Speciation and Bioavailability of Trace Metals (Cd, Cu and Pb) in Marine Sediment Samples from Placer Bay near Manila Mining Corporation, Surigao City, Philippines

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ABSTRACT

Chemical speciation by sequential extraction method of Cd, Cu and Pb of sediment samples from Manila Mining Corporation is described in this study. Different extractants was employed during the extraction process to each of the defined host fractions; exchangeable, carbonate-bound, organic bound, Fe-Mn bound and residual metal fractions. Moreover, excellent metal recoveries for sequential extraction protocol as compared to the total digestion method were observed for Pb and Cd, while low metal recovery for Cu. Results on the speciation and bioavailability of the studied trace metals in the sediment samples showed that the extractability (potentially available metal fraction) follows the order as Pb > Cu » Cd. Likewise, the average residual fraction (nonavailable metal fraction) in all sampling stations was found to be equal to 29 %, 38 % and 88 % for Pb, Cu and Cd, respectively. Importantly, Pb was found to be widely distributed in all of the host fractions across all sampling stations, while Cu was more associated in the following fractions: Fe-Mn oxide bound metal fraction (33 %), organic-bound metal fraction (28 %) and in the residual fraction (38 %). Similarly, Cd metal was found to be significantly adsorbed in the residual fraction which suggests that Cd metal is less available in all of the sediment samples being studied. Results of this study may serve as a basis to the administrators in conceptualizing an effective framework for proper remediation and rehabilitation program in the area.

Keywords: chemical speciation, fractionation, total metal concentration

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1   Introduction

Environmental quality of coastal ecosystem is an issue of growing international concern and its quality can be assessed by the quality of its sediment. Studies have shown that contaminants in aquatic systems often have a strong tendency to associate with particulate material suspended in the water-column (Duruibe et al., 2007; Holum, 1995; Honeyman and Santchi, 1988; Mundo and Nacpil, 1998; Salbu and Steinnes, 1995; Tessier et al., 1979). Likewise, under appropriate hydrological conditions, this particulate matter can accumulate on the bed of coastal waterways, thereby representing a contaminant sink. The contamination of sediments by trace metals is of particular importance because of their widespread use and their non-degradable nature (Calmano et al., 1992). Importantly, the determination of heavy metals in sediments in their various physico-chemical forms can provide information essential to proper assessment of risks and long term conservation and management of a natural water system (Capangpangan et al., 2016). The different mechanisms by which trace metals can be introduced into the aquatic environment facilitate adsorption and accumulation into the sediments. Since metals released into an ecosystem tend to accumulate in sediments through various adsorption and precipitation processes, the analysis of sediments can provide information as to the type and amount of pollutants present in such systems. In both ecological and environmental perspectives, the chemical speciation of heavy metals in marine sediments is an important area of research, since chemical species that can potentially harm marine or aquatic life can be determined precisely. To a larger extent, data obtained from chemical speciation can also address the reactivity and bioavailability of metal species, which in turn can impact the environment. Reactivity is dependent on the metal's physico-chemical form which in turn will dictate its interaction with other components in the biota, and consequently, its bioavailability and toxicity. Hence, the availability of the metal species from sediments can be assessed by chemical speciation. Metal ions, in its various forms, are associated with different spots in the sedimentary matrix: adsorbed in superficial particles, carbonates, iron and manganese hydroxides; trapped by organic material or silicates; deposited as sulfides; etc (Tessier et al., 1979). As a result, most of the heavy metals present in the aquatic system are retained in sediment matter, where they accumulate and initiate participation in several chemical and biological cycle, and might affect the water column and trophic chain. The concentration and bioavailability of heavy metals in sediment samples would depend upon their chemical partitioning. Knowledge of the concentrations of these components provides a baseline data against which to interpret results of chemical analysis of heavy metal. Such information is also useful also in determining the sediment history for proper management and planning in the specific study area. Moreover, metal speciation information could provide also an insight into the possible metal-microbial interaction, since the measurements of total concentrations of these heavy metals provide inadequate data to allow the assessment of the bioavailability and toxicity of these metals. Importantly, fractionation and bioavailability data is essential to the environmental planners and administrators of

Manila Mining (MM). With such information available, proper remediation procedure can be easily implemented for clean-up or rehabilitation of the contaminated waters. In this study, different physico-chemical forms and bioavailability of lead (Pb), cadmium (Cd) and copper (Cu) in marine sediment samples in Placer Bay, near Manila Mining Corporation, Placer, Surigao del Norte, Philippines were assessed and evaluated. Also, this study aims to chactacterize and assess the extent and degree of trace metal contamination and to present a practical baseline assessment on the total concentrations and the availability of the identified heavy metals.

Materials and Methods

General

All chemicals and reagents used in this study were all analytical grades and were being used as received; HNO_3 (J.T. Baker), $MgCl_2$ (JT Baker), CH_3COOH (Lab-Scan), $NH_2OH.HCl$ (Scharlau), H_2O_2 (Scharlau), HCl (Lab-Scan) and HF (Merck). Doubly-deionized water was used in the preparation of all the reagents. All the materials were acid-washed for 24 hours before use to ensure that the reagents were free from contamination. Likewise, analysis of total Pb, Cd and Cu, as well as its corresponding metal fractions were analyzed using Flame Atomic Absorption Spectrophotometer (AAS - Perkin Elmer AAnalyst200) with an air/acetylene flame. Calibration curve was plotted by measuring the metal ion content of known external standard solutions (of the desired metals). To evaluate the method's reproducibility and efficiency metal spiking (percent recovery) and sample/control blanks were performed.

Study Area

Manila Mining Corporation (MMC) is the major industry in the province of Surigao del Norte and it operates and controls almost 2,000 hectares (has.) of prime mineral lands in the said province. It is situated in the municipality of Placer covering an area of 22,849 populations out of 70, 000 populations from the four municipalities of the said province. The area is characterized by low to high relief. The low relief areas are located near the shoreline and valleys while the high reliefs are those are covered by volcanic plugs and limestone outcrops. However, the southwestern and southeastern part of the area is defined by moderately high relief which reaches up to 400 m above sea level (asl.). The highest peak in the area of concern is west of Barangay Anislagan, Placer that shows 301 m above sea level (Figure 1).

Sampling

Sediment samples were obtained within the proximity of the mining site in Placer Bay from eight (8) sampling stations (pre-chosen by MMC) as shown in Figure 2. Surface sed-

iment samples (0-10 cm) were collected along the shoreline of Manila Mining Corporation by random pick-up (grab sampling) using a rust-free shovel from the eight sampling stations (marked S1-S8). Samples were emptied into a polythene bags and then immediately transported to the laboratory where large and plant debris were manually removed from the samples. The sediment samples were then wet-sieved through a sieving machine using 0.850 mm screen. It was then allowed to settle for 2 days before overlying, and the water was removed by suction and thoroughly mixed before analysis. Samples were stored at 4 °C before analysis.

