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Heavy metal concentration in sediments and muscles of mud clam *Polymesoda erosa* in Butuan Bay, Philippines

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Abstract

The study assessed the mercury (Hg), lead (Pb), nickel (Ni), cadmium (Cd) and chromium (Cr) levels in sediments and the muscles of mud clam (*Polymesoda erosa*) in selected mangrove wetland stations of Butuan Bay, Philippines. Hg, Pb, Ni, Cd and Cr in sediments and Pb, Ni, Cd and Cr in the muscles of *Polymesoda erosa* were analysed using Atomic Emission Spectrometer Method adopted from Environmental Protection Agency (EPA). Hg in the muscles of *P. erosa* was analysed using Cold Vapor Atomic Spectrometry. Results revealed that the mean concentrations of the different heavy metals from sediments across three sampling stations in Pagatpatan is in the order of Hg > Cr > Ni > Pb > Cd, with Hg, Cr, and Ni exceeding their respective allowable limits. Similarly, heavy metals in Camagong is in the order of Ni > Cr > Pb > Cd > Hg, with Ni and Cr exceeding their respective allowable limits. For the bivalve muscles, the mean concentrations of the different heavy metals across three sampling stations in Pagatpatan is in the order of Cr > Ni > Hg > Pb > Cd, with Pb exceeding the respective allowable limits. Heavy metals in Camagong is in the order of Ni > Cr > Pb > Hg > Cd with Pb exceeding the allowable limit. These baseline findings imply that further study should be conducted to validate the cause of heavy metal accumulation to the physical and biological aspect of the environment.

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Introduction

Heavy metals and toxic pollutants from industrial and mining industries could be the probable source to water pollution in Caraga Region. Significant surface water quality issues in Butuan Bay is brought by mining, palm and coconut oil processing and wood-based industries, domestic wastewater, solid waste, and agro-chemical use (DPWH, 2003).

There are two gold mining industries existing in the Agusan River Basin. One of which is within the upper catchment in the Diwalwal gold rush area in Compostela Valley where small and medium-scale illegal mining operations have been going on unregulated for the last 20 years until State control has been in late 2002.

According to Department of Environment and Natural Resources-National Water Resource Board (2008) monitoring results of DENR-EMB XI until 2002 that in Naboc River, about 5km downstream of the Diwalwal mine site, Hg concentration in the water were often more than ten times the national standard of 0.002 mg/L. In Agusan River, about 30km from the confluence with Naboc River, tHg levels in the water were as high as four times the allowed limit. After the resume of mining in 2003, Hg levels in water once more soared to as high as 750 to 900 times the acceptable level. Likewise, in the Agusan River Basin, extensive palm oil plantations are found in its middle catchment, notably in Rosario, San Francisco, Trento and Veruela in Agusan del Sur. These industries were perceived by communities to have been responsible for the incidence of fish kills in the Basin (DENR-NWRB, 2008).

Match and plywood factories concentrated near the mouth of Agusan River in Magallanes pose potential problems to surface water quality of Agusan River as well as Butuan Bay due to the high level of organic wastes, and chemical effluents disposed into these river systems. Other non-operational saw mills have abandoned waste stockpiles that are washed out during flood events (DENR-NWRB, 2008).

This study determines the levels of Hg, Pb, Ni, Cd and Cr mainly present in the muscles of *P. erosa* and sediments in two mangrove areas in Butuan Bay. The information generated in this study will have an important impact on the possible risk of bioaccumulation of heavy metals by consumption of *P. erosa*. The result could be utilized in policy decisions to strengthen the Butuan Bay integrated coastal resource management in addressing other environmental issues of Agusan River basin considering mud clam an important food resource in the area.

Material and methods

Study site

Along Butuan Bay, two mangrove ecosystems were utilized as study areas namely; Pagatpatan Mangrove Wetland located in Barangay Pagatpatan, Butuan City and Camagong Mangrove Wetland located in Barangay Camagong, Nasipit, Agusan del Norte (Fig. 1).

