

Full Length Research Paper

Uptake of potentially toxic metals by vegetable plants grown on contaminated soil and their potential bioavailability using sequential extraction

Olayinka K. O.¹, Oyeyiola A. O.^{1*}, Odujebi F. O.¹ and Oboh B.²

¹Department of Chemistry, University of Lagos, Akoka-Yaba Lagos, Nigeria.

²Department of Cell Biology and Genetics, University of Lagos, Akoka-Yaba Lagos, Nigeria.

Accepted 8 June, 2011

In order to estimate plant available fraction of metals in three soils, three vegetable samples: *Amaranthus viridis*, *Celosea argentea* and *Corchorus olitorius* were grown on contaminated soils. Matured plants were harvested and analyzed for their metal concentration. The soil samples were collected before and after planting, acid digested and analyzed to determine the pseudo total metal concentration, and quantification was done using flame - atomic absorption spectroscopy (FAAS). To correlate metal accumulation by these plants with potential bioavailability of metals in soils, sequential extraction (SE) using the modified BCR technique was performed on the soils. A secondary soil reference material (GLAURM) was used for quality control. The results of the reference material showed that the found values were within three standard deviations of the target values for the elements determined in the *aqua regia* digests and for most steps of the SE. It was observed that each metal differed considerably in uptake. Of the plants investigated, *A. viridis* had the highest tissue concentration of Cd, Cr, Pb and Zn from the soils with concentration of 1.4, 5.2 and 3.2 mgkg⁻¹ of Cd, 76.5, 96 and 43.0 mgkg⁻¹ of Cr, 49.5, 20.0 and 27.4 mgkg⁻¹ of Pb and 78.1, 112 and 96.9 mgkg⁻¹ of Zn in the soil from sites A, B and C respectively. Results of SE showed that Cd and Zn were mostly present in the acid exchangeable and reducible fractions, where these metals were mostly taken up. Soil-to-plant transfer factor values decreased in the order Zn > Cd > Cr > Pb.

Key words: Potentially toxic metals, plant uptake, bioavailability, phytoremediation, sequential extraction.

INTRODUCTION

Potentially toxic metal (PTM) contamination in soil are widespread and contamination could be from geological sources or from or anthropogenic sources. The sources of these PTM (e.g Cd, Cr, Pb and Zn) include soil parent material, volcanic eruptions, fertilizers, pesticides sewage sludge, power station, automobiles, incineration of waste and waste disposal, metal smelting plants, mines etc (Ruley et al., 2006). The contamination of these toxic metals in agricultural land is a major concern. Potentially toxic metals in soil can bioaccumulate in plants and are transferred to the food chain where they raise human and

animal health concern. Once these potentially toxic metals are taken up by plants, they can enter the food chain and may be taken up by humans and animals leading to adverse health effect. Cd, Cr and Pb are of concern because they are toxic to plants and animals even in small concentrations; however Zn is an essential trace metal for plants and animal but can be dangerous at high concentrations (Wolnik et al., 1983). At high concentrations these metals exhibit chronic toxicity or carcinogenicity as well as fatality (Wolnik et al., 1983; Blaylock and Huang, 2000; Monni et al., 2000; Reeves and Baker, 2000).

It has been established that the total concentrations of metals in soil does not provide information about its bioavailability (Oyeyiola et al., 2010). The chemical form, in which such metals are present in soil, as well as the

*Corresponding author. E-mail: aaderonke8@yahoo.com. Tel: +2348033371174 or +23418521973.

physical and chemical characteristics of the environment can affect mobility, reactivity and availability for vegetal absorption. The accurate estimation of metal bioavailability in soils and solid wastes is becoming more important in risk assessments. Remediation efforts also acknowledge that total metal concentrations may not be the best predictors of metal bioavailability and phytoavailability (Menzies et al., 2007). Bioavailability of Cd, Cr, Pb and Zn to plants in metal polluted soils from dumpsites has rarely been investigated. As the concentration of metals increase, the chances of a phytotoxic response also increase. It is therefore important to estimate the concentration of bioavailable metals present in soils and correlating this to total metals in plants (Anawar et al., 2008).

