Evaluation of potential ecological risk and contamination assessment of heavy metals in sediment samples using different environmental quality indices – a case study in Agusan River, Caraga Philippines

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Abstract
The contamination and potential ecological risk posed by heavy metals from thirteen (13) sediment samples from different sampling sites along the lower portion of Agusan River were analyzed and assessed using different pollution indices. The results obtained shows that the total digest concentrations of different heavy metals under investigation have the following order: Cd < Pb < Zn < Mn, for both first sampling periods. The speciation and distribution pattern have shown that significant amounts of all metals are present in the residual fraction. Similarly, oxide-bound and organic-bound fractions were found to be highly important for Zn and Mn while Cd and Pb were significantly associated in the residual and exchangeable fractions. The results of different pollution indices moreover, showed that among all the heavy metals being studied, Cd posed the highest environmental risk across all sampling stations in both sampling periods and Mn metal was highly enriched and abundant in all of the sampling stations. Importantly, PCA results suggest that Zn, Mn and Pb may have the same origin while Cd might be coming from different sources, and this is corroborated well with the cluster analysis results. The results obtained from this work provide baseline data on the assessment of heavy metal pollution in the lower portion of Agusan River. Importantly, the acquired environmental indices will certainly help safety managers in assessing and interpreting the potential risk of the sediment associated chemical status that might adversely affect aquatic organisms in the selected sampling sites.

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Introduction

The growing global concern about heavy metal contamination and environmental pollution has prompted the scientific community to do extensive research to address this problem (Zerbe et al. 1999, Abdallah 2012, Zhao et al. 2012, Vega and Weng 2013, Aktaruzzaman et al. 2014, Zhuang and Gao 2014, Xu et al. 2015). Human activities, as a result of rapid industrialization and economic development have resulted to a rapid discharged of different heavy metals in various point sources (Wang et al. 2011, Yuan et al. 2013). Most of these heavy metals were reported to be indestructible and have toxic effects on living organisms, especially when the permisible limits were exceeded (MacFarlane and Burchett 2000, Ekeanyanwu et al. 2011, Jayaprabha et al. 2014. As being known, heavy metals from different anthropogenic sources will be transported thru various processes and being deposited in the aquatic sediments. Studies have shown that metal ions often have a strong tendency to associate in the particulate matter in the water column and may exists in different forms which dictates their reactivity and toxicity (Tessier and Campbell 1987, Ideriah et al. 2012). Hence, the study on the speciation pattern of these heavy metals call for a special attention and was considered as one of the important research areas in environmental studies. Furthermore, establishing considerable evaluation measures in terms of the possible contamination and potential ecological risks posed by different heavy metals based on different environmental quality indices is of paramount importance. Certainly, these evaluation measures is of significant help for development, evaluation and converging raw environmental data to decision makers and managers for public interest (Caeiro et al. 2005, Qingjie et al. 2008).

Accumulation of metal contaminants in sediments can pose serious environmental problems to the surrounding areas (Zakir et al. 2008, Kumar and Patterson Edward 2009, Ong and Kamaruzzaman 2009, Wang et al. 2010). Trace metal contamination in sediment matrices have been reported to affect the water quality and the likelihood of bioaccumulation into aquatic organisms, resulting in potential long-term implications on human health and ecosystems (Fernandes et al. 2007, Li et al. 2012, Abdel-Baki et al. 2013. However, the total metal concentration provides a poor indication of environmental risk since this information is not sufficient in understanding their chemical behavior. More often than not, trace metals undergo numerous changes in their speciation during their transport due to dissolution, precipitation, sorption and complexation phenomena (Akcay et al. 2003, Odukoya and Abimbola 2010).

This study presents a baseline data on the assessment of heavy metal pollution in the lower portion of Agusan River, Caraga Philippines. As of this writing, there has no documented report which deals into specific investigation on the speciation of heavy metals in sediment samples which is a significant factor in the transport of heavy metals in the chosen study sites. The extent of heavy metal pollution in Agusan River has great impacts on the different settlements along the area. Thus, the main purpose of this work was to demonstrate and evaluate different environmental indices (multi-element indices) derived from total content-based and speciation-based indices in interpreting the potential risks posed by heavy metals in the chosen sampling sites.

