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Levels of cadmium, copper, lead, nickel and mercury in the muscles of Guama *Johnius borneensis* (Bleeker, 1850) and sediments in lower Agusan river basin, Pagatpatan, Butuan city, Philippines

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Abstract
Muscles of *Johnius (Johnieops) borneensis* and sediments from Pagatpatan, Lower Agusan River Basin, Butuan City were tested for the determination of Cadmium (Cd), Copper (Cu), Lead (Pb), Nickel (Ni) and total mercury (tHg) from April to May 2015. Samples were analyzed, and the results show that the mean concentrations of the different heavy metals across three sampling sites are in the order of Pb> Ni> Cu> Cd> tHg. For the sediment samples, the mean concentrations of the different heavy metals across three sampling sites are in the order of Ni> Cu> Pb> Cd> tHg. Pb, Cd, Ni, and tHg exceeded their standard allowable limits for fish muscles and sediments established by USEPA and FDA. Results of the Pearson-correlation of fish muscles vs. Sediments show a slightly positive (r = 0.5960) value which suggests that heavy metal concentrations in the fish muscles are not directly influenced by the sediments.

Keywords: Guama, bioaccumulation, methylmercury, lower Agusan River basin

1. Introduction

The Agusan River Basin forms three sub-basins in the northeastern portion of Mindanao. It is popularly referred to as the Lower Agusan River Basin (downstream watershed), running along the downstream reach from Talacogon in Agusan del Sur to Butuan Bay; Middle Agusan River Basin (midstream watershed), spanning the area along the reach between Talacogon and Sta. Josefa in Agusan del Sur Province, including the Agusan wetland; and, Upper Agusan River Basin (upstream watershed), which is the area along the upstream reach from Sta. Josefa in Compostela Valley Province [1].

The Agusan River passes through Butuan City in the northeast part of Mindanao where it empties into Butuan Bay [2]. Over these years, the Agusan River has become threatened due to the increased anthropogenic activities. A recent study by MSU Naawan[3] placed the Agusan River under the polluted category due to mercury pollution. It is mainly due to the presence of small- scale mining activities in Diwalwal, Compostela Valley that are located upstream of the Agusan River. The creeks surrounding Diwalwal flow onto the Mamunga and Naboc Rivers, which in turn drains to the Agusan River located 24 kilometers away from the mining area. The impact of mercury pollution due to small scale mining activities extends far beyond the site. Affected areas include the provinces of Agusan del Sur and Agusan del Norte where the river traverses, and all the way down to Butuan Bay where the river drains [3].

The croaker fish *Johnius (Johnieops) borneensis*, locally known as Guama is a brackish water fish abundant in Agusan River. Since there are few studies on the bioaccumulation of heavy metals in this species here in the country, this study is of great significance in knowing the levels of concentration of certain heavy metals in the muscles of this fish and in sediments from selected sampling sites whether they are at safe levels or not. Since Guama is consumed by the residents, it is important to know if it is still safe for human consumption.

The study aimed to assess the present status of heavy metal pollution in the Lower Agusan River Basin. The results of this study will be of great importance to the local community and to the environmental planners in the management and conservation of the Lower Agusan River Basin.

2. Materials and Methods

Study Area

The study was conducted in three selected sampling sites in Pagatpatan, Butuan City. These areas are considered to be part of the Lower Agusan River Basin, in which part of it starts from the Municipality of Talacogon, Agusan del Sur running down to Butuan Bay where the Agusan river empties. Pagatpatan is situated near the river mouth, and part of it runs along the Agusan River (Fig. 1) [1].