According to Helgesen and Larsen (1998) to assess bioavailability of potentially toxic metals from a contaminated soil to a crop plant, the selection of an extractant which stimulates the plant-available fraction of the metal is important. A number of extractants have been applied for single extractions (McGrath, 1996; Quevauviller, 1998; Lu et al., 2003; Gupta and Sinha, 2006b; Menzies et al., 2007; Anawar et al., 2008), while a few for sequential extractions (Tessier et al., 1979; Rauret et al., 1999; Mossop and Davidson, 2003; Brunori et al., 2004; Joksic et al., 2005). Extractants used in single extractions are mainly to evaluate the exchangeable fractions of elements in soils and they include EDTA, DTPA, NaNO_3 , NH_4NO_3 , CaCl_2 etc. According to Menzies et al. (2007), neutral salt extractants such as 0.01 M CaCl_2 and 0.1 M NaNO_3 provide the most useful indication of metal phytoavailability, while Gupta and Sinha (2006a) observed a better correlation between extractable metals in different amendments of tannery sludge on soil using EDTA and metal accumulation in *Sesamum indicum* compared to NaNO_3 and CaCl_2 .

The application of sequential extraction has also been useful in the prediction of bioavailability and plant accumulation of metals in soils. The procedure involves the use of series of reagents to sequentially extract metals from soils. Different sequential extraction schemes have been proposed, and most of these include a number of steps between 3 and 8. One of the first and most applied sequential extraction procedure proposed is a five-step procedure published by Tessier et al. (1979). Several other procedures followed the Tessier procedure, and there is now a wide variety of sequential extraction procedures available based on different sequence of extractants and different operational conditions. The most recent sequential extraction protocol which also has a reference material is the 4-fraction modified BCR protocol by the SM & T program of the European Union. These potentially toxic metal fractions may be selectively affected by plant accumulation and a change in their proportions may give an idea of the mechanism responsible for metal uptake in soils (Gupta and Sinha, 2006a). This paper aims to (a) determine the potential bioavailability of Cd, Cr, Pb and Zn from soils (collected from dumpsites)

using the modified BCR sequential extraction scheme (b) determine the plant- available fraction of these metals and (c) determine the correlation between the potential bioavailable fraction and plant-available fraction.

MATERIALS AND METHODS

Instrumentation and reagents

Analyses were carried out on a flame atomic absorption spectrophotometer fitted with a hollow cathode lamp (Perkin-Elmer AA Analyst 200) using an air-acetylene flame. Sequential extraction was performed using 50-ml polytetrafluoroethylene centrifuge tubes, an IKA HS 260 basic reciprocating shaker, and an R-8C laboratory centrifuge. All chemicals used were of analytical grade and were obtained from VWR international (BDH). The working standard solutions for the calibration of the FAAS were prepared daily, from stock standard solutions (1,000 $\mu\text{g} / \text{ml}$) and distilled water was used for all dilutions. All the glassware used were soaked in 5% HNO_3 overnight and rinsed with distilled water before use. For quality control, a reference material, GLAURM, an urban soil secondary material prepared by participants in the EU URBSOIL project (Davidson et al., 2006) was used.

Soil sample preparation and digestion

Surface soils (0 to 5 cm) were collected from three sites in Lagos, Nigeria. Soil A was collected from a site where automobile spare parts and metal rods are sold, welding and smelting of metals also conducted. Soil B was collected from the Department of Metallurgy and Materials' University of Lagos dumpsite, while soil C was taken from a foundry in Ikeja where a lot of metal works, fabrication and moulding is carried out. The soils were collected randomly, mixed to obtain composite samples for each of the sites, into polyethylene containers. Each was homogenized, sub samples taken, air dried, and sieved to pass through a 2 mm stainless steel sieve (Endecott test sieve). The remaining soils were used for planting vegetable samples which were harvested after 8 weeks of planting. The pH of the soils in CaCl_2 was determined according to BS ISO 10390 (1995), using a Mettler Toledo pH meter and the organic matter content (%OM) was estimated by Walkley and Black (1934) method. The pseudo total metal concentration of Cd, Cr, Pb and Zn in the soils before and after planting were determined by digesting with aqua regia (3 HCl:1 HNO_3) on a hot plate. One gram of the soil was digested with 20 ml aqua regia for 2 h. After cooling, the digests were filtered into a 100 ml flasks and stored in plastic bottles prior to the analyses. The quantification of the analytes was done by FAAS (Perkin Elmer A Analyst 200 instrument with air-acetylene flame) under optimal conditions.

