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Full Length Research Paper

Prediction of extractable metals (Cd, Pb, Fe Cu, Mn and Zn) in sewage

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Sewage sludge are residues resulting from the treatment of wastewater released from various sources including homes, industries, medical facilities, street run off and businesses. It consists of nutrients and organic matter that can provide soil benefits and are widely used as soil amendments. Over several years, the inability to determine metal species in sewage hampers efforts to understand the mobility, bioavailability and fate of contaminant metals in environmental systems, to assess health risks posed by them and to develop methods to remediate metal contaminated sites. In an attempt to investigate the partitioning of some heavy metals (Cu, Pb, Fe, Cd, Mn and Zn) in sewage, the four stages of the Tessier sequential extraction method were employed. The effect of extractant concentration was also studied and found to affect extractability across the various fractions (reducible, oxidizable and residual). High level of total organic carbon coupled with the neutral pH of the sewage supports their potential agricultural benefits for agricultural land application. Across all samples, the sequence follows the pattern Cu>Fe>Zn>Pb>Mn>Cd. Greater percentage of all the metals occur in the oxidizable and residual fractions. The study shows that the metal ion binding to the biotic surface such as sewage is pH dependent and that there is an optimum pH for bioavailability of metals. The influence of the sludge pH and organic matter was observed only on copper ($r = -0.992$ at $p < 0.01$) and cadmium ($r = -0.970$ at $p < 0.05$) respectively with both displaying negative correlation. The concept might be a valuable tool in predicting quantitatively the metal ion sorption to plants present in a complex system and to predict the relative change in availability due to environmental changes.

Key words: Sewage sludge, heavy metals, sequential extraction, bioavailability.

INTRODUCTION

The high demand for agricultural products due to increased population has resulted in exploiting various means at improving on agricultural productivity. One economic way of achieving this involves the use of organic manure such as sewage. Sewage is generally characterized by an abundance of organic matter and nutrients it is often used in agriculture (Hani, 1991; Taylor et al., 1995; Sajwan et al., 1995; Basta, 1995, 2000). While applying sewage sludge, its advantageous effect on the physical and chemical soil properties is taken into account, including the increase in bioavailability of phosphorus, the increase in cation exchange capacity and supplying the soil with exchangeable ions of Ca^{2+} and Mg^{2+} (Cavallaro et al., 1993).

Even with the numerous roles that sewage could play in productivity, the use and addition of the sludge to soil for agricultural purposes is strictly limited by the presence of toxic compounds in its chemical composition, including some toxic heavy metals (Otabbang et al., 1997). Adequate knowledge to establish the degree of their solubility and mobility in the environment is possible only by taking into account various physical and chemical forms created by these elements. In this way it is also possible to determine their availability for plants uptake and potential for contamination of groundwater (Iwegbue et al., 2007).

The mobility of heavy metal combinations in sludge in terms of the rate of their release and potential negative effect on soil and plants is assessed using the sequential chemical extraction procedures; a scheme collectively referred to as speciation. Previous studies on heavy metals toxicity have based their report on total metals. Though, total metal content is a critical measure in assessing risk of a contaminated site, total metal content alone does not provide predictive insights on the bio-

Table 1. Chemical reagents and analytical conditions for the sequential extraction.

Step[a]	Operational Definition	Chemical reagents and conditions
1	Acid-extractable (AE)	To a 1-g aliquot, 40 ml of 0.11 M HOAc was added and shaken for 16 h at 22 ± 5°C; extract was separated from the solid residue by centrifugation at 3000 Xg for 20 min.
2	Reducible (RD)	To step 1 residue, 40 ml of 0.5 M $NH_2OH.HCl$ from a 1 - 1 solution containing 25 ml of 2 M HNO_3 (pH 1.5) was added and shaken for 16 h at 22 ± 5°C,then centrifuged at 3000 Xg for 20 min.
3	Oxidizable (OX)	To step 2 residue, 10 ml of H_2O_2 (pH 2 - 3), for 1 h at room temperature was added and heated to 85 ± 2°C for 1 h; a further 10 ml of H_2O_2 was added and heated to 85 ± 2°C for 1 h; 50 ml of 1 M NH_4OAc (pH 2) was also added and shaken for 16 h at 22 ± 5°C; The solution was centrifuged at 3000 Xg for 20 min.
4	Residual (RS)	To step 3 residue, 3 ml of distilled H_2O, 7.5 ml of 6 M HCl and 2.5 ml of 14 M HNO_3 was added and left overnight at 20°C. The final solution was later boiled under reflux for 2 h, cooled and filtered.