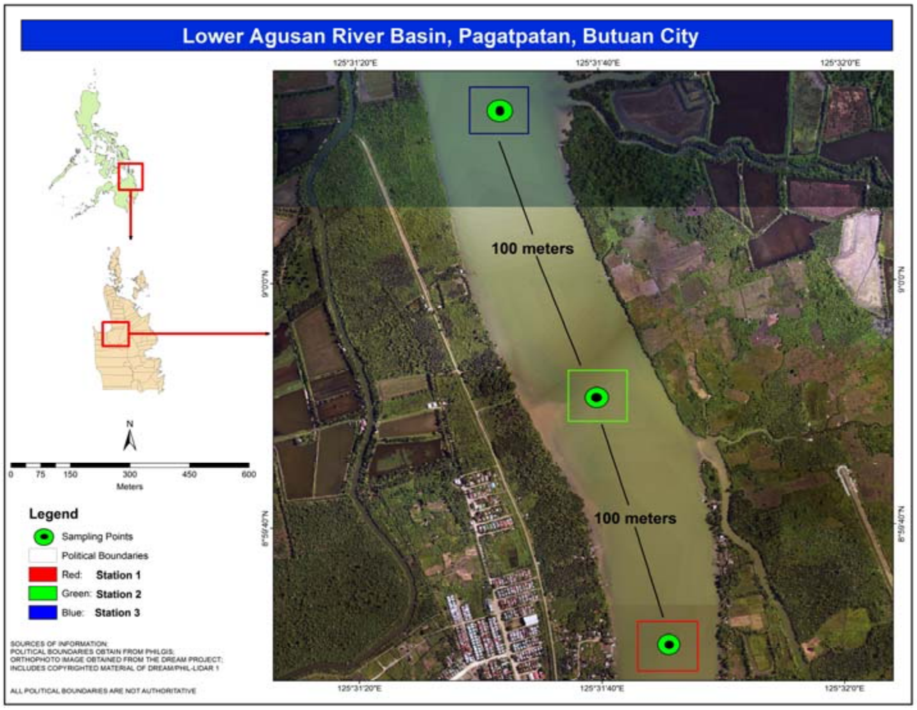

Fig 1: Map of Lower Agusan River Basin, Butuan City, and the sampling sites

Description on Fish Collection

Sampling of fishes was completed in a duration of two months (April to May 2015) along three sampling sites in Pagatpatan with three replicates per site. Fishing nets were used with the help of a hired fisherman. Selection of Guama (Fig. 2) as sample medium was based on their abundance in the area. This species forms part of the daily consumption for the people living near the riverbanks. The sampled fishes were placed in an ice bucket and taken to the laboratory. Samples were weighed on a digital weighing scale, and the total body length (TL) was measured using a ruler. Dissection was done in the laboratory. Samples caught were matured individuals, sizes ranging from 16-19 cm TL (17.9 ± 0.3114) with the body weight ranging from 52-94 grams (76.11 ± 4.524), unsexed. The mean body weight of all samples from the three sampling sites is 76.11 g, while the mean TL is 17.9 cm.

Fig 2: *Johnius (Johnieops) borneensis* (Guama) caught from Pagatpatan.

Sediments Collection

About 500 g of sediments were collected from each of the three replicates at each of the three sampling sites where the fishes were caught in Brgy. Pagatpatan using pre-cleaned 100 ml wide-mouthed disposable plastic containers. The samples were placed in zip lock containers with proper labels indicating where it is collected and the number of replicates per area. Samples were stored in tightly closed containers, coded with necessary information in preparation for the analysis of Ni, Cu, Pb, Cd, and tHg.

Digestion Preparation for Ni, Cu, Pb, Cd and tHg

Fish muscles and sediment samples were first digested before they were sent for analysis. Homogenized (skinned) fish muscles were oven dried for 3 hours at 100 °C. Dried samples were then ground by mortar and pestle and placed in plastic containers labeled with sampling site number and replicate number and sealed to prevent contamination. Sediment samples were sun-dried before grounded by mortar and pestle. Coarse sediments were refined using the standard wire mesh #100. Refined sediment samples were then placed in properly labeled plastic containers and sealed to avoid contamination.

Digestion of Fish Muscles

(*Method adopted from the Analytical Methods for Atomic Absorption Spectroscopy by PerkinElmer Inc*.,).

About 1 g of the powdered fish muscle was weighed using a digital weighing scale and placed in 100 ml beaker with label. The labeled beakers were placed on the hot plate under the fume hood covered with a watch glass. 5 ml of concentrated Nitric acid (HNO_3) and 5 ml Sulfuric acid (H_2SO_4) were added to the samples consecutively. When the reactions slow, the temperature was set to 600 °C for 30 minutes. 10 ml of concentrated Nitric acid (HNO_3) was added and heated to 1200 °C for another 30 minutes. The temperature was then increased to 1500 °C for 45 minutes. After the samples become brown, the temperature was then reduced to 300 °C. 5 ml concentrated

Hydrogen Peroxide (H_2O_2) was added continuously until the samples became clear or pale yellow. It was allowed to cool for about 30 minutes. The resulting fish digested samples were then filtered using Whatman No. 80 filter paper and diluted to 50 ml volumetric flask added with distilled-deionized water. All glasswares were pre-treated in 10% Nitric Acid solution.

