

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/299565490>

Sequential extraction procedure for fractionation of Pb and Cr in artificial and contaminated soil

Article in *Main Group Metal Chemistry* · April 2016

DOI: 10.1515/mgmc-2016-0001

CITATIONS

0

READS

55

5 authors, including:



Ibrar Khan

Hazara University

21 PUBLICATIONS 31 CITATIONS

SEE PROFILE



Yangguo Zhao

Ocean University of China

46 PUBLICATIONS 162 CITATIONS

SEE PROFILE



Farhana Maqbool

Hazara University

26 PUBLICATIONS 73 CITATIONS

SEE PROFILE



Changsheng Peng

Ocean University of China

90 PUBLICATIONS 1,178 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Bioremediation [View project](#)

All content following this page was uploaded by [Ibrar Khan](#) on 08 June 2016.

The user has requested enhancement of the downloaded file. All in-text references [underlined in blue](#) are added to the original document and are linked to publications on ResearchGate, letting you access and read them immediately.

Sadia Qayyum, Ibrar Khan, Yangguo Zhao, Farhana Maqbool and Changsheng Peng*

Sequential extraction procedure for fractionation of Pb and Cr in artificial and contaminated soil

DOI 10.1515/mgmc-2016-0001

Received January 4, 2016; accepted March 29, 2016

Abstract: Metal contamination of soil is due to mining, manufacturing and use of synthetic products (e.g. pesticides, paints, batteries, industrial waste and industrial or domestic sludge) which is a serious environmental problem. Hence, determining chemical forms of metals in soils is important to evaluate their mobility or bioavailability. Both artificial and contaminated soils were sequentially extracted to fractionate metals into the water soluble fraction (WSF), exchangeable fraction (EF), bound to carbonate fraction, bound to metal oxide fraction, organically bound fraction (OBF) and residual fraction (RF). In the case of contaminated soil, Pb and Cr are found to be associated with the carbonate fraction while in artificial soil, Pb bound to WSF and Cr with the Fe/Mn fraction. Chemical properties such as pH, electrical conductivity (EC) and textural classification of concerned soils were also analyzed. Percentage recovery was calculated to check the reliability of processes both in Pb and Cr, and it was found to be more in Cr (66% and 84%) in both artificial and contaminated soil than Pb (5% and 34%) in both soils. Analyses of extracts were carried out by atomic absorption spectrometry (AAS). Results were interpreted in terms of environmental mobility or bioavailability of metals.

Keywords: metals; mobility factor; sequential extraction; soil.

Introduction

The presence of metals in soil with different chemical forms influences their reactivity and thus their mobility or bioavailability. The list of sites contaminated with metals is growing every year due to anthropogenic impacts, presenting a serious health problem and a formidable danger to the environment (Adaikpoh, 2011). Mining, manufacturing activity (Kabala and Singh, 2001) and the use of synthetic products (e.g. pesticides, paints, batteries, industrial waste and land application of industrial or domestic sludge) can result in metal contamination of urban and agricultural soils (Adeniyi and Okedeyi, 2004; Yusuf, 2006). Metals also occur naturally, but rarely at toxic levels. Most of the previous work in the early eighties has been limited to the determination of total concentration of the metals only. Although the total concentration of trace elements in the soil provides some indication of the level of enrichment, depletion or pollution, it provides no insight into the element bioavailability of potentially toxic metals or mobility (Shuman, 1991). For many geochemical applications, total metal content of soils is useful, but often the fractionation like bioavailability or mobility of these metals is more of an interest in terms of what is biologically extractable (Zimmerman and Weindorf, 2010). Fractionation is defined 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 (Tack and Verloo, 1995).

Metals in soil may be found in one or more of the following forms: (a) dissolved (in soil solution), (b) exchangeable (in organic and inorganic components), (c) as structural components of the lattices of soil minerals, (d) as insoluble precipitates with other soil components. Typically, metals of anthropogenic inputs tend to reside in the first two fractions and metals found in the residual fraction (RF) are of natural occurrence in the parent rock (Kabala and Singh, 2001). The first two forms are available to the plants, while the other two are potentially available in the longer term. Understanding the mechanisms by which a heavy metal element changes from one form to another, and the speed at which it does so, is

*Corresponding author: Changsheng Peng, The Key Laboratory of Marine Environmental Science and Ecology, Ministry of Education, Ocean University of China, Qingdao 266100, China, e-mail: pcs005@ouc.edu.cn

Sadia Qayyum and Yangguo Zhao: The Key Laboratory of Marine Environmental Science and Ecology, Ministry of Education, Ocean University of China, Qingdao 266100, China

Ibrar Khan: UNESCO Chinese Center of Marine Biotechnology, Ocean University of China, Qingdao 266003, China

Farhana Maqbool: Department of Microbiology, Hazara University Mansehra, KPK 23100, Pakistan

imperfect but improving. In general, the concentration of an element in the soil solution is dependent on the equilibrium between the soil solution and solid phase, with pH playing the decisive role (Lindsay, 1979). The ability of the soil to immobilize metals increases with rising pH and peaks under mildly alkaline conditions. Metal mobility is related to its immobilization in the solid phase.

Apart from pH, other soil properties, such as electrical conductivity (EC), organic matter content, quantity and type of clay minerals, the content of the oxides of iron (Fe), aluminum (Al), and manganese (Mn), and the redox potential determine ability of the soil to retain and immobilize metals. When this ability is exceeded, the quantities of heavy metals available to plants increase, resulting in the appearance of toxicity phenomena.

