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Bioaccumulation of lead, mercury and nitrates of *Macrobrachium rosenbergii* (Shrimp) as indicator of water pollution in Iponan River, Cagayan de Oro

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Abstract

This study aimed to examine the mercury (Hg), lead (Pb), and nitrate (NO3) accumulation on *Macrobrachium rosenbergii* (shrimps) of Iponan River. The samples were analyzed using Cold Vapor Atomic Absorption Spectroscopy (CVAAS) for the heavy metals (Hg & Pb) and Bruccine Colorometric Method (BCM) for nitrate. One-way Analysis of Variance (ANOVA) and Standard Deviation analysis were used to test the significant difference in the concentration of the analytes on shrimps between sampling periods. Statistically, based on the results, there is significant difference on the concentration of lead, mercury and nitrates among sampling periods. *M. rosenbergii* contained high measured values of nitrate (NO3) analyte within two (2) sampling periods with concentrations of 120>32.8 ppm and 842>32.8 ppm respectively which exceeded the EPA standards. Lead concentration exceeds the BFAR standard (1.36>0.5) ppm during the second sampling while Hg content is present within specified standards. Results suggest that *M. rosenbergii* is a bioaccumulator of mercury (Hg), lead (Pb), and nitrates (NO3) and can be used to monitor pollution level in a river. This result could be used as basis for strengthening policies regarding water quality management and monitoring of Iponan River.

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Introduction

Surface waters often serve as sink for various toxicants of the environment through natural and anthropogenic processes. Heavy metals and nitrates received major attention because of their persistent toxic effects and their ability to be accumulated within compartments of the environment. This can lead to deleterious effect to benthic organisms, shrimps, and potentially on human health through ingestion of metals and nitrates in compound enriched sediment and through uptake from the river (Apodaca, David & Lagrana, 2011).

The Iponan River Watershed has a total area of about 405 square kilometers, one of the major watershed in Region 10, Northern Mindanao. It is located at coordinates 8˚30'30" North Latitudes and 124˚ 22' 43" to 124˚ 38' 16" East Longitudes. The mainstream of the river watershed is about 80 kilometers and classified as "Class A" river in late 70s, its current water quality has deteriorated so much that the need to reclassify it as "Class C" is most urgent (Dagoc, 2012).

Water quality of the river can be deteriorated with contributing impacts that affect its physical and chemical properties such as sewage effluent, existence of agricultural farms, urban development and quarrying and mining activities along the river. There are some news report which says that hydraulic mining and quarrying is prevalent in Iponan River for over a decade and that mercury has been used for a long time in the refining method to separate gold from its ore. People's livelihood living nearby the river is either doing farming or into mining. Farming practices on the other hand also uses commercial fertilizers in which excess amount could be carried as run off to the Iponan River (Borlongan *et al.,* 2010).

In view of the adverse effects of mining activities and the use of excess fertilizers on farms, this research focuses on the extent of bioaccumulation of nitrates (NO_3) and heavy metals specifically mercury (Hg) and lead (Pb), and enumerates the factors that influence accumulation from the immediate environment to benthic macro invertebrates.

This study generally assess the accumulation of heavy metals (Hg & Pb) and nitrates on *macrobrachium rosenbergii.* Specifically, the study attempts to determine if there is significant difference on the accumulated heavy metals (Hg & Pb) and nitrates on *microbrachium rosenbergii* between sampling stations and sampling periods and to assess if the measured values are within the EPA and FDA standards.

Materials and methods

Research Design

The study used a descriptive-comparative assessment method. Since the study encompasses the analysis of the concentration of the shrimp parameters in terms of its physico- chemical properties of the river. The researcher chooses to differentiate the results from different sampling periods and compared to the standard sets by the Environmental Protection Agency (EPA), Bureau of Food and Drugs (BFAD), Bureau of Fishery and Aquatic Resources (BFAR). One – Way Analysis of Variance (ANOVA) is used as one of the statistical treatment in finding significant difference at five (5%) percent level of significance to the measured values of the analytes on *macrobrachium rosenbergii* in different sampling periods.