Analysis

A previously reported standard sequential chemical extraction (Tessier et al., 1979) was employed in this study for the determination of the metal fractions. Wet weight samples were accurately determined and dried at 105-110 °C to determine moisture content (for mass correction). To determine the total metal content of Pb, Cd and Cu, approximately 0.5 g of dried sediment sample was digested with 20 mL aqua regia and then heated for 3 hours. Twenty millilitres (20 mL) of 0.08 M HNO_3 was then added to this solution and heating was continued until sample was nearly dry. The solution was then filtered and diluted to 100 mL with doubly deionized water. The sequential extractions on wet weight samples and the corresponding metal fractions (Figure S1) were directly conducted in a 50-mL polypropylene centrifuge tubes and centrifugation was done at ca. 6,000 rpm for 15 minutes. Washings of the residue were combined with supernatant and the total volume of each fraction was then made up to 50.0 mL with 0.08 M HNO_3 (to preserve the solution). Detailed procedure for each metal fraction extraction is reflected in the supplementary data provided (Figure S1).

Quality Assurance

Analyses of Pb, Cd and Cu were performed using Flame AAS (Perkin Elmer AAnalyst200) with an air/acetylene flame for all of the metals under study. Calibration curve was achieved and plotted with the prepared external standard solutions (of the desired metals) via the external calibration approach against which sample concentrations were calculated. To evaluate the method's reproducibility and efficiency, various quality assurance measures were observed. Each sediment sample was subdivided and subjected into three replicates sequential extraction as well as total digestions. Likewise, for method validation, metal spiking (percent recovery) (Supplementary data, Figure S2) was performed, of which a known volume of metal standards (Pb, Cd and Cu) were then digested following the same procedure as outlined in the digestion process for total metal analyses. Importantly, reagent blank was used throughout the experiment.

Results and Discussion

The results from the sequential extraction process can provide information on the possible chemical forms of the desired heavy metals. It appears that the extraction process used in this study is based on the five operationally defined host fractions: exchangeable, carbonate, Fe-Mn oxides, organic and residual. In agreement with the previous report (Tessier et al. 1979) with the assumption that bioavailability is related to solubility, the order of metal bioavailability follows the order: exchangeable > carbonate > Fe-Mn oxide > organic > residual.

Total Concentrations of Cd, Cu and Pb

The set of samples under study showed varied results on the total heavy metal concentrations as being determined by total digestion method. The variation in the concentration of all the metals under study (Pb, Cd and Cu) was shown in Figure 3. The total Pb concentrations in all of the different sampling stations were almost comparable, except for stations 6 and 4, which have 1251 mg/kg of Pb and 1110 mg/kg Pb, respectively.

Other sampling stations have varied Pb concentrations results, ranging from 485.1 mg/kg, 501.0 mg/kg, 532.2 mg/kg, 568.0 mg/kg, 890.8 mg/kg and 925.4 mg/kg for stations 1, 2, 7, 5, 3 and 8, respectively. The obtained results, moreover, corroborates with the fact that lead metal (Pb) has become ubiquitous in the environment (Drever 1997). Most often, elevated amounts of Pb in all of these sampling stations can also be attributed to the past inputs of Pb derived from various mining activities performed within the said areas, and may have been due to urban run-off and in any other anthropogenic sources as well; automobile fuels, paint and plumbing, batteries, alloys, among others. All sampling stations had considerable similar amount of Cu with the exception of station 2, (Cu content: of 131.4 mg/kg) (Figure 2). Station 7 had the highest Cu content of 793.0 mg/kg, followed by station 3 (780.3 mg/kg), station 4 (688.2 mg/kg), station 1 (683.2 mg/kg), station 6 (342.6 mg/kg), station 8 (335.1 mg/kg) and station 5 (330.3 mg/kg). Similar to the total Pb concentration, the elevated amount of Cu content in these sampling stations may had been due to the previous mining activities; mine drainage and all other anthropogenic sources: alloys, casting, tubing, gas and water piping.

Similarly, total Cd concentrations thru total digestion approach have also been performed. Generally, concentrations of Cd in all of the sampling stations were relatively similar. Results obtained revealed that in all of these stations, it has considerable smaller amount of cadmium metal as compared to the other two metals being studied. Further, station 1 had relatively higher cadmium content among all other sampling sites with 36.69 mg/kg Cd. Correspondingly, the order according to decreasing amount of Cd content in all of the sampling stations was of the order: 31.84 mg/kg > 30.09 mg/kg > 24.08 mg/kg > 20.24 mg/kg 18.55 mg/kg > 17.79 mg/kg > 13.10 mg/kg for stations 4, 6, 3, 8, 5, 2 and 7, respectively.

Chemical Partitioning Study of Cd, Cu and Pb

Assessment of trace metals in benthic sediments is necessary for risk and environmental monitoring, as it plays a major role in developing a sound management strategies for aquatic ecosystems; however, the total contaminant concentrations often provide a poor indication of environmental risk and insufficient in understanding the contaminant's behaviour. Knowing this fact provides the basis for conducting the study to understand the behaviour and partitioning of the heavy metals under study based on speciation measurements; identifying and quantifying the different species, forms or phases present in the sediment samples.

Metal Recovery for Sequential Extraction Method

The efficiency of the sequential extraction process was assessed by comparing the total digest concentrations of each metal; Pb, Cd and Cu to the sum of each individual fractions. Ideally, the total digest metal concentrations should be equal to the sum of the individual fractions of each metal. Shown in Figures 4(a), (b) and (c) are the comparisons of the results from the total digestion process and the sequential extraction method for Pb, Cd and Cu, respectively. There was an excellent agreement between the total and the sum of fractions of Pb and Cd sequential analysis as shown in the figure (Figure 4a-c). However, it was evident from Figure 4(c) that the extractability using the total digest approach and the sum of the individual fraction of Cu do not have an excellent correlation. The main reasons for this may have been due to the re-distribution of the Cu metal during the extraction process and incomplete dissolution of the target phase during extraction and the possibility of re-adsorption between extractions. To validate the reliability and accuracy of the method used, spiking (% recovery) with a known standard metal ion was done. A known amount of standard metal ion was added to the sample, and analyzed in the same manner as the un spiked sediment samples. The results showed that the amount of the standard metal ions that were being added in the solution was excellently recovered; $108.7 \pm 1.75\%$, $97.41 \pm 1.14\%$ and $95.10 \pm 2.84\%$ for Pb, Cd and Cu, respectively. These results demonstrate the accuracy of the sequential extraction process being adopted in this study (Supplementary Data, Figure S2).