In cases of soil contaminated by metals, it is important to identify the available and unavailable forms of the metals to ensure that the soil is managed in such a way to prevent the unavailable forms from becoming available. The most common and simple way to identify the forms in which metals are found in soils is to use sequential extraction methods, which are based on the rational use of a series of more or less selective reagents chosen to solubilize successively the different mineralogical fractions for retaining the larger part of metals (Gleyzes et al., 2002), in which components loosely held by the soil are extracted first, followed by those more tightly bonded. Much research on fractionation has been conducted on metals contamination in soils from various anthropogenic sources such as industrial wastes (Kakulu and Obanjo, 1988; Ikem et al., 2003; Adeniyi and Okedeyi, 2004; Yusuf, 2006), automobile emission (Ma and Rao, 1997; Arowolo et al., 2000), mining activity (Ramos et al., 1994; Dudka et al., 1995; Kabala and Singh, 2001) and agricultural practice (Chlopecka et al., 1996).

One commonly used sequential extraction procedure is designed to fractionate different trace metals based on their chemical nature. Of the many procedures

in publication, some are designed to operate within specific parameters while others are designed for more broad applications (Yusuf, 2006). The modified procedure (Tessire et al., 1979) is the most commonly used to determine both the actual and potential mobility of trace elements in soils and sediments. This consists of six steps in which metals are fractionated among different phases. The filtrate from each step is then analyzed depending on the project requirement (Jennifer, 1993). This multi-step procedure assures that all the metals of concern are completely extracted and quantified from the sample. Factors such as pH of the acid use for adjustment, temperature and duration of extraction are the critical factors that control the concentration of metal extracted from the sample (Ryan et al., 2008). The various forms of the metals sequentially extracted can be classified as dissolved, exchangeable, carbonate bound, organically-bound, or bound to oxides. These are also often referred in the literature as exchangeable, weakly absorbed, hydrous-oxide bound, organic bound, and lattice material components, respectively (Maiz et al., 2000). Typically metals of contaminated soil tend to reside in the first four fractions and metals found in the RF are of natural occurrence in the parent rock (Ratuzny et al., 2009).

In our study we examined a six-step sequential extraction process recently referenced in the literature (Maiz et al., 2000) by comparing fractions in both artificial and contaminated soil and predicted the bioleaching capacity of metals in the contaminated soil.

Results and discussion

Soil characterization

Soil characterization is given in Table 1 including its EC, organic carbon content which ranged from 6.1% to 6.2%

Table 1: Soil sample characteristics.

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Code	Soil location	pH	Electrical conductivity	Organic carbon content	Metal concentration
Artificial soil					
Cr	OECD (1984)	6.2	9.1 mS·cm ⁻¹	6.23%	44.42 mg·kg ⁻¹
Pb	OECD (1984)	6.1	8.1 mS·cm ⁻¹	6.15%	36.38 mg·kg ⁻¹
Contaminated soil					
Cr	Weifang ZTE Battery Co., Ltd	9.24	3.08 mS·cm ⁻¹	3.15%	1574.50 mg·kg ⁻¹
Pb	Weifang ZTE Battery Co., Ltd	7.53	1.16 mS·cm ⁻¹	5.48%	2521.60 mg·kg ⁻¹

for artificial soil and from 3.15% to 5.48% for contaminated soil and total metal contents in both soils measured by atomic absorption spectrometry (AAS). Soil pH provides various clues about soil properties and is easily determined. In present study, the pH on the 14th day of incubation of the artificial soil ranged from 6.2 for Cr and 6.1 for Pb indicating acidic, while pH of contaminated soil ranged from 9.24 for Cr and 7.53 for Pb indicating slightly alkaline (Table 1). The effect of soil pH is great on the solubility of minerals or nutrients. The pH of artificial soils is low with values of six or less. The low pH values are possibly due to the presence of high metal contents. Low pH values may reduce the availability of most micronutrients. This rather low pH can easily be overcome by using organic fertilizer. Continuous application of organic fertilizer can increase the pH to a more favorable level for crop growth, as well as increase the organic contents of the soil (Shamshuddin and Paramanathan, 1980). The difference in pH of the soils highlights the displacement of the ions H^+ adsorbed on the exchange sites of the absorbing complex from soil towards the soil solution.

pH is one of the factors which influence the bioavailability and the transport of metal in the soil and according to Smith (1996), metal mobility decreases with increasing soil pH due to precipitation of hydroxides, carbonates or formation of insoluble organic complexes. Metals are generally more mobile at $pH < 7$ than at $pH > 7$. The amount of metals mobilized in a soil environment is a function of pH, properties of metals, redox conditions, soil chemistry, organic matter content, clay content, cation exchange capacity and other soil properties (Mukhejee, 1998; Marr et al., 1999; Saure et al., 2000).

Figures 1–4 show the particle size distribution and textural classification. The textural analysis of the soils shows that clay>sand>silt in artificial soil and sand>silt>clay in contaminated soil. The textural classification shows dominantly clay in artificial soil, while it shows sandy loam in contaminated soil.

Metal fraction

Figure 5 shows the flow diagram for the fractionation of metals in contaminated soils into six steps. Table 2 shows the concentrations of soil Pb and Cr in the individual sequential extraction fractions.

Pb (contaminated soil): carbonates>organic>water soluble>exchangeable>residual>Fe-Mn oxide.

Cr (contaminated soil): carbonate>Fe-Mn oxide>exchangeable>water soluble>organic>residual.

Pb (artificial soil): water soluble>organic>exchangeable>residual>carbonate>Fe-Mn oxide.

Cr (artificial soil): Fe-Mn oxide>water soluble>carbonate>exchangeable=organic=residual.

