DEVELOPMENT OF A FASTER TECHNIQUE FOR THE SPECIATION OF POTENTIALLY TOXIC METALS IN SOIL

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Abstract

The impact of potentially toxic metals (PTM) on humans and other organisms depends on their concentration, toxicity and bioavailability, which in turn, depend in part on reactivity and solubility. The total concentrations of PTM do not determine the mobility, bioavailability and uptake by plants but the fraction of these metals in soils or sediments, hence the development of sequential extraction method which has been widely used for speciation or fractionation studies. The limitations and problems of long extraction time associated with BCR (Community Bureau of Reference) method of extraction has led to the development of a faster and rapid Ultrasonic Assisted method of extraction. The species of the metals in soils was determined using both the conventional BCR and ultrasonic assisted methods of extraction, and quantification was done with Flame Atomic Absorption Spectrophotometer (FAAS). Though it was observed that the results obtained in the ultrasonic assisted method of extraction were slightly higher than those obtained in the BCR method of extraction, statistically (p<0.95) there was no significant difference in the two methods for all the studied metals. Prior to comparison, the analytical performance of the laboratory was tested with a secondary reference material-GLAURM, using both procedures and good recoveries were observed in most cases (80.6-104%). This research showed that that the ultrasonic method with a much shorter extraction time happens to be a valid alternative to the conventional BCR method.

Keywords: BCR Sequential extraction; potentially toxic metals; speciation; ultrasonic assisted method;

INTRODUCTION

Potentially toxic metals are released into the environment by both natural and anthropogenic sources. The presence of elevated metal concentrations in the environment is related to man's activities such as agriculture, mining, smelting, construction and industrial processes (Lee et al., 2005). The manner in which an element is bound to the solid components of environmental solids, such as soils or sediments, influences the mobility and ultimately, the bioavailability and toxicity of

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the metal to organisms (Ure and Davidson, 2002). The growing interest in having available information about mobility and bioavailability of potentially toxic metals present in the environmental samples has led to the widespread use of sequential extraction schemes in providing relevant information about the samples (Rauret et al., 1989).

Speciation can be defined as the process of identifying the different defined species, forms or phases present in a material or the description of the amounts and kinds of these species, forms or phases present (Ure *et al.*, 1993; Ure and Davidson, 2002). It is also defined by Tack and Verloo (1995) as the identification and quantification of the different, defined species, forms or phases in which an element occurs and is essentially a function of the mineralogy and chemistry of the soil sample examined. Heavy metal speciation studies continue to be of great value in environmental monitoring because not only the concentration of the heavy metals was determined, but more critically their forms existing in the environment which determines their toxicity, mobility and bioavailability (Bhogal *et al.*, 2003). Sequential extraction is an analytical process that chemically leaches metals out of soil, sediment and sludge samples. The purpose of sequential extraction is to mimic the release of metals into solution under various environmental conditions. Different sequential extraction schemes have been proposed, (Tessier et al, 1997) but the most recent is that proposed by Community Bureau of Reference (BCR), which may apparently turn out shorter and simplified

(consisting on three stages) when compared to previous extraction schemes (Quevauviller et al., 1993). The BCR scheme has been extensively studied in the analysis of trace metals including Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in both river sediments and marine sediments, dusts, compost and soils (Ahnstrom and Parker, 1999; Greenway and Song, 2002; Ho and Evans, 1997; Oyeyiola et al., 2011; Tokalioglu et al., 2003). A major limitation of BCR sequential extraction scheme is that it is time-consuming. For example, the procedure involves three periods of overnight shaking (16hrs), together with *aqua regia* digestion of the residue, and analysis of extracts and digest, this means that an entire week may be required to obtain results from a batch of samples – a fact hardly likely to encourage widespread use of the approach in busy, environmental monitoring laboratories (Bacon and Davidson, 2008).

Ultrasonic processing applies intense, high-frequency sound to liquids, producing intimate mixing and powerful chemical and physical reactions (Marin *et al.*, 2001). The process of "cavitation" is, in effect, "cold boiling" and results from the creation of chemical and physical reactions, such as those of surfactancy, which is why ultrasonic processing may be a preferred extraction technique.