Sequential extraction of soils

This was carried out according to the method of Rauret et al. (1999):

(i) Step 1 (exchangeable and acid soluble fraction): 40 ml of 0.11 mol l^{-1} CH_3COOH was added to 1 g of air-dried soil in a 50 ml centrifuge tube, stoppered, and extracted by shaking at a speed of 23 rpm for 16 h at room temperature (overnight). The extract was separated from the solid residue by centrifugation at 3,000 rpm for 20 min, and the supernatant liquid decanted into a polyethylene container. The extract was stored in a refrigerator at a temperature of 4°C prior to the analysis. The residue was washed with 20 ml distilled water, shaken for 15 min and centrifuged for 20 min at

3,000 rpm. The supernatant was carefully discarded (without discarding any of the solid residues).

(ii) Step 2 (reducible fraction): 40 ml of 0.5 mol l⁻¹ hydroxylamine hydrochloride (adjusted to pH 1.5 by addition of a fixed amount of HNO₃) was added to the residue from step 1, and the extraction performed as in step 1 above.

(iii) Step 3 (oxidizable fraction): 10 ml of 8.8 mol l⁻¹ hydrogen peroxide was added in aliquots to the residue from step 2. The vessel was covered loosely and the contents digested at room temperature for 1 h with occasional agitation. It was then placed in a water bath, the contents digested at 85°C and the volume reduced to less than 3 ml. Another 10 ml of the hydrogen peroxide was added, and further heated to near dryness. Thereafter, 50 ml of 1.0 mol l⁻¹ ammonium acetate (adjusted to pH 2 with HNO₃) was added, and the extraction performed as in the previous steps.

(iv) Step 4 (residual fraction): The residue from step 3 was transferred into a suitable vessel and the metal content digested with *aqua-regia* (as described above for pseudo total metal content).

Planting of vegetables

Seeds of three vegetable plants –*Corchorus olitorius*, *Amaranthus viridis* and *Celosia argentea* were purchased from local markets and grown in plastic pots which contained 1 kg of soil. The seeds were grown in the three different soil samples and were watered 3 times a week with distilled water. The plants were allowed to grow for 8 weeks after which the matured vegetable plants were carefully harvested. The leaves were separated and thoroughly washed with distilled water. They were dried in the oven at a temperature of 35°C and the concentrations of Cd, Cr, Pb and Zn in them were determined. Soil samples beneath the roots were collected after harvesting the plants and digestion of the soils as well as sequential extraction were carried out.

Plant digestion

1 g of each portion of leaves of the vegetable samples (grown on the different soils) was weighed and digested with 10 ml of concentrated nitric acid. The digestion was carried out for 2 h after which it was allowed to evaporate to near dryness. The residue was then taken up in 1 M HNO₃ and allowed to cool. After cooling, the sample was filtered with Whatman filter paper (11 cm) into a 25 ml volumetric flask and made up to mark.

RESULTS AND DISCUSSION

Quality control

Secondary reference material GLAURM was used to assess the analytical performance during pseudo total digestion and sequential extraction. GLAURM is an urban soil secondary material prepared by participants in the EU URBSOIL project (Davidson et al., 2006). The found values (values obtained from experiments) (Table 1) were within three standard deviations of the target values for all elements determined in the *aqua regia* digests and for most steps of the sequential extraction except in a few cases like Cr, where the found values were below the detection limit of the instrument. The fractionation pattern of Cd in the secondary reference material was not

determined because the pseudototal metal concentration was found to be low. The precision of results was < 10% for pseudo total digestion as well as the sequential extractions, except for Cr (in steps 3 and 4) where the RSD values were 19 and 18% respectively.

Pseudo-total metal concentrations

The pseudo total metal concentrations in the sediment samples are presented in Table 2. The pH of the soil was observed to be in the range of 6.0 to 6.9, which indicates slight acidity, while the percentage organic matter was found in the range of 2.8 to 4.4. The concentration of metals found was in the range of 9.1 to 55.5 mgkg⁻¹ for Cd, 486 to 1080 mgkg⁻¹ for Cr, 583 to 903 mgkg⁻¹ for Pb and 685 to 969 mgkg⁻¹ for Zn. The relatively higher concentration of metals in soil B could be as a result of the higher organic matter content and the activities taking place at that site. Organic matter has been found to influence PTM adsorption in sediments. This effect is probably due to the high cation exchange capacity of organic material (Maxwell, 1986). The relative standard deviation was <10% in most cases, except for Cd in soil B and Pb and Zn in soil A which were 11, 12 and 13% respectively. The results indicate that the metals were evenly distributed and suitable for plant growth. The concentrations of Cd, Cr, Pb and Zn in soils A, B and C were higher than the recommended upper limits of metals in soils (Table 3).