[a]Steps 1 - 3 are based on the protocol given in Rauret et al. (1999); for the residual fraction (Step 4) the source of the procedure used in this study was Ross and Filip (2003).
Step 4 differs slightly from the aqua regia digestion suggested by Rauret et al. (2000), which included 7.0 ml of 12 M HCl and 2.3 ml of 15.8 M HNO_3.

availability, mobility and fate of the metal contaminant (Hooda and Alloway, 1994; Shiowatana et al., 2001; Morillo et al., 2004; Burton et al., 2005; Zhang et al., 2007). For instance, a site with a total metal concentration of 300 mg Pb kg^{-1} is significantly more toxic than one which is 30 mg Pb kg^{-1}. However, suppose that the only species of Pb in the 300 mg Pb kg^{-1} sewage was galena (lead sulfide, PbS; $K_{sp} = 10^{-28.1}$), while the only species of Pb in the 30 mg Pb kg^{-1} sewage was cerussite (lead carbonate, $PbCO_3$; $K_{sp} = 10^{-13.13}$). Absolutely, from simple thermodynamic calculations alone, a greater concentration of Pb is likely to be both mobile under water transport and bioavailable in soluble form to organisms in the soil with the lower Pb concentration, because cerussite is vastly more soluble than galena.

Therefore, mobility of metals is clearly dependent on chemical form; an important factor in assessing human health or ecological risks. Speciation analyses have become an important topic of present day analytical research. From the perspective of risk assessment, speciation analyses are important for several reasons. It is part of an overall approach to understand the complex chemistry and behavior of contaminants in environmental and biological systems. A very good understanding of speciation result can be redirected into the design, selection, optimization and monitoring of remediation strategies applied to clean up a site, if necessary. As a result of the variation in the physico-chemical properties of sewage from various sources, differences do occur in metal composition (He et al., 2004; Wang et al., 2006).

The aim of the study is to determine the presence of some important heavy metals and forms of bioavailable metals in sewage and to evaluate what proportion of the metals measured are in the bioavailable fractions following the application of the four phase extraction procedures described by Tessier et al., (1979) and modified by Ross and Filip (2003). Although iron is not a heavy metal but it is equally determined in order to simplify the presentation of the results.

MATERIALS AND METHODS

Sample collection and preparation

Sewage samples were collected from four locations; two from Adekunle Ajasin University campus, that is, hostel (SH) and two from the University host community (TW) in Akungba, Ondo State, Nigeria. Sludge samples were taken from each location from heaps using an auger. After 5 - 8 individual samples were taken from one heat, they were all mixed together and one average sample was composed for analysis. The samples were preserved inside a polyethylene bags that were previously soaked in 10% HNO_3 for 24 h and then allowed to drain to dryness outside the laboratory environment. The collected material was prepared by drying, ground to pass through a 1.0 mm stainless steel sieve and stored at 4˚C for further analysis. The sewage sludge was analyzed for basic physico-chemical properties using standard procedures: pH - was measured with a glass electrode in 1 mol • L^{-1} KC1 (1:2.5 ratios) and Organic carbon - by wet dichromate oxidation with sulphuric acid.

