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# Levels of Heavy Metals in Fish and Sediments from Different Salinity Gradients of Lower Agusan River to Butuan Bay, Caraga, Philippines

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## Abstract

While numerous studies have evaluated levels of metals across different fish species, very few were actually done based on salinity gradients and trophic levels. Levels of Cd, Cr, Cu, Pb, Ni and Hg in muscles of fishes and sediments from different salinity gradients of Lower Agusan River to Butuan Bay, Philippines were determined. Freshwater fishes (*Oreochromis niloticus, Cyprinus carpio, Channa striata*), brackish (*Johnius borneensis, Pseudocoranx dentex, Scatophagus argus*) and marine fishes (*Lutjanus malabaricus, Nemipterus japonicus, Selar crumenopthalmus*) were collected from Brgy Buhangin, Butuan City, Brgy Pagatpatan and Brgy Dahekan, Magallanes, Agusan del Norte respectively. Highest Cd concentrations (17.5 ± 4.25 ppm) were detected from *L. malabaricus*, Cr (26.5 ± 12.87 ppm) from *J. borneensis,* and Cu (414.17 ± 255.89 ppm) from *N. japonicus*. Highest Pb (18.67 ± 3.77 ppm) was detected from *S. argus* and Ni (200.17 ± 131.98 ppm) from *L. malabaricus*. These concentrations were found to be above the standard values set by FAO, FEPA and WHO (Cd = ≤ 0.05 ppm, Cr = ≤ 0.01 ppm, Cu = ≤ 0.15-1.0 ppm, Pb = ≤ 0.5 ppm, Ni = ≤ 2.0 ppm). The total mercury (tHg) concentrations were below detection in all tested fishes. In sediments, highest Cr concentrations were found in the order: marine (98.33 ± 1.16ppm)> freshwater (77.56 ± 0.40 ppm) > brackish (76.67 ± 0.69 ppm) respectively. These concentrations were found to be above the standard values recommended by US-EPA and WHO (≤ 25 ppm) for soils. The results show no direct relationship regarding the accumulation of heavy metals in fish muscles and the three salinity gradients except for the higher Ni and Cu concentrations in fish from the brackish and marine stations. Comparison of heavy metal concentrations between trophic status show overall higher metal concentrations (except for Pb) in piscivores than non-piscivores and may pose health problems with frequent consumption of contaminated fish from the areas studied.

***Keywords:*** Butuan Bay; Heavy metals; Salinity gradients; Bioaccumulation

## 1. Introduction

Salinity limits the tolerance of certain fish species due to osmoregulation, hence, limiting their territory and possible exposure to certain pollutants. In the Philippines, studies that could significantly impact avoiding potential heavy metal contamination based on nature of diet and salinity differences among freshwater, brackish and marine environments are wanting.

One of the noted sources of mercury contamination in Agusan River Basin was attributed to artisanal small-scale gold-mining activities in Mt. Diwalwal (Appleton *et al.*, 1999). Minor tributaries of the Mamunga River from Diwalwal subsequently joins the north to Agusan River. In the past twenty years, several artisanal small scale gold mines have been sporadically observed upstream of Agusan Marsh and Agusan River further contributing to the load of heavy metals, especially mercury, often used in amalgamation, into the river (Breward, 1996; Appleton *et al*., 1999; Israel and Asirot, 2000).

Heavy metal contamination in Agusan River, Philippines has been previously reported through studies from the sediments and fish muscles of *Mesopristes cancellatus* (Cabuga *et al*., 2016), *Johnius borneensis* (Velasco *et al*., 2016), and *Mugil cephalus* (Cabuga *et al*., 2017) where cadmium, copper, lead, nickel, mercury and chromium were found beyond safe limits set by the international standards for fish muscles. Lead was also beyond safe limits in the muscles of the mud clam *Polymesoda erosa* collected from Butuan Bay (Elvira *et al*., 2016).Roa *et al.* (2010) reported 2.20 to 1256.16 mg/kg for Pb, 0.05 to 44.46 mg/kg for Cd and 2.85 to 341.06 mg/kg for Hg in some stations of Agusan River. Similarly, the study of Demetillo and Goloran (2017) also confirmed that Hg concentrations varied from 0.17-0.26 mg/kg from the sediments in Agusan River. Capangpangan *et al.* (2016) reported that cadmium and manganese were both in high levels among of sampling stations in Agusan River.