Bioaccumulation of metals by vegetable plants

Table 4 shows the mean concentration of metals in the leaves of three vegetable samples grown on the three different soils (A, B and C). It was observed that the metal differed considerably in uptake from each other. Similar observation was reported by Sinha et al. (2006) who reported that accumulation of metals in plants grown on contaminated soil collected from Jajmau varied from one plant to another.

A. viridis was observed to have accumulated the highest concentration of the metals studied (except in a few cases) followed by *C. argentea*. The higher bioaccumulation of metals in *A. viridis* could be because of its larger leaves and shoot as compared to the other vegetables, hence there are more sites for absorption of metals. Mobility of metals by plants can be determined by calculating the transfer factor. According to researchers (Sauerbeck, 1991; Gray et al., 1999; Cui et al., 2004; Chojnacka et al., 2005; Intawongse and Dean, 2006):

$$TF = \frac{C_{\text{plant}}}{C_{\text{total-soil}}}$$

Where C_{plant} is the concentration of a metal in the plant

Table 1. Results of analysis of secondary reference materials GLAURM (mgkg⁻¹).

Result		Cd	Cr	Pb	Zn
Pseudo total	Target	<0.74	43.2 ± 3.0	387 ± 25	177 ± 11
Pseudo total	Found	<1.2	46.4 ± 5.0	340 ± 40	199 ± 15
Sequential extraction					
Step 1	Target	*	0.64	25.1	26.3
	Found	*	<4.3	21.3 ± 1.4	41.5 ± 2.7
Step 2	Target	*	3.14	235	15.4
	Found	*	<4.83	247 ± 22	17.6 ± 0.9
Step 3	Target	*	9.12	52.9	27.2
	Found	*	12.8 ± 2.4	61.4 ± 5.6	25.1 ± 1.5
Step 4	Target	*	27.8	73.3	118
	Found	*	18.9 ± 3.6	51.2 ± 4.5	93 ± 7.3

Found results are mean values ± standard deviation for n = 3 (pseudo total digestion) and n = 2 (sequential extraction); < indicates a result below the detection limit; * = not determined.

Table 2. Physicochemical properties and pseudototal metal concentration (mgkg⁻¹) of soil samples before planting.

Soil samples	pH	%OM	Cd	Cr	Pb	Zn
A	6.9	3.7	9.1 ± 0.3	524 ± 22	583 ± 72	685 ± 88
B	6.2	4.4	44.9 ± 5.2	1080 ± 48	903 ± 12	969 ± 25
C	6.0	2.8	55.5 ± 2.8	486 ± 42	605 ± 8	889 ± 36

Table 3. Total metal concentration of metals in uncontaminated soil (mgkg⁻¹).

Metal	Typical total metal content in uncontaminated soil	Recommended upper limit
Cd	0.5	3.0
Cr	2.0	400
Pb	5.0	250
Zn	80	300

DOE/NWC (1981), ADAS (1987).

material (dry weight basis) and $CF_{total-soil}$ is the total concentration of the same metal in the soil (dry weight basis) where the plant was grown. The higher the value of the TF, the more mobile/available the metal is. The transfer factor values for the metals for the different vegetables studied varied between the plants and the different soils (Table 5). Generally, the transfer factors were low. This may be because only the accumulation of metals in the leaves were studied more metals could have accumulated in the root. According to various researchers, the concentration of most metals studied were found to be more in the lower part (root) of the plants compared to the upper part (shoot) and this could be due to the complexation and sequestration of metals

in cellular structures (e.g., vacuole) in the plant and unavailable for translocation to the shoot (Singh and Sinha, 2005). Amongst the metals studied and based on the transfer factor, accumulation of metals in plant leaves was observed to decrease in the order Zn > Cd > Cr > Pb. This is similar to the findings of Sauerbeck (1991) and Intawongse and Dean (2006).

Fractionation pattern of metals in soils before and after planting of vegetable plants

Sequential extraction procedures as well as single extraction procedures have commonly been used to study

Table 4. Concentration of metals (mgkg^{-1}) in the leaves of vegetable samples.