Digestion of Sediment Samples

About 1 g of dried sediments was weighed using a digital weighing scale and placed in a 100ml beaker with label. The labeled beakers were placed on the hot plate under the fume hood covered with a watch glass. 20 ml Aqua Regia was added to the samples. The temperature was set to 2400 °C for 3 hours. A pinch of calcium perchlorate ($KClO_3$) and 20 ml (0.08M) Nitric acid (HNO_3) were added to the samples respectively. The temperature was set to 2000 °C and heated to near dryness for about 20 ml. The resulting digested sediment samples were then filtered using a Whatman No. 80 filter paper and diluted to 50 ml in a volumetric flask added with distilled-deionized water. All digested samples were placed on plastic capped containers with parafilm placed before capping to prevent contamination.

Ni, Cu, Pb, Cd and total Hg Analysis

Analysis of Ni, Pb, Cd and tHg for both fish and sediment samples was done by the DA Regional Soils Laboratory at Taguibo, Butuan City using Agilent Technologies 14 MY14300001 Atomic Emission Spectrophotometer with a detection limit of 0 ppm for Cd, Pb, Ni and tHg. Sodium borohydride ($NaBH^4$) was used as reducing agent for samples.

Analysis of Cu for both fish and sediment samples was done at CSU-Chemistry Laboratory using Perkin Elmer AAnalyst 200 Atomic Absorption Spectrophotometer with a detection limit of 0.012 ppm. Procedures were based on the official methods set by AOAC International. The preparation of solutions of copper were as follows: About 200–250 mL of 1% v/v HNO_3 to use as blank solutions and about 200 – 250 mL each of the three standards in 1% v/v HNO_3. The suggested standards of copper are 0.5 ppm (Standard 1), 1 ppm (Standard 2), 1.5 ppm (Standard 3) and 1 ppm (Reslope).

Data Analysis

One-way analysis of variance (ANOVA) was used for comparing the different means of concentration of tHg, Ni, Cu, Pb and Cd in the fish muscle and sediments. Pearson-correlation was used to compare the heavy metal concentrations between fish muscles and sediments. Data was presented as mean ± standard error mean (SEM) and was calculated using Paleontological Statistics and Software (PAST®). All data was analyzed using Graph Pad Prism 6®.

3. Results and Discussion

Total Mercury, Nickel, Copper, Lead and Cadmium in Fish

The results heavy metal concentrations of the digested fish muscle samples were analyzed through PAST® and GraphPad Prism 6®. The recommended safe limits set by international agencies for fish muscles and sediments heavy metal concentrations are presented in Table 1 for comparison.

Table 1. Recommended safe limits of heavy metals in fish muscles and sediments established by international agencies.

Heavy Metal	Recommended Safe Limits (ppm)			
	Fish Muscles	Agency	Sediments	Agency
tHg	0.5	USEPA [4] and FDA [5]	0.1	FAO [6]
Ni	70-80	USFDA [5]	75	FAO [6]
Cu	30	FAO [6]	4300	FAO [6]
Pb	0.5	USEPA [4] and FDA [5]	420	FAO [6]
Cd	0.05	USEPA [4] and FDA [5]	85	FAO [6]

One way ANOVA shows that the means of the different heavy metal concentrations across three sampling sites were of no significant difference ($p<0.05$). Ni also had high concentrations but did not exceed the recommended safe limit by USFDA [5]. Mercury, Lead, and Cadmium have concentrations that exceeded the recommended safe limits for fish muscle. These heavy metals are very hazardous in high amounts and are further hastened by bioaccumulation.

Table 2: Data of tHg, Ni, Cu, Pb and Cd in *J. borneensis* muscles caught from Pagatpatan, Lower Agusan River Basin, Butuan City.

Heavy Metal	Concentration (ppm)			
	Site 1	Site 2	Site 3	Mean
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
tHg	3.245 ± 0.251	2.596 ± 0.429	2.596 ± 0.601	2.812 ± 0.036
Ni	30.867 ± 0.817	28.467 ± 0.372	29.533 ± 1.854	29.622 ± 0.247
Cu	9.533 ± 1.711	7.467 ± 0.235	6.617 ± 1.392	7.872 ± 0.861
Pb	30.617 ± 1.719	31.5 ± 1.176	28.767 ± 2.640	30.294 ± 0.985
Cd	8.05 ± 0.35	7.733 ± 0.136	7.783 ± 0.159	7.856 ± 0.181

Note: USEPA and FDA tolerance limit for tHg in fish muscles: 0.5 ppm, Pb: 0.5 ppm, Cd: 0.05 ppm; FAO tolerance limit for Cu: 30 ppm; USFDA maximum limit for Ni: 70-80 ppm. BDL-Below Detection Limit.