Fish muscle is an important tissue for routine monitoring of environmental heavy metal contamination (Jezierska and Witeska, 2006; Sow *et al*., 2019; Havelkova *et al*., 2008; Mole *et al*., 2001; Silva and Shimizu, 2004). Fish is a major part of the diet in many communities near Agusan River, however, no data exist in comparing levels of accumulated metals in fish muscles based on salinity gradients and the trophic nature of fishes assessed. This study aims to determine the levels of cadmium (Cd), chromium (Cr) copper (Cu), lead (Pb), nickel (Ni) and mercury (Hg) in selected fish species and sediments collected from different salinity gradients of lower Agusan River to Butuan Bay, Philippines. The outcome of the study will be an important source of information for the management of heavy metal contamination in the Agusan River Basin and Butuan Bay and its implication towards the fish-consuming community in the area.

## 2. Materials and Methods

### *2.1 Study Area*

The study stations were established along Lower Agusan River to Butuan Bay, Caraga, Philippines based on salinity gradients as determined by a salinometer. The freshwater station was located downstream of Brgy. Buhangin, Butuan City (N08$^{0}$56.272’ E125$^{0}$32.782’) (mean salinity 0.27 ± 0.04 ppt). The water column is murky and soil is mostly clay type. The riparian vegetation is composed of fully-grown deciduous and perennial trees. A plywood factory is situated in the area. The brackish station was located in Brgy. Pagatpatan, Butuan City (N09$^{0}$ 00.569’ E125$^{0}$31.208’) (mean salinity 5.7 ± 2.12 ppt). The water is mostly muddy. The riparian flora is composed of various mangrove and shrub species. Numerous households can be seen near the banks, with a local piggery situated near the area. The marine station was located in Brgy. Dahekan, Magallanes, Agusan del Norte (N09$^{0}$02.075’ E125$^{0}$30.832’) (mean salinity 20.23 ± 2.78 ppt).The water appears to be clear while the soil is of sandy type. A match factory along with a cluster of households can be seen near the area while a local wet market is located near the coast of the municipality. Scarce vegetations, mostly of mangrove species, characterize the banks of this station (Figure 1).

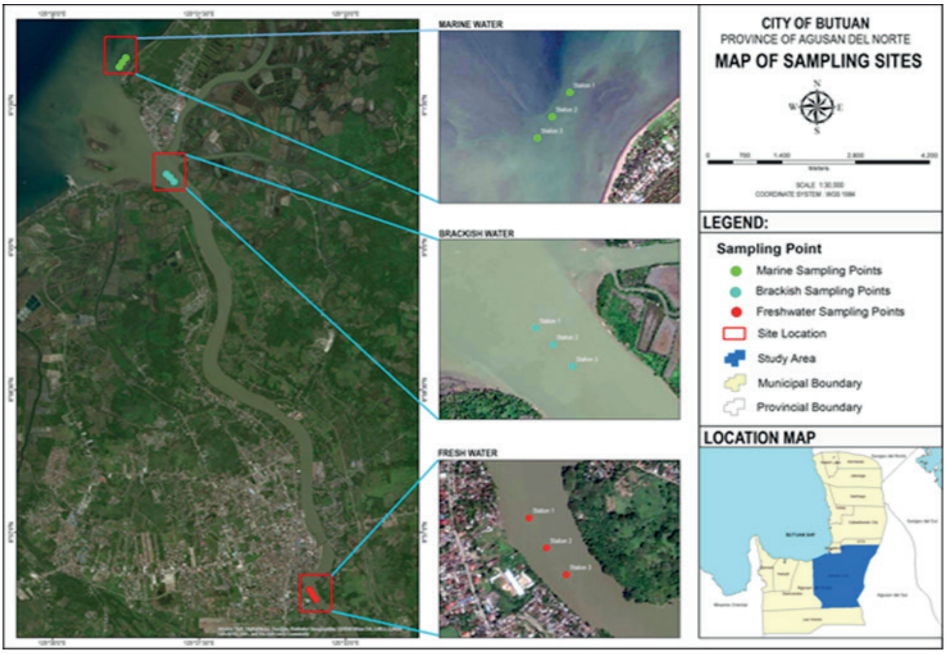


**Figure 1.** Map of the study areas in three different salinity gradients of Lower Agusan River to Butuan Bay, Caraga, Philippines