The researchers chose to get the samples in three (3) zones of Iponan River and sampling were done by composite method. Site one (1) is located at Zone 6, Bulao; Site two (2) at Zone 4 Dampil; and site three (3) near Barra Bridge. Samples of similar sizes from three sites were collected, placed in an iced bucket and digested for analysis.

Research Setting

Fig. 1 shows the map of Barangay Iponan, well-known of its huge and lengthy river that serves as boundary to other barangays of Cagayan de Oro City. This river consisted of highly turbid and brownish in appearance. This is one of the major estuaries in the region, traverses towns in Cagayan de Oro, Iligan City, and Misamis Oriental. Iponan River crosses and drains to Macajalar Bay. Base from the Office of Barangay Iponan council, the area is considered residential (with a population of 9,827 as of 2002) although some part of the upper portion of Iponan River is also agricultural. Watershed is situated in the Cagayan de Oro – Western Misamis Oriental segment of the North Bukidnon-Lanao Plateau of the Central Visayas Physiographic Sub province. It has a drainage density of 0.33 kilometre per square kilometre it means that every higher order of stream it has about 0 lower orders of tributaries above it and signifies possible soil erosion. The study is conducted within the Iponan River with the selected zone of area.

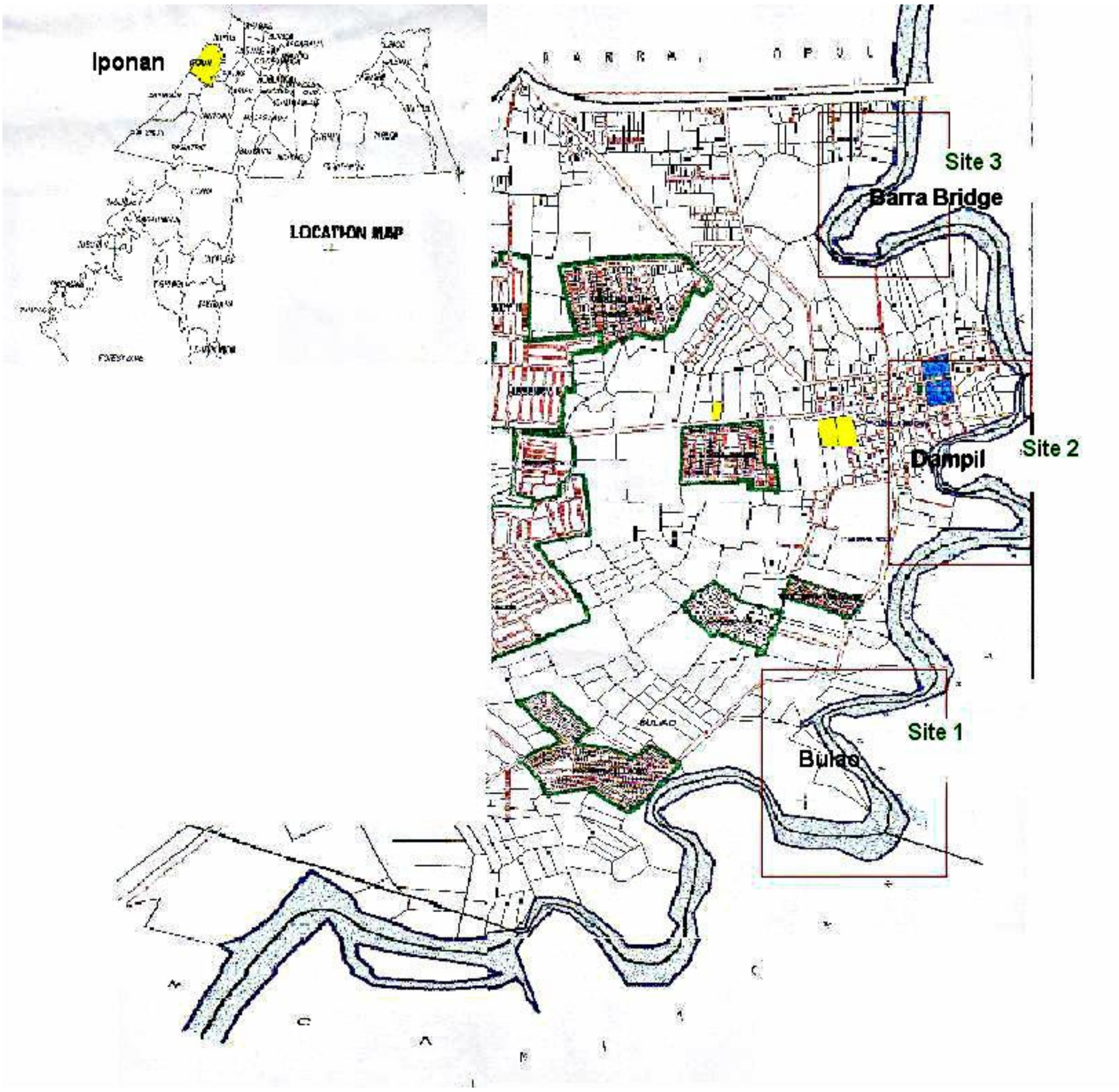

Fig. 1. Map of Iponan River.

Entry Protocol and Ocular Inspection

The researchers observed proper entry protocol to the local government officials of barangay Iponan before the actual sampling was conducted. Formal letter that expressed the objectives of the study is sent to the office of barangay Iponan. Local guide accompanied the researchers during the ocular inspection to establish actual sampling sites. The selection of sampling sites is based on distance, about 1.5 km between sampling sites that also covers zone 1 to zone 6.

Establishment and Description of Sampling sites