Materials and methods

Description of the Sampling Sites

Agusan River is the third largest basin of the Philippines (2008). It is also the draining majority of Caraga Region and some parts of Compostela Valley province with major gold mining and/or processing operations which can be considered as culprits of heavy metal pollution.

Sediment samples were strategically collected in 13 chosen sampling sites which represent the whole lower portion of the river. Mostly, the selected sampling stations were chosen in areas with noted tributaries in the main river (Fig. 1). Likewise, these sites were characterized by some manufacturing
industries, mixing factories with residence and with agricultural activities nearby. Moreover, mining industries upstream of the river were reported to be in operation that may contribute for the heavy metal load in the lower portion of the river. Exact locations for all sampling sites were determined using a global position system and entered into the geographical information system for processing.

![Fig. 1. Graphical representation of all the sampling sites in Agusan River.](image)

**Sample collection and preparation**

About 1 kg of sediment samples were collected, stored in a polyethylene bottle/container and transferred to the laboratory for analysis. Sample treatment, extraction and subsequent analytical determinations are being performed in a clean laboratory and with acid-washed glassware’s. The sequential extraction method for the analysis of heavy metal speciation is being analyzed according to the outlined method. Quantitative amount of the heavy metal fraction and free heavy metal (total heavy metal content) were analyzed using the Atomic Absorption Spectroscopy (AAS).

**Total concentration of Mn, Zn, Cd and Pb**

Sediment samples were digested using a concentrated HNO₃ and HCl (aqua regia). In brief, 20.0 mL aqua regia was added to 1.0 g of dry sediment sample and was digested for 3 hours. Then, about 20 mL of 0.08 M HNO₃ was added and it was heated continuously to near dryness. The supernatant solution was then filtered and diluted to 50 mL with doubly-deionized water.

**Chemical Fractionation**

A modified sequential chemical extraction protocol as described by previous literature (Tessier and Campbell 1987) was employed in this study for the determination of the different operationally-defined metal fractions (Fig. 2). All reagents were prepared from reagent-grade chemicals and doubly-deionized water. Similarly, triplicate trials were performed in all of the analyses.

**Environmental quality indices**

Quantitative interpretation on the potential impacts of different metal ions is an integral part as far environmental assessment is concerned. Various
pollution calculation methods were usually used in order to assess the heavy metal enrichment and the degree of contamination in sediments and other biota. In this particular study, eleven (11) different environmental quality indices were applied to assess the level of contamination of the four (4) sediment-associated metals in the lower portion of Butuan River. These indices covered the speciation/total content and individual and multi-element approaches. Calculations of these indices were adopted from these reported literatures (Muller 1969, Buat-Menard et al. 1979, Hakanson 1980, Perin et al. 1985, Ikem et al. 2003, Priju et al. 2006, Qingjie et al. 2008, Yaqin et al. 2008, Rabee et al. 2011, Varol 2011, Zhao et al. 2012, Banu et al. 2013, Nwankwoala 2013, Asrari 2014, Aktaruzzaman et al. 2014, Zhuang and Gao 2014).

Results and discussion
Small and coarse particles were observed to be present in all of the sediment samples. The sediment particle sizes were monitored by sieving it using 0.850 mm mesh sieve. It has also been observed that some samples were made up of sand-particles, as expected, due to deposition of siliceous sand derived from long-shore water current. As noted, sediments are generally the sinks for metals in aquatic environment and constitute in an enriched metal aquatic environment and comprises an enriched metal pool that can be potentially accumulated by the benthic animals (Campbell et al. 1988).

**Fig. 2.** Schematic diagram showing the extraction and fractionation process.

**Total digest concentrations of Cd, Pb, Zn and Mn**
The total digest concentration of all metals (Cd, Pb, Zn and Mn) expressed in mg/kg is graphically represented in Fig. 3. As shown, the total digest concentrations of Cd across all sampling stations are almost comparable which ranges from 0.622 to 2.44 mg/kg and 0.733 to 2.48 mg/kg for sampling 1 and sampling 2, respectively. On the other hand, similar results were obtained in the total digest concentration of Pb across sampling stations for both sampling 1 and 2 except in stations 2 (33.1 mg/kg) for second sampling period and station 5 (39.1 mg/kg) for the first sampling period. Similarly, the total concentration of Zn and Mn were noted to have similar results as well and doesn’t show any significant difference, except in station 11 (1.89 × 10³
mg/kg) for the second sampling of the total digest concentration of Mn.