The aim of this research is to develop a method of extraction using ultrasound with a view to shorten the extraction time in each of the stages corresponding to the sequential extraction method proposed by the BCR,

and also to compare the two techniques statistically.

EXPERIMENTAL
Instrumentation

Determination of metals in the extracts was carried out using a flame atomic absorption spectrophotometer (Perkin-Elmer AA Analyst 200) fitted with a hollow cathode lamp and using an air acetylene flame. Sequential extractions were performed using 50 ml polytetrafluoroethylene centrifuge tubes, an IKA HS 260 basic reciprocating shaker, and an R-8C laboratory centrifuge (0-6000 rpm, timer 0-60 min, 220/50 Hz). The ultrasonic extractions were carried out with an ultrawave sonicator (timer 0-30 min, 220-240 V, 50-60 Hz, Maximum temperature 90°C, fuse T2.0A).

Reagents

The extracting solutions were prepared from high quality reagents. Stock metal solutions (1000 μg/ml) were prepared from pure metal salts and calibration solutions were prepared daily by appropriate dilution of the metal stock solutions using distilled water. All the glasswares used were soaked in 5% HNO_3 overnight and rinsed properly with distilled water before use. For quality control, a secondary reference soil material -GLAURM (an urban soil secondary material prepared by participants in the EU URBSOIL project) was used to check the analytical performance of the laboratory as well as that of the analyst (Davidson *et al.*, 2006).

Sampling and Sample Preparation

Soil samples were collected randomly at two dump sites in Lagos State (Nigeria). The soil samples were collected using small plastic cups at a depth of 2.5 cm from the soil surface, transferred into clean polyethylene bags and taken to the laboratory. At the laboratory, the soil samples were spread on plastic trays and allowed to dry at ambient temperature for a few days. Large pieces of stones were removed before the samples were further homogenized. The samples were sieved to pass through a 2 mm stainless steel sieve and stored in clean polyethylene bags.

Physicochemical Parameters and Pseudo-total Metal Concentrations

The pH of the samples was determined according to British Standard ISO 10390 (2005), total organic content by Walkley-Black method and pseudo-total metal concentration was determined using the conventional aqua regia ($3HCl:1HNO_3$) on a hot plate. 1 g of the soil sample was digested with 20 ml aqua regia for 2 hrs. After cooling, the digest was filtered into a 50 ml standard flask (using Whatman filter paper, 11 cm) and stored in plastic bottles at a temperature of 4 °C prior to analysis. The metals were determined by FAAS under optimal conditions.

BCR Sequential Extraction Procedure
Step1: 40 ml of 0.11 M acetic acid was added to 1 g of air-dried soil sample in a 50 ml centrifuge tube, covered, and shaken in end-over-end mechanical shaker for 16 hrs at a speed of 30 rpm. The mixture was

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centrifuged at 3000 rpm and extracts decanted.

Step 2: 40 ml of freshly prepared 0.5 M hydroxyl ammonium chloride adjusted with 2M nitric acid to pH 1.5 was added to the residue from step 1 in the centrifuge tube and extracted as in step 1. The extracts from the solid residues were separated as in step 1.

Step 3: 10 ml of 8.8 M hydrogen peroxide was added carefully in aliquots (to avoid losses due to possible violent reactions) to the residue from step 2 and digested at room temperature for 1 hr with occasional manual shaking. The digestion was continued for 1 hr at $85 \pm 2°C$ with occasional agitation for the first 30 min in a water bath and reduced to a volume of < 3 ml. 10 ml of hydrogen peroxide was further added to the digest and concentrated to about 1 ml. Then, 50 ml of 1.0 M ammonium acetate was added and the extraction performed as above.

Between each extraction steps the residue was washed with distilled water, shaken for 15 min on a shaker and centrifuged for 20 min at 3000 rpm. The supernatant was decanted and discarded carefully to avoid the discard of residue. This procedure was followed in order to remove any remaining reactants and metals present in the residues from the previous extracts. All the solution obtained were transferred to 50 ml polyethylene tubes and stored at 4°C prior analysis.