Each of the sampling sites was further subdivided into three stations (≥ 50 and ≤ 100 meters apart) with three replicates of samples respectively. The sampling was done during the wet season; the weather was rainy due to the prevailing intertropical convergence zone where samples were collected during low tide. Both areas were characterized as a mangroved ecosystem.

Area one is along Mantangue Creek in Barangay Pagatpatan, which is one of the tributary creeks of Agusan River while area two is located adjacent to the river mouth of Agusan River. Mangrove crown cover in Pagatpatan and Camagong area was dominated mostly by Pagatpat (*Sonneratia alba*) and *Avicennia* spp.

Collection procedures of sediments

Sediment collection was done using the Targeted or Judgemental Sampling Designs adopted from US EPA (2002) where the stations were selected based on prior knowledge of the other factors such as contaminant loading.

Three (3) sediment samples replicated three times were collected in every sampling station of the two areas using 2 feet long PVC pipe with a minimum of 1 kg of sediments per replicate. The collected sediment samples were secured in zip lock pouches and labelled with necessary data such as the date, time and place where the sample was collected.

The overlying was removed by gently decanting sediment sample while removing foreign or unrepresentative materials such as roots, shells, and stones or wood chips using a pre-cleaned spoon. Sample homogenization was done right after accomplishing all sediment collection activity.

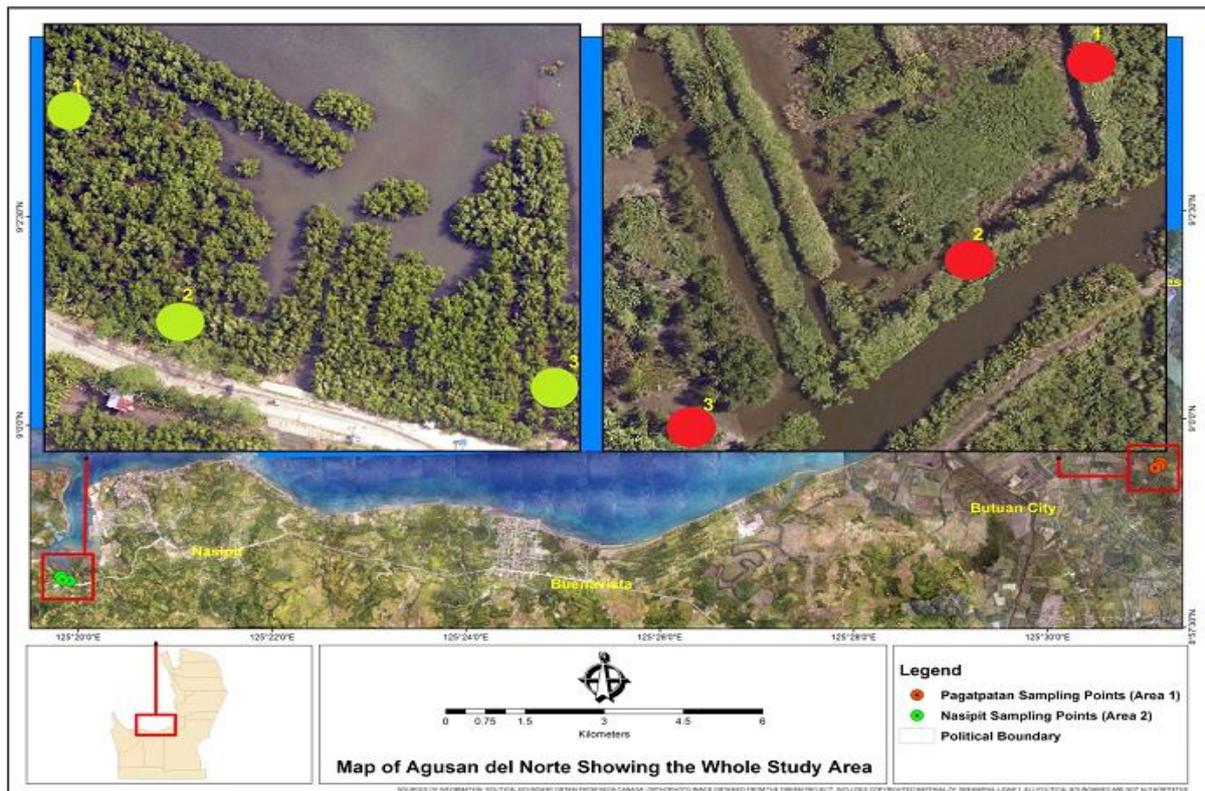


Fig. 1. Map of Butuan Bay showing the study area.