It is also obvious in the figure that the second sampling period has notably higher values than the first sampling period for all metals across sampling stations. Plausibly, this may be due to the difference in weather conditions between sampling periods. It should be noted that the typical season during the first sampling period was moderately dry, while maximum rain period is experienced starting December (Sampling 2). As reported (Gaur et al. 2005), high concentrations of all the metals were noticed in water and sediment in rainy season compared to summer and winter. Probably because in rainy season runoff from open contaminated sites, agricultural field and industries, directly comes into the river without any treatment.

Significant amount of total Cd was likewise recorded in sampling stations 6, 7 and 8 for both sampling periods. It is a known fact that cadmium commonly contaminates the environment due to the presence of cadmium-containing materials in household and industrial wastes and due to bio-industrial manure. Farming was also noted to be one of the sources of cadmium contamination due to the application of phosphate fertilizers (Nicholson et al. 2003, Sundaray et al. 2011, Ling et al. 2012). In particular, farming activity was found to be the major activity in all of these sampling stations. Too, piggery and poultry establishments were known to be one of the major sources of income of the community in station 6 as well, which can be considered as a possible source of Cd, hence corroborates to the fact that considerable higher Cd results were obtained in the mentioned sampling stations.

Fig. 3B also showed that the total digest concentration of lead is maximum at station 5 (first sampling) and station 2 (second sampling). Station 5 (Bit-os) has an existing pumping station aside from few households with farming as a major activity, while station 2 (Bitan-agan) has at least 40 households with farming and fishing as the main source of income of the residents in the said area. To some extent, peeling-off the lead-based paint used in
the protective film inside the PVC pipes may degrade due to inappropriate disinfection and can cause the lead metal to leach into the river system. Also, galvanic corrosion on lead, copper and bronze pipes can contribute lead contamination. Similarly, use of fertilizers, irrigation, and early pesticides (lead arsenate) may increase the soil’s toxic load of elements and salts. Lead contamination can also be attributed as well from spill of leaded gasoline from fishing boats which then be distributed in the sampling sites (Akan et al. 2010, Ling et al. 2012, Selanno et al. 2014). On the other hand, significant amount of the total digest concentration of manganese (Fig. 3D) was recorded at station 13 (first sampling) and station 11 (second sampling). As noted, station 13 (Maug) is a residential area near Celebes Coconut Corporation, while station 11 (Bading) has an existing storage area of finished plywood (Pramac Plant). Likewise, it has been reported that the main anthropogenic source of manganese release to air are industrial emissions and released to water thru waste discharge from industrial facilities or as leachate from landfills and soil (Howe et al. 2004).

Fig. 4. Speciation and distribution pattern of (A-D) and (E-H) Cd, Pb, Zn and Mn for both 1st sampling and 2nd sampling periods, respectively.

Speciation and distribution pattern of Cd, Pb, Zn and Mn
The extraction process adopted in this study is the sequential extraction method modified from the reported extraction method (Tessier et al. 1979). This is basically based on the five operationally-defined host fractions: exchangeable, carbonate, Fe-Mn oxides, organic and residual fractions. The results from the sequential extraction process can provide information on the possible chemical forms of the desired heavy metals. The order of metal bioavailability is in the order: exchangeable > carbonate > Fe-Mn oxide > organic > residual with the assumption that the bioavailability is related to solubility (Tessier et al. 1979).