Determination of heavy metals in sewage by sequential extraction

The method of sequential extraction used in this study followed literature guidance (Tessier et al., 1979; Rauret et al., 1999; Ross and Filip, 2003). The heavy metals were partitioned into four operationally defined fractions: acid extractable, oxidizable, reducible and residual fraction. The extraction was carried out in polypropylene centrifuge tubes of 50 ml capacity with an initial mass of 2.5 g oven dried (105˚C) fine fraction (< 1 mm) of the samples. The scheme below (Table 1) summarizes the procedure employed for the sequential extraction of metals in the sewage sample. In addition to the scheme below (Table 1), slight modifica-

Table 2. Total contents (µg/g) and standard deviation of metals in sewage (N = 3).

Site	pH	TOC (%)	Cd	Pb	Cu	Mn	Fe	Zn
SH-1	7.5 ± 0.1	37.3 ± 2.2	0.82 ± 0.13	0.71 ± 0.23	4.92 ± 1.20	1.46 ± 0.34	5.81 ± 1.04	1.82 ± 0.44
SH-2	7.7 ± 0.1	39.2 ± 2.4	0.73 ± 0.21	0.58 ± 0.23	4.36 ± 1.02	1.41 ± 0.41	5.87 ± 1.21	2.31 ± 0.52
TW-1	7.2 ± 0.2	44.6 ± 2.7	0.61 ± 0.11	0.88 ± 0.17	6.13 ± 1.01	1.11 ± 0.12	7.81 ± 1.24	4.72 ± 1.01
TW-2	7.3 ± 0.1	48.2 ± 2.9	0.59 ± 0.15	0.94 ± 0.18	5.91 ± 1.21	1.17 ± 0.13	7.11 ± 1.22	4.34 ± 1.04

tions were made on the reducible fraction on the concentrations of trioxonitrate V acid (HNO_3). This is due to the fact that reducible fractions of heavy metals constitutes potential risk to living, especially, because of solubility, in aquatic environment (Boughriet et al., 1992). Three different concentrations; 1.5 M, 2.0 M and 3.0 M of HNO_3 were used during the study. This was to evaluate the impact of pH on the bioavailability of metals in sewage especially when used in different soil samples as manures.

After each successive extraction, the supernatant was collected after centrifugation at 3000 r/min for 20 min, filtered through a 0.45 m membrane filter, and diluted to volume. The residue was washed with 10 ml of de-ionized water by shaking and centrifugation without loss of solids. Total metal content from a separate sample was analyzed to evaluate the performance of sequential extraction by digesting it with HF and $HClO_4$ (5:1). Heavy metal concentrations of all extracts were determined by flame atomic absorption spectrophotometry (Alpha 4AAS, Chemical Tech. Analytical Euro). All extractions were performed in triplicates and the mean values are presented with standard deviation. The obtained data were subjected to statistic analysis to evaluate the correlation coefficient (R) at two levels of significance ($p < 0.01$ and $p < 0.05$).

Quality Assurance (QA) / Control (QC) protocol prescribed by the U. S Environmental Protection Agency (EPA) for metal analysis was used. In this study, analytical precision through replicate runs and the use of certified standard reference material PACS-2 (heavily contaminated marine sediment available from the Natural Research Council, Canada) was employed. The results indicated that the percent recoveries are quite reasonable with 84% being the least recovered.

RESULTS AND DISCUSSION

Results

A summary statistics of the pH and TOC along with those of total metal contents in sewage sludge from four different sites are presented in Table 2. The pH across the study sites are above 7.2 while the TOC (%) ranges from 37.3 – 48.2 with the higher percentage in sewage from town. The results of the analysis shows that of all the metals examined in the sewage, the following sequences was obtained Cu>Fe>Zn>Pb>Mn>Cd. Similar trend was found in sewage across the various sites. The range in mean concentration (in µg/g) of the sequential extraction results of the sewage from hostel (town in brackets) are; acid extractable: 0.02 (0.04) – 0.21 (0.30); reducible: 0.12 (0.10) – 0.86 (1.13); oxidizable: 0.20 (0.15) – 1.90 (2.25); and residual: 0.13 (0.18) – 1.65 (2.14). The average percentages of extractable metals (at different concentrations of HNO_3 in the reducible fraction (that is, 1.5 M HNO_3, 2.0 M HNO_3 and 3.0 M HNO_3) belonging to each fraction are presented in a bar diagram in Figures 1 – 4.