Collection procedures of P. erosa

Individuals of *P. Erosa* were collected randomly from the sampling site. About 25-30 of similar-size individuals of *P. erosa* from each sampling site were used for the metal analyses. To avoid differences in metal content because of size or reproductive stage, only the commercial size individuals of *P. erosa* were collected (Saavedra *et al.*, 2004).

Digestion procedure of sediments

Because of the extreme sensitivity of the analytical procedure and the omnipresence of mercury, care was taken to avoid unnecessary contamination. Sampling devices and sample containers were ascertained to be free of mercury.

The samples were not exposed to any condition in the laboratory that could result in contact or air-borne mercury contamination.

Moisture of sediment samples was removed using an oven dry at a temperature of 100°C. No observed mercury losses by using this drying step. The dried samples were pulverized and thoroughly mixed before weighing the aliquot.

About one g of dried sediments was considered using a digital weighing scale and placed in 100ml beaker with a label. The labeled beakers were then transferred on the hot plate under the fume hood and covered with a watch glass. 20 ml Aqua Regia was added to the samples.

The temperature was set to 240°C for 3 hours. A pinch of Calcium per Carbonate (CaCO₃) and 20 ml 0.08M Nitric acid (HNO₃) were added to the samples respectively. The temperature was set to 200°C and heated to near dryness for about 20ml. The resulting digested sediment samples were then filtered using a Wattman No. 80 filter paper and diluted with distilled-deionized water to 100ml in a volumetric flask.

Digestion procedure of P. erosa

Samples of homogenized gut muscles of mud clam were dried in a laboratory oven at 100°C for about 3 hours. The dried samples were pulverized using ceramic mortar and pestle. After crushing the samples, it was then placed in a zip lock containers with coded labels of the area and the number of replicates.

About one g of the homogenized gut muscles of mud clam was weighed using a digital scale and placed in a 100ml beaker. The beakers were then transferred on the hot plate under the fume hood and covered with a watch glass. A mixture of 5ml Nitric acid (HNO₃) and 5ml Sulfuric acid (H₂SO₄) was added to the samples respectively. The reaction of the chemicals and samples were allowed to take place. The temperature was set at 60°C for 30 minutes. Ten ml of concentrated Nitric acid (HNO₃) was added and heated at 120°C for 30 minutes. The temperature was then increased to 150°C for 45 minutes. When samples became darker in appearance, the temperature was then reduced to 30°C. Five ml concentrated Hydrogen Peroxide (H₂O₂) was added continuously until the samples become clear or pale yellow. It was then allowed to cool down the temperature for 30 minutes. The resulting mud clam digested samples were then filtered using Wattman paper No. 80 and diluted to 50ml using volumetric flask added with distilled-deionized water (AOAC International, 2012).

Analysis of digested sediment samples

Sediment samples were delivered to Regional Soils Laboratory, Department of Agriculture, Regional Field Office, Capitol Site, Butuan City. Mercury (Hg), lead (Pb), nickel (Ni), cadmium (Cd) and chromium (Cr) analysis for sediment samples was determined using Atomic Emission Spectrophotometer.

Analysis of Digested Muscles of P. erosa

The samples were delivered to Regional Soils Laboratory, Department of Agriculture, Regional Field office, Capitol Site, Butuan City for lead (Pb), nickel (Ni), cadmium (Cd) and chromium (Cr) analysis.

Mercury (Hg) analysis for the muscles was analyzed to FAST Laboratories, Cagayan de Oro City using Cold Vapor Atomic Spectrometry adopted from the Official Method of Analysis of AOAC International, 19th edition, 2012.