The pattern of the concentration of the heavy metals in the fish muscles is in the order of Pb> Ni> Cu> Cd> tHg (Table 2A).

The Pb, tHg and Cd concentrations have exceeded the USEPA [4] and USFDA [5] recommended safe limit of ≤0.5 ppm and ≤0.05 ppm respectively. Cu did not exceed the FAO [6] recommended safe limit of 30 ppm.

Mercury has no known biological importance and is very toxic. Both of its inorganic and organic forms can cause neurological disorders and total damage to the Central Nervous System [7].

Cadmium is tolerable at extremely low levels, but it can be exceptionally toxic to humans. It accumulates in the human body and may induce damage to the skeleton, renal dysfunction, and deficiencies to the reproductive organs [8]. It can also act as a human carcinogen [9].

The common source of Lead is from mining and burning of fossil fuels [10]. It is also present in high amounts in fish organs and muscles. When it accumulates in the human body, it replaces calcium in the bones and may lead to effects like neurotoxicity [11, 12]. Together with Cd, Pb can significantly reduce semen quality and damage DNA [13].

Nickel is very abundant on the earth's surface. It is emitted by volcanoes and is usually present in the soil [14]. It is biologically essential, but very toxic in high concentrations [10].

Heavy metals may exert beneficial or harmful effects on plant, animal and human life depending upon their concentration [15]. Some of these heavy metals are lethal to living organisms even at small concentrations. However, others are biologically important and can become deadly at relatively high concentrations. When consumed in excess amounts, heavy metals associate with the body's biomolecules, like proteins and enzymes to form stable biotoxic compounds, thus altering their structures and deterring them from their functions [7].