Three sampling sites were established along Iponan River. The river is an inland water body characterized by mixture of pollutants both from point and non-point sources specifically from residential and agricultural run- offs. The sampling sites consist of vegetation at the side of the river. From the selected zone, majority of the species are shrimps. Site one (1) is shown in Fig. 2 located at Zone 6, Bulao, Barangay Iponan, Cagayan de Oro City. The area is residential and agricultural. Site 1 to site 2 approximately measures a distance of 2.80 kilometres. This site is characterized with more vegetation along the river bank and the surrounding area is also utilized as farmlands. The water channel is composed of rocky substrate and the water is highly turbid.

Fig. 2. Zone 6, Bulao, Iponan.

Fig. 3 shows sampling site two (2) located at Zone 4, Dampil, Barangay Iponan, Cagayan de Oro City. The area is both residential and agricultural and the site is characterized with more vegetation along the river bank and the surrounding area is also utilized as farmlands. The area is also characterized having mostly quarrying /dredging activity and surrounded by housing subdivisions. The area is also highly vegetated with shrubs seen along the river bank and water channel is highly composed of rocky substrate. Site 2 to site 3 approximately measures 4.80 kilometers. Site three (3) as shown in Fig. 4 is located at Zone 1, Barra Opol Bridge, National Highway of Opol, Misamis Oriental. The river water is more turbid compared to the upstream part and the middle stream portions of the river. Most residents live along the sides of the river in which some of them used to catch shrimps and fish for their consumption.

Data Collection Method and Sampling Scheme

The collections of shrimp were manually done using fish net. Local residents were involved in the catching of shrimps with the guidance of researchers. Collection of samples were done across the river and in all directions in each selected zones (from zone 1 to zone 6) done by composite sampling. Every sampling was conducted after it rains to increase the water level of the river and the possibility of high shrimp catch.

Fig. 3. Zone 4, Dampil, Iponan.

