



## FRACTIONATION OF POTENTIALLY TOXIC ELEMENTS (PTE) IN SOILS IRRIGATED WITH WASTEWATER USING MODIFIED BCR SEQUENTIAL EXTRACTION PROCEDURE

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### ABSTRACT

*Irrigation using wastewater is a very common practice in most of the developing countries. Excessive build-up of Potentially Toxic Elements (PTE) in the soils through this process may not only result in soil pollution, but also lead to higher PTE uptake by crops, and thus affecting food quality and safety. This uptake depends on the bioavailability and mobility of these elements which can be evaluated using the sequential extraction method. In this work the modified European Community Bureau of Reference (BCR) sequential extraction protocol was used. The results of the pseudototal extraction showed that the concentrations of all the PTE determined were below the standard guideline values given by the UK Contaminated Land Exposure Assessment (CLEA) soil guideline values (SGV) and the Dutch Soil Intervention Values. The sequential extraction results showed that Cu, Fe, Ni and Pb were predominantly in the residual fraction which made them the least mobilised among the PTE studied, whilst substantial amounts of Cr, Mn and Zn were released in step 1 which made them the most mobile of the PTE and most likely to be taken up by the plants grown on these soils.*

**Keywords:** Potentially toxic elements; Sequential extraction; BCR sequential extraction; Inductively Couple Plasma Atomic Emission Spectrometry, CRM 601

### INTRODUCTION

Some potentially toxic elements (PTE) are biologically essential for living organisms including humans (Alloway, 1995), but the presence of these elements at a higher concentration may cause toxic effects if human population is exposed to them (Davydova, 2005). Due to rapid urbanisation, anthropogenic activities are becoming more intense thereby increasing the amount of PTE in the environment. Human beings are exposed to the risk of these elements through a direct link via inhalation (Schmidt, 2010), oral ingestion (Luo, 2012) and dermal contact (Siciliano, 2009). These elements can also be taken in through the indirect paths; the element can be taken up by plants from contaminated soils and thereby entering into the food chain.

In many developing countries such as Nigeria, industrial and municipal waste waters are used for irrigation purposes (Singh *et al.*, 2004, Hossain *et al.*, 2015) and these contain large amounts of PTE. Prolonged irrigation using waste water leads to accumulation of these elements in the soils and plants grown on them (Zehra *et al.*, 2009, Khan *et al.*, 2008). The total content of PTE in soil is not enough to fully assess the environmental impact of element on the environment since the toxicity of an element is dependent on the mobility, transformation and the potential bioavailability to plants and so into the food chain. Sequential extraction methods have been widely used in an attempt to quantitatively estimate PTE chemical forms that strongly affect their mobility, reactivity and availability to plants. In this technique, the soil is subjected to a series of chemical reagents

of increasing reactivity, with phytoavailability and mobility of PTE decreasing in the order of the sequential extraction step. In this way, the amount of PTE extracted from the more bioavailable fractions gives an idea of the size of the pool that might be depleted by a plant during the growing period. Many sequential extraction schemes were developed using different steps, reagents and extraction conditions (Rauret *et al.*, 1988; Mossop and Davidson 2003). Due to the operationally-define nature of sequential extraction, comparing results obtained from these schemes became difficult and, in 1987, the European Community Bureau of Reference BCR (now called the Standards, Measurements and Testing Programme, SMT), launched a project to harmonise the measurements of extractable metal content in soils and sediment (Quevauviller *et al.*, 1994). This led to the development of a harmonised 3-step sediment sequential extraction protocol together with the production of a certified reference material (CRM 601). There were reports of problems of irreproducibility of the BCR scheme especially the step 2, consequently a revised version, known as the modified BCR procedure was recommended (Rauret *et al.*, 1999). In this modified scheme, it was recommended that an additional step be added, in which the residue from step 3 is digested using *aqua regia* and the sum of all the steps be compared with the result of a separate *aqua regia* of the whole soil (pseudototal content). This serves as an internal check to assess the overall effectiveness of sequential extraction and element recoveries.

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In Kano metropolis, just like in other cities of the developing countries, wastewater irrigation is very common, and recently a number of articles have been published on the levels of PTE in soils and vegetables irrigated with wastewaters in Kano urban areas (Chiromawa *et al.*, 2014; Dawaki *et al.*, 2013; Lawal and Audu 2011; Awodee *et al.*, 2008; Audu and Peacock, 2005) and all the results indicated elevated amount of PTE in the substrates. However, there was no attempt to investigate the various forms in which these elements exist.