DISCUSSION

Sewage characteristics and total metals

The results of this study showed that the investigated sewage sludges are characterized by varying chemical composition. Two major trends were observed; those from the hostel displayed similar trend while those from the community are equally similar. From the point of view of their agricultural usage, the contents of organic carbon and pH are essential. The pH values vary between 7.2 and 7.7 with sludges SH-1 and SH-2 rendering higher values compared with sludges TW-1 and TW-2. The levels of organic carbon varied considerably with the highest and lowest values being 48.2% and 37.3% respectively from TW-2 and SH-1. The pattern and levels in TW-1 is similar to those of TW-2. Similarly, SH-1 and 2 displayed very closely related levels of organic carbon, though slightly less than those of TW-1 and 2.

The increased level in TW-1 and 2 over SH-1 and 2 could be associated with the complexity of wastes emanating from the community compared to those found within the student hostel. The higher percentage of organic carbon from the community demonstrates higher rate of biodegradation; an important tool for increased organic matter content. Considering the role of organic matter and organic carbon in soil amendments, the high level as recorded in this study coupled with neutral pH support the viewpoint that sewage sludge has high potential agricultural benefits for land application (Walter et al., 1994; Fuentes et al., 2004). The influence of the sludge pH and organic matter was observed only on copper ($r = -0.992$ at $p < 0.01$) and cadmium ($r = -0.970$ at $p < 0.05$) respectively with both displaying negative correlation.

Several groups of elements are present in sewage sludge. However, heavy metals are undoubtedly the most crucial and at the same time the most controversial ones as those responsible for pollution. Data presented in Table 2 indicate that total content of Cu, Pb, Cd, Fe, Mn and Zn showed significant differences between the investigated sludge samples. Three of the metals; Cd, Pb and Mn are recorded at levels less than 1.0 µg/g. The differences in the metal concentrations were most pronounced in SH-1 and 2 which was characterized by