Distribution of metal showed that Pb and Cr in contaminated soil are largely associated with carbonate, organic and Fe-Mn oxide forms. Fe/Mn oxides exist as nodules, concretions, cement between particles or as coatings on particles and are excellent trace element scavengers (Ikem et al., 2003). In agreement with these results, several other workers have also reported the dominance of the Fe-Mn oxides bound in Zn (Ramos et al., 1994; Karczewska, 1996; Ahumada et al., 1999; Narwal et al., 1999). The carbonate fraction is influenced by pH and the

>	5	2	1.25	1	0.7	0.5	0.355	0.25	0.18	0.125	0.1	<
5	$\frac{5}{2}$	$\frac{2}{1.25}$	$\frac{1.25}{1}$	$\frac{1}{0.7}$	$\frac{0.7}{0.5}$	$\frac{0.5}{0.355}$	$\frac{0.355}{0.25}$	$\frac{0.25}{0.18}$	$\frac{0.18}{0.125}$	$\frac{0.125}{0.1}$	$\frac{0.1}{0.063}$	0.063
							6.8	4.8	20.4	7.9	10.6	49.5

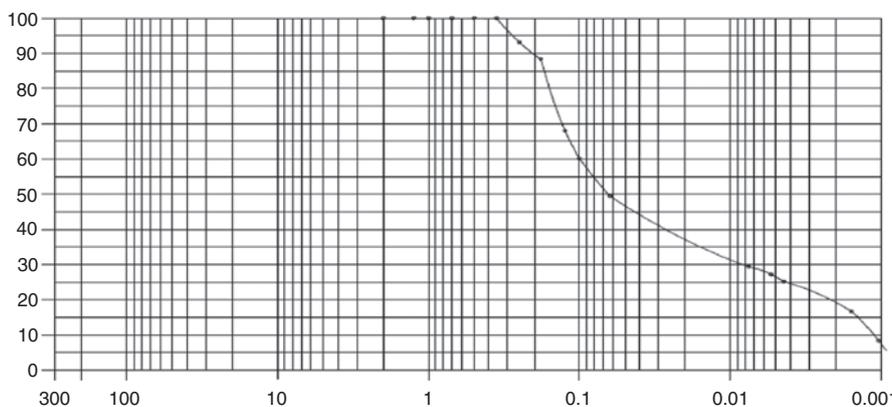


Figure 1: Particle size distribution of $K_2Cr_2O_7$ (artificial soil).

>	5	2	1.25	1	0.7	0.5	0.355	0.25	0.18	0.125	0.1	<
5	2	1.25	1	0.7	0.5	0.355	0.25	0.18	0.125	0.1	0.063	0.063
							9.1	6.1	22.9	8.4	10.0	43.5

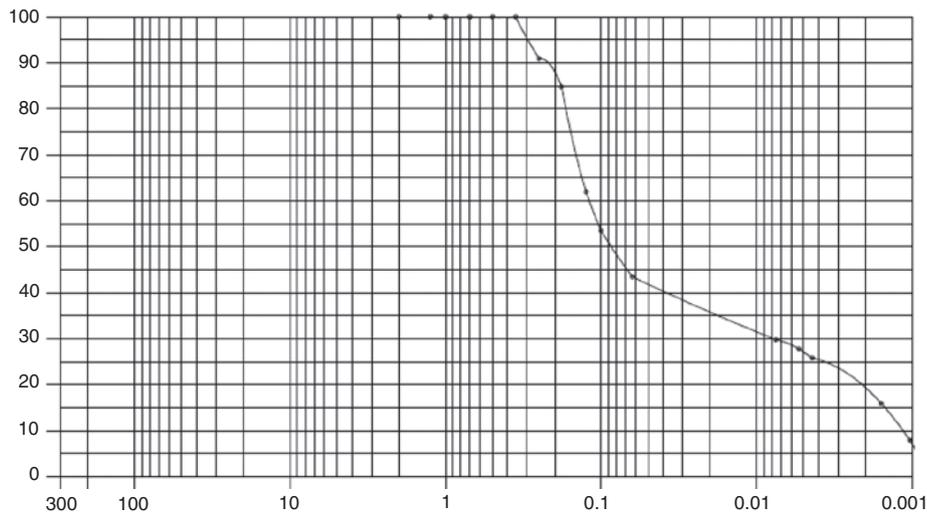


Figure 2: Particle size distribution of Pb(NO₃)₂ (artificial soil).

>	5	2	1.25	1	0.7	0.5	0.355	0.25	0.18	0.125	0.1	<
5	2	1.25	1	0.7	0.5	0.355	0.25	0.18	0.125	0.1	0.063	0.063
		2.2	0.1	3.2	4.0	6.0	6.7	1.8	8.7	4.3	9.2	53.8

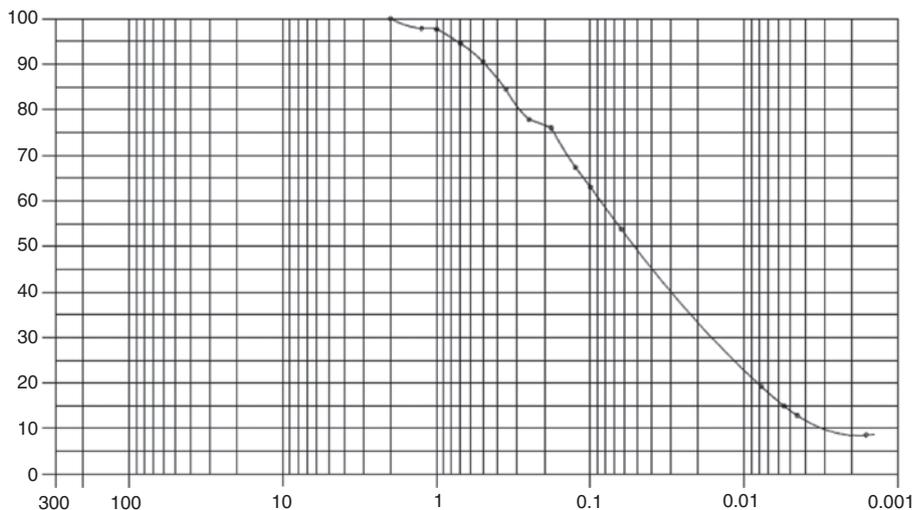


Figure 3: Particle size distribution of Cr (polluted soil).