Data analysis

The result of the laboratory analysis was encoded and was analyzed using computer-based statistical program PAST and Graph Pad Prism 6. The mean value of the level of heavy metals in sediment and *P. erosa* was analyzed using the measure of central tendency and graph and also the physicochemical and the abundance of *P. erosa* in the different sampling area. The mean curve was presented in the histogram graph.

The T-test was used to test the significant difference on the level of heavy metals and the physicochemical analysis in different areas. To compare the level of significance between each sampling area, an independent sample T-test was used. Also, T-test was used for the abundance of *P. erosa* in the different sampling area.

On the other hand, for the ecological profile, a qualitative analysis was used based on ocular survey and secondary data. The presentation of all statistical information was through the mean ± Standard deviation (SD).

Results and discussion

Heavy metals in Sediments

The mean value of Hg in sediments in Pagatpatan was 257889±57798.60 ppm while in Camagong, the value was below the detection limit of the instrument (Table 1).

Results revealed that Pagatpatan area is above the standard limit based on CBSOG SQG (2003), New York sediment criteria and sediment quality criteria guideline (1992) for the total mercury in sediments (Table 3, 4, 5, 6).

Table 1. Heavy metals of sediments in the two mangrove area in Butuan Bay.

Metal	Pagatpatan	Camagong	Significant Difference (P<0.05)
Hg	257889±57798.60	BDL	
Pb	8.22±0.36	8.56±1.16	Yes
Ni	162.44±3.69	290.11±21.66	Yes
Cd	0.89±0.11	0.22±0.15	Yes
Cr	162.44±4.19	269.89±15.18	Yes

Note: BDL- Below detection limit.

The higher Hg in Pagatpatan area is expected since the mouth of the Agusan River Basin is along this area. Hence, it can be anticipated that higher sediment deposition is possible. The presence of small and large scale mining operations along Agusan River basin could probably have contributed to the elevated Hg level in Pagatpatan area.

These mining activities were in proximity to the river system and could probably be dumping the wastes in the river. According to Veiga and Baker (2004), the small scale mining operations uses mercury because it is an efficient magnet for gold. Although the use of Hg is prohibited, it is often preferred by operators since it is inexpensive and available in the market.

Table 2. Heavy metals in the muscles of *P. erosain* the two mangrove area in Butuan Bay.

Metal	Pagatpatan	Camagong	Significant Difference (P<0.05)
Hg	6.72±1.62	1.67±0.83	Yes
Pb	3.44±0.056	4.50±0.35	Yes
Ni	8.50±0.61	10.72±0.63	Yes
Cd	BDL	BDL	
Cr	9.50±2.27	9.61±2.92	Yes

Note: BDL- Below detection limit.

Most of the small-scale gold mining operations do not have proper waste disposal facilities, thus, there is a high probability that wastes containing mercury will be dumped directly into the river and could settle in sediments and eventually reach downstream of the Agusan River system. It was estimated during the height of gold rush that about 140 tons of mercury were disposed into the environment in 1986 to 1988 alone, another estimated 26 tons of mercury were dumped in the water bodies yearly and ultimately drained in Agusan River Basin to Butuan Bay (DENR-NWRB, 2008).

Smale-scale gold mining processors generate mercury of about 71.4%, and 28.6% is coming from industrial mining companies. Compostela, Boringot, Tagum, Masara and Diwalwal's gold mining activities have the greatest contribution of total mercury disposal which account for more than 90% (Breward, 1996).

Mean Pb concentrations in the sediments of the two areas are lower than the presented standards for Pb concentration in sediments (Table 1). There is no significant difference on the level of Pb in the two areas based on the results.

However, accumulation could increase if major sources of Pb exposure are continuously disposed of in the area through time. The atmosphere, aquatic and terrestrial environments are the major means where Pb is released from different natural and anthropogenic sources. It can be from a single high-level exposure or the cumulative effect of repeated high or low-level exposure.

The t-test shows that Ni in sediments is significantly higher in Camagong however, both areas has already exceeded the prescribed tolerable limits on Ni (Table 3, 4, 5 and 6).

Pagatpatan and Camagong area are severely polluted based on the EPA Standard, heavily polluted based on CBSOG SQG (2003) sediment quality standard; severe effect range based on New York Sediment Criteria and high effects range (ISQG-high) in Sediment Quality Criteria Guideline (1992).