Exchangeable Metal Fraction

To know the amount of the most mobile form of the metals, the exchangeable amount of sorbed metals in the sediments was determined. As shown in Figure 5, considerable amount of Pb was present in this fraction, which indicated that more Pb metals could be readily released to the environment and may be exchanged with various cations present in the sample matrix. On the other hand, there were undetectable or negligible amounts of Cd and Cu from this fraction. In the context of toxicity and environmental risk, this information can lead to an interpretation that Pb has a greater chance of polluting the environment

more readily than other either Cd or Cu. Though this may be true at some given conditions, the fact that trace metals retained in soil and sediments via electrostatic attraction to negatively charged surfaces must compete with major cations for outer-sphere sorption sites (Silveira et al. 2003, Violante et al. 2010) must also be taken into account in interpreting results such as herein reported. Taking into consideration the abundance of some major cations in seawater; Mg^{2+}, Ca^{2+}, Na^+, K^+, it is not surprising to note that negligible proportions of these metals (Cu and Cd) to be associated in this fraction. Importantly, other factors, such as ionic strength, salinity, and analytical operations involved in the sequential extraction procedure, that can possibly affect the observed non-conformance of the obtained results to the established theoretical concept, can also contribute to some losses of precision.

Carbonate-Bound Metal Fraction

Trace metals recovered in this fraction are associated with carbonate materials present in the sediments, hence, may be an important indicator of weakly sorbed metals on the surface of clay minerals, organic matter, and oxide minerals, as well as materials co-precipitated with carbonate minerals (Burton et al. 2005, Gleyzes et al. 2002). Figure 6 shows the distribution of trace metals being extracted from this fraction. It was evident that Pb dominated in this fraction, with insignificant amounts of Cd and Cu. Previous workers have found that Pb was found mainly as $PbCO_3$ in sediments (Zerbe et al. 1999). Further, the results suggest that the negligible amount (for Station 1) of Cd and Cu in this fraction was due to less bioavailability of these metals in the sediment samples, which was in good agreement with the reported studies (Burton et al. 2005, Xiangdong et al. 2000).

Fe-Mn Oxide-Bound Metal Fraction

The amount of toxic metal ions (Pb, Cd, Cu) bound on metal oxides (Fe and Mn) fraction on the aquatic sediment is an indicator of highly weathered samples that are rich in Fe and Mn-oxyhydroxides (Xiangdong et al. 2000). As shown in the graph (Figure 7), this fraction contains significant amounts of Pb and Cu in all sampling stations, which indicates that this sediment fraction shows strong preference to Cu and Pb. On the other hand, the amount of Cd in the metal oxide fraction was negligible. According to the reported studies (Benjamin and Leckie 1981, Bilinski et al. 1991, Burton et al. 2005, Forstner and Wittmann 1979) the predominant metal sorbents in aquatic systems are the oxide minerals along with organic matter. Further, it has been reported that the amorphous metal oxides have relatively larger surface area (with several orders of magnitude) as compared to the carbonate minerals. The high affinity of Cu and Pb in this sediment fraction might be due to the selective preference of Mn-oxyhydroxides to Cu thru covalent interaction (Nascimento et al. 2003) and the diffusion of the surface species into the micropores and their entrapment in microporous soild for Pb (Domanska 2008). This probably explains the

high affinity of Cu and Pb metals to the amorphous oxide minerals and the opposite case happened in Cd metal. Most of the studies dealing with the sequential extraction schemes failed to discuss deeply on the microscopic explanation pertaining to the adsorption of trace metals in the amorphous metal-oxide fractions. Henceforth, it is possible to explore on the quantitation of the affinity of these trace metals in the amorphous and crystalline metal-oxide fractions.

Organic-Bound Metal Fraction

Organic-bound trace metals are indicators of strongly bound metal ions in organic matters. As shown in Figure 8, organic-bound metal fraction contain significant amounts of Cu and Pb, but not Cd, indicating that trace Cu and Pb were strongly associated to organic materials in the sediment samples. The high amount of Pb and Cu in this fraction implied that such metals can easily complex with organic matter. Such strong affinity of Pb and Cu might be due to the high formation constants of the organic-Cu/Pb complexes (Ahmed et al., 2015; Holm 1990). Moreover, Cu can also form stable sulfide complexes (Nascimento et al., 2003; Xiangdong et al., 2000). Looking closely at Figure 8, samples from station 4 (S4) contained strongly adsorbed Pb in the organic-bound fraction, which corroborates with the nature of sediment sample where it was sampled. Sediment sample from this station was obtained from a mangrove area, which apparently contains high organic materials. The distribution of Cu in aquatic systems is mainly affected by natural organic matter such as humic materials (Tessier et al., 1979; Xiangdong et al., 2000). The strong adsorption of Pb in the organic- fraction (Figure 8) could be attributed to the greater stability of the organic-Pb complexes (Ahmed et al., 2015; Rafique et al., 2011). In contrast, the observed non-association behavior of Cd to organic materials is not well understood. However, the obtained result is in good agreement with some other reported paper stating that Cd was not detected in organic-bound metal fraction (Iwegbue et al., 2007). To better understand this physical phenomenon, thermodynamic studies on the association-dissociation of these metals with soils and or sediment samples with variations in organic materials content must be explored.

Residual Fraction

This fraction represents the metals occluded within the crystal structure of recalcitrant minerals. This phase corresponds to a lattice bound metal which cannot be remobilized at least under normal polluting circumstances (Forstner and Wittmann 1979). In the present study (Figure 9), significant amounts of Pb and Cu and moderate amount of Cd are associated in the residual fraction. It has been reported that this metal fraction is not available for biological or diagenetic processes except for over a period of years (Burton et al., 2005). 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 amount of trace metals present in this fraction, the less chances of pollution effect will be, because of the difficulty in the mobilization of trace metals that are occluded in the crystal lattice under normal condition.