observed pH for contaminated soil ranged from pH values for Pb (~7.5) and Cr (~9.2). The predominance of the Fe-Mn oxide is in broad agreement with the result reported by Kabata-Pendias (1992) for the affinity of Pb to soil organic matter and Ramos et al. (1994) that found that most Pb associated with the oxide fraction in their study of contaminated Spanish soils, with only very low amounts in the exchangeable fraction (EF). Similar findings were also

reported by other groups (Sposito et al., 1982; Dudka et al., 1995; Karczewska, 1996; Ahumada et al., 1999). In general, it seems that the oxide fraction is able to scavenge Pb in natural and contaminated soils (Xian, 1989) while in artificial soil, Pb is associated with the water soluble fraction (WSF) and Cr is associated with the Fe-Mn oxide fraction. The WSF (i.e. Pb) is certainly the most biologically active. The WSF has the highest potential of contamination of

>	5	2	1.25	1	0.7	0.5	0.355	0.25	0.18	0.125	0.1	<
5	2	1.25	1	0.7	0.5	0.355	0.25	0.18	0.125	0.1	0.063	0.063
	3.0	2.8	1.1	1.7	1.6	1.7	1.3	0.4	1.3	0.8	17.5	66.8

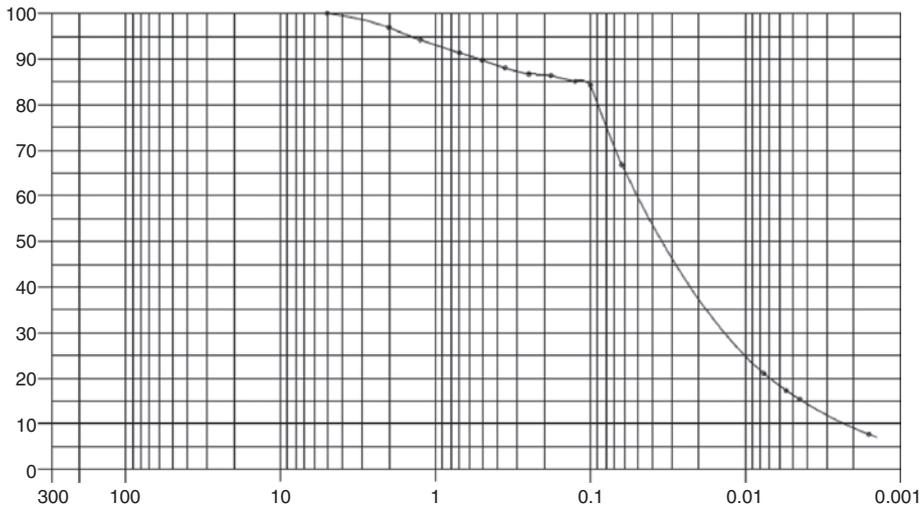


Figure 4: Particle size distribution of Pb (polluted soil).

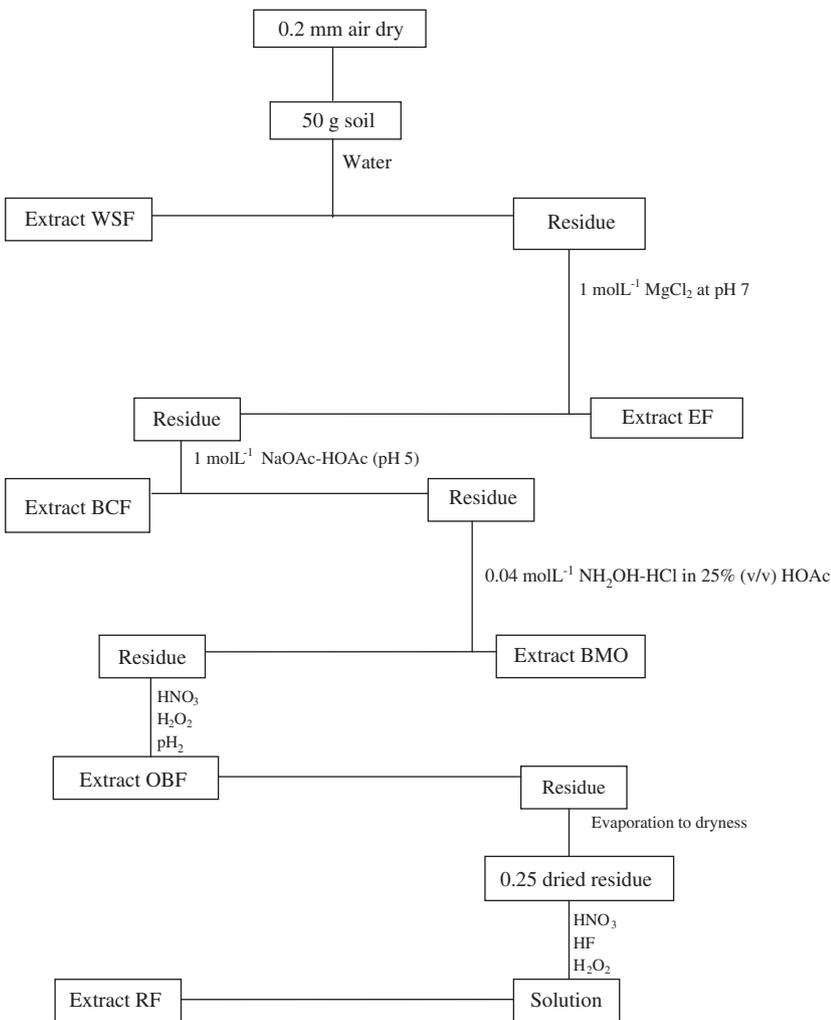


Figure 5: Flow diagram for the fractionation of metals in contaminated soils (Uduma and Jimoh, 2013).

Table 2: Concentration of soil (Pb and Cr) in individual sequential extraction fraction in mg·kg⁻¹.