Table 3. Prescribed tolerable limits of selected metals in sediments based from EPA Standard.

Metal	EPA Standard		
	Non Polluted	Slightly Polluted	Severely Polluted
Hg	-	-	-
Pb	<40	40-60	>60
Ni	<20	20-50	>50
Cd	-	-	>6
Cr	<25	25-75	>76

The result on the level of Ni in sediments is alarming, and it needs to have local intervention to regulate the mining activities along Butuan Bay such operation of San Roque Metal Incorporated located in Tubay, Agusan del Norte. Mitigation measures are necessary to reduce the environmental impact that may lead to fishery resources of Butuan Bay depleted.

But in the New York Sediment Criteria Camagong area exceeded the criteria for the lowest effect range of 0.6 ppm and Sediment Quality Criteria Guideline (1992) for lowest effects range (ISQG-low).

Results revealed a significant difference in the value of Cd on sediments, but Cd in Pagatpatan and Camagong area were within tolerable limits based on EPA Standard and CBSOG SQG (2003) sediment quality standard.

Cd in the environment is widely disseminated at low levels; the sources for human exposure are air, water, food and tobacco, with food representing the major route of uptake to the general public (Anon, 1993). Thus, the accumulation of Cd in sediments may be taken up by dwelling organisms in the aquatic environment and magnify to the other organisms including humans that could lead to a serious environmental problem.

Table 4. Prescribed tolerable limits of selected metals in sediments based from CBSOG SQG*(2003).

Metal	CBSOG SQG*(2003)		
	Non Polluted	Moderate Polluted	Heavily Polluted
Hg	<0.18	0.18-0.64	>0.64
Pb	<40	40-70	>70
Ni	<23	23-36	>36
Cd	<0.99	0.99-3	>3
Cr	<43	43-76	>76

The two areas already exceeded sediment chromium tolerable limits based on EPA Standards indicating that both are severely polluted. Also, both areas had already exceeded the CBSOG SQG (2003) sediment quality standard.

Compared with the New York Sediment Criteria, both Pagatpatan and Camagong areas also exceeded greater than the range for severe effect range and Sediment Quality Criteria Guideline (1992) for high effects range (ISQG-low).

Anthropogenic activities cause the increasing content of chromium in the environment. The hazards of global pollution by chromium does not yet exist, although its local emissions into the atmosphere may cause its excessive inclusion in biochemical circulation, consequently, posing a danger to the aquatic resources of Butuan Bay, as well as the health of the people living along the bay.

Heavy metals in the muscles of P. erosa

Table 2 shows the significant difference of Hg in the muscles of *P. erosa* in Pagatpatan area.

However, the mean value of *P. erosa* from two areas did not exceed the allowable limit. This result, however, reflects only in the *P. erosa* samples used in the study which was collected in the month of July 2015 in a single year during a wet period using the muscles for analysis as this usually is the part eaten by the consumers.

Table 5. Prescribed tolerable limits of selected metals in sediments based from New York Sediment Criteria.

Metal	New York Sediment Criteria	
	Lowest Effects Range	Sever Effect Range
Hg	0.15	1.3
Pb	32	110
Ni	16	50
Cd	0.6	9
Cr	26	110

The feeding habit of *P. erosa* that filters sediments could be the main reason these species accumulate mercury in their muscles. Because of their capability in filtering water and being persistent in the same place makes them good bio-indicator of marine ecosystems (Chiaravalle, 2013). The Hg in *P. erosa* samples taken near river mouth shows a varying degree of concentrations. Accumulation of metals in the muscles of these organisms have aspects known to

effect such as bioavailability of heavy metals, climatic condition and season of sampling, hydrodynamic of the environment, species maturity and sexual characteristics, alterations of the composition of bivalve tissue and cycle of propagation (Boyden and Phillips, 1981). If no mitigating measures are taken to lessen the impact of mercury contamination in the mangrove ecosystem, not only the *P. erosa*, but other fishery resources in the Bay might be affected.