Comparisons on the Different Metal Fractions of Pb, Cd and Cu

Comparison of the metal fractions in each metal is shown in Figure 10. Pb metal has uniform distribution across all metal fractions (Figure 10(a)), from F1–F5. Moreover, it is evident that sampling station 3 (S3) showed the highest Pb concentration associated in the carbonate-metal fractions. In all sampling stations, trace Pb was detected in the residual metal fraction of the sediment samples. As mentioned, this might be caused by the formation of stable complexes on the carbonaceous materials in the sediment sample. It is notable also from the Figure that residual metal fraction for trace Pb metal is uniformly distributed in all sampling stations. This will reflect on the rate of which Pb metal will be mobilized and will readily be released in the environment causing serious environmental problems. Furthermore, the results suggest that a considerable amount of Pb is tightly bound and occluded in a crystal lattice. Thus it is difficult for this trace metal (Pb) to be remobilized at a normal condition. Importantly, high amount of this metal present in this fraction reflects its geochemical origin. Similarly, the trace Cd (Figure 10(b)) in this fraction indicates that a significant amount of this metal is associated and obstructed in the crystal lattice-bound metal (residual fraction). On the other hand, undetectable and negligible amount of Cu metal has been extracted (Figure 10c) in all sampling stations. This result suggests that Cu metal is strongly sorbed in the sediment and or soil samples via electrostatic interaction in the outer-sphere sorption sites. Likewise, it is evident that insignificant amount of Cu metal has been extracted in the carbonate-bound metal fraction. Cu metal is believed to be strongly sorbed in the sediment sample by electrostatic interactions (Violante et al., 2010), thus it is less bioavailable. It is supposed that Cu metal forms unstable complexes with the carbonaceous material in the sediment sample and that significant amount of this metal has been widely distributed in the organic-bound metal fraction. This can probably be explained by knowing the fact that Cu metal has been known to be strongly adsorbed in an organic materials and forming a stable organic-Cu complexes.

Distribution and Bioavailability of Trace Metals in Sediment Samples

The summary of results in terms of the % extractability (distribution) of trace metals across all sampling stations and across all metal fractions is shown in Figure 11. The amount of potentially available Pb seemed to be evenly distributed across all sampling stations (Figure 11a). Likewise, significant amounts of potentially available Cu metal are present across all sampling stations (Figure 11c). Most of the fractions of Cu metal are being associated in the reducible fraction (F3) and in the organic fraction (F4). Previous

reports stated that Cu is strongly adsorbed in the amorphous mineral oxide. Likewise, Cu can easily complex with organic matters and have high formation constants of organic-Cu complexes. On the other hand, with the exception of station 1 (S1), negligible amount of potentially available Cd was found in all sampling stations, This results suggests that Cd metal is not readily available, because almost all trace Cd metal has been occluded in the crystal lattice in the residual fraction (F5, Figure 11b).

Conclusions

Sequential extraction protocol successfully provides information on the mobility and bioavailability of trace metals. The total digest metal concentration reveals high metal enrichment across all sampling stations, specifically in Pb and Cu while low metal enrichment has been observed for Cd. On the other hand, excellent metal recoveries for sequential extraction protocol compared to the total digestion method were observed for Pb and Cd, while low metal recovery for Cu. This suggests that the amount of remobilizable or potentially available metal as estimated by its chemical extractability was shown to considerably differ from the total metal content of the sediment sample. The speciation and distribution results, moreover, have shown that the order of extractability in the sediments from Placer Bay is shown: Pb > Cu >>> Cd. Likewise, the residual (non-available metal fraction) was found to be equal to 29% for Pb, 38% for Cu and 88% for Cd. Furthermore, Pb was found to be evenly distributed in all host fractions across all sampling stations, while Cu was more associated in the following fractions: Fe-Mn oxide bound metal fraction (33%), organic-bound metal fraction (28%) and in the residual fraction (38%). Likewise, significant amount of Cd was found to be more adsorbed in the residual fraction. It is noteworthy, that the use of sequential extraction protocol successfully provides information on the mobility and bioavailability of trace metals. This is one of the important considerations that need to be considered in assessing the ecotoxicological effect of the trace metal on the soil and or sediment samples. The results of this work can be of great help for environmental managers of Manila Mining for proper evaluation on the potential risk of the various metal host fractions of different metal ions. The potential environmental risk associated with different metal fractions can provide detailed information as to the potential mobility and toxicity of metal ions for proper risk evaluation and for appropriate remediation measures.

Acknowledgement

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Fig. 1: Map showing the entire Manila Mining Corporation
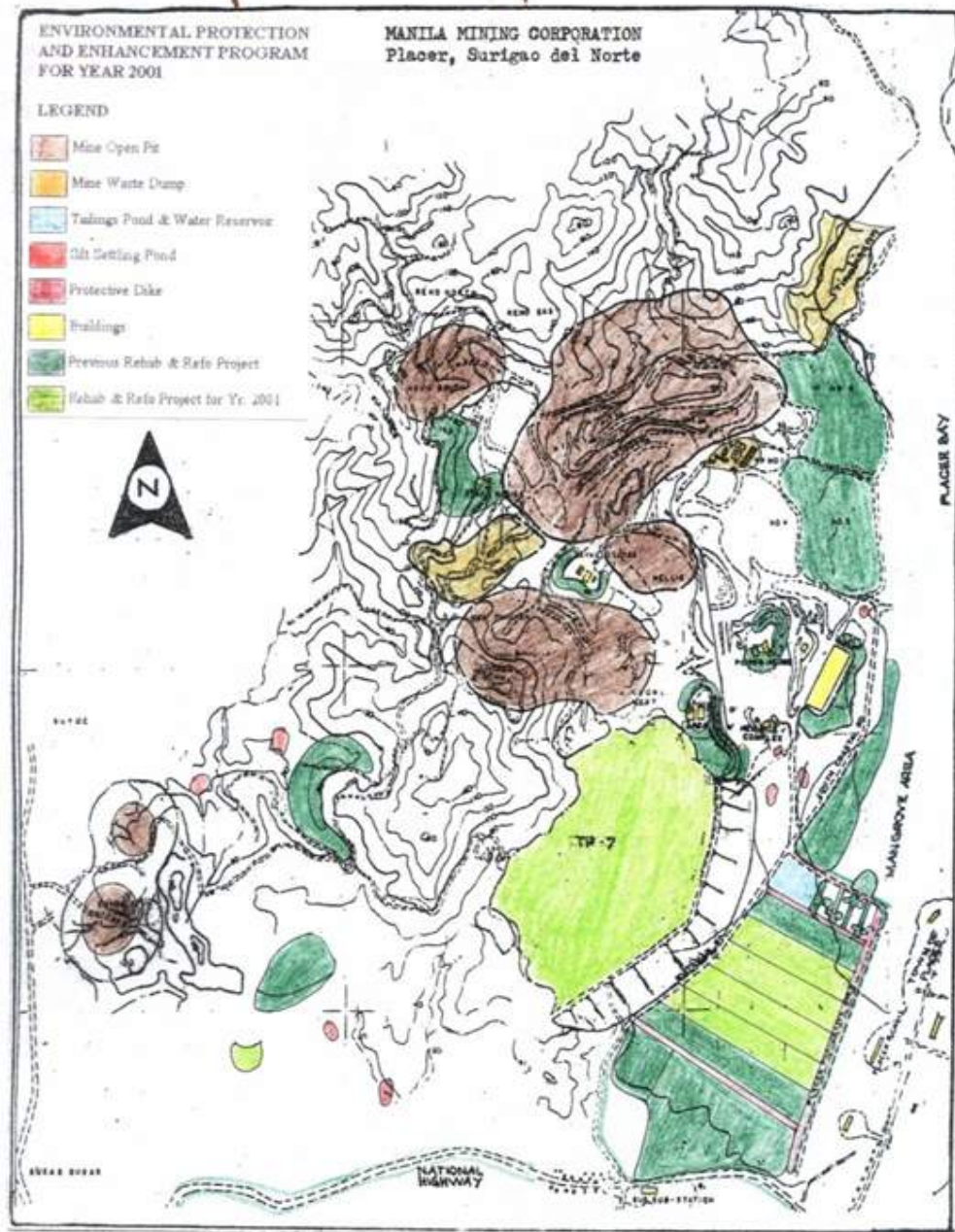
Fig. 2: Map showing the location of the different sampling sites