Metals	Soil A	Soil B	Soil C
Cd			
<i>Amaranthus viridis</i>	1.4 ± 0.3	5.2 ± 2.2	3.2 ± 1.6
<i>Celosea argentea</i>	0.54 ± 0.24	4.1 ± 1.9	3.5 ± 3.2
<i>Corchorus olitorius</i>	0.78 ± 0.17	4.2 ± 0.8	4.2 ± 2.0
Cr			
<i>Amaranthus viridis</i>	76.5 ± 12	96 ± 9.6	43.3 ± 0.5
<i>Celosea argentea</i>	20.1 ± 11	79 ± 11.3	49.6 ± 12.2
<i>Corchorus olitorius</i>	21 ± 13	12 ± 6.1	17.7 ± 5.6
Pb			
<i>Amaranthus viridis</i>	49.5 ± 7.2	20.0 ± 4.8	27.4 ± 23.4
<i>Celosea argentea</i>	21.1 ± 2.5	6.1 ± 3.2	26.6 ± 8.9
<i>Corchorus olitorius</i>	36.2 ± 5.7	16 ± 8.9	11.4 ± 5.7
Zn			
<i>Amaranthus viridis</i>	78.1 ± 12.4	112 ± 28.5	96.9 ± 6.7
<i>Celosea argentea</i>	45.2 ± 15.2	91.8 ± 26.5	103 ± 14
<i>Corchorus olitorius</i>	38.2 ± 5.9	78.3 ± 25.5	53.6 ± 13.8

Table 5. Ratio of concentration of metal in plants to metal in soil (Transfer factor).

Metals	Soil A	Soil B	Soil C
Cd			
<i>Amaranthus viridis</i>	0.15	0.11	0.06
<i>Celosea argentea</i>	0.06	0.09	0.06
<i>Corchorus olitorius</i>	0.09	0.09	0.08
Cr			
<i>Amaranthus viridis</i>	0.15	0.09	0.09
<i>Celosea argentea</i>	0.04	0.07	0.10
<i>Corchorus olitorius</i>	0.04	0.01	0.04
Pb			
<i>Amaranthus viridis</i>	0.08	0.02	0.05
<i>Celosea argentea</i>	0.04	0.01	0.04
<i>Corchorus olitorius</i>	0.06	0.02	0.02
Zn			
<i>Amaranthus viridis</i>	0.11	0.11	0.11
<i>Celosea argentea</i>	0.07	0.10	0.12
<i>Corchorus olitorius</i>	0.06	0.09	0.06

metal mobility and availability in soils. There has been several reports on the use of single extraction methods to correlate plant available metals in soils (Brun et al., 1998; Gupta and Sinha, 2007; Anawar et al., 2008), but very little has been done on the use of sequential extraction to

correlate plant availability of metals. The fractionation patterns of the potentially toxic elements in the soils before and after planting the three vegetable plants, using the revised BCR sequential extraction are shown in Figure 1.