Ultrasonic Bath-assisted Extraction

Davidson and Delevoye (2001) reported that the length of time and power used in sonication was important and these conditions were designed to give maximum extraction in the shortest possible time, whilst avoiding an increase in the temperature of the samples. In this experiment, the temperature of ultrasonic bath was fixed at $30 \pm 2°C$ by cooling water of bath. After optimizing the parameters of the sonicator, the sequential extraction was carried out for on the soil samples and the reference soil material GLAURM.

Step 1: 40 ml of 0.11M acetic acid was added to 1 g of air dried sample in centrifuge tubes and placed in an ultrasonic bath for 5 min after which it was removed, centrifuged, and the supernatant separated from the residue. To check the maximum recovery time, the sonication time was varied using 5, 10 and 15 mins.

Step 2: 40 ml of 0.5 M hydroxyl ammonium chloride adjusted to pH 1.5 with nitric acid was added to the residue obtained from step 1 after different sonication time, and the extraction was performed as described in step 1.

Step 3: Hydrogen peroxide digestion was performed according to the conventional BCR procedure. Then, 50 ml of 1.0 M ammonium acetate adjusted to pH 2 with nitric acid was added and the extraction in ultrasonic bath was performed as described above.

Blank samples were also performed simultaneously, blank tubes (containing reagent but no samples) were also taken through each complete procedure. The

results obtained for the optimisation of the reference material GLAURM are shown in Figure. 1. As an internal check on the procedure, the residual materials from step 3 of both sequential extraction methods were digested with aqua regia, and the total amount of metal extracted (i.e., the sum of step 1 + step 2 + step 3 + residue) was compared with that obtained by the aqua regia digestion of a separate 1 g sample of the soil.

RESULTS AND DISCUSSION
Physico-chemical Properties of Samples
The results of the physico-chemical properties of the soil samples used for the comparison of the sequential extraction techniques as well as the concentration of the PTM (Cd, Cr, Cu, Pb, and Zn) determined in the contaminated soils (A and K) and reference soil material GLAURM are summarized in Table 1. Sample A was found to be nearly neutral while sample K

was slightly alkaline. The organic matter content of sample A was found to be 1.2% while that of sample K was found to be 12.1%. This result was in accordance with the the research carried out by of O'Neill (1985) that soil high in organic matter tends to form under wet or cold conditions where decomposer activity is impeded by low temperature or excess moisture.

The samples had varying concentrations of potentially toxic metals. The results showed that Cd had the lowest concentration for both soil samples while lead had the highest. In order to assess the quality of the data obtained, a secondary reference material GLAURM was used as internal quality control. The results obtained showed that a good agreement was found between the target and found values. This is an indication of a good analytical performance of the laboratory and the method.

**Table 1:** Result of the physico-chemical parameters of soil samples and reference material (GLAURM)

	pH	OM (%)	Cu (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Cr (mg/kg)	Cd (mg/kg)
A	7.7	1.2	150.8	312.2	148.4	85.5	8.8
K	8.2	12.0	1978	4287.2	1393.8	165.4	22.9
GLAUR M (f)	4.2	11.2	97.2	270.0	144.3	40.4	6.0
(t)	4.2	11.7	111.0	387.0	117.0	43.2	5.7

Optimisation of sonication time and comparison of the conventional and ultrasonic method of extractions on the reference material and soil samples

In order to determine the optimal extraction time, the sonication time was varied using 5, 10 and 15 mins for the secondary reference material. The results are shown in Figure 1. With the exceptions of Cr in step 1 and Zn and Cd in steps 1 and 2 of the sequential extraction scheme, the amount of metals extracted increased with increase in sonication time with 15 mins having the highest concentration of metals extracted. Vaisanen et al. (2002) reported that the uptimal time for ultrasonic extraction was 9 mins, while Seon-Suk et al.(2007) reported 12 mins as the best extraction sonication time for releasing metals. In this study, a sonication time of 15 mins was used in the comparism of the two sequential extraction methods.