Fig. 3: Total Digest Concentration of Pb, Cd and Cu
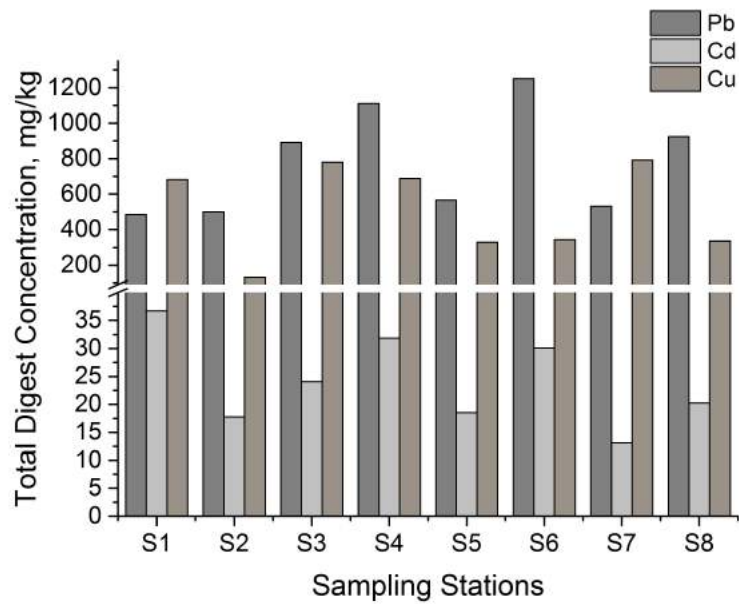
Fig. 4: Comparison of the total digestion method to the sequential extraction for (a) Pb; (b) Cd and (c) Cu
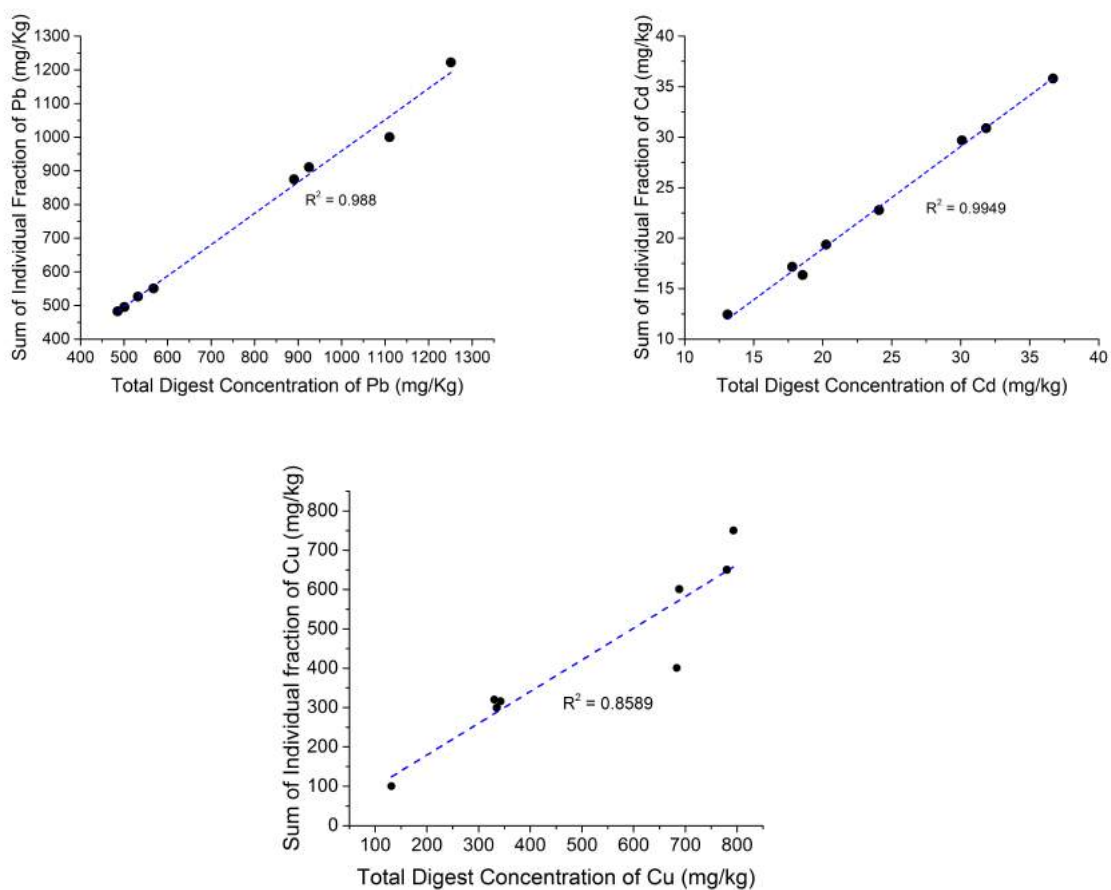
Fig. 5: Exchangeable metal fraction
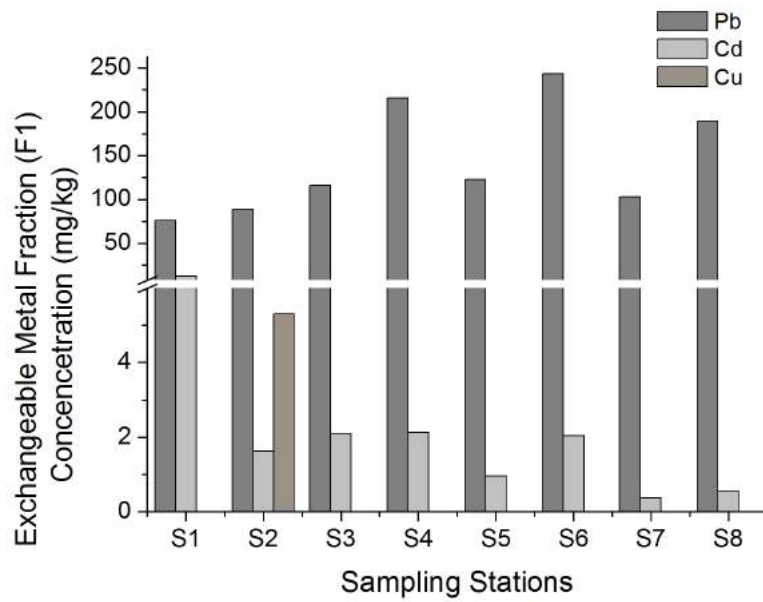
Fig. 6: Carbonate-bound metal fraction
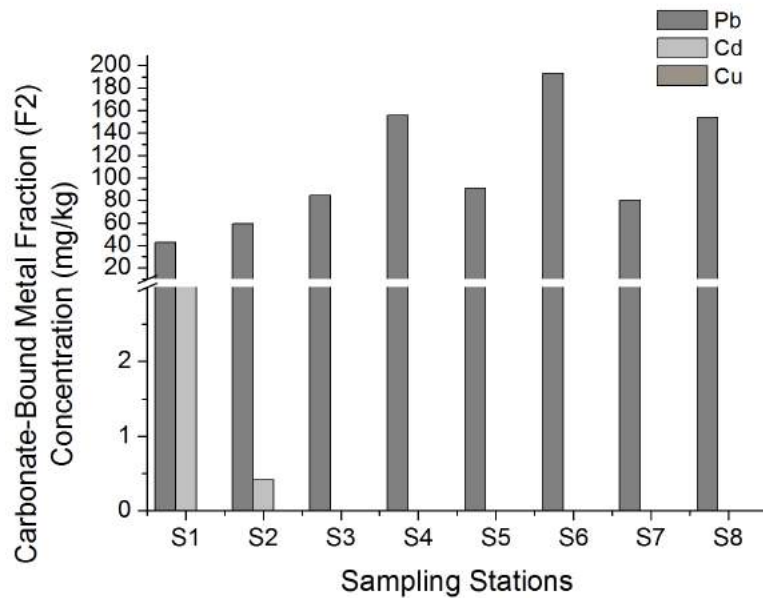
Fig. 7: Fe-Mn Oxide-Bound Fraction (F3)
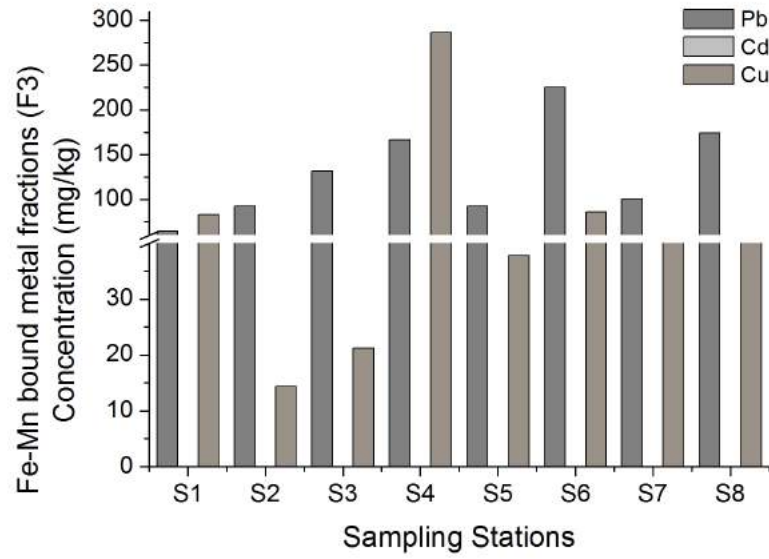
Fig. 8: Organic-bound metal fraction
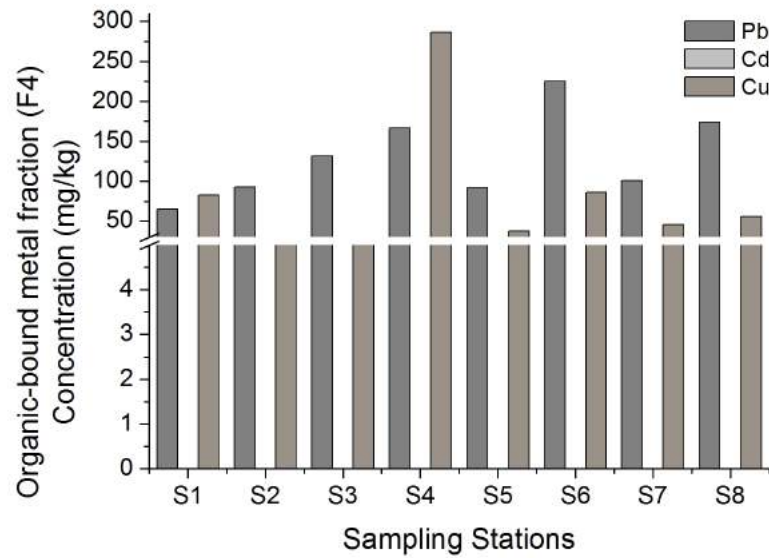
Fig. 9: Residual Fraction
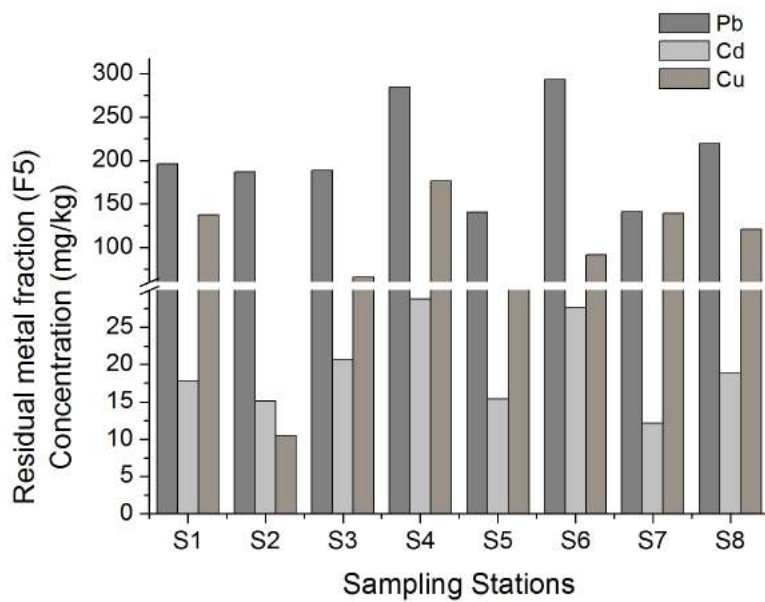
Fig. 10: Comparisons on the different metal fractions for (a) Pb, (b) Cd and (c) Cu
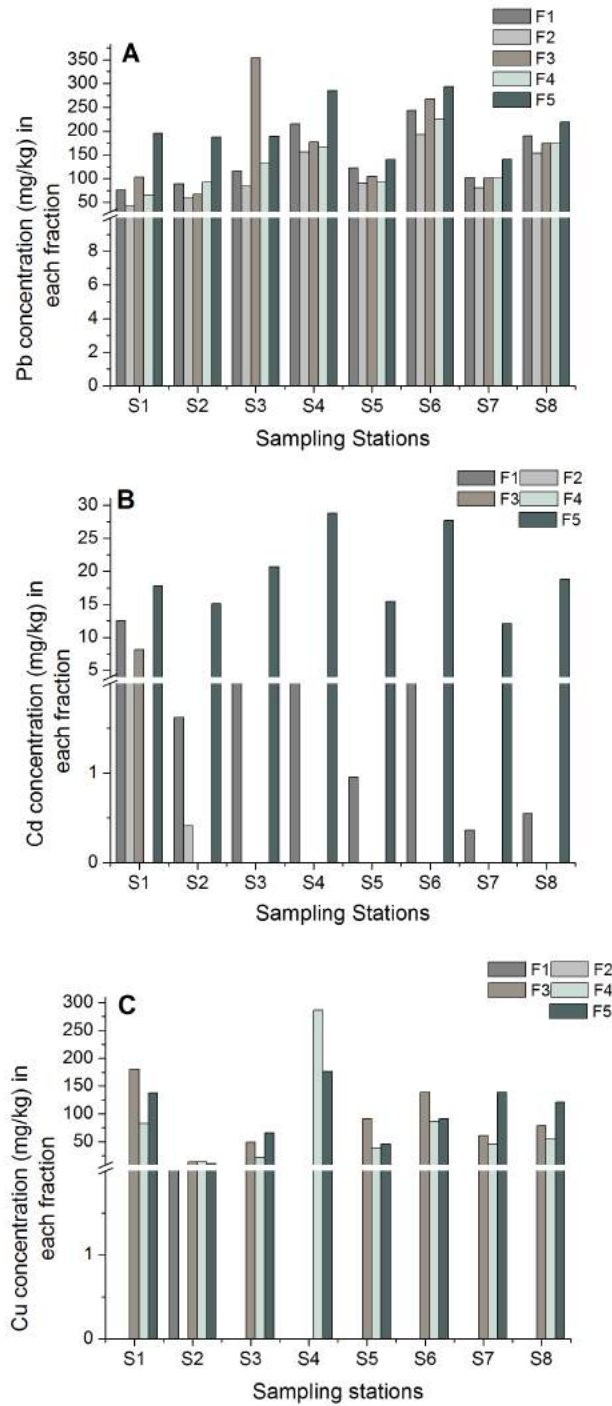
Fig. 11: Distribution and availability of metal fractions for (a) Pb, (b) Cd and (c) Cu
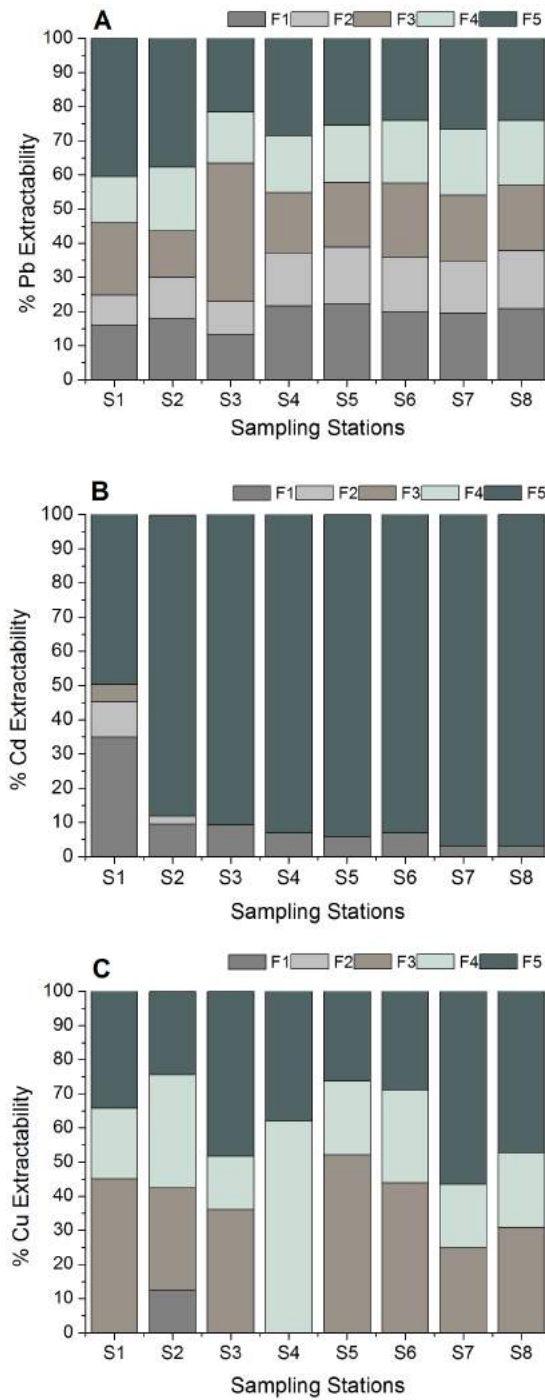
Fig. S1: Detailed schematic process for the extraction of five different metal fractions

```
                    1.0 g of Sediments sample
                              │
                    Polypropylene centrifuge tube