Fig. 4. Zone 1, Barra Bridge, Iponan.

There were seven (7) zones in barangay Iponan, however only selected zone sites of the river are being considered. The climate was characterized by wet season with the average temperature of 26.23°C. There were three (3) sampling periods considering wet seasons (September to December). From the combined samples of shrimps representative samples of almost the same size were considered. After gathering the samples, it was put on a sealed polyethylene bags, labelled, and documented then put in an iced bucket and transported to the laboratory.

Inside the lab area shrimp samples of the same size / weight were selected for digestion and analysis. Fig. 5 shows typical shrimp size caught during sampling from Iponan River.

Fig. 5. Sample species of *Macrobrachium rosenbergii* from selected zones in Iponan River.

Digestion and Sample Analysis

The concentration of mercury possibly accumulated on the shrimp biomass was tested by using Cold Vapor Atomic Absorption Spectroscopy (CVAAS). Fresh sample of shrimp was used to blend for the digestion. Five (5) grams of the blended samples were weighed and placed in three 250ml COD flask. About 0.05 ug and 0.1 ug of Hg standards were added to two of the three samples being weighed. Four glass beads and 1ml 2% of sodium molybdate was added. Then, 25ml of concentrated sulfuric acid and 20ml 1+1 nitric acid were added. After one (1) hour, the condensing unit was refluxed, connected and cooled for 15 minutes and added 20ml 1+1 nitric- perchloric acid through the condenser. The condenser was turned off and the solution has appeared. Heating was continued for 10 minutes, cooled and carefully added with 10ml water while swirling. Again, it is boiled for 10 minutes then removed from the heat and washed with three 15ml portions of water, cooled and diluted to 100ml. The sample solutions were aspirated into a flame and atomized. Then the absorbance of the atomic vapor generated by these methods was measured. Cold Vapour Atomic Absorption Spectroscopy (CVAAS) is used for heavy metal concentration analysis specifically mercury (Hg) and lead (Pb). For nitrate concentration, Electrode Method is used. Average data from the laboratory analysis is considered in the study.

Data Analysis Procedure

In understanding the data gathered, the researchers used statistical tools in the form of mean and standard deviations. Hypotheses tested at 0.05 significant levels and one- way analysis of variance (ANOVA) is used to determine if there's a significant differences in the concentration of each parameters among sampling periods and towards its standards.

Results and discussion

Water Analysis of Mercury, Lead and Nitrates

The researchers examined the water quality in Iponan River to consider the possible changes after the occurrence of heavy rains which may have the distribution of mercury, lead and nitrates concentration. Based on table 1, the presence of mercury and lead in water do not exceed the standard value while the presence of nitrate exceeds the standard values per DENR-AO No. 34-35. The data used to compare each parameter between sampling periods and comparison of the parameters per period. It indicates that the accumulation of heavy metals in water is gradual. Although, the concentration of heavy metal was tolerable at the present, but this would serve as a threat to human if the mining activity still continue.

Table 1. Heavy metal and compound analysis of Shrimps samples from Iponan River.

Sampling Period	Hg (ppm)	Pb (ppm)	NO_3 (ppm)
Sampling 1	<0.05	<0.05	120
Sampling 2	<0.05	1.36	28.85
Sampling 3	<0.05	0.3	842
Average	<0.05	0.56	330.28
Food Standard	0.5*	0.5*	200**
* - BFAR			
** -FDA			

Shrimp Analysis of Mercury, Lead and Nitrates

The data obtained in this study were based on the samples taken from Iponan River during wet season. Shrimp samples of similar sizes were examined and compared in terms of accumulation level on the parameters namely total mercury (THg), lead (Pb), and nitrates (NO_3). Water sampling conducted for one sampling period were analyzed for the presence of the same parameters mentioned above. Results from shrimp analysis obtained different concentration on mercury (Hg), lead (Pb), and nitrates (NO_3) from different sampling periods. The mean values in each sampling periods and each parameters were then compared statistically, in order to determine which periods and parameters obtained much higher concentrations on the accumulation of the species *Macrobrachium rosenbergii.*