The objectives of this study are: to determine the pseudototal concentrations of Cr, Cu, Fe, Mn, Ni, Pb and Zn in soil samples irrigated with wastewater, to

ascertain their mobility and bioavailability by fractionation of these elements using the modified BCR sequential extraction protocol.

**MATERIALS AND METHODS**

*Study Area*

This has been described in details elsewhere (Imam, 2012), soils from gardens irrigated with wastewaters from six sampling points (drainage) were collected. Sampling Site A: Jakara River, Sampling Site B: Getsi River, Sampling Site C: Magami (Confluence), Sampling Site D: Bela (Entry Point into Wasai reservoir), Sampling Site E: Barwa (Spillway), Sampling Site F: Wasai (Outlet) (Figure 1).

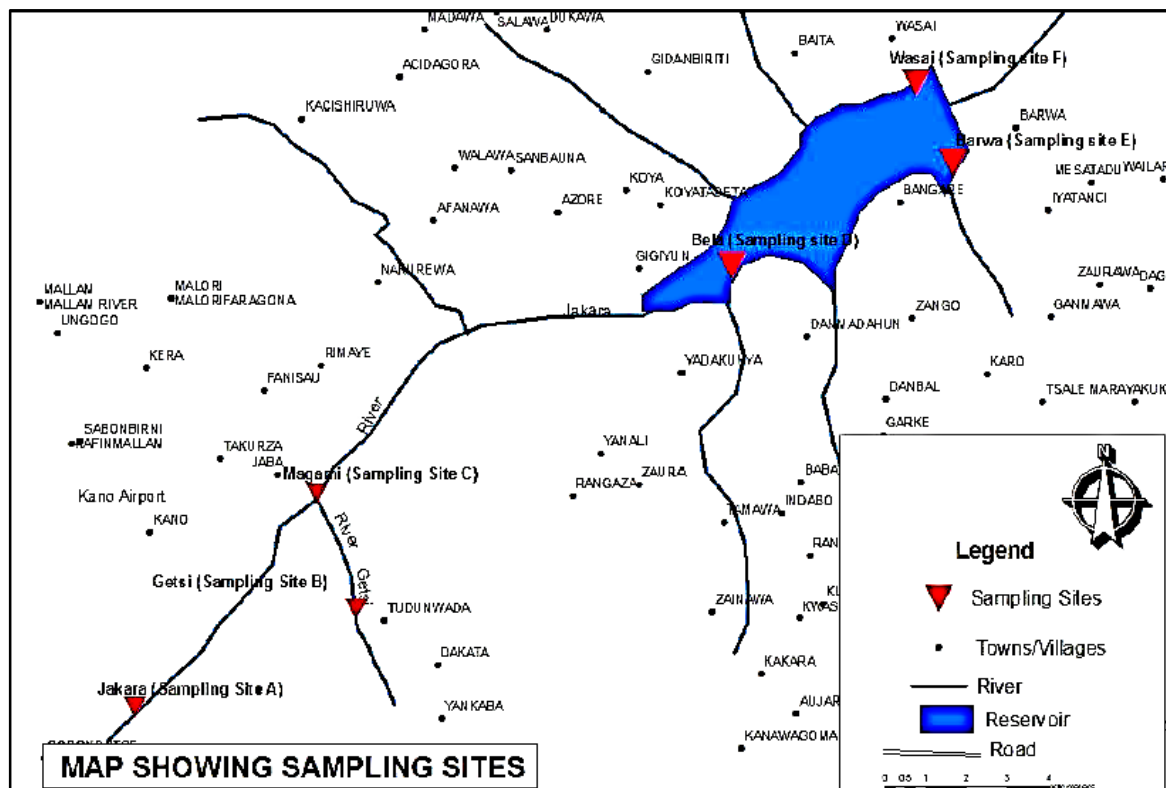


Figure 1: Study Area: Bompai-Jakara Catchment Basin, Kano State, Nigeria and Sampling Sites (Source: Google Earth, 2008).