**Table 1.** Fish species collected from Agusan River to Agusan Bay, Philippines

| Species | Family | Trophic level |
|---|---|---|
| **Freshwater** | | |
| *Oreochromis niloticus* | Cichlidae | Herbivore |
| *Cyprinus carpio* | Cyprinidae | Benthic Invertivore |
| *Channas triata* | Channidae | Piscivore |
| **Brackish** | | |
| *Johnius borneensis* | Sciaenidae | Piscivore |
| *Pseudocaranx dentex* | Carangidae | Piscivore |
| *Scatophagus argus* | Scatophagidae | Detritivore |
| **Marine** | | |
| *Lutjanus malabaricus* | Lutjanidae | Piscivore |
| *Nemepterus japonicus* | Nemipteridae | Piscivore |
| *Selarcrumenoph thalmus* | Carangidae | Piscivore |

### *2.2 Fish Collection*

The collected fishes varied from piscivorous (fish-eating fish) and non-piscivorous trophic levels (Table 1) based on their abundance in the study areas during the sampling period from April-May, 2018. All fishes are known resident species in the area. Three species in triplicates of similar (adult) size ranges were collected per study station/ salinity gradients using gill nets, hook and line, seines and fish traps.

### *2.3 Collection and Processing of Sediment Samples*

Bottom sediment samples were collected from the same point where the fish samples were taken. Sediment samples were taken using an improvised PVC scoop sampler. The samples were then kept in ziplock plastic containers and placed in a chilled container during transport. Sediment samples were air-dried, ground into fine powder and sieved through a no.100 mesh sized metal sieve.

*2.4 Digestion of Fish Muscles and Sediments for Cd, Cr, Cu, Pb and Ni*

Fish samples were processed and the muscle tissues were ash-dried and digested via acid digestion process following the Official Method for Analysis of AOAC (2002). Briefly, about 10 mL of $HNO_3/H_2SO_4$ (1:1 v/v) was added to the samples (dried, 1 g) and the mixture was heated initially to 60 °C for 30 minutes to prevent vigorous reactions. Then, 10 mL of concentrated $HNO_3$ was added to the mixture and the temperature was increased to 120 °C for another 30 minutes and later set to 150 °C for another 45 minutes. Lowering the temperature to 30 °C was carried after the color of the mixture turned brown. A 5 mL 30% $H_2O_2$ was then added until the sample becomes clear or pale yellow. The mixture was then cooled to room temperature and then filtered by filter paper (Whatman No. 80) and diluted to 50 mL volumetric flask. The filtrates were then stored at 4 °C until metal determination by AAS.

*2.5 Digestion of Fish Muscle and Sediments for total Hg*

The digestion process was adopted from the Official Method of Analysis of AOAC (2002). Briefly, dried fish samples (2.5 g) were placed in a digestion flask and 25 mL 9M $H_2SO_4$ , 20 mL 7M $HNO_3$, 1 mL 2% sodium molybdate solution were added and the resulting mixture was heated gently under reflux (with continuous flow of water). The heat was then removed and allowed to stand for about 15 minutes. The 20 mL $HNO_3$-$HClO_4$ (1+1) was added into the condenser. Boiling the flask was carried out with continuous flow of water until white fumes appear and continuously heated to about 10 minutes. Then, the mixture as allowed to cool and about 10 mL of water was added through the condenser while swirling the flask. The mixture was heated again for 10 minutes. The solution was then allowed to cool to room temperature, filtered and transferred to the volumetric flask and diluted to 100 mL. The solution was then stored to 4 °C until metal determination by Cold Vapor AAS.

*2.6 Heavy Metal Analysis (Cd, Cr, Cu, Pb, Ni, and tHg) in fish muscles and Sediment sampl.*

The concentrations of Cd, Cr, Cu, Pb, and Ni were determined by Flame Atomic Absorption Spectrometry (Spectra AA 700) by using an air acetylene flame. Similarly, tHg concentration was determined using Cold Vapor -AAS technique (Shimadzu AAS 7000). For tHg analysis, $SnCl_2$ was used as a reducing agent. Calibration curve and standardization was carried out for every specific metal of interest using the corresponding standard solutions (with different concentrations).

*2.7 Quality Control*

The determination of the analytical concentration of the different heavy metals were all blank-corrected. Laboratory quality assurance process was ensured to guarantee the quality of the analytical data; following the standard operating procedures, analysis of reagent and sample blanks, the use of calibration standards, recovery of the spiked samples and analysis of replicates (at least 3 replicates). The accuracy and precision of the analytical procedures were evaluated by the recovery measurements on the spiked sample. Statistical analyses such as the evaluation of precision and the relative standard deviation were also evaluated and the final results were expressed as the mean.

*2.8 Statistical Analysis*

PAST (Paleontological Statistics Software) was used in testing the difference of means of the concentration and levels of Cd, Cr, Cu, Pb, Ni and Hg in the muscles and sediments. One-Way Analysis of variance (ANOVA) was used for testing the significant difference of the muscles and sediments. T-test was used to compare metals between piscivorous and non-piscivorous fishes. Data were presented as mean ± standard error mean (SEM).