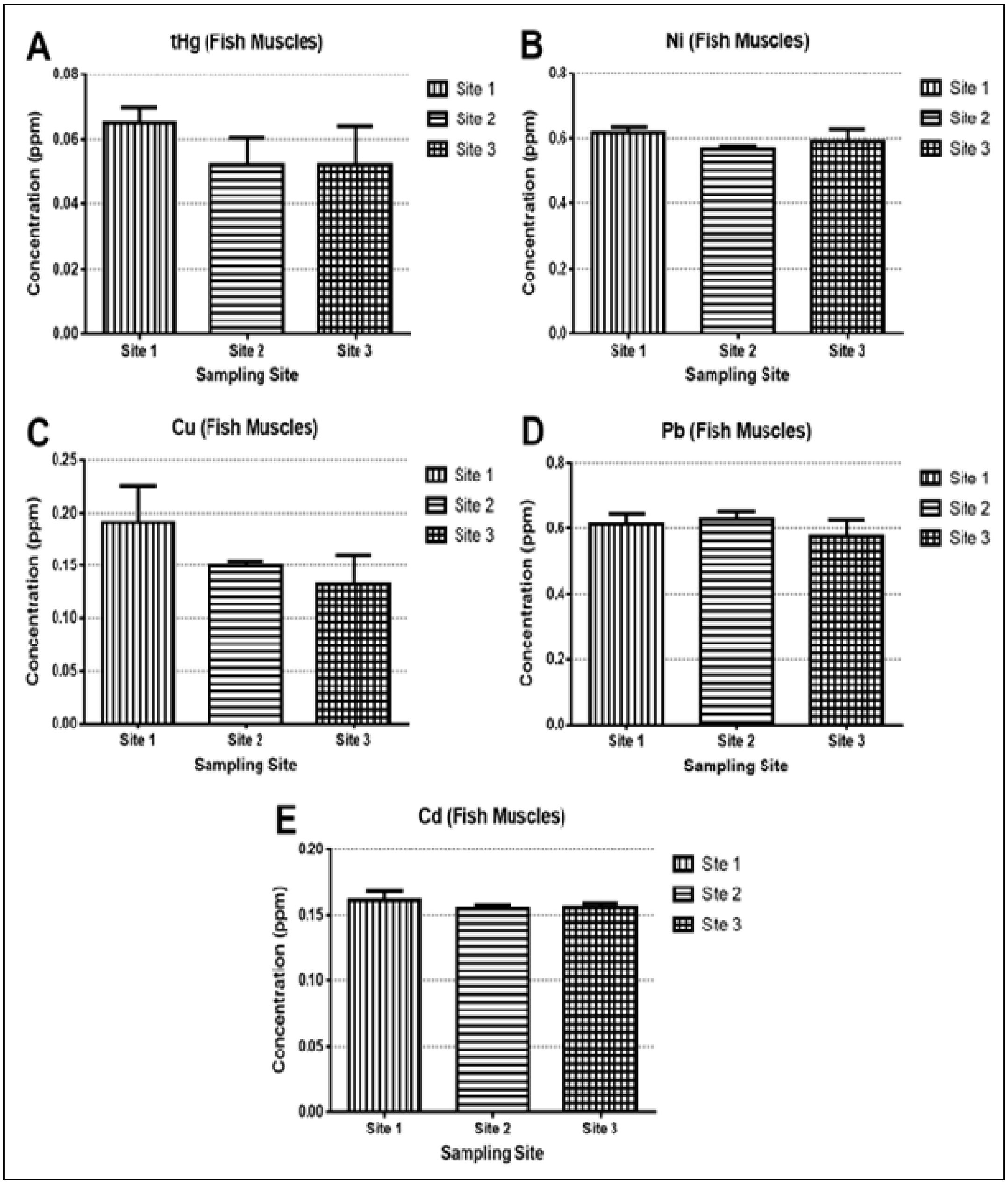

Fig 3: Concentration (ppm) of A. Total Mercury (tHg), B. Nickel (Ni), C. Copper (Cu), D. Lead (Pb), and Cadmium (Cd) in *J. borneensis* across stations in Pagatpatan, Lower Agusan River, Butuan City, Philippines.

Total Mercury, Nickel, Copper, Lead and Cadmium in Sediments

Results from concentrations of tHg, Ni, Cu, Pb and Cd were analyzed (Table 3). The data shows that the order of the concentration of heavy metals in the sediments is Ni> Cu> Pb> Cd> tHg.

The mean value of Ni being the highest, exceeded the USEPA recommended safe limit of ≤75 ppm [4]. As for tHg and Cd, they have the lowest concentrations across the three sampling sites, but tHg concentrations in Site 1 (0.122 ± 0.081) and Site 2 (0.109 ± 0.021) have exceeded the recommended safe limit. The rest of the heavy metal concentrations did not exceed their respective recommended safe limits as issued by FAO [6] and USEPA [4].

One-way ANOVA shows that the mean concentrations of the heavy metals in sediments across three sampling sites were of no significant differences except for Cd, having a *p*-value of 0.0002 (p<0.05). Based on the results, heavy metal concentrations in sediments were higher than those of the fish muscles (Figure 4) but none exceeded the permissible limits established by FAO [6] and USEPA [4] except for Ni and tHg. In the aquatic environment, sediments act as reservoirs or important sinks for metals and other aquatic pollutants [16]. Sediment contamination by heavy metals affect water quality and could lead to bioaccumulation of heavy metals to aquatic organisms, which results to potential long-term implication on human health and ecosystem [17].

Table 3: Data of tHg, Ni, Cu, Pb and Cd in sediments from Pagatpatan, Lower Agusan River Basin, Butuan City, Philippines.

Heavy Metal	Concentration (ppm)			
	Site 1	Site 2	Site 3	Mean
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
tHg	0.122 ± 0.081	0.109 ± 0.021	0 ± 0	0.077 ± 0.031
Ni	121.167 ± 3.180	123.167 ± 7.797	119.833 ± 1.878	121.389 ± 2.845
Cu	67.65 ± 19.970	77.667 ± 4.740	58.733 ± 1.751	68.017 ± 7.079
Pb	33.833 ± 0.726	31.167 ± 1.691	31.667 ± 0.667	32.222 ± 0.294
Cd	24 ± 3.055	1.5 ± 1.5	0 ± 0	8.5 ± 1.398

Note: FAO tolerance limit for tHg in sediments: 0.1 ppm; USEPA tolerance for Ni: 75ppm; Cu: 4300 ppm; Pb: 420 ppm; Cd: 85 ppm. BDL-Below Detection Limit

