 ppm concentration of Lead (Pb) and the rate of adsorption of 15 grams of Kambu-ay shell in three-hour duration of lead removal.

The standard deviation further suggests that the variation of the results was small. This strongly suggested that Kambu-ay Shell was an excellent biosorptive material by adsorbing almost all the lead that is dissolved in 100 mL water.

Table 3.4. Mean and standard deviation of the remaining amount of 50 ppm solution of lead (Pb) per set up after treated with 15 grams of Kambu-ay Shell for 3 hours

<table>
<thead>
<tr>
<th>Heavy Metal Removal</th>
<th>Lead (Pb left in ppm)</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Adsorbed by Kambuay Shell (ppm)</th>
<th>Adsorbed by Kambuay Shell (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtrate 1</td>
<td>0.55</td>
<td>0.57</td>
<td>0.08</td>
<td>49.45</td>
<td>98.90</td>
</tr>
<tr>
<td>Filtrate 2</td>
<td>0.66</td>
<td></td>
<td></td>
<td>49.34</td>
<td>98.68</td>
</tr>
<tr>
<td>Filtrate 3</td>
<td>0.50</td>
<td></td>
<td></td>
<td>49.50</td>
<td>99.00</td>
</tr>
</tbody>
</table>

All set ups had almost the same rate of adsorption. Filtrate 3, however, had the most amount of removed Pb. Fig 3.4 showed graphically the amount of Pb that was removed by the shell.

These results signified that the Kambu-ay shell can be used as a monitor of lead accumulation since the shell can significantly remove and accumulate lead even though it has already inherited lead content.

3.4.2 Hg Removal Capability of Kambu-ay

Table 3.5 shows the remaining amount of mercury (Hg) after being treated with 15 grams of Kambu-ay Shell for three hours. This also shows the average remaining amount of mercury which is almost 90%.
Table 3.5. Mean and standard deviation of the remaining amount of 50 ppm solution of mercury (Hg) per set up after treated with 15 grams of powdered Kambu-ay Shell for 3 hours

<table>
<thead>
<tr>
<th>Heavy Metal Removal</th>
<th>Mercury (Hg) left in ppm</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Adsorbed by kambuay shell (ppm))</th>
<th>Adsorbed by kambuay shell (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtrate 1</td>
<td>6.51</td>
<td>6.12</td>
<td>1.45</td>
<td>43.49</td>
<td>86.98</td>
</tr>
<tr>
<td>Filtrate 2</td>
<td>4.51</td>
<td></td>
<td></td>
<td>45.49</td>
<td>90.98</td>
</tr>
<tr>
<td>Filtrate 3</td>
<td>7.34</td>
<td></td>
<td></td>
<td>42.66</td>
<td>85.32</td>
</tr>
</tbody>
</table>

Fig 3.5 showed graphically the amount of Pb that was removed by the shell. It can be noticed that Kambu-ay Shell was an excellent biosorptive material adsorbing great amount of mercury that was dissolved in 100 mL water. All set ups have almost the same rate of adsorption. While the shell had already a 0.22 ppm inherited level of Hg in the environment of Agusan Marsh, it still was able to remove and accumulate more.

The results indicated that the shell can be used as an indicator of Hg accumulation given it has a capability of adsorbing high amounts of mercury despite its inherited mercury content.

3.4.3 Removed Hg by Kambu-ay Shells (in %)

Figure 3.6 showed the percentage of Hg adsorption of Kambu-ay shell. The shell had adsorbed almost 100% of the 50-ppm lead solution. The three set-ups had different rate of adsorption. Filtrate 2 had the highest rate of removal. This indicated that the adsorptive capacity of Kambu-ay shell in 50-ppm solution was high even though it had already inherited level of mercury in the water of Agusan Marsh.

The results implied that the shell is also a good candidate for local monitoring of Hg.

3.4.4 Removed Pb by Kambu-ay Shells (in %)

Figure 3.6 showed the percentage of Pb adsorption of Kambu-ay shell. The shell had adsorbed almost 100% of the 50-ppm mercury solution. The three set-ups have different rate of adsorption. Filtrate 3 had the highest rate of removal. This indicated that the adsorptive capacity of Kambu-ay shell in 50-ppm solution was high even though it had already inherited level of lead in the water of Agusan Marsh.
The results implied that the shell is also a good candidate for local monitoring of Pb.

3.4.5 Comparison of Removed Pb and Hg by Kambu-ay Shells (in %)

Graph 3.7 showed the comparison between the rate of adsorption of Kambu-ay shell in three replicates of heavy metal removal process of 50 ppm lead and mercury solutions. It showed that the adsorption rate of Kambu-ay shell in 50 ppm lead solution was much higher than that of the 50 ppm mercury solution.

Despite these results, Kambu-ay shell still exhibited that it was excellent biosorptive material by adsorbing great amount of heavy metals in both solutions. Considering that it had already inherited heavy metals from the water of Agusan Marsh, the shells can be used as indicator for higher level of Hg and Pb accumulation.

4. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Heavy metals negatively affect people’s health. With scarce public recognition for the harm they cause, these toxic metals do untold damage to people’s lives.

In Agusan Marsh, transport industries such as pump boats used leaded gasoline. Mining played an important role in the economy of Agusan and Compostela however it is one of the leading sources of toxic wastes and improper disposal of these toxic wastes is becoming a concern in the area. Thus, the water in Agusan Marsh should be monitored.

Kambu-ay shells were subjected to Direct-Air-Acetylene Flame AAS to check the inherited level of lead and Cold Vapor AAS to determine the inherited level of mercury. The shells were further tested for accumulation and elimination of these heavy metals under 50 mg/L-set-up laboratory condition to determine if biosorption was still possible.

Based in the findings, the researchers present the following conclusions:
1. There is an inherited level of Lead (Pb) which is 220 ppm and 0.22 ppm of Mercury adsorbed by powdered large Asiatic apple snail’s (Pila ampullacea) shell.
2. Large Asiatic Apple Snail’s shell can accumulate Hg and remove almost 90% of Hg in the water.
3. Large Asiatic Apple Snail’s shell can accumulate Pb and remove almost 100% of Pb in the water.
RECOMMENDATIONS

For further research and development, the researchers recommend the following:
1. Determine the Pb and Hg content of the meat of Kambu-ay.
2. Conduct a study about Hg and Pb accumulation in the fishes.
3. Monitor the increase of Hg and Pb in the area using Kambu-ay shells.

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