## 3. Results and Discussion

The total length (cm) and body weight (g) of the collected fish species indicate that samples were within of adult size ranges (Table 2). Essentially, age-size ratio plays a key role for bioaccumulation of pollutants as length-weight relationship influences the build-up of toxic metal in the tissues (Canli and Atli, 2003). Fish sizes and age may also enhance contaminant levels in different tissues and may not be dependent to the exposure (Henry *et al*., 2004).

### *3.1 Levels of Cd, Cr, Cu,Pb, Ni and Hg in Fish Muscles*

*Freshwater Fish Species*

Among freshwater fish species, *C. carpio* has accumulated heavy metals in the following order: Cr > Ni > Cd > Cu > Pb > Hg; *O. niloticus* with Cd > Cr > Pb > Cu > Ni > Hg and *C. striata* with Cd > Cr > Pb > Ni > Cu > Hg. Among the six (6) heavy metals analyzed in the muscles of three freshwater species, Cd has the highest concentration in *O. niloticus*, followed by Cr in *C. carpio* and *C. striata* (Table 3). Highest Ni mean was observed in *C. carpio*. Highest Pb and Cu concentrations were both observed in *O. niloticus*. The tHg concentrations were below detection limit (BDL) among freshwater species analysed. No significant difference ($p > 0.05$) between the levels of Cd, Cr, Cu, Pb, Ni and Hg among the freshwater fish species.

*Brackish Fish Species*

Among the brackish fish species, *J. borneensis* has accumulated heavy metals in the following order: Cu > Cr > Ni > Pb > Cd > Hg; *P. dentex* Cu > Ni > Pb > Cr > Cd > Hg and *S. argus* Cu > Ni > Pb > Cr > Cd > Hg. Among the six heavy metals determined in the three marine species, Cu has the highest mean concentration followed by Ni and Pb all, from *S. argus* (Table 3). High concentrations of Cr and Cd were both observed in *J. borneensis*. All brackish species assessed registered BDL for tHg. The Cd, Cr, Cu, Pb, Ni and Hg levels were significantly higher in the muscle samples of *P. dentex* ($p = 0.0389$) and *S. argus* ($p = 0.0082$). However, there were no significant differences between levels of Cd, Cr, Cu, Pb, Ni and Hg in *J. borneensis*.

*Marine Fish Species*

Accumulation of metals for *L. malabaricus* was in the order: Ni > Cu > Cd > Cr > Pb > Hg; In *N. japonicus:* Cu > Ni > Cd > Cr > Pb > Hg and in *S. crumenophthalmus*: Cu > Ni > Cd > Cr > Pb > Hg. Cu has the highest mean concentration from *N. japonicus* followed by Ni from *L. malabaricus*. High Cd, Cr and Pb were all observed from *L. malabaricus* (Table 3). Similar with the observations from freshwater fishes, Hg concentrations were below detection limit (BDL). Comparison of Cd, Cr, Cu, Pb, Ni and Hg levels were significantly higher ($p < 0.05$) between the marine species assessed.

**Table 2.** Total length and body weight of fish species utilized for heavy metal analysis from Lower Agusan River to Butuan Bay, Philippines Data were presented as mean ± standard error mean (SEM)

| Fish | N | Total length (cm) | Body weight (g) |
|---|---|---|---|
| *Oreochromis niloticus* | 3 | 21.5 ± 0 (21.5) | 215 ± 5.77 (205 - 225) |
| *Cyprinus carpio* | 3 | 25.5 ± 0 (25.5) | 226.67 ± 14.47(210 - 236) |
| *Channas triata* | 3 | 26 ± 1.16 (24 - 28) | 171.33 ± 19.64 (210 - 236) |
| *Johnius borneensis* | 3 | 19.33 ± 0.17 (19 - 19.5) | 101.33 ± 2.19 (97 - 104) |
| *Pseudocoranx dentex* | 3 | 15.67 ± 0.667 (15 - 17) | 59 ± 9 (50 - 77) |
| *Scatophagus argus* | 3 | 13.5 ± 1.01 (13.5 - 17) | 107 ± 20.31 (74 - 144) |
| *Lutjanus malabaricus* | 3 | 22 ± 0.58 (21 - 22) | 159.67 ± 7.06 (149 - 173) |
| *Nemepterus japonicus* | 3 | 20.33 ± 0.67 (19 - 21) | 114.67 ± 8.95 (99 - 130) |
| *Selarcrumenoph thalmus* | 3 | 20.5 ± 0.29 (20 - 21) | 114.33 ± 4.70 (105 - 120) |