The distributions of different metal fractions are graphically shown in Fig. 4. As shown, significant
amounts of Mn was detected in exchangeable metal fraction (F1) for both sampling periods, while insignificant amount of Pb, Zn and Cd were detected in this fraction in all sampling stations. It has been widely accepted that metals in this fraction is considered to be labile, highly toxic and the most bioavailable fraction (Wang et al. 2010), which only shows that Mn metal can be considered to be more labile and most bioavailable as compared to other metals mentioned. Sources of Mn metal, as well as the other metals under study can be attributed to small-scale mining activities and smelting of the ore in the upper portion of the river, which includes acid mine drainage with soluble Pb (water pollutant), tailing from ore dressing (particulate water pollutant and soil pollutant) which oxidizes to release the soluble Pb (Salbu and Steinnes 1995). This fraction also includes the portion which is held by the electrostatic adsorption as well as those specially adsorbed (Sharmin et al. 2010). The amount of metals in this fraction indicated the environmental conditions of the overlaying water bodies. Also, metals in this fraction are considered to be the most mobile and easily available for biological uptake in the environment (Singh et al. 2005, Zakir et al. 2008).

However, the carbonate metal fraction (F2) was of negligible importance for Mn, Cd and Pb, while dominant contribution of Zn metal in this fraction was observed. The trend in Zn metal amount corroborates to the noticeable significant variations on the results obtained; particularly on the results for the first and second sampling periods. Variation in the obtained results might be due to rain-fed floods which substantially increase the potential toxicity of river water (Byrne et al. 2009). The principal source of metals and acidity during flood events is most likely the dissolution of efflorescent salts from small-

Fig. 5. Contamination and Ecological risk of individual metal in the sediment samples along the lower portion of Agusan River (A) Individual Contamination Factor (ICF), (B) Index of geoaccumulation (Igeo), (C) Enrichment Factor (EF), (D) Contamination Factor (CF), (E) Risk Assessment Code (RAC) and (F) Ecological Risk (ER).
scale mining at the upper portion of the river. The data obtained, moreover deviated from the previous reported study (Zerbe et al. 1999) which demonstrated that Pb metal was strongly bound to the carbonate fraction. Plausibly, there were other concomitant reasons that can be accounted for the obtained results; nature of the sediment matrix, environmental condition, laboratory protocols, among others. Perhaps, the calcite has a strong affinity to Zn metal but not for other metals under study (Pb, Cd and Mn). On the other hand, the obtained results agree with reported studies (Zerbe et al. 1999, Sobczyński and Siepak 2001) of which negligible amounts of Cd have a strong affinity with the carbonate fraction.

![Graphs showing comprehensive contamination and ecological risks of a given site (A) Global Contamination Factor (GCF), (B) Degree of Contamination (DC), (C) Pollution Load Index (PLI), (D) Global Risk Index (GRI) and (E) Risk Index (RI).](image)

The mineral oxide-bound metal fraction (F3) was observed to be highly important for Zn and Mn but insignificant to Cd and Pb metal. The results were in good agreement with the previous studies (Zerbe et al. 1999, Burton et al. 2005). This probably explains the high affinity of Zn and Mn metals to the amorphous oxide minerals and opposite case happened in Cd and Pb metals. In addition, this fraction was considered as one of the most important geochemical phases that affects the mobility and behavior of trace metals (Swallow et al. 1980, Petersen et al. 1993). The figure also showed that significant affinities of Mn and Zn were noted in this fraction. In contrast, Cd and Pb metals were found to be insignificant in this fraction. As discussed previously (Bordas and Bourg 2001, Sharmin et al. 2010), metals in this phase are sensitive to anthropogenic inputs and are unstable under reducing conditions. These conditions resulted to the release of metal ions to the dissolve fractions. Moreover, this fraction has been considered as a significant sink for trace metal in the aquatic system through various co-precipitation and adsorption mechanisms. The same trend was observed for the organic-bound metal fraction (F4). As shown, Zn and Mn metals were observed to be strongly bound in organic matters compared to Cd and Pb metals. Reason for the observed non-association behavior of
Cd and Pb metals to organic materials is not well understood. The result obtained is in contradiction with the results from some of the studies conducted stating that Cd is strongly adsorbed in the organic fraction and this can be possibly be attributed to some factors occurred during the analysis. In contrast, Zn and Mn metals showed significant association with the organic materials present in the sediment samples. It is therefore, believed that these metals may possibly form stable organic complexes.