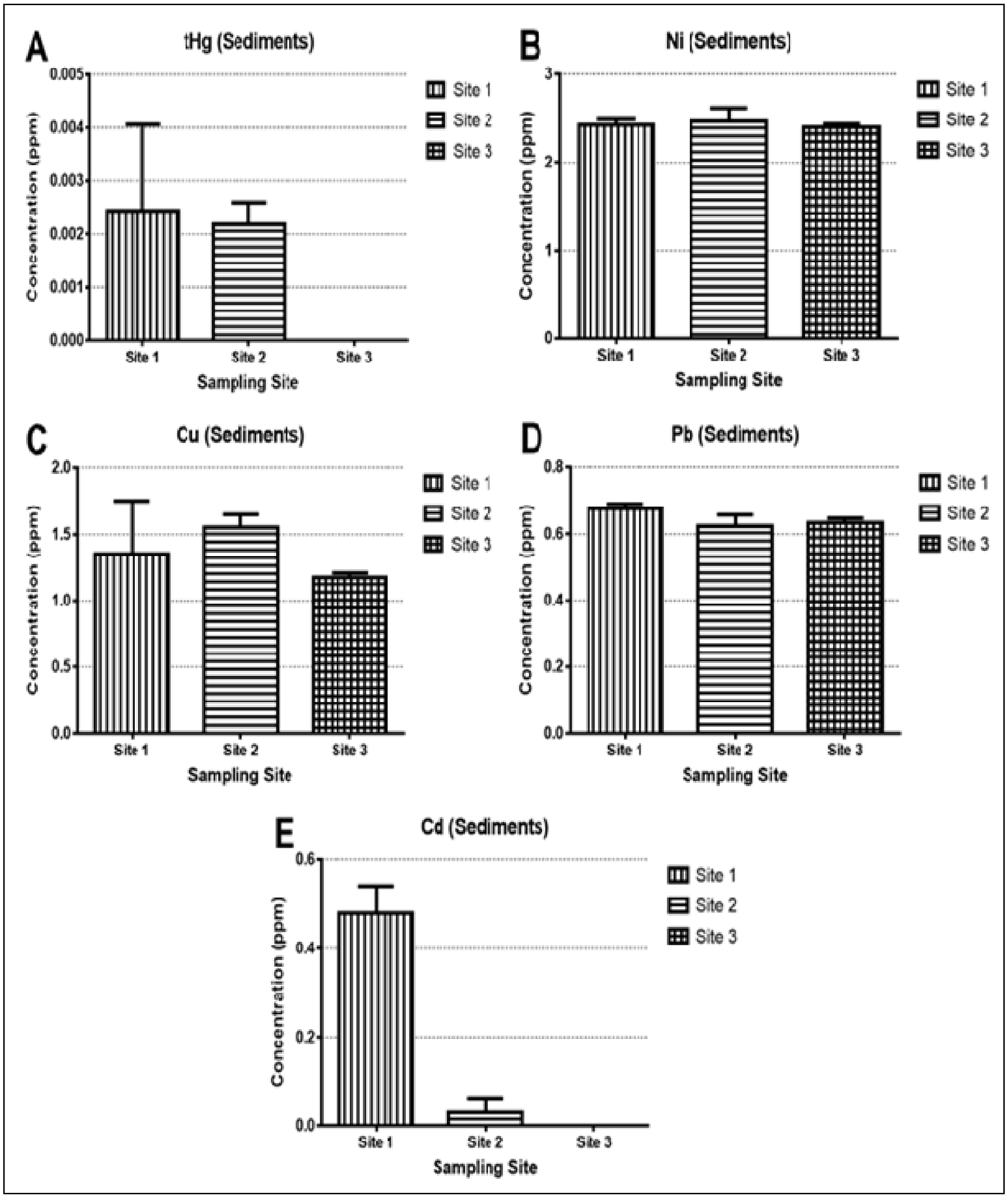

Fig 4: Concentration (ppm) of tHg, Ni, Cu, Pb and Cd in sediments across stations in Pagatpatan, Butuan City, Philippines. Error bars represent SEM.