Fig. 6 shows the comparison of mercury (Hg) and lead (Pb) absorption on the species with its food standard set by the EPA, BFAR and FDA. Mercury absorption was consistently low in three sampling periods (October to December); it is because during sampling, majority of the collected shrimps were in juvenile stage. Based from one study, juveniles showed lower mercury (Hg) concentration than adults (Bianchini *et al.,* 2007). The time of exposure on shrimp into polluted water could be the possible factor of obtaining low concentration of metals. According to the study of Kargin *et al.,* 2001 on the accumulation of some trace metals in whole body of fresh water shrimp, the seasonal variations of the element concentrations, and the relationship between element concentrations in males and females were also a factor. It has been found that season affects concentration on shrimps and it also showed that female shrimps contained higher THg than male shrimps (Yilmaz, 2007). This can be explained by its feeding mechanisms because female species has higher feeding rates than male shrimp species and that females tend to accumulate more fat than males. Mercury when it is in living organisms is converted to methyl mercury (CH3-Hg) due to bacterial activity with the help of environmental factors such as temperature, salinity, hardness, and seasons.

The possible reason why lead (Pb) absorption on *Macrobrachium rosenbergii* in second sampling was much higher with 1.36ppm, compared to the other sampling periods (see Fig. 6) could be due to its water characteristics, during this time the water quality of lead (Pb) was less than 0.01 (see table 2) which is below DAO-34 standard. Based from one study heavy metal absorption in aquatic ecosystem are usually monitored by measuring their concentrations in water, sediments and biota (Camusso *et al.*, 1995). Biota tends to absorbs more heavy metals, when the water quality exists in low level and higher sediment concentration, have the tendency to accumulate in various organs of marine organisms, especially fish, which may enter into human metabolism through consumption causing serious health hazards (Mansour and Sidky, 2002).

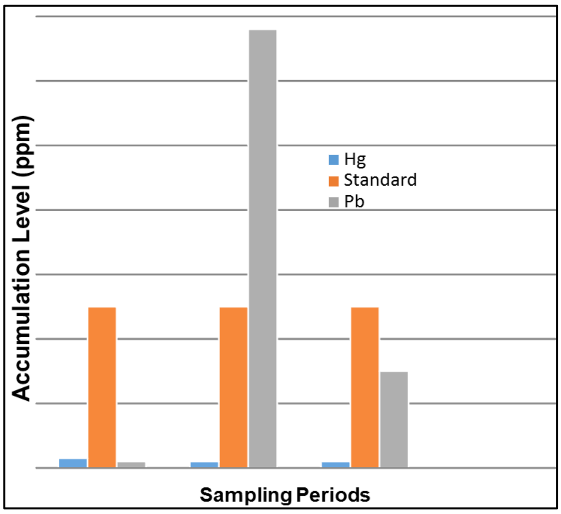

Fig. 6. Graph presentation on mercury (Hg) and lead (Pb) accumulation on *M. rosenbergii.*

Table 2. Shows the water analysis (mercury, lead and nitrate) a single period.

Sample source	Hg (mg/L)	Pb (mg/L)	NO_3 (mg/L)
Surface water	<0.001	<0.01	8.05
Water Standard (DAO-34)	0.002	0.05	0.1

Table 3. One-way Analysis of Variance (ANOVA) on nitrates.

Source	Sum of Squares	Degrees of Freedom (df)	Mean Square	F-Computed value	F-value
Periods	217793.8	2	108896.913	1.646	0.269
Parameters	396936.1	6	66156.018		
Total	614729.9	8			

It was clear from the Fig. that lead value during second (2nd) and third (3rd) sampling were above the standard. The possible reason for this increase could be due to runoff during heavy rains.