*Apparatus and Reagents*

A Perkin Elmer Optima 3000 ICP-AES instrument (Perkin Elmer, Bucks, UK) equipped with radial-view plasma and an AS-91 autosampler, A G.F.L. 3040 mechanical end over end shaker (G.F.L, Burgwedel, Germany), ACL 4237 centrifuge (CAMLAB Ltd., Cambridge, UK), and MARS Xpress microwave digestion system (CEM Microwave Technology Ltd., Buckingham, UK). All reagents were obtained from Fisher Scientific, Loughborough, UK and VWR, Leicestershire, England, UK. Multi element reagent matched calibration solutions (i.e. made up in 20% *aqua regia*, 0.11 mol/l acetic acid, 0.1 mol/l hydroxylammonium chloride or 1.0 mol/l ammonium acetate) were prepared by serial dilution of 1000 mg/l Spectrosol standard solutions (Merck, Poole, UK)

of Cr, Cu, Fe, Mn, Ni, Pb and Zn. All the glassware and plastic vessels were soaked in 5%(v/v) HNO<sub>3</sub> for 24 hours and then rinsed with distilled water before use.

**Pseudototal extraction**

About 1.0 g of the soil sample was weighed and placed into a high pressure vessel and 20 ml of freshly prepared *aqua regia* was added and left to stand in a fume cupboard overnight, this was to allow all the vigorous reactions to subside. The pressure vessel containing the digestion mixture was placed into a MARS 5 microwave digestion system (CEM Corporation, Buckingham Industrial park, UK) and digested using the following program: operating temperature 160°C, power 1600 watts, ramp time 10 minutes and 20 minutes holding time.

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The vessels and the contents were allowed to cool and the digested samples were filtered (Fisher brand FB 59023) into 100 ml volumetric flasks and were washed several times with distilled water, the filtrates were made up to mark. These solutions were kept in a polyethylene bottles at 4 °C prior to ICP-AES analysis.

**BCR sequential extraction procedure**

The PTE were fractionated into different forms using the modified BCR sequential extraction procedure as described by Rauret *et al.*, (1999). This procedure consisted of 3 steps and a residual extraction and is summarized below:

**Step 1 (exchangeable fraction)**

A 1.0 g sub-sample was weighed into a 100 ml centrifuge tube and was shaken for 16 hours with 40 ml of 0.11 mol l<sup>-1</sup> acetic acid. The sample was then centrifuged at 3000 g for 20 minutes to separate the supernatant liquid from the solid residue. The liquid was decanted into a polyethylene bottle and refrigerated at 4 °C before analysis. The residue was washed by adding 20 ml of distilled water and shaken for 15 minutes. After centrifuging the wash supernatant was decanted and discarded.

**Step 2 (reducible fraction)**

40 ml of freshly prepared 0.5 mol l<sup>-1</sup>hydroxylammonium chloride solution adjusted to pH 1.5 by the addition of 2 mol l<sup>-1</sup> HNO<sub>3</sub> was added to the washed residue from step 1 in the same centrifuge tube. The sample was then shaken, centrifuged and stored as in step 1.

**Step 3 (oxidisable fraction)**

10 ml of 8.8 mol l<sup>-1</sup> hydrogen peroxide solution was added slowly in small aliquots to avoid vigorous reaction to the washed residue from step 2. The centrifuge tube was covered loosely with its lid and left to digest for 1 hour with occasional manual shaking. The digestion was continued for a further 1 hour in a water bath at 85 ± 2 °C, before removal of lids and reduction of volume to less than 3 ml. Another 10 ml of hydrogen peroxide solution was carefully added and the covered samples heated for a further 1 hour at 85 ± 2 °C. After removal of the lids the volume was reduced to less than 1 ml, with care not to take to complete dryness. The resulting solution was then allowed to cool before adding 50 ml of

1 mol l<sup>-1</sup> ammonium acetate solution adjusted to pH 2 with concentrated HNO<sub>3</sub>. The centrifuge tube was then shaken and centrifuged, the solution decanted and stored, and the residue washed as in step 1.

**Step 4 (residual fraction)**

20 ml of *aqua regia* was used to wash the residue from step 3 into a pressure vessel and digested using the microwave digestion procedure as in the pseudototal extraction described above.

**Quality control of the analytical data**

An urban soil reference material (URM), which is an in-house reference material, was used to validate the pseudototal extraction method while a lake sediment (CRM 601) certified reference materials obtained from the Community Bureau of Reference of the European Union (Brussels, Belgium) was used to validate the BCR sequential extraction procedure. Also blank determinations were performed in triplicate as well as sequential extractions of samples throughout all the experiments.