The results of fractionation of the reference material were also compared to the target values as seen in Table 2 using both extraction schemes. The results obtained by the two methods of extractions were comparable except for lead in step 1 where the result was slightly higher. The results obtained for each fractions were compared statistically ($P < 0.95$) and no significant difference was observed.
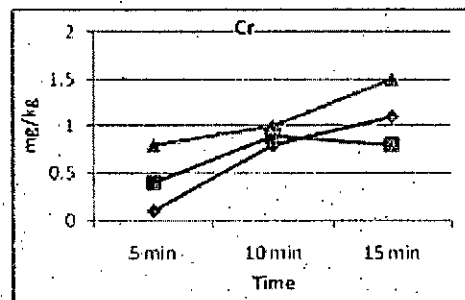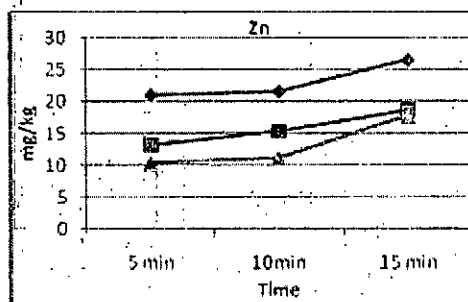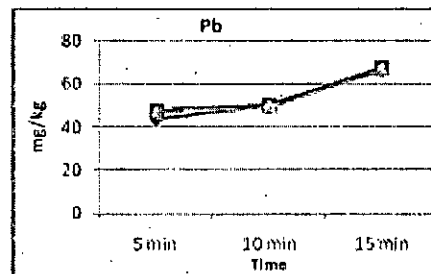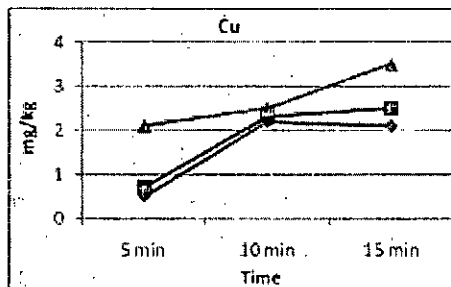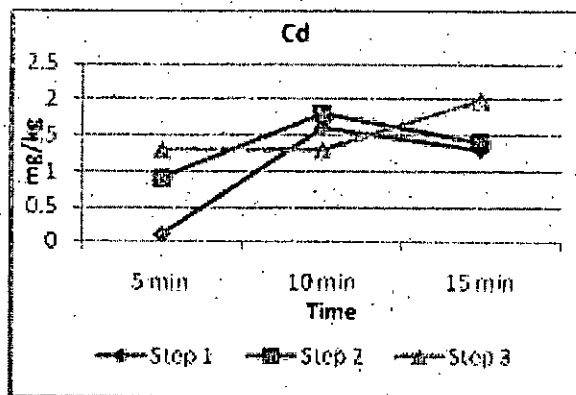
Figure.1. Effect of sonication time on the extraction of heavy metals on reference material GLAURM

Table 2

Comparison of results obtained from conventional and ultrasound extraction methods in GLAURM (mg/kg)

Step	Target value (mg/kg)	Found value BCR method (mg/kg)	Found value Ultrasonic method (mg/kg)	% recovery BCR method (mg/kg)	% recovery Ultrasonic method (mg/kg)
Step 1					
Cu	9.2	8.2	9.1	89.1	98.9
Pb	25.1	24.4	25.5	97.2	102
Zn	15.4	14.1	13.8	91.6	89.6
Cr	0.6	ND	ND		
Cd	*	1.2	1.1		
Step 2					
Cu	28.3	25.2	25.5	89.0	90.1
Pb	23.5	24.8	23.8	105	101
Zn	26.3	24.7	24.2	93.9	92
Cr	3.1	2.8	2.5	90.3	80.6
Cd	*	1.0	1.1		
Step 3					
Cu	33.2	32.9	32.0	99	96.4
Pb	52.1	50.2	48.0	96.4	92.1
Zn	27.2	22.4	23.1	82.4	84.9
Cr	9.7	9.3	8.8	95.9	90.7
Cd	ND	1.8	1.7		
Step 4					
Cu	28.5	30.8	30.4	119	118
Pb	73.3	71.2	70.0	97	95.5
Zn	118	82.8	83.0	70	70
Cr	27.8	28.0	28.9	101	104
Cd	*	2.1	2.0		

*= Not determined, ND= Not detected

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Generally, a good recovery was found for all the metals in all steps (80.6- 104%) with the exception of Zn in step 4 which had 70.2 and 70 % recovery for the BCR and ultrasonic method respectively and Cu in step 4 which has 119 and 118 % recovery for the BCR and ultrasonic method respectively. This showed a good analytical performance of the laboratory.