S/No.	WSF	EF	BCF	BMO	OBF	RF	Mobility factor
Contaminated soil							
Pb	18.58	3.96	74.61	0.42	26.64	3.21	17.69
Cr	110	180	340	320	80	10	27.88
Artificial soil							
Pb	6.86	1.35	0.24	0.15	2.65	1.21	65.68
Cr	10	2.5	7.5	12.5	2.5	2.5	33.33

BCF, Bound to carbonate fraction; BMO, bound to metal oxide fraction; OBF, organically bound fraction; RF, residual fraction.

food chain, surface water and ground water (Leita and Nobility, 1991; He et al., 1992).

Mobility or bioavailability of metals

The mobility of metals in soil samples may be assessed on the basis of absolute and relative content of fractions weakly bound to soil components. The mobility factor (MF) in the case of contaminated soil gave average values of 27.8 and 17.69 for Cr and Pb, respectively, while in artificial soil these values were 33.33 and 65.68 (Table 2). In contaminated soil the high MF values of Cr as compared to Pb and in artificial soil high MF values of Pb as compared to Cr may be interpreted as symptoms of relatively high liability and biological availability of the metals in soil (Ma and Rao, 1997). Cr and Pb metals have toxic effects on living organisms because they are not essential nutrient elements. Toxicity and biological activity of many elements is dependent not only on their quantities, but also on their chemical forms, so in this regard study on fractionation of metals is very important (He et al., 2005).

Element recoveries

Analytical results validity was tested by recovery experiments, because there was no standard reference material, which is more preferential, or needed to control the accuracy of the method studied, in our laboratory.

The analytical results acquired for percentage recovery are depicted in Figure 6. Recovery values of the tested elements for the scheme were calculated according to Eq. (2) and generally agreed with each other, although some recoveries deviated from acceptable values. For example, low recoveries were obtained for contaminated soil samples, i.e. 5% in Pb and 66% in Cr, while applying

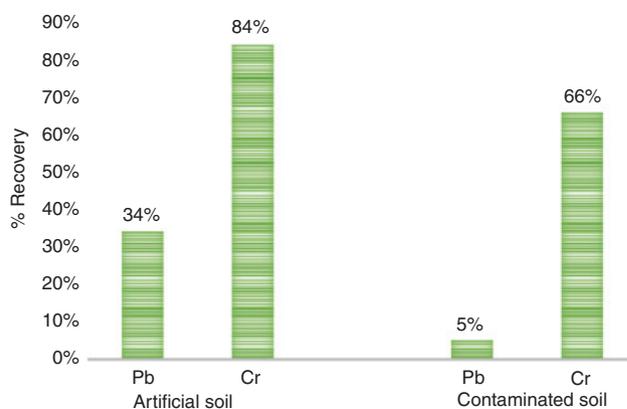


Figure 6: Overall recovery rates for soil samples calculated from the sum concentration of the 6 extraction steps compared to the recommended value for the total concentration.

sequential extraction procedures for fractionation of metals, sample contamination or loss could occur during the extraction steps. The reason for obtaining low recovery rate in the case of contaminated soil may be that Cr and Pb are more mobile in this environment. This phenomenon may cause the experimental errors, i.e. obtaining low or high recovery.

Results from the sequential extraction procedure provided information on the potential mobility of the studied elements in both artificial and contaminated soil in this research. The distribution of heavy metals in the sample allows us to predict their mobility (Haung et al., 2007). It shows the potential of a particular metal from the soil matrix to enter the soil solution from which it can be absorbed by plants or by the production of organic acid by fungi. The metal concentrations, MFs of all the sequential extraction steps including the residual phase fractions, determined at each extraction step in Table 2, indicate that each metal has a characteristic distribution pattern in both soils.

Conclusions

To characterize contamination intensity, the total content of contaminated elements in the soil can be a useful parameter. The pH in artificial soil is 6.1–6.2 for Pb and Cr, while in contaminated soil it is 7.5–9.2 for Pb and Cr, respectively. However, the fractionation of metals with selective extracting agents gives additional information about the fundamental reactions governing the behavior of the metals in soils. The chemical fractionation of the metals studied revealed the geochemical nature of these two heavy metals and their probable association with different chemical

forms in the soils around the smelting industrial area. The results showed that in contaminated soil, Cr and Pb binds to the carbonate fraction, which explains their greater mobility and availability to plants. This may present a real threat as Cr and Pb are transferred into the food chain from soil contaminated by these two metals, while in the case of artificial soil, Fe/Mn and WSFs predominated. This overwhelming importance of the carbonate fraction in this study illustrates clearly the difficulty of distinguishing between background and anomalous levels of heavy metal contamination when only total metal analyses are performed. Mobility of metals is controlled by the sorption process. The adverse effects of mobility are that their persistence in soil causes accumulation of heavy metals in the food chain when they reach the favorable environment, which is suitable for conversion of an unavailable form into an available form. Indigenous microbes which may dissolve an amount of metals from soil, i.e. bioremediation, are the most important technique to clean the contaminated soil environment.

Experimental section

The contaminated soil samples were collected from Weifang ZTE Battery Co. Ltd, which was built in 1954 and located in Weifang City, Shandong Province, China. It is a smelting industry which mainly produces and sells lead-acid batteries and accessories. During the lead-acid battery production and assembly, such as transport of raw material, the leaking of the production process and stacking behavior may cause contamination of the site with lead; the main sources are lead contaminated wastewater, lead dust and lead scrap ointment. Artificial soil samples were prepared according to the Organization for Economic Cooperation and Development (OECD) guideline 207. Artificial soil in this work is used only to compare fractions in both soils.