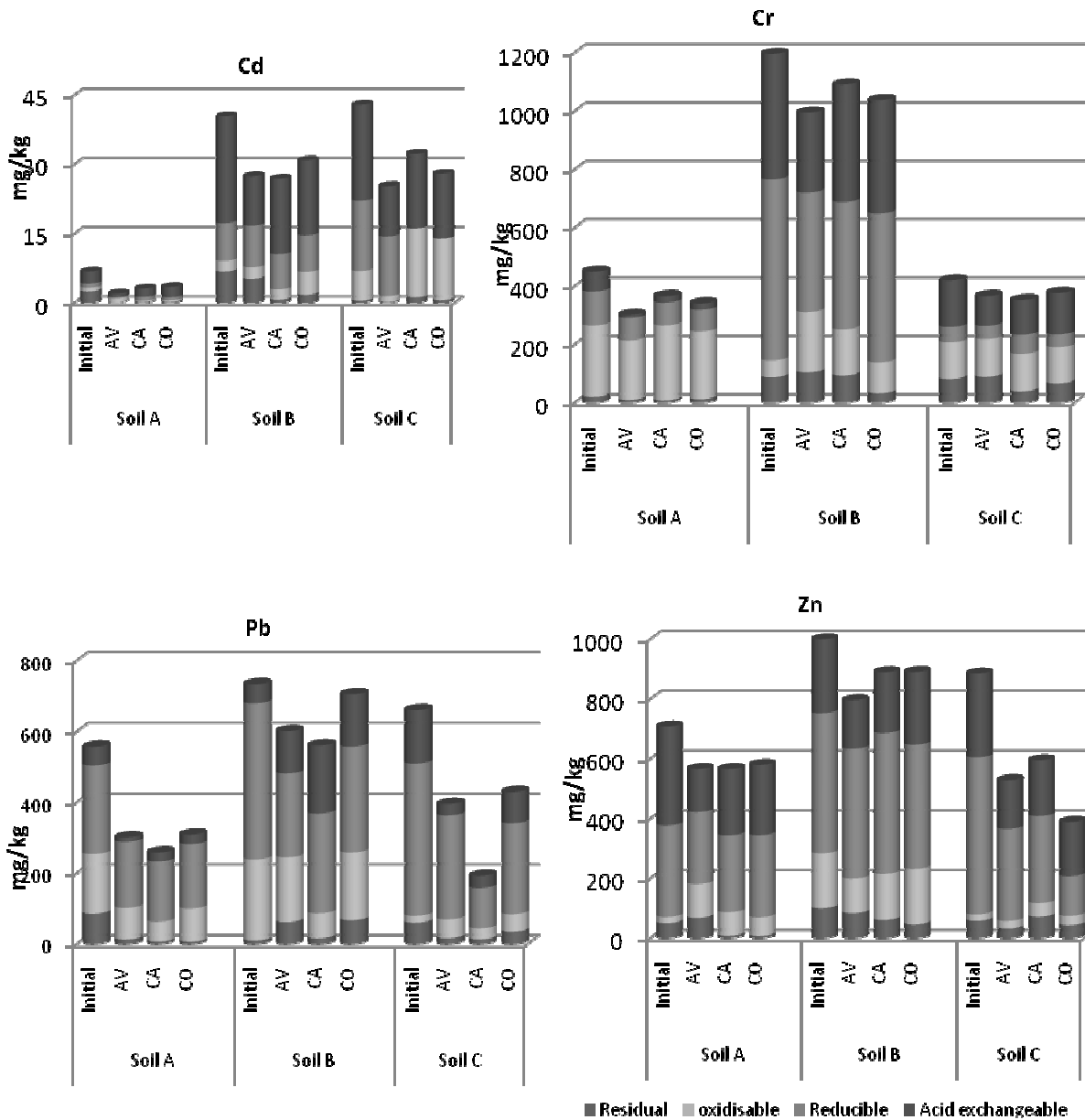


Figure 1. Fractions of metals in soil before and after planting of vegetable samples. Initial = SE of metals in soils before planting, AV = SE of metals in soils after planting *Amaranthus viridis*, CA = SE of metals in soils after planting, *Celosea argentea*, CO = SE of metals in soils after planting *Corchorus olitorius*.

For all the soils before planting, Cd was found predominantly associated with the exchangeable phase, but was also present in step 2, the reducible phase (Figure 1). This is of considerable environmental concern. The vegetable plants were observed to remove more Cd from these two phases. Cd being predominantly present in these two phases probably accounts for its higher accumulation in plants as compared to the other metals. Metals in these two phases have been said to be more mobile and bioavailable (Bacon and Davidson, 2008). There was a slight increase in the level of Cd in the

oxidisable fraction for some of the soil samples after planting the vegetables thus indicating a shift in the fractions. There was a shift of Cd from the residual phase to the oxidisable phase, thus becoming more bio-available.

In soil A before planting, Cr was observed to be mostly present in the reducible and oxidisable fractions while it was mostly present in acid exchangeable and reducible fractions in samples B and C. In the initial soils before planting of the vegetable plants, Pb was found to be associated with the four fractions, but the proportion varied

between the different soil samples. In all the soil samples, the highest percentage of lead was found to be mostly present in the reducible phase, though there was a significant percentage present in the oxidisable fraction for sample A and B and in acid exchangeable fraction for sample C. In sample A, the vegetable plants removed Pb mostly from the exchangeable fraction and the residual fraction, while in samples B and C, Pb was mostly taken up by the plants from the oxidisable fraction. A shift in fraction was observed for Pb in sample B. The amount of Pb in the exchangeable fraction of soil B, increased after the planting, thus making it more bioavailable and of environmental concern.

Zn was observed to be mostly present in the reducible fraction, but also significant in the exchangeable fraction. Just like Cd, the vegetable plants studied were observed to remove more Zn from these two fractions. Zn being predominantly present in these two phases probably accounts for its higher accumulation in plants as compared to the other metals. Generally, the amount of metals in the oxidisable fraction of the soil after planting the vegetable samples reduced a little or increased due to a shift in the fractions. This may be because this fraction is associated with organic matter which is a factor that reduces the availability of metals to plants due to metal-organic complexation (Gupta and Sinha, 2006a). According to researchers, metal organic complexation decreased metal mobility in soils (Van Erp and Van Lune, 1991; Udom et al., 2004). It was observed generally that there was no correlation between the plant accumulation and bioavailability as determined by sequential extraction.