**RESULTS AND DISCUSSION**

*Validation of the methods*

The quality of the analytical data obtained for the pseudototal extraction was checked with an urban soil reference material (URM), which is an in-house reference material. The URM was digested in parallel with our samples. The results obtained and the indicative values are shown in Table 1. The extractable results obtained are in good agreement with indicative values (recoveries ± 10%); this indicates good accuracy of the analytical method. The quality of extraction using the modified BCR sequential extraction was verified using a CRM 601. The results of the fractionation of the certified reference material by the modified BCR sequential extraction protocol and the indicative values are shown in Table 2. The recoveries obtained for Cr, Cu, Ni Pb and Zn when compared with the indicative values were within ± 10% (except Cr and Ni for step 3) indicating good accuracy of the extraction. The relative standard deviations of the means of three replicate measurements were less than 10% which was regarded as a satisfactory precision.

Table 1: Results of the pseudototal content of URM (mg/kg)

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Target values (n=34) **	43.2	111	30600	442	48.8	389	177
Values obtained (n=3)	47.4	116	32400	445	52.3	349	193
% Recovery	110	105	106	101	107	89.7	109

\*(Davidson *et al.*, 2006)

Table 2 The recovery (%) values for the content of various extractable PTE (mg/kg) in BCR 601 certified reference material

	Cr	Cu	Ni	Pb	Zn
<b>Step 1</b>					
Indicative value	0.35 ± 0.08	10.5 ± 0.8	7.82 ± 0.84	2.28 ± 0.44	260 ± 13
Obtained value	0.33 ± 0.01	10.9 ± 1.2	8.42 ± 0.55	2.07 ± 0.12	257 ± 9
Recovery (%)	94	104	108	91	99
<b>Step 2</b>					
Indicative value	10.6 ± 0.9	72.8 ± 4.9	10.6 ± 1.2	205 ± 11	266 ± 17
Obtained value	9.90 ± 0.7	70.6 ± 7.5	11.1 ± 1.7	221 ± 26	271 ± 19
Recovery (%)	93	97	105	108	102
<b>Step 3</b>					
Indicative value	14.4 ± 2.60	78.6 ± 8.9	6.04 ± 1.27	19.7 ± 5.80	106 ± 11
Obtained value	12.5 ± 1.80	73.6 ± 3.1	3.36 ± 0.11	18.1 ± 4.21	103 ± 16
Recovery (%)	87	94	56	92	97
<b>Step 4</b>					
Indicative value	78.2 ± 6.5	60.4 ± 4.9	50.5 ± 6.1	38.0 ± 8.7	161 ± 14
Obtained value	80.2 ± 6.9	56.1 ± 2.9	52.7 ± 2.9	40.4 ± 2.7	168 ± 11
Recovery (%)	103	93	104	106	104

Indicative values are n= 7; obtained values are n= 3

**DISCUSSION**

*Pseudototal concentrations of the elements*

The pseudototal concentrations of Cr, Cu, Fe, Mn, Ni, Pb and Zn of the samples A to F are shown in Table 3. The concentrations of Cr, Ni and Pb in all the soil samples are below the existing UK Contaminated Land Exposure Assessment (CLEA) soil guideline values (SGV) of 130 mg/kg, 230 mg/kg and 450 mg/kg for residential gardens and allotments respectively (DEFRA-EA, 2002, 2009). Also the concentrations of Cu and Zn obtained for all the samples do not exceed

the Dutch Soil Intervention Values of 190 and 750 mg/kg respectively (VROM, 2009). Although these results indicated that none of the elements exceeded these standard guideline values, but continuous irrigation using this waste water may lead to elevation of these elements above the guideline values. Previous work by Imam (2002) revealed that the concentrations of all the elements investigated in the wastewaters used for irrigating these soils have exceeded the limits set by FEPA, WHO, AWWA, CCME.