                              │
              Extract with 8.0 ml 1.0 M MgCl₂ at pH 7.0
                              │
           Agitate frequently at room temperature for 1 hour
                              │
               Centrifuge for 15 minutes at 6000 rpm
                              │
   Residue ◄──────────────────┴──────────────────► Supernatant

              Add 8.0 mL 1 M NaOAc, pH adjusted to 5.0        Fraction 1
                              │                        (water-soluble or exchangeable)
           Agitate for 5 hours with HOAc at room temperature
                              │
               Centrifuge for 15 minutes at 6000 rpm
                              │  Decant
        Supernatant ◄─────────┴─────────► Residue

          Fraction 2                Add 20 mL 0.04 M NH₂OH in 25% (ᵛ/ᵥ) HOAc
     (carbonate-bound                         │
      metal fraction)          Agitate in boiling water (95°C) for 5 hours
                                              │
                               Centrifuge for 15 minutes at 6000 rpm
                                              │  Decant
                      Residue ◄───────────────┴───────────────► Supernatant

        Add 5 mL, 30% H₂O₂ and 2 mL 2 M HNO₃                   Fraction 3
                    │                                   (Fe-Mn oxide metal fraction)
        Agitate for 3 hours at 85 °C
                    │
        Add 5 mL 3.2 M NH₄OAc in 20% (ᵛ/ᵥ) HNO₃
                    │
        Centrifuge for 15 minutes at 6000 rpm
                    │  Decant                           Residue
   Supernatant ◄────┴────►                       (Oven dry at 80 °C)

   Fraction 4              Add 2 mL HF and 16 ml Aqua Regia
                                        │
  (Organic Bound)      Heat in a water bath (85 °C) for 2 hours (under hood)
                                        │
                                   Cool and filter
                                        │
 Note:                    Dilute to 50 ml with 0.08M HNO₃
  1. Volume was made to 50ml               │
     volumetric flask with 0.08M HNO₃    Fraction 5
     (to preserve the sample fraction)
```