**Table 3.** Mean Concentrations of Heavy Metals (ppm) in the muscles of fish species collected in Agusan River to Butuan Bay, Philippines. Data were presented as mean ± standard error mean (SEM)

| Fish | Cd | Cr | Cu | Pb | Ni | Hg | Studies |
|---|---|---|---|---|---|---|---|
| **Freshwater** | | | | | | | |
| *Cyprinus carpio* | 1.67 ± 0.67 | 4.67 ± 2.42 | 0.33 ± 0.33 | 0.33 ± 0.33 | 2.17 ± 0.33 | BDL | Present study |
| *Oreochromis niloticus* | 12.5 ± 1.76 | 3.5 ± 1.80 | 1.83 ± 0.73 | 2 ± 0.29 | 1 ± 0.29 | BDL | Present study |
| *Channas triata* | 8.5 ± 0.87 | 4.67 ± 0.93 | 0.5 ± 0.5 | 1.5 ± 1.26 | 1 ± 0 | BDL | Present study |
| **Brackish** | | | | | | | |
| *Johnius borneensis* | 3.83 ± 1.01 | 26.5 ± 12.87 | 32.67 ± 9.38 | 15.33 ± 1.30 | 20 ± 1.65 | BDL | Present study |
| *Pseudocoranx dentex* | BDL | 3.5 ± 0.5 | 75.67 ± 33.08 | 13.83 ± 2.33 | 17.67 ± 4.0 | BDL | Present study |
| *Scatophagus argus* | 3.17 ± 1.17 | 8 ± 2.08 | 137.67 ± 75.06 | 18.67 ± 3.77 | 45 ± 33.15 | BDL | Present study |
| **Marine** | | | | | | | |
| *Lutjanus malabaricus* | 17 ± 4.25 | 6.17 ± 2.24 | 124 ± 83.09 | 3.83 ± 0.67 | 200.17 ± 31.98 | BDL | Present study |
| *Nemepterus japonicus* | 9.5 ± 1.04 | 3.83 ± 1.09 | 414.17 ± 25.89 | 3.5 ± 0.76 | 155.17 ± 21.22 | BDL | Present study |
| *Selarcrumenoph thalmus* | 13.17 ± 0.88 | 5.33 ± 1.59 | 78.83 ± 7.97 | 2 ± 1 | 59 ± 9.74 | BDL | Present study |
| ***Other studies*** | | | | | | | |
| *Mesopristes cancellatus* | 0.5 ± 0.35 | | 6.69 ± 0.42 | 30.05 ± 0.47 | 0.5 ± 0.10 | 0.88 ± 0.32 | Cabuga et al., 2016 |
| *Johnius borneensis* | 7.86 ± 0.18 | | 7.87 ± 0.86 | 30.294 ± 0.99 | 29.62 ± 0.25 | 2.81 ± 0.04 | Velasco et al., 2016 |
| *Mugil cephalus* | 0.5 ± 0 | | | 4.11 ± 0.11 | | | Cabuga et al., 2017 |
| *Glossogobius giuris* | | | | | | 0.24 | Roa, 2001 |
| *Johnius vogleri* | | | | | | 0.19 | Roa, 2001 |
| *Ambassis commersonii* | | | | | | 0.78 | Roa, 2001 |
| *Sillago sihama* | | | | | | 0.58 | Roa, 2001 |

Note: The detection limit of the analyzer is 0.1 ppm except for Hg is 0.02 ppm. Recommended safe limits in fish Cd = ≤0.05 ppm (US EPA, 2000; FAO, 1983; FAO, 1999; WHO, 1985) Cr = ≤0.01 ppm (US EPA, 2000) Cu = ≤0.15 - 1.0 ppm (FAO, 1983; FEPA, 2003; WHO, 1985) Pb = ≤0.5 ppm (US EPA, 2000) Ni = ≤2.0 ppm (FAO, 1983& 1999; FEPA, 2000; WHO, 1985) Hg = ≤0.5 ppm (US EPA, 2000)

In general, Cu had the highest mean value exceeding the recommended safe limits of ≤0.15-1.0 ppm for food set by FAO (1983), FEPA (2003) and WHO (1985) indicating that fish, particularly *N. nemepterus* may not be suitable for prolonged, daily consumption as it is toxic at high concentrations (Silva and Shimizu, 2004).The muscles of *Mesopristes cancellatus* locally known as Pigok collected in Agusan River also showed higher Cu concentration (6.694 ± 0.417 ppm) that exceeded the recommended safe limits (Cabuga *et al*., 2016). Cu naturally occurs in the water bodies due to the sources coming from agricultural and domestic sewages (Edward *et al*., 2013).