Comparison of the Results Obtained from Ultrasound Extraction with that Obtained by the Conventional Extraction Method in Samples A and K
The results of the ultrasound method of extraction and the conventional method in samples A and K are shown in Table 3. The results obtained in most cases were comparable. There were however a few exceptions to this. In step 1, the results obtained by the ultrasonic extraction were higher than those obtained by the conventional extraction for all the metals under investigation. In step 2, the results obtained by the ultrasonic extraction were lower than those obtained by the conventional method except for copper and chromium. In step 3, the results obtained by the ultrasonic extraction were lower than those from the conventional method except for zinc and chromium. The differences in the results could be partly be explained by losses of small quantities of metals when rinsing between steps. It must be highlighted that lead and zinc are the most abundant metals found in first and second fractions and that is dangerous because they are bioavailable fractions for plants. However, one possible explanation for the modification

of metal fractionation (especially that of Cd and Pb) could be according to Sonia *et al.*, (2005) readsorption phenomenon. This occurs mainly during the first two fractions of the extraction procedure. Metals released in these steps may be readsorbed on the remaining solid phases. The greatest modification was observed for Cd fractionation, suggesting that Cd affinity for clay surfaces is higher than that of Pb. This result is consistent with McBride work (1994), who reported that the adsorption of heavy metals on mineral adsorbent is based on electrostatic interactions, placing Cd high and Pb low in the affinity sequence. The major portion of cadmium in sample K was extracted in the reducible fraction with respect to pseudo-total concentration of cadmium. For other metals (Cu, Pb, Zn and Cr) the operational speciation determined with the ultrasonic bath was little lower to those obtained from conventional extraction.

Table 3

Comparison of the results obtained from ultrasound extraction with that obtained by the conventional extraction method in samples A and K (mg/kg)

Step	A		K	
	BCR (mg/kg)	Ultrasonic Extraction (mg/kg)	BCR (mg/kg)	Ultrasonic Extraction (mg/kg)
Step 1				
Cu	38.4	39.2	1090	1120
Pb	47.6	49.1	2540	2760
Zn	99.5	100	487	514
Cr	2.7	2.8	24.2	25.3
Cd	2.2	2.3	5.9	6.3
Step 2				
Cu	32.5	34.8	250.7	254
Pb	269	250	382	353
Zn	26.5	25.1	324	308
Cr	8.0	7.0	9.1	9.4
Cd	2.4	2.2	7.9	7.2
Step 3				
Cu	44.3	47.5	90.5	87.1
Pb	12.4	12.6	88.8	85.2
Zn	18.9	20.1	124	125
Cr	58.3	54.4	41.9	46.3
Cd	ND	ND	ND	ND
Step 4				
Cu	34.6	29.0	550	511
Pb	17.1	7.2	1280	1090
Zn	4.0	4.3	470.6	434
Cr	17.3	21.0	92.5	85.1
Cd	4.5	4.0	9.5	10.3

ND- Not determined

CONCLUSION

Sequential extraction methods are usually employed to evaluate the possible mobility and bioavailability of heavy metals present in environmental samples. In general, they are slow and tedious, little used for routine analysis, because of their lengthy treatment time. Ultrasonic extraction represents a valid alternative to the conventional shaking system and allows us to reduce substantially the extraction time required in the sequential extraction method proposed by the BCR. In the first and second fractions, the shaking time had been reduced from 16 hrs to 15mins. Recoveries for all heavy metals are acceptable as compared to the values obtained by conventional extraction methods except for Cd where nothing was detected in steps1, 2 and 3 for both samples. This suggests that, isolation of Cd can be improved by changing different ultrasonic bath parameter of extraction procedures.

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