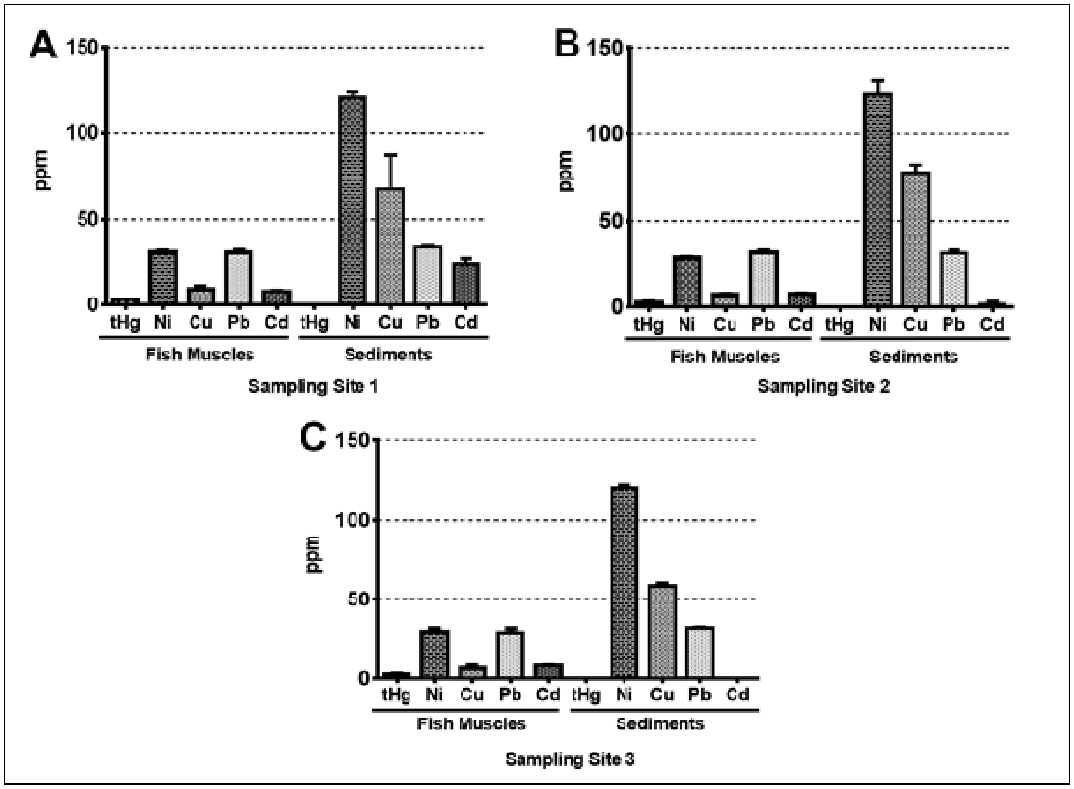

Fig 4: Comparison of heavy metal concentrations across stations and comparisons between fish muscle and sediments. Error bars represent SEM.

Correlation between Fish and Sediment Samples

The Pearson-correlation between the fish muscles and sediments shows a slightly positive (r= 0.5960) (Figure 5) and very low significant correlation. This suggests that the heavy metal concentrations on the fish muscles is not mainly influenced by the sediments and could be attributed to other factors not determined in the study.

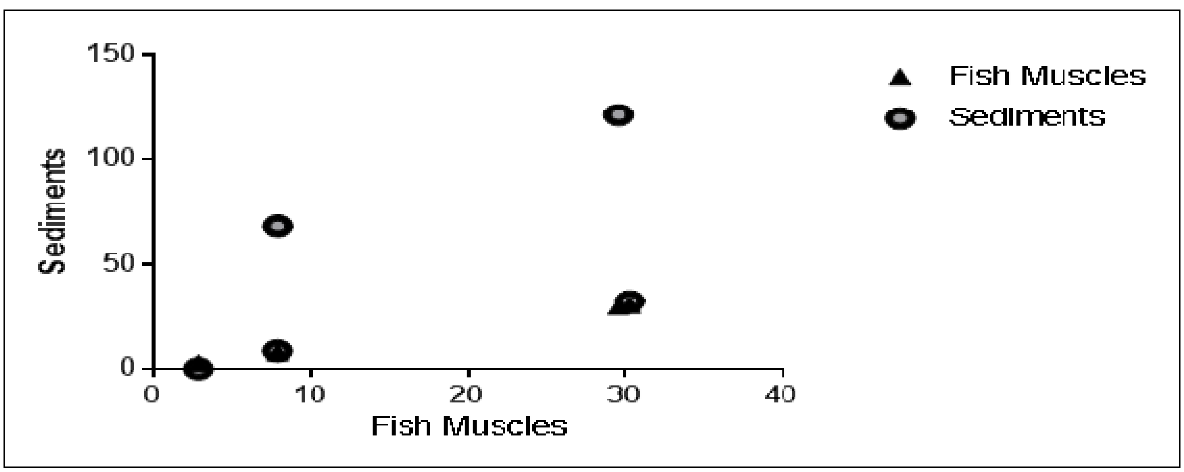

Fig 5: Scatter plot showing correlation of *J. borneensis* and sediments in Pagatpatan, Lower ARB, Butuan City.