Table 6. Prescribed tolerable limits of selected metals in sediments based from Sediment Quality Criteria Guideline (1992).

Metal	Sediment Quality Criteria Guideline (1992)	
	Lowest Effects Range (ISQG-low)	High Effects Range (ISQG-high)
Hg	0.2	2
Pb	31	250
Ni	16	75
Cd	0.6	10
Cr	26	110

The t-test shows that Pb is significantly higher in Camagong. The threshold limits of Pb in the Mollusk/Bivalve Muscle based on international standards (FAO/WHO, 1984) is 1.5 ppm and has exceeded the allowable limit for Pb in muscles. The accumulations of Pb in the muscles of *P. erosa* are useful as sentinel organisms for assessing the bioavailability of metal contaminants in aquatic ecosystems.

Frequently, muscle metal concentrations are used by environmental monitoring studies to evaluate potential exposure and effects scenarios. However, *P. erosa* may accumulate lead, to a significant extent in shells. Lead, in particular, is a metal whose environmental concentrations are often poorly correlated with tissue concentrations and adverse effects (Elder and Collins, 1991; Mason and Jenkins, 1996).

Lead is also known to be deposited in the shells of bivalves along with cadmium, copper, manganese, mercury, and strontium (Swinehart and Smith, 1975; Sturesson, 1978; Imilay, 1982).

Although the level of Ni in the two areas did not exceed the allowable limits, it is still alarming since the discharges from anthropogenic activities like mining operations that contribute to nickel in the area are continuing.

The main source of Ni in waterways is coming from domestic waste water (Nriagu and Pacyna, 1988). The Ni species commonly found are nickel silicate, nickel subsulfide, and nickel chloride associated with combustion, incineration, and metals smelting and refining are often nickel complex oxides, nickel sulfate, and metallic nickel, and in more specialized industries (EPA, 1985a).

Table 7. Prescribed tolerable limits of selected metals in bivalves.

Metal	Mollusk/Bivalve Muscle World Standard (FAO/WHO, 1984)
Hg	13.0 mg/kg
Pb	1.5 mg/kg
Ni	80.0 mg/kg
Cd	0.5 mg/kg
Cr	13.0 mg/kg

The level of cadmium in the muscles of *P. erosa* was not detected below the 0.04 ppm standard detection limit. Cadmium merits distinct attention among many other heavy metal contaminants due to its possible threats to aquatic biota and to human beings as well (Mayer *et al.*, 1991; Barber and Sharma, 1998; Groten and Van Bladeron, 1994; Vanderpool and Reeves, 2001). Results revealed that *P. erosa* from Pagatpatan mangrove area and Camagong mangrove area is free from cadmium contamination. But even at the small level of concentration of cadmium in natural water, it is known to be a common aquatic pollutant that is highly toxic to most marine and freshwater organisms (Lovert *et al.*, 1972).

Results revealed that Pagatpatan and Camagong area did not exceed the allowable limit of chromium. Although the level of Cr in the two areas did not exceed in the world standard, the results are still alarming since the anthropogenic activities are still present in the area. Besides natural sources (erosion of rocks, volcanic emissions), Cr is one of the heavy metals in the environment from activities associated with mining, manufacture and fossil fuel combustion which released in large quantities. Mining operations in Caraga region are present, and this could eventually increase the accumulation of chromium through time.

Conclusion

The study revealed that Pagatpatan area is already contaminated with Hg, Ni and Cr at the level above the standard for sediments while detected levels of lead and cadmium are below the allowable limit. Barangay Camagong stations have no mercury contamination while nickel and chromium were detected above the standard for sediments while lead and cadmium is below the allowable limit. Stations in Pagatpatan and Camagong are already contaminated with mercury, nickel and chromium, but these concentrations are below the standard limits for bivalves. Levels of lead in the muscles are above the standards while cadmium analysis revealed no contaminations in the muscles of *P. erosa* in the two areas.

There is a need for further studies to determine the specific cause of Hg, Pb, Ni, Cd and Cr accumulation in the physical and biological component of the environment. Further studies should also be conducted on the correlation of the abundance and growth development of *P. erosa* as factors for heavy metal contamination.

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