1.0 g of Sediments sample

Polypropylene centrifuge tube

Extract with 8.0 ml 1.0 M MgCl₂ at pH 7.0

Agitate frequently at room temperature for 1 hour

Centrifuge for 15 minutes at 6000 rpm

Residue — Supernatant

Add 8.0 mL 1 M NaOAc, pH adjusted to 5.0

Fraction 1 (water-soluble or exchangeable)

Agitate for 5 hours with HOAc at room temperature

Centrifuge for 15 minutes at 6000 rpm

Decant

Supernatant — Residue

Fraction 2 (carbonate-bound metal fraction)

Add 20 mL 0.04 M NH₂OH in 25% (ᵛ/ᵥ) HOAc

Agitate in boiling water (95°C) for 5 hours

Centrifuge for 15 minutes at 6000 rpm

Decant

Residue — Supernatant

Add 5 mL, 30% H₂O₂ and 2 mL 2 M HNO₃

Fraction 3 (Fe-Mn oxide metal fraction)

Agitate for 3 hours at 85 °C

Add 5 mL 3.2 M NH₄OAc in 20% (ᵛ/ᵥ) HNO₃

Centrifuge for 15 minutes at 6000 rpm

Decant

Supernatant — Residue (Oven dry at 80 °C)

Fraction 4 (Organic Bound)

Add 2 mL HF and 16 ml Aqua Regia

Heat in a water bath (85 °C) for 2 hours (under hood)

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Cool and filter

Dilute to 50 ml with 0.08M HNO₃

Fraction 5

Note:
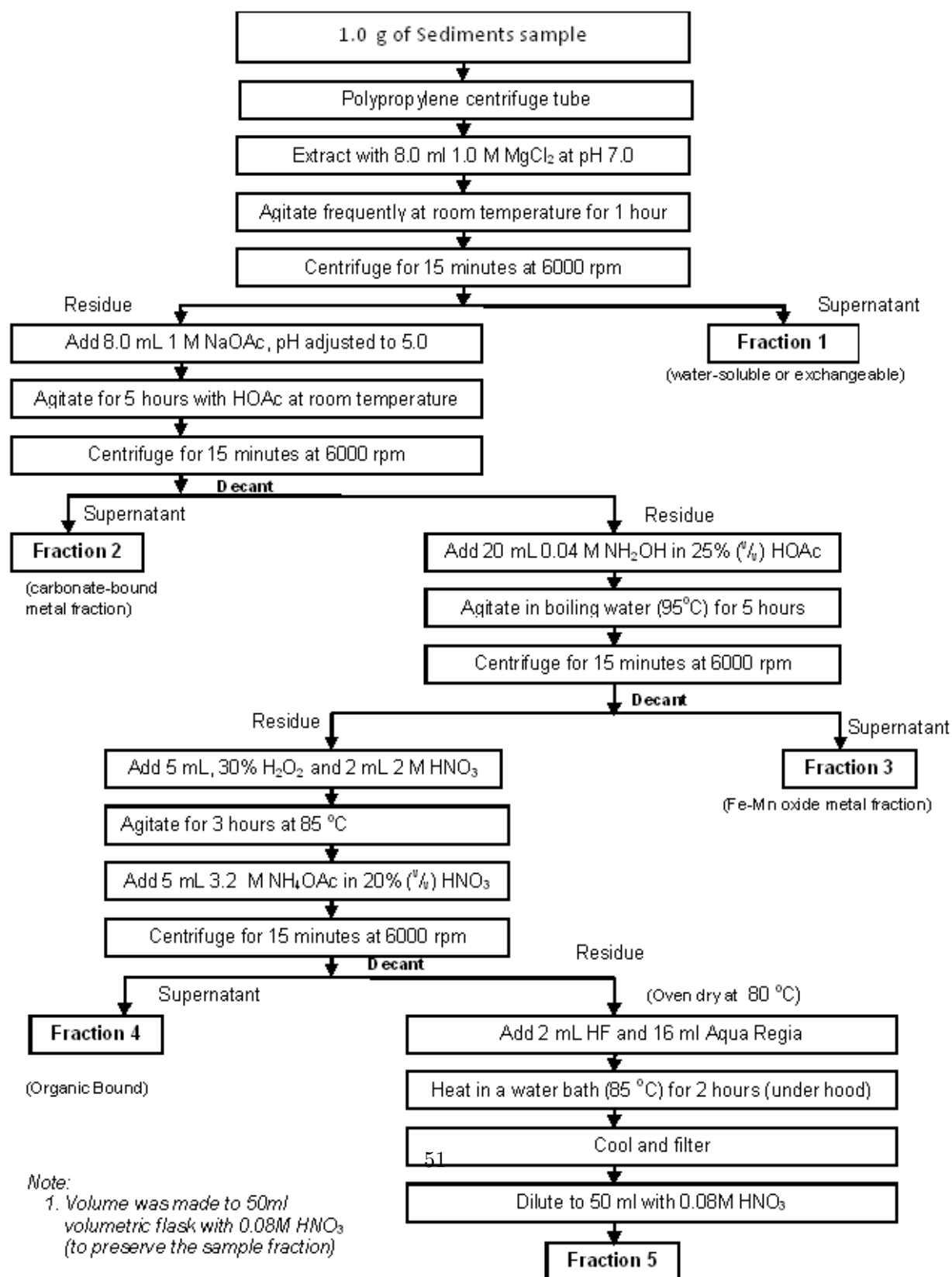 1. Volume was made to 50ml volumetric flask with 0.08M HNO₃ (to preserve the sample fraction)

Fig. S2: Percent recovery of Pb, Cd and Cu