Reagents

Analytical grade chemicals and deionized water were used for preparing all solutions (purchased from Zhengzhou Sino Chemical Co., Ltd, China). Stock solutions containing 1000 mg L⁻¹ of all the analytes were prepared from nitrate and chromate salts of Pb and Cr in 1% HNO₃ into 1 L calibrated flasks. Working standard solutions were prepared in 1 mol L⁻¹ HNO₃ by appropriate dilutions of the stock solutions. Blank determinations were run by using the same reagents in equal quantities as described in the analysis procedure throughout the experiments. Solutions of 3.2 mol L⁻¹ NH₄OAc were prepared in distilled water and 0.04 mol NH₂OH·HCl in 25% HOAc. The concentrated 30% H₂O₂ solution (pH=2, adjusted with HNO₃) in acidic medium and 3.2 mol L⁻¹ in 20% HNO₃ is often preferred for oxidizing the organic substances in analysis of soils. The digestion was performed with 7 mol L⁻¹ HNO₃.

Quality assurance

All chemicals used were of analytical grade and ultra-pure deionized water was used throughout the experimentation. Plastic wares were pre-cleaned by soaking in 10% HNO₃. Procedural blanks, preparation of standard solutions under a clean laboratory environment, calibration of the American Thermo Electron Corporation M6 (AAS) using certified standards and the analyses of calibrated standards after 10 samples to ensure that the instrument remained calibrated were taken during the experiment to ensure quality of the results.

Sample collection

Two types of soil were used for this study. The artificial soil used in this study was developed by the OECD (1984). This medium, which consists of a mixture of 70% fine (silver) sand, 20% kaolin clay and 10% coarse ground Sphagnum peat with powdered calcium carbonate added to adjust soil pH (for further details of preparation of test soil see Van Gestel et al., 1989 and Spurgeon et al., 1994). The dry soil was moistened with distilled water to obtain approximately half of the final required water content. Metal salts were added to artificial soil and then soil was incubated for 2 weeks in polyethylene bags.

Another soil sample was contaminated soil, which was sampled at 0–20 cm (ploughing layer) with a sharp edged plastic spatula, directly transferred into a labeled polyethylene homogenization container and mixed thoroughly to obtain a homogenous sample representative of the entire sampling interval. The labeled homogenization polyethylene bags were closed tightly and returned to the laboratory for pretreatment and analysis.

The contaminated soil samples were air dried under laboratory conditions for 2 weeks, ground, sieved through a 0.2 mm polyethylene sieve, dried to a constant mass in an oven at 75°C and kept in a desiccator for further analysis. A size fraction of about 0.2 mm was taken for sequential extraction process. Total chromium and lead contents were determined after digestion with both aqua regia (1 g soil per 2.5 mL nitric acid [HNO₃], 7.5 mL hydrochloric acid [HCl] and 0.5 mL 30% hydrogen peroxide [H₂O₂]) (Kilburn, 2000) and 2 M nitric acid (5 g soil per 100 mL concentrated nitric acid) (UN/EC, 1998). The amounts of elements were determined by AAS.

Some properties like pH, EC and textural analysis were done initially. Soil pH was measured potentiometrically in 1 M KCl with a soil/extractant ratio of 1:5 in three replicates per sample. The particle size composition (sand, silt and clay) of the soil samples was determined by the hydrometer method. The texture of the soil samples was established by charting the percentages of sand, silt and clay fractions with a textural triangle. The AAS analyses for the determination of the total metal contents of the soil samples were done. All analysis was done in triplicate for accuracy of the method.

Instrumentation

An atomic absorption spectrometer, Model American Thermo Electron Corporation M6 AAS was used to determine total metal concentrations and total amount recovered from all sequential extraction steps for Pb and Cr. Hollow cathode lamps were used as the excitation source. Lamp intensity and bandpass were used according to the

manufacturer's recommendations. Acetylene and air flow rates for all elements were 2–4 L min⁻¹, respectively. A centrifuge was used for complete separation of the extracts from the residues; an end-over-end shaker (Model American Crystal Technology and Industries Inc. IS-RDS3C stackable incubator shaker), a pH meter and an electrical heater were used throughout all the experiments. Borosilicate tubes having a volume of 50 mL were used for dissolving the soil samples.

Analytical extraction procedure

Determinations of metal speciation or distribution in both soils were made by (Salbu and Oughton, 1998). The sequential chemical extraction scheme is a modified version of Tessier et al. (1979). The six sequential extraction procedure used in this study was designed to separate metals into:

- a. WSF (F1)
- b. EF (F2)
- c. Bound to carbonates fraction (F3)
- d. Bound to metal oxide fraction (F4)
- e. Organically bound fraction (F5)
- f. RF (F6).

WSF (F1)

De-ionized water (50 mL) was added to 50 g of soil samples in 100 mL polypropylene centrifuge tubes with screw caps. The extraction was performed by shaking in a mechanical, end-over-end shaker (Model American Crystal Technology & Industries Inc. IS-RDS3C Stackable Incubator Shaker) at a speed of 300 rpm and at a room temperature of 28°C for 5 h and left overnight. The solution was centrifuged at 5000 rpm for 15 min and the supernatant was filtered into a plastic container through No. 1 Whatman filter paper and stored in a refrigerator at 3–4°C for analysis.

EF (F2)

Some 50 mL of 1 mol L⁻¹ MgCl₂ (pH 7) were added to residue from the WSF in 100 mL polypropylene centrifuge tubes with screw caps and allowed to stand for 1 h at room temperature. Centrifugation and filtration were performed according to the above procedure.

Bound to carbonates fraction (F3)

The residue from Step 2 above was extracted with 50 mL of 1 mol L⁻¹ NaOAc-HOAc (pH 5) and allowed to stand for 5 h at room temperature. Centrifugation and filtration were performed according to the above procedure.

Bound to metal oxide fraction (F4)

The residue from Step 3 was extracted with 50 mL 0.04 mol L⁻¹ NH₂OH·HCl in 25% (v/v) HOAc and allowed to stand for 6 h at 85°C

in a water bath. Centrifugation and filtration were performed according to the above procedure. Iron and manganese oxides which can be present between particles or coatings on particles are excellent substrates with large surface areas for absorbing trace metals. Under reducing conditions, Fe (III) and Mn (IV) could release adsorbed trace metals. The extracted metal solution was decanted from the residual soil which was used for the next extraction.