Lead is accumulated preferentially in the non-edible fraction of shrimps, in the exoskeleton and in the muscle. Lead is mostly concentrated in the sediments in the river, and shrimps being a bottom feeders could increase lead concentration on its biomass (Lee *et al.,* 2000).

Fig. 7 shows the comparison of nitrate (NO_3) absorption on the species compared to EPA standard. It was shown from the Fig. that nitrate value during first (1st) and third (3rd) sampling were above the standard. Anthropogenic factors play a major role in contributing to the pollution of rivers. Anthropogenic factors such as agricultural development, population growth, urbanization and industrialization have been identified as the root causes of water pollution (UNEP, 2006). It has been observed that most residents live nearby the river and their daily activities could potentially affect the quality of the river water.

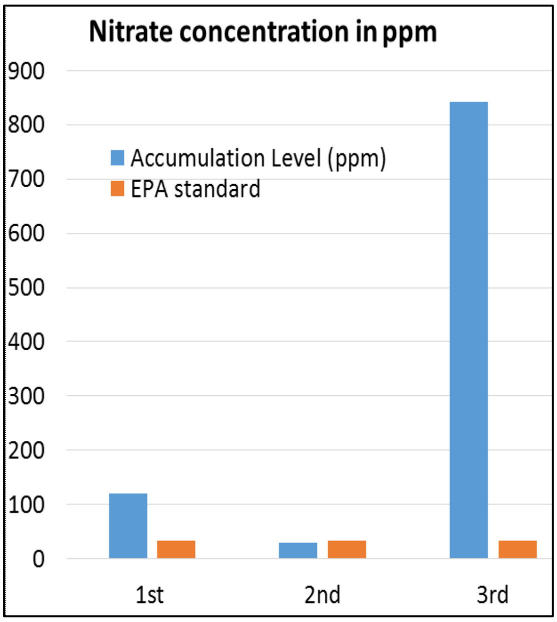

Fig. 7. Graph presentation on nitrates (NO_3) accumulation on *M. rosenbergii.*

Agriculture in some settings across the earth requires the use of fertilizers and the application of pesticides. The application of such chemicals lead to the release of toxins as Nitrogen (N) and Phosphorus (P). These toxins leach into soils to contaminate underground water and surface waters which lead to the eutrophication of water systems (Hayakawa, Shimizu, Woli, Kuramochi, Hatano, 2006). Another reason for the increase in nitrate level could be due to heavy siltation following heavy rains before sampling. According to (EPA, 2012), excess nitrates in a water body can cause hypoxia (low levels of dissolved oxygen) and can become toxic to warm-blooded animals at higher concentrations (10mg/L) or higher under certain conditions. The natural level of ammonia or nitrate in surface water is typically low (less than 1 mg/L) for many of aquatic organisms to survive.

Heavy rains prior to sampling which bring high runoffs containing nitrate concentration from soil surface to rivers could be one of the reasons for high nitrate level in the water sample. One study revealed, bioaccumulation of chemicals in biota may be a prerequisite for adverse effects on ecosystem (Burkhard, 2003).

Sedimentation on the river banks can also contribute to increase in the sediments of the river which may contain fertilizers or organic materials. Since, the slope percentage of Iponan has 3 to 8 land feature (see Fig.. 8). The tributaries of Iponan River have a severe to moderate erosion capability which greatly influences the turbidity of the river (see Fig.. 9); in that case the sediments tend to increase nitrates for this aspect naturally present in soil, water and bottom sediments. Nitrate concentration can reach high level due to agricultural runoff.

Agricultural lands are present in the area (see Fig. 10). During heavy runoff, steep slopes can introduce debris, sediments and nutrients that may affect color, turbidity and the growth of algae (Letterman, 1999).