Higher Ni concentration was observed in the muscles of *L. malabaricus* and was significantly higher ($p$=0.043) when compared among fish species, exceeding the recommended safe limits of ≤ 2.0 ppm in food set by FAO (1983), FEPA (2003) and WHO (1985). High intake of Ni through diet could result in skin defects, i.e., dermatitis, eczema, and erythema (Gawkrodger *et al*., 2000; Cempel and Nikel, 2005). While possible build-up of Ni in fish could also be due to its general occurrence in an aquatic system even low concentrations (Kumar *et al*., 2011), levels are likely to increase when anthropogenic activities such as industrialization, urbanization and agricultural activities are occurring (Giguere *et al.*, 2004; Gupta *et al*., 2009).

Overexposure to chromium would result in acidosis, kidney failure, acute tubular necrosis and even death as it is recognized as a potential carcinogen (USEPA, 2009). Among many possible sources, Pb could be attributed from smelting and mining activities and also from an automobile exhaust by combustion of petroleum fuels (Edward *et al*., 2013).

It has been reported that Cd accumulation in the fish varies from different tissues including muscle however the main site of higher concentration was in kidney, liver, gonads and gills (El-Nemr, 2003;Van Aardt and Erdman, 2004; Kargin, 1996). High Cd content in the human body could cause increased blood pressure, kidney failure, and in men testicular tissue destruction (Gupta *et al*., 2009).

All concentration in the fish muscles of assessed in this study were BDL for tHg. Mercury is known human carcinogen and poisoning affects the central nervous system (CNS) and the areas associated with the sensory, visual, auditory, and coordinating functions. Nonetheless, previous studies indicated detectable levels of tHg in the muscles of *M. cancellatus* and *J. borneensis* (Cabuga *et al*., 2016; Cabuga *et al*., 2017 Velasco *et al.,* 2016) and in Polymesoda erosa in Agusan River (Elvira *et al*., 2016).

The study shows no direct relationship in metal accumulation in fish muscles between salinity gradients however, there is evidence of higher Ni and Cu concentrations in the brackish (Ni : 82.67 ± 27.56; Cu: 82 ± 30.46) and marine (Ni:138.11 ± 41.63; Cu: 205.67 ± 105.06) stations probably also due to elevation and trophic nature of the fishes utilized. While the concentrations of heavy metals in the fish muscles were found higher among piscivorous fishes compared to non-piscivorous (except for Pb), t-test comparisons show these are not significantly different between trophic levels (Table 4). Nonetheless, highest concentrations were found in the marine fishes and followed by brackish fishes (Figure 2). The piscivores in this study mainly feed on small fishes, crustaceans and bottom invertebrates. This may suggest that feeding habits constitute enough for the increase of heavy metals accumulation that could improve through the food chain pathways. In a related study by Mole *et al.,* (2001), the highest mercury concentrations were found among piscivores *Serrasal musrhombeus* (0.35 µg/g1) and *Cichla ocellaris* (0.39 µg/g1) than of non-piscivore species. Fishes mostly acquired highest form of heavy metal concentrations due to feeding habits, age-size ratio and intake of organic matters present in the water (Kamaruzzaman, 2008; Zheng *et al*., 2007). Species, age, size structure and trophic levels highly influence heavy metal accumulations (WHO, 1990).

**Table 4.** Heavy metal concentrations in fish muscles across piscivores and non-piscivores fishes collected from three different salinity gradients of lower Agusan River to Butuan Bay, Philippines Data were presented as mean ± standard error mean (SEM)

| Metals | Piscivore | Non-Piscivore | P value |
|---|---|---|---|
| Cd | 13.17 ± 2.42 | 5.78 ± 2.77 | 0.2678 |
| Cr | 8.33 ± 3.34 | 5.39 ± 1.10 | 0.5426 |
| Cu | 123.75 ± 55.89 | 46.61 ± 7.17 | 0.3668 |
| Pb | 6.67 ± 2.32 | 7 ± 4.78 | 0.9435 |
| Ni | 75.5 ± 30.79 | 16.05 ± 11.82 | 0.2036 |
| Hg | BDL | BDL | ---- |