Organically bound fraction (F5)

The residue from Step 4 above was extracted. Then 4.5 mL of 0.02 mol L⁻¹ HNO₃ and 7.2 mL of H₂O₂ were added and allowed to stand for 2 h at 85°C in a water bath. Then, 4.5 mL of H₂O₂ (pH 2 with HNO₃) was allowed to stand for 3 h at 85°C in a water bath and finally 8.8 mL of 3.2 mol L⁻¹ NH₄OAc was added in 20% (v/v) HNO₃ and allowed to stand for 30 min at room temperature.

RF (silicates) (F6)

The residue from Step 5 above was evaporated for dryness. A 0.25 g sub-sample was taken and this was analyzed by tri-acid mixture digestion with 3 mL of 1 mol L⁻¹ HNO₃, 2 mL of 2 mol L⁻¹ HF and 1 mL of 8.8 mol L⁻¹ (40%) H₂O₂ solution in a platinum crucible. After the acids had been digested and evaporated, 40 mL of 7 M HNO₃ was added and warmed on a sand bath and filtered into 50 mL labeled plastic containers and filled to volume with the 0.25 M HNO₃ solution. Total metal content of the original soil samples was similarly determined earlier using 0.25 g of soil samples, to estimate the total amount of Pb and Cr recovered from all the sequential extraction steps.

The digested residue samples were stored in the refrigerator at 3–4°C for analysis. The mobility of samples was determined according to the equation below.

MF of metals

The MF of metals in soil samples may be assessed on the basis of percentage absolute and relative content of fractions weakly bound to soil components and it is calculated on the basis of the following Eq. (1) (Salbu and Oughton, 1998; Narwal et al., 1999; Kabala and Singh, 2001):

$$MF = \frac{F_1 + F_2}{F_1 + F_2 + \dots + F_n} \times 100 \quad (1)$$

where MF is the mobility factor and F₁, F₂ and F_n are the first, second and nth fractions, respectively.

Percentage recovery

An important consideration in the reliability of sequential extraction data is the percentage recovery relative to a single digestion using a mixture of strong mineral acids or generally a mixture of strong acids at the digestion of the residual phase of the sequential extraction protocol employed (Smith, 1996). Recovery is defined as follows:

$$\text{Recovery} = \left[\frac{\sum n \text{ sequential extraction procedure}}{\text{single digestion with strong acids}} \times 100 \right] \quad (2)$$

Where n is the concentration of a given element and the single digestion with strong acids used for reference was a mixture of strong acids used in the RF digestion (Boch et al., 2000).

Acknowledgments: This work was supported by the State Key Laboratory of Environmental Criteria and Risk Assessment (No. SKLECRA2013FP12) and the Cultivation Fund of the Key Scientific and Technical Innovation Project, Ministry of Education of China (Grant/Award Number: 708060). We are also thankful to an anonymous reviewer for helpful comments.