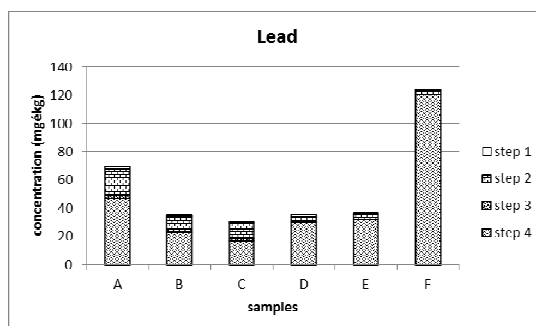
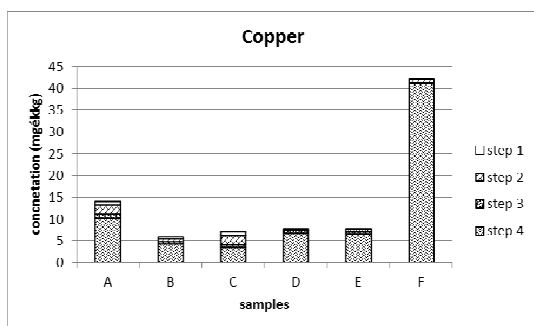
Table 3: Pseudototal (*aqua regia* soluble) PTE contents (mg/kg dry weight) (*n=3*)

Sample	Cr	Cu	Fe	Mn	Ni	Pb	Zn
A	37.31±0.71	15.25±1.96	11085±912	135.87±2.72	4.65±0.25	77.05±2.97	59.46±4.10
B	67.77±2.93	9.13±1.04	6492±539	150.06±15.30	5.01±0.15	48.87±1.14	46.67±2.48
C	48.13±2.06	9.96±1.14	6885±762	111.58±6.08	4.66±0.56	52.20±7.59	60.90±6.73
D	10.41±0.63	7.71±0.38	6053±239	122.18±4.11	3.03±0.22	43.16±4.84	18.53±2.27
E	11.54±0.22	7.05±0.58	5402±471	59.22±1.62	3.11±0.43	48.48±2.42	18.70±1.11
F	30.85±1.08	44.25±3.66	37089±3112	73.89±3.2	0.97±0.71	152.61±12.48	24.10±2.42

*Partitioning of metals*

The fractionation outline of the PTE in the soil samples A to F, are given in Figure 2. Chromium was fairly distributed in all the fractions except the reducible fraction. The presence of Cr in step 1 in these soils indicated that Cr was in a mobile fraction and can readily be mobilised in the environment and can be easily taken up by the plants grown on these soils. This is also an indication that it may likely come from anthropogenic activities such as the tanning industries from Bompai industrial area. Copper was predominantly in the residual fraction which indicated that it may not likely pose any danger to the environment as it was in an immobile phase. Iron was also predominantly in the residual fraction, this was expected as its minerals formed a major component of soils. A similar result was obtained in the studies of some garden soils (Tokalioglu *et al.* 2006). Manganese was predominantly in exchangeable and residual fractions. Its presence in the residual fraction may indicate the lithogenic nature of Mn as it is one of the most abundant elements in soils. The presence of Mn in step 1

showed high mobility of Mn in the soil, Tokalioglu and Kartal (2006) in their speciation studies of heavy metals in street dust samples from the Organized Industrial District in Kayseri (Turkey) obtained a similar result. As in the case of Fe and Cu, Ni was predominantly in the residual fraction which showed that in an immobile fraction and not likely to pose any human risk. Nickel is lithogenic in nature associated with the silicate matrix (Kartal *et al.* 2006). Some studies (Kartal *et al.* 2006; Tokalioglu and Kartal, 2006) have suggested that Ni is predominantly found in the residual fraction in soil. Lead is predominantly in the residual fraction, although in samples A, B and C, lead is in an appreciable amount in the reducible fraction. Lead in these soils may not likely pose any human risk as it will not be available for the plants to take them up. Zinc was found in the exchangeable, reducible and residual fractions. The presence of Zn in the two most labile fractions (exchangeable and reducible) makes Zn the most mobile of the elements determined in this work. There is high chance that it may be taken up by the plants grown on these soils thereby posing danger of going into the food chain.