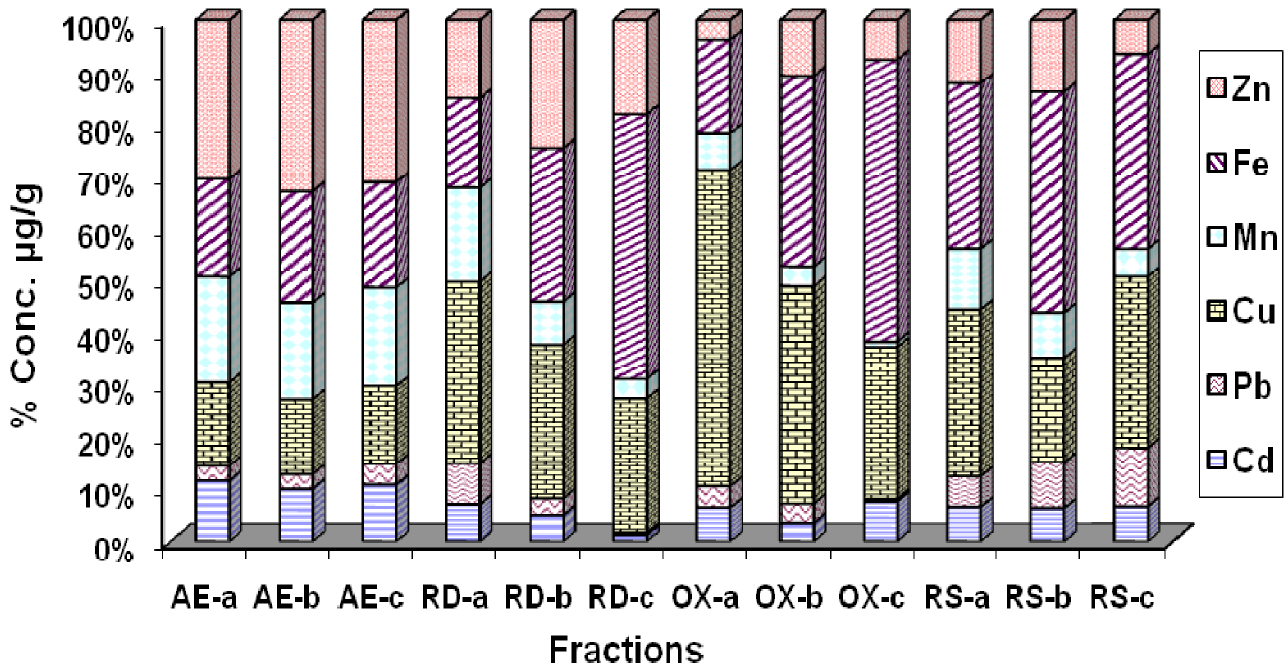

Figure 1. The distribution of various fractions of heavy metals SH-1 with pH- dependent concentrations of the reducible fraction. a, b and c represents respective concentrations when 1.5 M, 2.0 M and 3.0 M HNO_3 was used in the reducible fractions during extraction.