References

- Adaikpoh, O. Sequential extraction of cadmium, lead, zinc in soil profiles of Ifom and Environs, Southwest Nigeria: submission for their availability to terrestrial organisms. *Ind. J. Sci. Technol.* **2011**, *4*, 339–403.
- Adeniyi, A.; Okedeyi, O. Assessing the speciation pattern of lead and zinc in surface water collected from Abegede Creek, Ijora, Lagos. *Pak. J. Sci. Ind. Res.* **2004**, *47*, 430–434.
- Ahumada, I.; Mendoza, J.; Ascar, L. Sequential extraction of heavy metals in soils irrigated with wastewater. *Commun. Soil Sci. Plant Anal.* **1999**, *30*, 1507–1519.
- Arowolo, A.; Bamgbose, O.; Odukoya, O. The chemical forms of lead in roadside dust of Metropolitan Lagos, Nigeria. *Global J. Pure Appl. Sci.* **2000**, *6*, 483–487.
- Boch, K.; Schuster, M.; Risse, G.; Schwarzer, M. Microwave assisted digested procedure for the determination of palladium in road dust. *Anal. Chem. Acta.* **2000**, *459*, 257–265.
- Chlopecka, A.; Bacon, R.; Wilson, J.; Kay, J. Forms of cadmium, lead and zinc in contaminated soils from Southern West Poland. *J. Env. Qual.* **1996**, *25*, 69–79.
- Dudka, M.; Piotrowaska, A.; Chlopecka, A.; Laptek, J. Trace element contamination of soil and crop plants by mining and melting industry in Southwest Poland. *Geochem. Explore* **1995**, *52*, 237–250.
- Gleyzes, C.; Tellier, S.; Astruc, M. Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedure. *Trends Anal. Chem.* **2002**, *21*, 451–467.
- Haug, J.; Haug, R.; Jiao, J. J.; Chen, K. Speciation and mobility of heavy metals in mud, in coastal reclamation areas in Chenzhen, China. *Environ. Geol.* **2007**, *53*, 221–228.
- He, T.; Trainer, J.; Logan, J. Chemical properties of municipal solid waste compost. *J. Environ. Qual.* **1992**, *21*, 318–329.
- He, L.; Xia, Y.; Stofella, J. Trace Element in Agro ecosystems and impacts on the environment. *J. Trace Elem. Med. Biol.* **2005**, *19*, 125–140.
- Ikem, A.; Egibog, O.; Newyork, K. Trace element in water, fish and sediment from Tuskegee Lake, Southeastern USA. *J. Water Air Soil Poll.* **2003**, *149*, 51–75.
- Jennifer, J. Sequential extraction method. A review and evaluation. *Environ. Geochem Health* **1993**, *15*, 2–3.
- Kabala, C.; Singh, R. Fractionation and mobility of copper, lead and zinc in soil profile in the vicinity of a copper smelter. *J. Quats.* **2001**, *30*, 485–492.
- Kabata-Pendias, A.; Pendias, H. Trace elements in soil and plants. 2nd Edition, CRC Press: Boca Raton, FL, 1992; pp. 365.
- Kakulu, E.; Obanjo, O. Trace heavy metal pollutional studies in sediments of the Nigeria. *Appl. Geochem.* **1988**, *315*, 322.
- Karczewska, A. Metal species distribution in top- and sub-soil in an area affected by copper smelter emissions. *Appl. Geo. Chem.* **1996**, *11*, 35–42.
- Kilburn, M. Aqua regia digestion for soils/sludges. Analytical methods manual. Method NO: H115, 03/07/00. **2000**.
- Leita, M.; Nobility, D. Water soluble fraction of heavy metal during compositing of municipal solid waste. *J. Env. Qual.* **1991**, *22*, 857–863.
- Lindsay, L. Chemical Equilibria in Soils, John Wiley & Sons: New York, NY, 1979.
- Ma, Q.; Rao N. Chemical fractionation of calcium, copper, nickel and zinc in contaminated soils. *J. Env. Qual.* **1997**, *26*, 259–264.
- Maiz, I.; Arambarri, R.; Garcia, E. Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. *Environ. Pollut.* **2000**, *110*, 3–9.
- Marr, K.; Fyles, H.; Hendershot, H. Trace metal in Montreal urban soil and the leaves of Taraxacum Officinale. *Can. J. Soil Sci.* **1999**, *79*, 385–387.
- Mukhejee, AB. Nickel. A review of occurrence, uses, emission and concentration in the environment in Finland. *Environ. Qual.* **1998**, *4*, 405–444.
- Narwal, P.; Singh, R.; Salbus, B. Association of cadmium, zinc, copper and nickel with component in naturally heavy metal rich soils studied by parallel and sequential extractions. *Commun. Soil Sci. Plant Anal.* **1999**, *30*, 1209–1230.
- OECD (Organisation for Economic Cooperation and Development) (1984) Earthworm, Acute Toxicity Tests, Guideline No. 207. OECD, Paris.
- Ramos, L.; Hernandez, M.; Gonzales, J. Sequential fractionation of copper, lead, cadmium and zinc in soils from Donena National Park. *J. Environ. Qual.* **1994**, *23*, 50–57.
- Ratuzny, T.; Gong, Z.; Wilke, M. Total concentrations and speciation of heavy metals in soils of the Shenyang Zhangshi irrigation area, China. *Environ. Monit. Assess.* **2009**, *156*, 171–180.
- Ryan, C.; Hillers, S.; Walk, X. Stepwise effects of the BCR sequential chemical extraction procedure on dissolution and metal release from common ferromagnesian clay minerals: a combined solution study. *Sci. Total Environ.* **2008**, *407*, 603–614.
- Salbu, T.; Oughton, H. Characterization of radioactive particles in the environment. *Analyst* **1998**, *123*, 843–849.
- Saure, S.; Henderson, W.; Allen, E. Solid–solution partitioning of metals in contaminated soils: dependence on pH, total metal and organic matter. *Environ. Sci. Tech.* **2000**, *34*, 1125–1131.
- Shamshuddin, J.; Paramanathan, S. Acidity and charge characteristics of marine alluvial soils from Carey Islands, Selangor. *Pertanika* **1980**, *11*, 15–23.
- Shuman, M. Chemical forms of micronutrients in soils. In: Micronutrients in Agriculture, SSSA: Madison, WI, 1991; pp 113–114.
- Smith, R. Agriculture Recycling of Sewage Sludge and Environment, CAB International: Walling Fork, UK, 1996; pp 382–390.
- Sposito, G.; Lund, J.; Chang, C. Trace metal chemistry in arid-zone soils amended with sewage sludge 1. Fractionation of Ni, Cu,

- Zn, Cd and Pb in solid phase. *Soil Sci. Soc. Am. J.* **1982**, *46*, 260–264.
- Spurgeon, D.J.; Hopkin, S.P.; Jones, D.T. Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. *Environ. Pollut. (Ser. A)*. **1994**, *84*, 123–130.
- Tack, G.; Verloo, G. Chemical speciation and fractionation in soil and sediment heavy metal analysis: a review. *Int. J. Environ. Anal. Chem.* **1995**, *59*, 225–238.
- Tessier A.; Campbell C.; Bisson, M. Sequential extraction procedure for speciation of particulate trace metals. *Anal. Chem.* **1979a**, *51*, 8–51.
- Tessire, A.; Campbell, C.; Bisson, M. Sequential extraction procedure for the speciation of soils. *Speciation Chemistry*. **1979b**, *51*, 844–85.
- Uduma, A.; Jimoh, L. Sequential extraction procedure for partitioning of lead, copper, cadmium, chromium and zinc in contaminated arable soils of Nigeria. *AJEEPR*. **2013**, *1*, 186–208.
- UN/ECE ICP on Integrated Monitoring. Manual. August 1998.
- Van Gestel, C. A. M.; van Dis, W. A.; van Breemen, E. M.; Sparenburg, P. M. Development of a standardized reproduction toxicity test with the earthworm species *Eisenia andrei* using copper, pentachlorophenol and 2,4-dichloroaniline. *Ecotoxicol. Environ. Saf.* **1989**, *18*, 305–312.
- Xian, X. Effect of chemical forms of Cadmium, zinc and lead in polluted soils on their uptake by cabbage plant. *Plant Soil* **1989**, *113*, 257–264.
- Yusuf, A. Evaluation of the three stage BCR (European Community Bureau of Reference) sequential extraction procedure to assess the potential mobility and toxicity of heavy metal in road side soil. *Pak J. Sci. Find Res.* **2006**, *49*, 181–188.
- Zimmerman, J.; Weindorf, H. Heavy metal and trace analysis in soil by sequential extraction. A review of procedures. *Int. J. Anal. Chem.* **2010**, *7*, 387803, doi: 10.1155/2010/387803.