Slope map (Fig. 8) shows the topography of Iponan of 3% to 8% slope. It is considered as one factor with high run off during rainfall carrying sediments and nutrients to the river making nitrates content higher than the standard value. Erosion map (Fig. 9) shows the Iponan River is slightly eroded that can be attributed from the terrain which affect the water bodies coming from different tributaries namely Tumpagon, Pigsag-an, Tuburan, Taglimao, Pagatpat, San Simon, Canitoan, Baikingon and other sources of wastes altering its water quality.

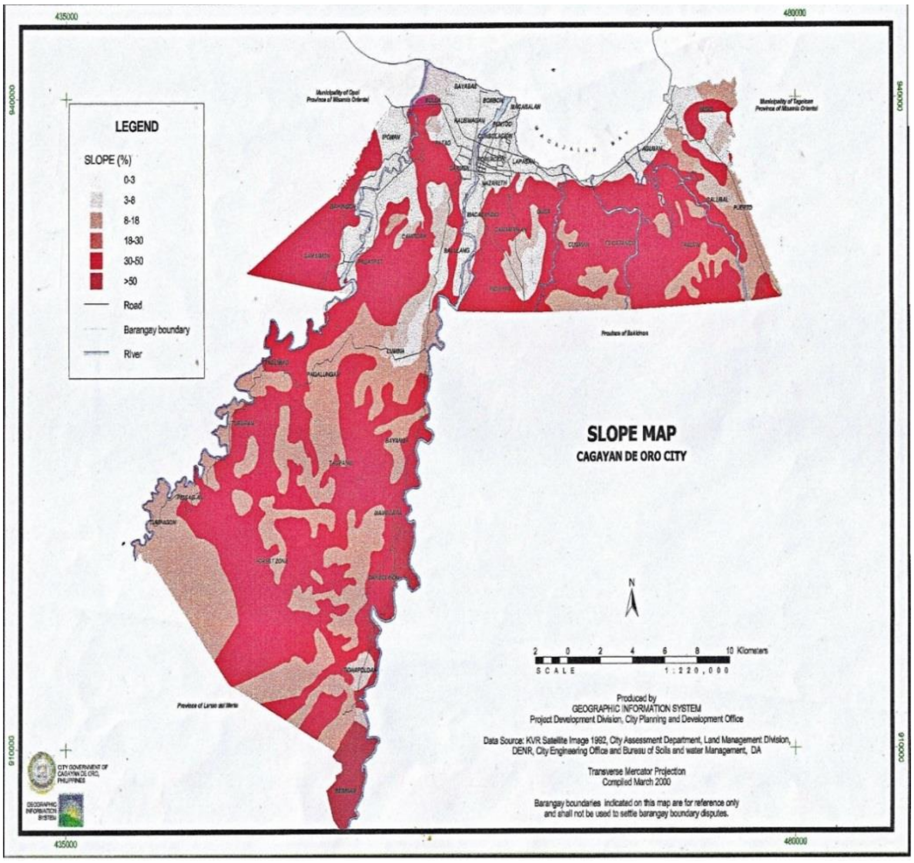

Fig. 8. Slope map of Iponan River.