Conclusion

Three vegetable plants planted on metal polluted soils were investigated for their metal accumulation. *A. viridis* was observed to have the highest uptake of the metals studied followed by *C. argentea*. It was observed that the higher the concentration of metals in soil, the higher the uptake of metals by the vegetables. The metal bioavailability from soil to plant based on the transfer factor was observed to decrease in the order $Zn > Cd > Cr > Pb$. Sequential extraction of soil before planting of the vegetable samples showed that Cd and Zn were the most potentially bioavailable as they were mainly present in the first two fractions (acid exchangeable and reducible fractions). There was no particular fractionation pattern for Cr and Pb, but the fractionation pattern depended on the soil. The vegetable plants studied were observed to remove more Cd and Zn from the first two fractions, thereby reducing the threat of the polluted soils to humans and surrounding water bodies. The amount of Pb in the exchangeable fraction of soil B, increased after the planting of the plants, thus making it more bioavailable and of environmental concern. Generally it was observed

that planting of the vegetable sample caused a change in the fractionation pattern of the metals in the soils investigated and there was no correlation between potential bioavailability and accumulation by plants. It was observed from this study, that individual plant types differ in their metal uptake.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Christine Davidson of the Department of Pure and Applied Chemistry University of Strathclyde, Glasgow for supplying the secondary reference material and also the assistance of Agidi Ruth, Idialu Ofure, and Sanusi James for their assistance in carrying out this research.

REFERENCES

- ADAS (1987). The use of sewage sludge on agricultural land. Booklet MAFF Publications, Alanwick. p. 2409.
- Anawar HM, Garcia-Sanchez A, Regina SI (2008). Evaluation of various chemical extraction methods to estimate plant-available arsenic in mine soils. *Chemosphere*, 70: 1459-1467.
- Bacon JR, Davidson CM (2008). Is there a future for sequential chemical extraction? *Anal.*, 133: 25-46.
- Blaylock MJ, Huang JW (2000). Phytoextraction of metals. In: Raskin, I., Ensley, B.D. (Eds.), *Phytoremediation of Toxic Metals: Using Plants to Clean Up Environment*. John Wiley and Sons, New York, pp. 53-70.
- Brun LA, Maillet J, Richarte J, Herrmann P, Remy JC (1998). Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils. *Environ. Pollut.*, 102: 151-161.
- Brunori CL, Galletti M, Cremisini C, Morabito R (2004). Comparison of three sequential extraction procedures (original and modified 3 steps BCR procedure) applied to sediments of different origin. *Ann. Chim.*, 94: 409-419.
- British Standard (BS) (1995). Determination of pH. BS 7755-3.2 1995 ISO 10390.
- Chojnacka K, Chojnacki A, Gorecka H (2005). Bioavailability of potentially toxic metals from polluted soils to plants. *Sci. Total. Environ.*, 337: 175-182.
- Cui YL, Zhu YG, Zhai RH, Chen DY, Huang YZ, Qiu Y, Liang JZ (2004). Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. *Environ. Int.*, 30: 785-791.
- Davidson CM, Urquhart GJ, Ajmone-Marsan F, Biasioli M, Costa Duarte A, Diaz-Barrientos, E et al (2006). Fractionation of potentially toxic elements in urban soils from five European cities by means of a harmonized sequential extraction procedure. *Anal. Chim. Acta*, 565: 63-72.
- DOE/NWC (1981). Report of the subcommittee on the disposal of sewage sludge to land. Standing Technical Committee report. Dept. Environ., London. p. 20.
- Gray CW, McLaren RG, Roberts AHC and Condron LM (1999). Cadmium phytoavailability in some New Zealand soils. *Australian J. Soil. Res.*, 37: 791-796.
- Gupta AK, Sinha S (2006a). Chemical fractionation and potentially toxic metal accumulation in plant of *Sesamum indicum* (L.) var. T55 grown on soil amended with tannery sludge: Selection of single extractants. *Chemosphere*, 64: 161-173.
- Gupta AK, Sinha S (2006b). Role of *Brassica juncea* L. Czern. (var. Vaibhav) in the phytoextraction of Ni from soil amended with fly ash: selection of extractant for metal bioavailability. *J. Hazard. Mater.*, 136: 371-378.
- Gupta AK, Sinha S (2007). Assessment of single extraction methods for the prediction of bioavailability of metals to *Brassica juncea* L. Czern.