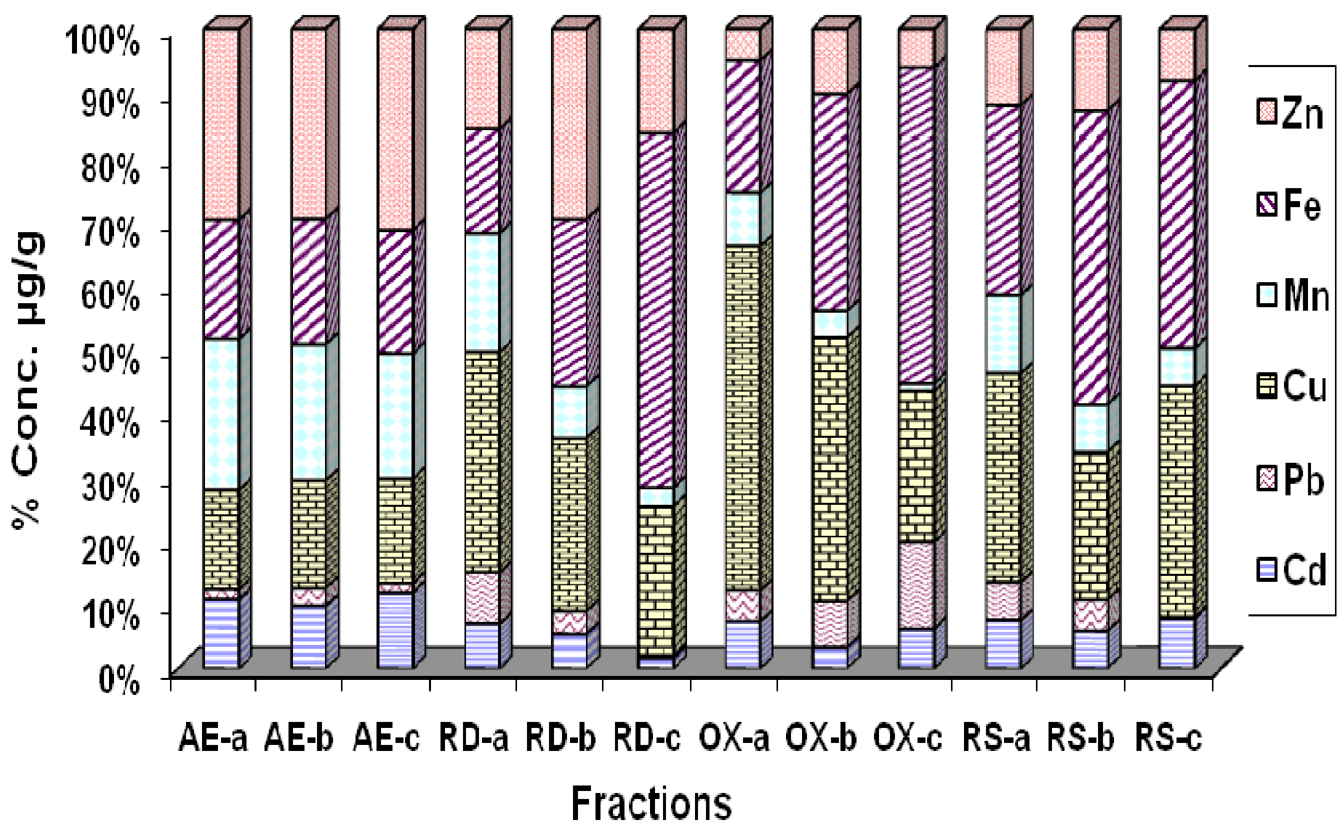

Figure 2. The distribution of various fractions of heavy metals SH-2 with with pH- dependent concentrations of the reducible fraction. a, b and c represents respective concentrations when 1.5 M, 2.0 M and 3.0 M HNO_3 was used in the reducible fractions during extraction.

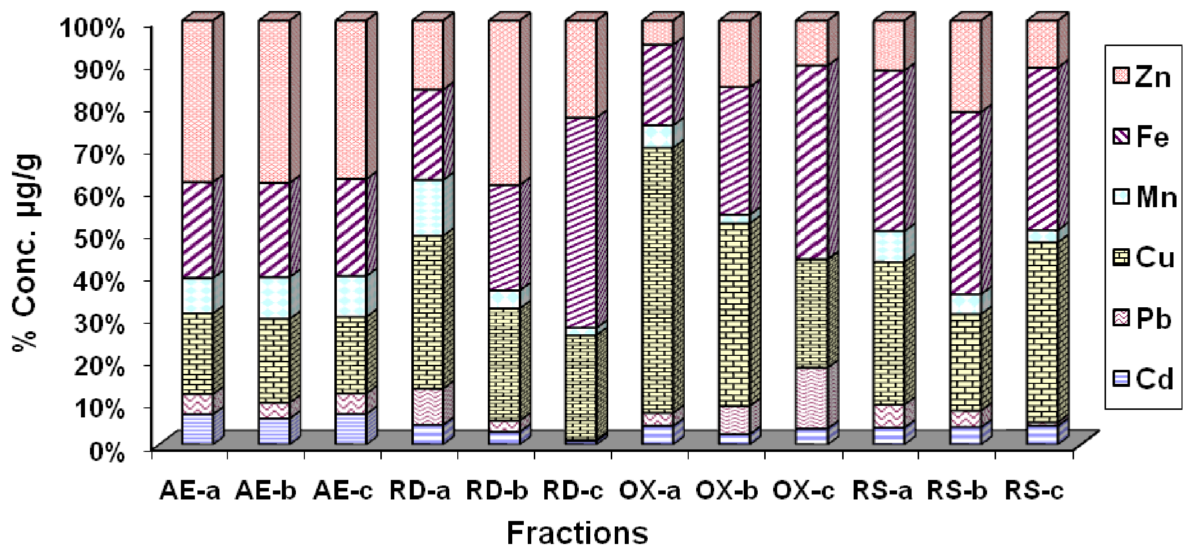

Figure 3. The distribution of various fractions of heavy metals TW-1 with pH- dependent concentrations of the reducible fraction. a, b and c represents respective concentrations when 1.5 M, 2.0 M and 3.0 M HNO_3 was used in the reducible fractions during extraction.