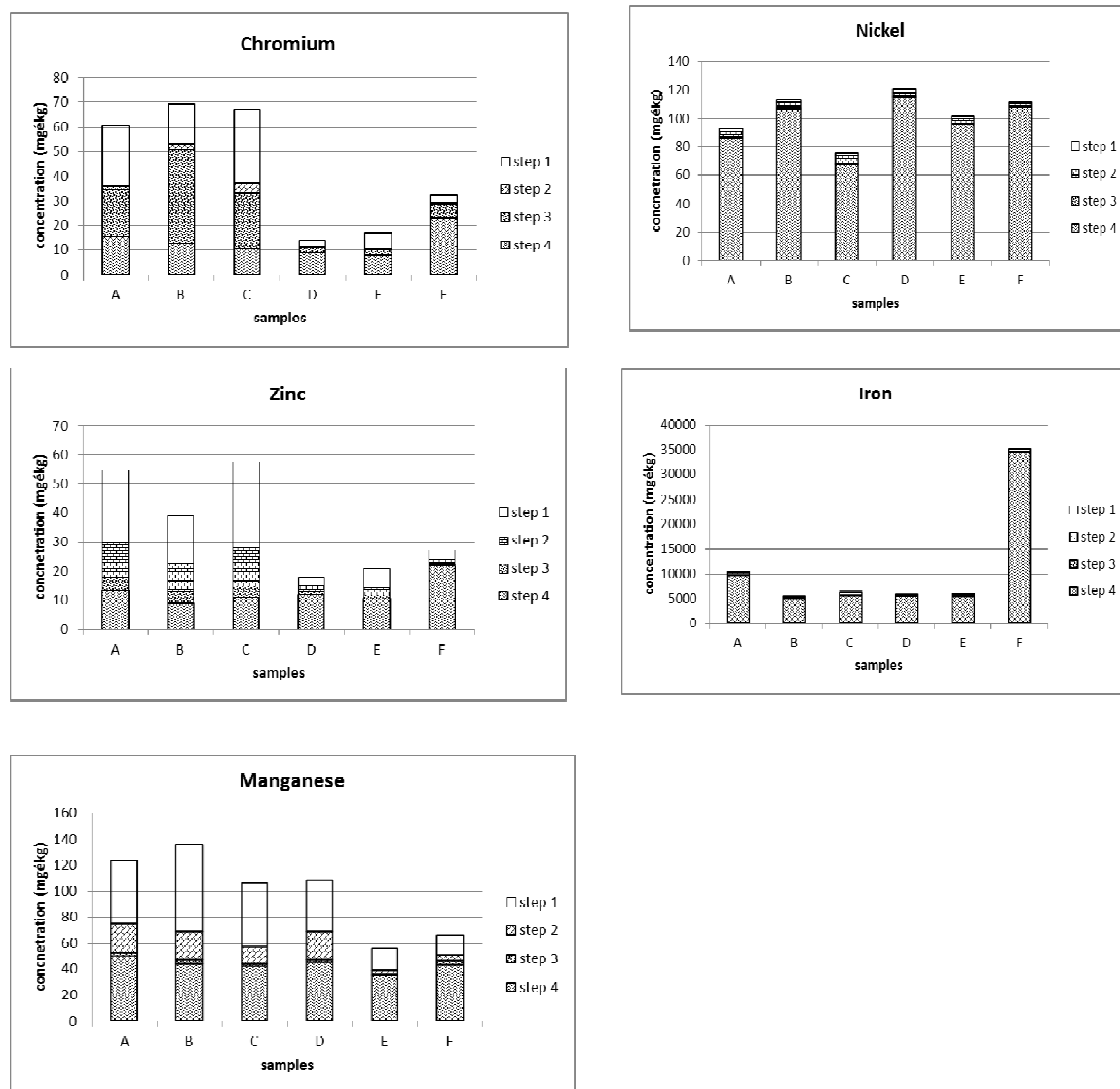


Figure 2 Fractionation of various PTE soil samples using the modified BCR sequential extraction

**CONCLUSION**

The concentrations of all the PTE determined do not exceed most of standard guideline values which shows that it safe to grow crops on these soils. However, previous studies of the waste water used in the irrigation has shown that the concentrations of these elements were above the standard values and therefore these PTE may accumulate in the soils if the source of pollution is not checked and it will gradually reach and subsequently pass the standard limit set. Thus serious steps are necessary to control this continuing accumulation.

The results of the fractionation indicated that Cu, Fe, Ni and Pb were predominantly in the residual fraction; this made them the least mobilise among the PTE studied. Environmentally they are of less impact as they would not be readily available to the environment, therefore they pose less danger. Substantial amountsof Cr, Mn and Zn were released in step 1 which made them the most mobile of the PTF

under study and most likely to be taken up by the plants grown on these soils.

**Contributions of Authors**

**Sagagi, B. S.** conducted the experiment, analysis as well as the write up of the research paper;

**Imam, T. S.** designed field sampling techniques, collected the soil samples for experimentation and reviewed the write up.

**Conflict of Interest**

There is no conflict of interest

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