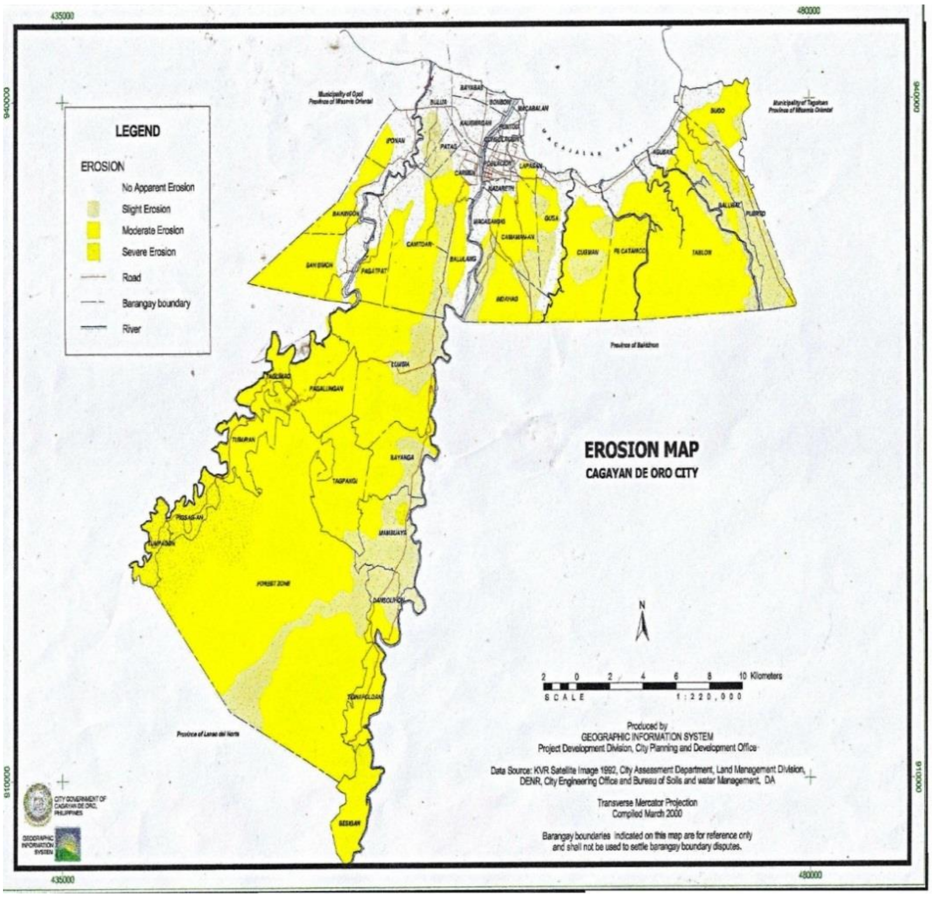

Fig. 9. Erosion map of Iponan River.

Fig. 10 shows the common land use in Barangay Iponan. The agricultural land position is perpendicular to the river basin which during rain fall more runoff water can be carried down into the stream. Most of the crops are vegetables (like kangkong, string beans, eggplants, corn, fruit trees like banana and coconut and other vegetation were also present. These agricultural activities can be a basis for the occurrence of nitrates (NO_3) since fertilizers are usually added to the crops for yield increase.

Fig. 10. Agricultural land near the river.

Conclusion and recommendation

The findings of this study showed that there is accumulation of mercury, lead and nitrate in Iponan River although the first two parameters is minimal and does not exceed with the given standard. The nitrates, on the other hand, has a value exceeding standard that is set by the Food and Drug Administration (FDA). Generally, there is a significant difference on the level of concentration on mercury (Hg), lead (Pb) and nitrate (NO_3) between sampling periods. Shrimps accumulate more on nitrates (NO_3) than mercury (Hg) and lead (Pb).

Based on the results of the study, the researchers would like to recommend the following: Another similar study on shrimps must be done to compare different accumulation levels on different parts of shrimp like comparing its concentration on the head part, the meat, and its outer covering. Then, compare the degree of accumulation between sizes (small, medium, large) of shrimps.

There should be a comparison on shrimp species between cook and raw to determine which of the two obtain high concentration of mercury (Hg), lead (Pb) and nitrate (NO_3). Bioaccumulation can be engaged in monitoring assessment in the area that exercise further supervision system consequently to the contamination of the aquatic environment. It would be of large significance in the study to perform in longer time to have a more defined examination set and in view of wet and dry seasons for a good comparison study. Further study on the other naturally-occurring species is of great consideration to identify other alternatives in remediating toxic heavy metals in the environment. For a follow-up study, it is recommended to use other process instead of bioaccumulation, such as bioavailability, bio-magnifications and bio-concentration.

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