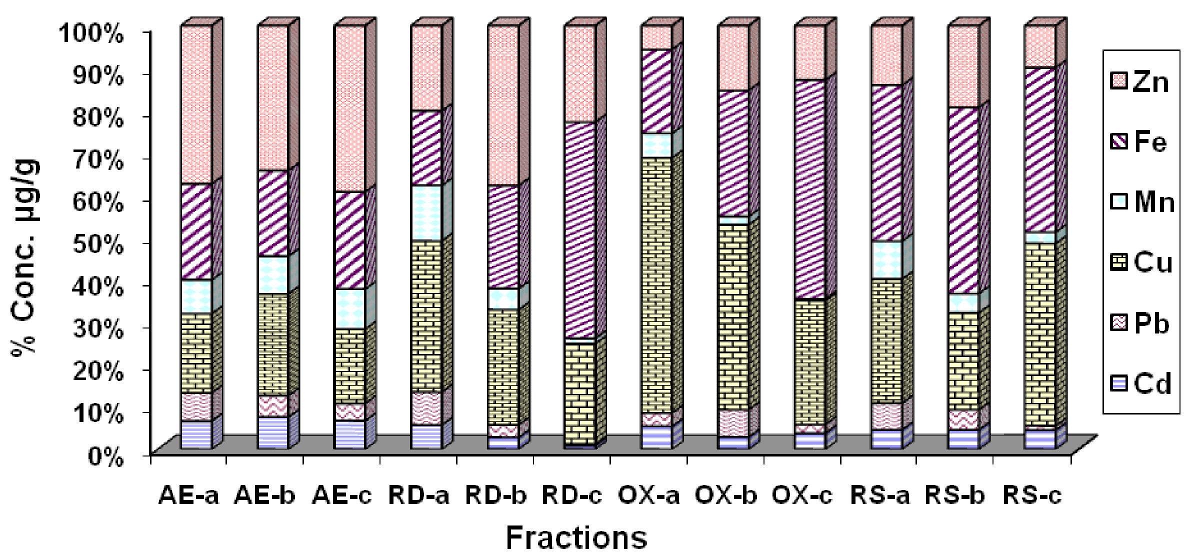

Figure 4. The distribution of various fractions of heavy metals in TW-2 with pH- dependent concentrations of the reducible fraction. a, b and c represents respective concentrations when 1.5 M, 2.0 M and 3.0 M HNO_3 was used in the reducible fractions during extraction.

high total contents of heavy metals

Sequential extraction

The conducted sequential analysis of metals revealed that in spite of the significant effect of the nature of these elements on the rate and direction of the formation of chemical combinations, the similarly dominant quantities of Cu and Fe were found in both the organic combinations as well as residual fraction. The preference for these two metals for organic matter is supported by the high stability constant of the metals complexes with organic matter (Gonzalez et al., 1994).

The metal levels in the oxidizable fraction which is associated with the organic matter are relatively high, particularly in Pb and Cu and they represent a large fraction of the average total metal concentrations in both SH and TW sewages. In this study, it is worthy to note that apart from Pb and Cu, the highest percentages of the extractable metals were recorded in the residual fractions from all the sewage sources and to a lesser extent in the exchangeable fractions in all the metals (Figures 1 - 4). Thus, the organic fractions in Zn, Fe, Mn and Cd account averagely for 21, 20, 21 and 34% respectively of the fractional sum of the metals without any significant difference ($p < 0.05$) between the various sample sources. In fact, regardless of the adjustment in the pH and sewage sludge sources, almost 70% of the concentrations occurred in the oxidizable and residual fractions (Figures 1 - 4).

The results suggest that majority of the metals might be linked to organic substances or be co-precipitated with oxides, carbonates and phosphates in sewage sludge (Zhang et al., 2007). A relatively higher percentage in the amount associated with carbonate for Mn and Fe and lower percentage for Cd and Zn as compared with the oxide fraction were recorded. It is concluded that Cd and Zn pollutants for both inputs may have been in both residual and oxidizable forms while those of Mn and Fe may probably be more in the residual and reducible forms. Among the various metals, the highest and least residual fractions were found in Fe and Cd respectively. This fraction of the metals represents the part that cannot be mobilized.

In addition, the based on the data in Figures 1 – 4, greater percentage of Cd and Cu are released under the strong oxidizing condition. This is very significant because, this fraction constitutes an important source of potentially available trace metals (Ure and Davidson, 1995). Significant positive correlation ($\alpha = 0.01$ and 0.05) were observed between some of the metals in samples from both the hostel and town (Table 3). Cadmium was found to be positively correlated with Pb, Cu and Mn while Pb equally displayed correlations with Cu, Mn and Mn.