Lastly, the residual metal fraction (F5) was found to be highly important to all metals under investigation. It has also been reported that this metal fraction is not available for biological or digenetic processes except for over a period of years. This fraction represents the metals which are notably fixed within the crystal lattice (Zakir et al. 2008) and expected to be chemically stable and biologically inactive (Sharmin et al. 2010). Likewise, metals associated in this phase were most likely to be occluded within the crystal structure of recalcitrant minerals (Burton et al. 2005) and corresponds to the lattice bound metal which cannot be remobilized at least under normal polluting circumstances (Pardo et al. 1990). Further, this metal fraction is very important geochemical phase for trace metal retention in uncontaminated settings (Burton et al. 2005). In ecotoxicological point of view, the greater the amount of trace metals present in this fraction, the smaller the chances that it can harm to the different biota because of the difficulty in the mobilization of trace metals that are occluded in the crystal lattice at normal condition.

Speciation results revealed that contamination of Zn metal is most likely due to anthropogenic sources, as significant concentrations were observed in the non-resistant fractions (sum of the first four fractions). In particular, noticeable amount of Zn metal was also reported that is strongly bound to carbonate fraction which indicates that pollution brought by this metal experienced a more recent pollution (Abdallah 2012). Likewise, Mn and Pb metals seem to be brought by a combination of lithogenic sources and anthropogenic sources while Cd maybe contributed from lithogenic in origin. Among of all heavy metals being investigated, much concern has been focused on the concentration of Cd metal due to its high toxicity (Qiao et al. 2013). Though, higher concentrations of
this metal have been found in the residual fractions, however, considerable amount of Cd metal has also been found in the exchangeable fraction (F1). This result suggests that appreciable amount of Cd could be remobilized in the environment due to minute changes of the environment conditions such as variations in pH or changes in the ionic strength, thus becoming more available to aquatic biota.

**Contamination Assessment and Evaluation of Potential Ecological Risk using Different Environmental Quality Indices**

The contamination and ecological risk degrees were summarized in Fig. 5. The contamination indices used were the individual contamination factor (ICF), index of geoaccumulation ($I_{geo}$), enrichment factor (EF) and contamination factor (CF). Similarly, risk assessment code (RAC) and potential ecological risk factor (ER) were used to evaluate the potential ecological risk posed by the different heavy metals being studied. As shown, Mn metal was found to be highly abundant in almost all of the sampling sites (Fig. 5A). As noticed, moreover, all of the sampling sites were notably contaminated with Cd metal (Fig. 5B-D).

In similar manner, RAC data (Fig. 5E) revealed that Mn, Pb and Cd metals posed low to high risk to the environment, while Zn was categorized to be at no risk. However, the results obtained from the ER (Fig. 5F) shows that among all the metals being studied; only Cd metal posed a very high ecological risk. It is noticeable, moreover that based on different indices being examined, it turns out that all the sampling sites were contaminated with Cd metal which is highly enriched and it showed very high ecological risk. To have a clear overview on the comprehensive risks of a given sampling site, global risk and pollution indices were also evaluated as shown in Fig. 6.

![Fig. 8. Clustering of different sampling stations.](image)

It can be observed that the results obtained from GCF (Fig. 6A) were different from that obtained from DC (Fig. 6B). Markedly, the GCF (speciation index) result shows that all sampling sites were only modestly (lowly) contaminated by the four metals, as the results were all below the critical line, except at station 7 (farming and fishing are the major activities and heavy populated area). On the other hand, DC (total content index) revealed that all the sampling sites were all moderately contaminated. The observed differences of the two indices have also been reported by other group (Zhao et al. 2012); however the results from DC are more severe over the GCF results. The same trend was also observed for the results obtained from GRI (Fig. 6D) of that obtained from RI (Fig. 6E). Results from GRI shows that all the sampling sites
were lowly contaminated but based on the speciation index, RI, some of the sampling sites, particularly sites 6, 7, 8, 11 and 13, were moderately contaminated. However, PLI data revealed that all sampling sites were highly contaminated by the four metals. This PLI is more often used index to evaluate the severity of pollution state in a given environment (Tomlinson et al. 1980, Wilson 2003, Priju and Narayana 2006, Rabee et al. 2011, Asrari 2014).