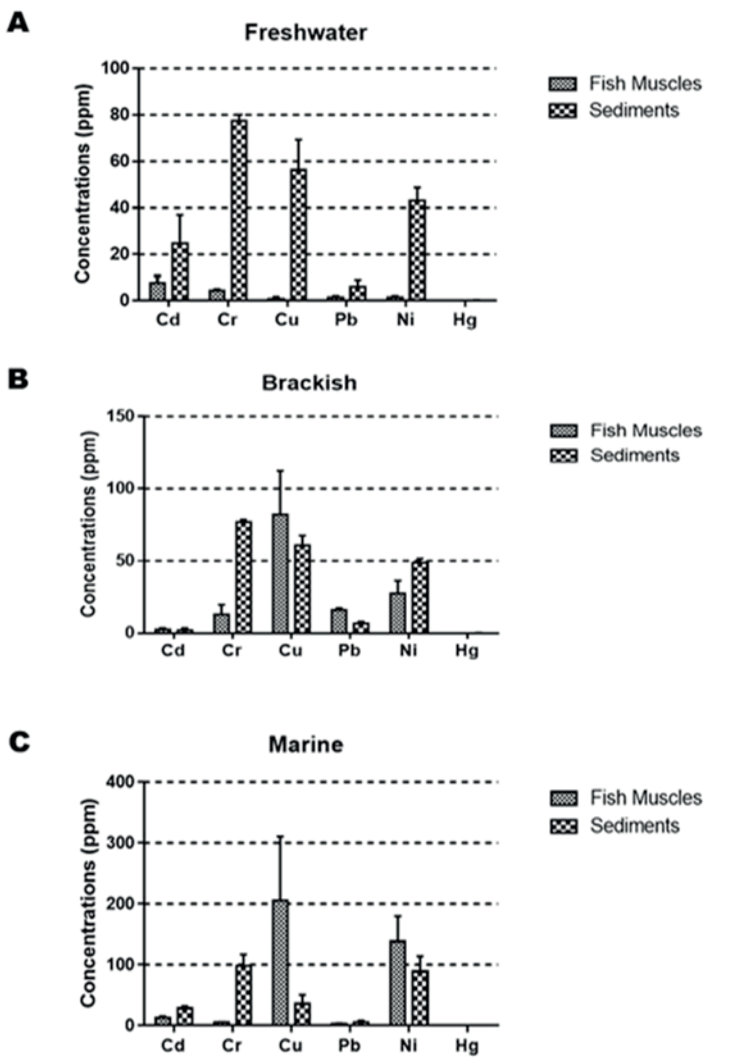


**Figure 2.** Comparison of heavy metal concentrations between fish muscles and sediments in three different salinity gradients of Lower Agusan River to Butuan Bay, Philippines A. Freshwater B. Brackish C. Marine

*3.2 Levels of Cd, Cr, Cu, Pb, Ni and Hg in Sediments*

Heavy metals in freshwater sediments were in the order of Cr>Cu>Ni>Cd>Pb>Hg (Table 5). Sediments from the brackish stations were in the order of Cr>Cu>Ni>Pb>Cd>Hg wherein Cr concentrations exceeded recommended limits for soil (25 ppm)set by USEPA (2000) and WHO (2004). The tHg in the brackish stations exceeded recommended safe limits (<0.1ppm) in soil set by FAO (1999). Marine sediments were in the order: Cr>Ni>Cu>Cd>Pb>Hg. Concentrations of Cr, and Ni exceeded from the recommended safe limits in soil set by USEPA (2000) and WHO (1999). Hg in marine sediments were below detection limit (BDL). No significant differences in the level of concentrations were observed for the all stations. Overall, Cr had the highest concentrations, exceeding recommended safe limits in soil set by FAO (1999) followed by Ni which also exceeded from the recommended safe limits in soil set by FAO (1999). The tHg in freshwater and brackish stations both exceeded recommended safe limits.

Rivers that transcend rural and urban areas carry metals, partially dissolved and adsorbed on suspended materials; and these materials accumulate and settle in the bottom sediments (Pintilie *et al*., 2007) as end result of environmental pollution due to the process of circulation and deposition of these toxic metals (Oyarzun *et al*, 2004).

The presence of Ni in the sediments might attributed from point and non-point sources of pollutants in the river system. Elevated level of nickel was associated to mettalurgical industries, geological weathering and municipal wastewater (Battarbee *et al*, 1988). The increased amount of Ni may also be related to hydrological factors, microbial activity and the mixture of physicochemical state in the water and sediments (Forstner 2004; Calmano *et al*, 1993; Zoumis *et al*, 2011; Munk and Faure, 2004; Chrastny *et al*, 2006).