Lead and Cd are two environmentally important heavy metals without any known biological function in plants, animals and humans. In this study, a very small proportion of Pb and Cd are found on exchangeable fraction while very high proportion lies within the oxidizable fraction. Thus, if pollutant Pb is being added to the soil in form of sewage as oxides, this suggests that some of this Pb is being adsorbed onto the organic material. These findings is consistent with previous reports on the behavior of Pb in contaminated soils, for which there is little evidence of downward movement, with accumulation on the surface (Thornton, 1981).

In a study of Spanish soils, Ramos et al. (1994) also found that most Pb was associated with the oxide fraction, with only very little amount in the exchangeable fraction. Similar findings were reported by Dudka et al. (1990) and Chlopecka (1993). It thus shows that the oxide fraction in sewage sludge is capable of scavenging Pb. This is indeed very good for farmers as it reduces the chances of Pb phytoextraction. However, this may also depend on other factors such as pH and organic matter.

The variability in concentrations of the reducible fractions due to changes in the pH of HNO_3 in this fraction displayed some trend in the concentrations of not only the reducible fraction but also the oxidizable and residual fractions. Iron demonstrated increase in concentrations with increased pH of the extractant (that is, HNO_3) in the reducible and oxidizable fraction across the entire sludge. However, Mn and Pb displayed a decrease in concentration with increased pH. No regular pattern was observed with the other metals in their reducible and oxidizable fractions. It is worth noting that not only were there differences among metals in the preferential formation of combinations, but also such phenomena occurred in the individual sludge (especially from those with similar sources) for the same element.

Table 3. Correlation matrix of heavy metals (N = 24).

	Cd	Pb	Cu	Mn	Fe	Zn
Cd	1	.765**	.744**	.495*	.379	.136
Pb	.678**	1	.553**	.636**	.527**	.302
Cu	.823**	.504*	1	.462*	.250	.133
Mn	.497*	.448*	.369	1	.124	.203
Fe	.428*	.477*	.341	.138	1	.521**
Zn	.197	.235	.221	.161	.613*	1

Significant /r/ *($P < 0.05$); **($P < 0.01$.).
Hostel and Town Pearson coefficients are shown above and below the diagonal line respectively, N = 24 in each case (correlated values are in bold form).

A critical assessment of Figures 1 - 4 showed that certain trend were also noticeable in the residual fractions. For Cd, Pb, Mn and Fe, the residual fractions were observed to increase with increase in pH. The pattern in Figures1 - 4 also shows that the impact of pH is not only dependent upon the variation in the pH value but also on the nature of the metal involved. The observation above equally demonstrated that there is an optimum pH for bioavailability of metals. The non-regularity of the oxidizable and residual fractions with pH across the various metals is also indicative of the partitioning nature of some metals in different chemical environment.

Analysis of exchangeable combinations and bioavailable form of metals is vital both for the ecological and agricultural points of view. Such data generally revealed the possibility of their fast transfer to the soil solution and thus their high potential activity in the soil environment.

Conclusion

Heavy metals are dangerous not only to plants and animals but also to the entire environment. The present study on metal partitioning is particularly important because of the significant role that sewage plays in our agricultural system towards improving crop yields. Though, some of the metals such as Cu, Zn and Fe considered in the study are generally referred to as nutritional metals, at high concentration especially in the bio-available form, they can become toxic. Most importantly, two of the metals, Pb and Cd, are very toxic metals with no known biological function. Their levels (that is, Pb and Cd) in this study may be a sort of concern because when the sludge is applied to soils, may enter the food chain through plants or animals, contaminate surface and ground water and thus cause health hazards. This attempt of a pH-dependent approach, particularly in the reducible fraction has further expanded the scope of sequential extraction. Only through this kind of study can information be derived on the partitioning of metals in sewage and their eventual bioavailability for uptake by plants. However, the interaction mechanism related to the release of bio-available metals should be further studied in plant grown in soil amended with sewage sludge. This is hope to give a better understanding of the basic chemistry of wastes and their interactions with soil which will help sewage managers make intelligent decisions on the use of sludges on lands.

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