The Pearson-correlation between the fish muscles and sediments shows a slightly positive (r= 0.5960) (Figure 5) and very low significant correlation. This suggests that the heavy metal concentrations on the fish muscles is not mainly influenced by the sediments and could be attributed to other factors not determined in the study.

Though the heavy metal concentrations in sediments were higher than that of the fish muscles, it doesn't have an impact on the latter. Therefore, the heavy metal concentrations in the fish muscles are not directly influenced by the heavy metal concentrations in the sediments.

Factors such as feeding habits, age, size, community structure and the length of the food-chain of the fish should also be considered in assessing possible bioaccumulation in a sample medium [18]. *J. borneensis* do not mainly feed on detritus found in bottom sediments, but are rather piscivorous [19].

Heavy metal pollution in the environment is prominent in mining and/or old mining areas, and pollution could increase depending on the rate and time of disposition in a water body. The mining areas in Mt. Diwalwal, where Hg concentrations are in great amounts, is located in the Upper Agusan River Basin, where the river originates, in Compostela Valley, which is very far from the sampling site in Pagatpatan, Lower Agusan River Basin in Butuan [20].

The present study is the most current follow-up of the results of Roa [3] confirming that levels of mercury in Agusan River have increased since its first report in 2001. The results have shown that even in Lower Agusan River Basin, concentrations of tHg in the fish muscles and sediments exceeded the tolerable limit which may imply that the Agusan River is already polluted with mercury.

Another contributing factor to the levels of mercury in fish is that bigger and older fishes can bioaccumulate heavy metals in their bodies from the smaller fishes they eat. Either metals are Transported as dissolved species in water (have the greatest potential of causing lethal effects) or as an integral part of suspended sediments [20, 21]. The concentrations of tHg, Pb and Cd in this study are relatively higher than permissible limits for fish muscles while in sediments Ni and tHg concentrations also exceeded tolerable limits. The rest were high but within standards. These heavy metals are toxic even in minute amounts [22].

The location of the sampling sites can also be attributed to the high levels of tHg, Ni, Pb and Cd concentrations in the fish muscles and sediments. Butuan City is located in Lower Agusan River Basin, where anthropogenic activities due to industrialization are very prominent. Geologic and Anthropogenic activities are the sources of heavy metal pollution [23]. The effects of these heavy metals could be toxic in nature (acute, chronic or sub-chronic) carcinogenic, neurotoxic and teratogenic [24].

4. Conclusion

Therefore, Pb has the highest mean concentration in fish muscles across three sampling sites (30.294 ± 0.985 ppm) which exceeded the ≤0.5 ppm USEPA and FAO recommended safe limit. Cd, being the fourth highest in concentration (7.856 ± 0.181 ppm), also exceeded the recommended safe limit of 0.05 ppm. One-way ANOVA indicate no significant difference in the concentrations across three sampling sites.

For sediment samples, the order of toxicity is Ni> Cu> Pb> Cd> tHg, where all values are within standards except for Ni (121.389 ± 2.845 ppm) and tHg in Site 1 (0.122 ± 0.081 ppm) and Site 2 (0.109 ± 0.021 ppm) that exceeded their recommended safe limits. One-way ANOVA results show that the mean concentrations of the five heavy metals in the sediment samples across three sampling sites have no significant difference except for Cd (p=0.0002) in the sediment samples. Results for the Pearson-correlation of fish muscles vs. sediments show a slightly positive (r= 0.5960) and the low significant correlation could suggest that heavy metal concentrations in the fish muscles may not be directly influenced by metals in the sediments. However, the tHg, Ni, Pb and Cd concentrations in the results of this study should raise awareness and concern. As for the other heavy metals within their acceptable limits, we should still consider the possibility of bioaccumulation depending on the frequency and quantity of consumption of contaminated fish.

5. Acknowledgement

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