**Table 5.** Mean concentrations of heavy metals (ppm) in sediments from three salinity gradients of LowerAgusan River to Butuan Bay Data were presented as mean ± standard error mean (SEM)

| Metals | Freshwater | Brackish | Marine | Source |
|---|---|---|---|---|
| Cd | 36.83 ± 1.36 | 5.33 ± 0.88 | 28.44 ± 1.06 | Present study |
| Cr | 77.56 ± 0.40 | 76.67 ± 0.69 | 98.33 ± 1.16 | Present study |
| Cu | 56.22 ± 0.73 | 60.78 ± 3.75 | 35.78 ± 1.25 | Present study |
| Pb | 8.83 ± 2.09 | 6.56 ± 0.11 | 7.67 ± 0.17 | Present study |
| Ni | 43.22 ± 0.22 | 48.67 ± 0.51 | 88.78 ± 0.68 | Present study |
| Hg | 0.25 ± 0.04 | 0.15 ± 0.04 | BDL | Present study |
| tHg | 0.21 | 0.077 ± 0.031 | | Velasco *et al.*, 2016<br>Demetillo and Goloran, 2017 |
| Ni | | 121.38 ± 2.84 | | Velasco *et al.*, 2016 |
| Cu | | 68.02 ± 7.08 | | Velasco *et al.*, 2016 |
| Cu | | 68.01 ± 7.07 | | Cabuga *et al*, 2016 |
| Pb | 2.20 - 1256 | 32.22 ± 0.29 | | Cabuga *et al*, 2016 |
| Cd | 0.05 - 44.46 | 8.5 ± 1.398, | | Cabuga *et al*, 2016,<br>Roa *et al*, 2010 |
| Cr | | 9.50 ± 2.27 | | Elvira *et al.* 2016 |
| Cd | 1.45 | 2.25 | | Capangpangan *et al.* 2016 |
| Pb | 18.5 | 20.0 | | Capangpangan *et al.* 2016 |

Note: The detection limit of the analyzer is 0.1 ppm except for Hg (0.02 ppm). Recommended safe limits in sediments. Cd = ≤ 85 ppm (FAO, 1999) Cr = ≤ 25 ppm (WHO 2004; US EPA, 1999) Cu = ≤ 100 ppm (FAO, 1999) Pb = ≤ 100 ppm (FAO, 1999) Ni = ≤ 50 ppm (FAO, 1999) Hg = ≤ 0.1 ppm (FAO, 1999) BDL = Below Detection Limit.

Concentrations of Cd, Cr, Ni and Cu in sediments were higher compared to fish muscles in freshwater stations while Cu and Pb were higher in the fish muscles for brackish stations. The Cu and Ni were higher in fish muscles in the marine stations (Figure 2). Nonetheless, there were no significant relationship seen between fish and sediments (Freshwater: r = 0.2759); Brackish: r = 0.3724); Marine: r = 0.1806). This would mean that the concentrations of heavy metal in the fish muscles are not directly associated with the concentration of heavy metals in the sediments. Most of the heavy metals being investigated showed elevated concentration in fresh water sediments. The results suggest that heavy metals in fresh water environment tends to be attracted to the organic and carbonate fraction in the sediments samples, thus making them immobile

## 4. Conclusions

This is the first report comparing levels of selected heavy metals in nine fish species and sediments across three salinity gradients in Agusan River Philippines. It was observed that Cd, Cr, Cu, Pb and Ni exceeded the safe limits in fish food across the nine fish species tested. Generally, Cu had the highest accumulations through *N. nemipterus* while Cu concentrations was significantly higher among heavy metals and among fishes exceeding the recommended safe limits indicating that fish from the study area may not be suitable for prolonged, daily consumption. Likewise, Cr, Ni and Hg exceeded the safe limits in sediments. Both freshwater and brackish gradients detected the highest load of metal concentrations in sediments.Heavy metal concentrations were found highest in piscivorous fishes than that of non-piscivorous fishes (except for Pb).

The obtained data show that there should be caution in prolonged, daily consumption of the fishes determined in this study to contain unsafe levels of heavy metals from Lower Agusan River up to Butuan Bay. The study recommends that consumption of non-piscivorous fishes may be the better option from the area as these were found to accumulate less heavy metals compared to piscivorous fishes. Determination of the other toxic heavy metals such as arsenic and cobalt and wider coverage of study stations are recommended in future studies. Finally, it is recommended that the extent other heavy metal concentration, especially MeHg be determined through the hair samples from the fish-eating community.

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