Principal component analysis (PCA) for heavy metals in sediment samples
To evaluate the interrelationships and the dynamics of the different heavy metals, PCA and clustering analysis was performed. Shown in Fig. 7 are the principal component loadings of different metals across sampling stations. As reflected in Fig. 7A, there were three components being extracted. As noted, Zn, Mn and Pb metals, particularly the one obtained in the second sampling period showed positive relation in the first component which constitute to 30.12% of the total variance. Similarly, PC2 which explained 21.69% of the total variance has a positive relation to Zn metal (first sampling) and Cd metal (second sampling) but having negative relation to Pb metal (obtained in first sampling period). The results suggest that an increase in concentration of other metals may lead to a decrease of Pb metal, particularly in the first sampling period. Variations in the obtained results have already been explained previously, as this maybe due to the difference in the weather conditions between the two sampling periods. Lastly, Mn metal (first sampling) showed positive relation while Cd metal (first sampling) showed negative relation in PC3 which explained 18.98% of the total variance. This means that this component increases when there is an increase in Mn concentration while there is a decrease in Cd concentration. Importantly, the result suggests that these two metals had different sources and geochemical activities.

On the other hand, the contribution of bioavailable fraction of metals in the sediment samples across sampling stations was also evaluated (Fig. 7B). As shown, there were also three components being extracted where PC1 explained the 38.27% of the total variance. This component revealed a very strong association of the non-bioavailable fractions and the total of bioavailable and non-bioavailable fractions (sum) of Zn and Cd metals. Likewise, PC 2 which explained 23.23% of the total variance showed strong positive relations to all fractions of Pb metals and also to the bioavailable fraction of Zn metal. However, this fraction showed negative relations to both the bioavailable fractions of Pb and Cd metals. Lastly, the significant variables in PC 3 (11.6%) which showed strong positive relations are the non-bioavailable and the sum fractions of Mn metal. Based on the component analysis, PC 2 is mostly comprises of the bioavailable fractions of metals, while PC 1 comprises the total fractions and non-bioavailable fractions. The results may further suggest that organic matters is of high significant effects in PC 2, which can also be explained that farming is the major activity in most of the stations that can be categorized in PC 2, such as Bit-os, Bitan-agan, Pangabugan and Bilay. As reported, organic matters not only solubilize the metal species by complexing the metal ions but also take out the metal ions from the solution (Sundaray et al. 2011). Similarly, hierarchical clustering analysis using nearest neighbor method produced five possible clusters (shown in the dendogram in Fig. 8). As noted, highly related stations were found on Groups I (stations 1 and 12) and II (stations 7 and 8). Possible contamination from a small mining operations and effluent sources from a saw mill establishment were commonly found in stations 1 and 12, respectively. Likewise, both stations 9 and 10 were located in highly dense residential and commercial areas while stations 7 and 8 were located in areas where farming is the major source of living. Similarly, stations 5 and 6 were residential areas and with pumping stations located nearby; stations 2 and 11 both have plywood manufacturing establishments. Lastly, Veneer Plant was situated near station 4; purely agricultural area with few households in station 3 and station 13 was located near at the mouth of Butuan Bay where local
Effluents input along the seashore were prevalent.

**Conclusion**

The current study showed that the total digest concentrations of heavy metals have varied results across sampling stations. It was noted, that the order of increasing total digest concentration of all the metals under study for both sampling periods 1 and 2 is in the following order (mg/kg): Cd < Pb < Zn < Mn. Further, the fractionation results showed that Cd metal has been observed to dominate in the residual fraction which follows the fact that the said trace metal lies mostly in the crystal lattice making it hard to be remobilized under normal condition. Likewise, Mn and Zn showed variable results; particularly in the residual and exchangeable metal fractions. On the other hand, most of the Pb metal was in the residual fraction and considerable amounts have also been found in exchangeable metal fraction.

Different environmental quality indices approach showed that Mn metal is highly abundant in both sampling periods across sampling stations while Cd metal was highly enriched among other metals being studied. Also, all sampling stations were highly contaminated as supported by the PLI data. Principal Component Analysis (PCA) revealed that Zn, Mn and Pb metals showed similarity in origin of which from anthropogenic or a combination of anthropogenic and lithogenic sources while Cd was from other origin, might be from purely lithogenic in origin. Contributions from anthropogenic sources were highlighted in the cluster analysis.

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