- (var. Vaibhav) grown on tannery waste contaminated soil. *J. Hazard. Mater.*, 149: 144-150.
- Helgesen H, Larsen EH (1998). Bioavailability and speciation of arsenic in carrots grown in contaminated soil. *Anal.*, 123: 791-796.
- Intawongse M, Dean J (2006). Uptake of potentially toxic metals by vegetable plants grown on contaminated soil and their bioavailability in the human gastrointestinal tract. *Food Addit. Contam.*, 23: 36-48.
- Joksic AS, Katz SA, Horvat M, Milacic R (2005). Comparison of single and sequential extraction procedures for assessing metal leaching from dredged coastal sediments. *Water, Air, Soil. Pollut.*, 162: 265-283.
- Lu A, Zhang S, Shan X-q, Wang S, Wang Z (2003). Application of microwave extraction for the evaluation of bioavailability of rare earth elements in soils. *Chemosphere*, 53: 1067-1075.
- Maxwell JA (1986). *Rock and mineral analysis*. Interscience, New York. p. 584.
- McGrath D (1996). Application of single and sequential extraction procedures to polluted and unpolluted soils. *Sci. Total Environ.*, 178: 37-44.
- Menzies NW, Donn MJ, Kopittke PM (2007). Evaluation of extractants for the estimation of the phytoavailable trace metals in soils. *Environ. Pollut.*, 145: 121-130.
- Monni S, Salemaa M, Millar N (2000). The tolerance of *Empetrum nigrum* to copper and nickel. *Environ. Pollut.*, 109: 221-229.
- Mossop KF, Davidson CM (2003). Comparison of original and modified BCR sequential extraction procedures for the fractionation of copper, iron, lead, manganese and zinc in soils and sediments. *Anal. Chim. Acta.*, 478: 111-118.
- Oyeyiola AO, Olayinka KO, Alo BI (2010). Comparison of three sequential extraction protocols for the fractionation of potentially toxic metals in coastal sediments. *Environmental Monitoring and Assessment* DOI 10.1007/s10661-010-1336-4.
- Quevauviller P (1998). Operationally defined extraction procedures for soil and sediment analysis I, Standardization. *Trends Analyt. Chem.*, 17: 289-298.
- Rauret G, Lopez-Sanchez JF, Sahuquillo A, Rubio R, Davidson C, Ure A, Quevauviller P (1999). Improvement of the BCR three step sequential extraction prior to the certification of new sediment and soil reference materials. *J. Environ. Monit.*, 1: 57-61.
- Reeves RD, Baker AJM (2000). Metal-accumulating plants. In: Raskin, I., Ensley, B.D. (Eds.), *Phytoremediation of toxic metals using to clean up the environment*. John Wiley & Sons, New York, pp. 193-230.
- Ruley AT, Sharma NC, Sahi SV, Shree SR, Sajwan KS (2006). Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in *Sesbania drummondii* grown in soil. *Environ. Pollut.*, 144: 11-18
- Sauerbeck DR (1991). Plant element and soil properties governing uptake and availability of potentially toxic metals derived from sewage sludge. *Water Air Soil Pollut.*, 57,58: 227-237.
- Singh S, Sinha S (2005). Accumulation of metals and its effects in *Brassica juncea* L. Czern. (Cv. Rohini) grown on various amendment of tannery waste. *Ecotox. Environ. Safety*, 62: 118-127.
- Sinha S, Gupta AK, Bhatt K, Pandey K, Rai UN, Singh KP (2006). Distribution of metals in the edible plants grown at Jajmau, Kanpur (India) receiving treated tannery wastewater: relation with physico-chemical properties of the soil. *Environ. Monit. Assess.*, 115: 1-22.
- Tessier A, Campbell PGC, Bisson M (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analyt. Chemist* 51: 844-851.
- Udom BE, Mbagwu JSC, Adesodun JK, Agbim NN (2004). Distributions of zinc, copper, cadmium and lead in a tropical ultisol after long-term disposal of sewage sludge. *Environ. Int.*, 30: 467-470.
- Van Erp PJ, Van Lune P (1991). Long-term heavy metal leaching from soils-sewage sludge and soil/sewage mixtures. *Environ. Sci. Technol.*, 25: 706-711.
- Walkley A, Black IA (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.*, 37: 29-37.
- Wolnik KA, Fricke FL, capar SG, Braude GL, Meyer MW, Satzger RD, Bonnin E (1983). Elements in major raw agricultural crops in the United States. Cd and Pb in lettuce, peanuts, potatoes, soybeans, sweetcorn and wheat. *J. Agric. Food Chem.*, 31: 1240 -1244.