Bull Environ Contam Toxicol (2009) 82:43–47
DOI 10.1007/s00128-008-9566-7

Estimation of Lead Bioavailability in Smelter-Contaminated Soils by Single and Sequential Extraction Procedure

Su Chen · Lina Sun · Lei Chao · Qixing Zhou · Tieheng Sun

Received: 20 December 2007 / Accepted: 24 September 2008 / Published online: 15 October 2008

Abstract In this study, the single extraction and sequential extraction procedure were used to separate Pb from an old smelter soil in Liaoning Province, China. At the same time, the root and the overground parts of six species plants in the smelter were harvested for analysis. The concentrations of Pb in the roots and the overground parts of the plants were then compared with the amounts of Pb extracted from the rhizosphere soil by range of conventional extractants. The speciation distribution of Pb was RES > OX > OM > WSA > SE. Assuming that metal mobility and bioavailability are related to their solubility and the contents in typical plants in the contaminated soil, Pb would be the potential risk to environment safety in the area.

Keywords Smelter-contamination · Lead · Single extraction · Sequential extraction · Bioavailability

S. Chen · L. Sun · T. Sun
Key Laboratory of Eco-remediation of Contaminated Environment and Resources Reuse, Shenyang University, Ministry of Education, Shenyang 110044, China

S. Chen · L. Sun (✉) · L. Chao · Q. Zhou · T. Sun
Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
e-mail: mailsydx@yahoo.com.cn

S. Chen · L. Sun
Ministry of Education Key Laboratory of Environmental Remediation and Ecological Health, College of Natural Resources and Environmental Science, Zhejiang University, Hangzhou 310029, China

L. Chao
Liaoning Academy of Environmental Sciences, Shenyang 110031, China

With the rapid development of industrial production in recent decades, anthropogenic activities such as mining, smelting, ore refining, and irrigation with sewage have led to increasing heavy metal contents in soils (Chen et al. 2007). Now the heavy metal contamination of soils derived from anthropogenic activities and the consequent ecological security problems have become a focus of world attention. Smelting activities generate a great deal of particulate emissions and waste slag enriched in heavy metals which contaminate the surrounding soil, water and air. Recently many smelters have been moved out of cities in view of the environmental problems in China; however, the abandoned land surface is contaminated by heavy metals and in great need of ecological risk assessment and remediation. The Shenyang Smelter (123°49′277″–123°49′655″ E, 42°07′476″–42°07′793″ N) was one of them, which was founded in 1936 and the area of the factory was 3.6×10^5 m^2 (Cui et al. 2007). It was located in the heavy industrial center of Shenyang, which is the capital of Liaoning Province. The main production of this smelter was lead (Pb). While the centuries-old smelter was responsible for more than 40% sulfur dioxide (SO_2) and 98% lead particulate emissions within the city. As a result of pollution problems, it was demolished in 2002 and most sites are seriously contaminated with heavy metals.

Most analytical measurements dealt with the total content of particulate metals in analysis samples. The use of total concentration as a criterion to assess potential effects of soil contamination is not sufficient, because fate and toxicity of heavy metals in a contaminated soil is greatly controlled by speciation in the soil (Guo et al. 2006). Therefore, total metal concentrations may not be the best predictor of metal bioavailability, and the accurate estimation of metal bioavailability in soils is becoming more important in ecological risk assessments. The aim of this work was to study the distribution of Pb speciation in the contaminated soil in the Shenyang Smelter, in order to determine bioavailability and contaminant mobility and to provide information for risk assessment on soil contamination.

Materials and Methods

Six species of plants were collected on August 2004 in the studied Shenyang Smelter. At least three individual plants of each species were randomly collected. At the same time soil samples were also collected among the roots of each plant for analysis (Wei et al. 2005). Plant samples were washed to remove soil particles attached to the plant surfaces. The roots and overground parts were separated and oven-dried at 105°C for 30 min, then 70°C to constant weight. The dried tissues were weighed and ground into powder for the determination of metal concentration. Soil samples were air-dried for two weeks, and then sieved through a 2 mm mesh. Some main soil properties for the samples are listed in Table 1. The soil used in this study is meadow burozem (silty clay loam). Soil pH (soil/water ratio of 1:2.5), organic matter and cation exchange capacity (CEC) were carried out following the standard methods of chemical analysis (Zhou 2003). For soil and plant samples, the concentrations of Cd, Pb were analyzed using the atomic absorption spectrophotometer after a wet digestion of the sample with the mixture of HNO_3 and $HClO_4$ (Guo et al. 2006).

All experiments were carried out at a room temperature of 25 ± 1°C. 1.000 g soil samples were weighed into 100-mL acid-cleaned polystyrene centrifuge tubes. Different extraction solutions were added and the tubes were shaken at ambient temperature on a variable speed reciprocal shaker at 220 strokes min^{-1} for 2 h (Maiz et al. 2000). After the extraction, the sample solutions were centrifuged at 5000 rpm for 15 min and then filtered. The supernatant was used for analysis. Experimental details are listed in Table 2.

The sequential extraction was developed from that of Tessier et al. (1979) using the same terminology. The defined chemical fractions were as follows: fraction 1, SE (water soluble plus exchangeable); fraction 2, WSA (bound to carbonate or weakly specifically adsorbed); fraction 3, OX (bound to Fe or Mn oxides); fraction 4, OM (bound to organic matter); fraction 5, RES (residual). After each successive extraction, separation was carried out. The supernatant was filtered and placed in a tube for measuring.

All chemicals used in the experiment were of analytical grade and deionized water was used to prepare solutions. All treatments were replicated three times to minimize experimental errors. The average of the three replicates for each treatment and standard deviation (SD) were calculated. Analysis of the heavy metal in the supernatants was carried out by a SpectrAA-200 atomic absorption spectrophotometer (AAS, made in Australian). Reference material (GBW07404) was used for quality control of the instrument's performance. The recovery and precision were found to be within 100 ± 10%. The statistical package used throughout this study was SPSS 11.0.

Results and Discussion

The general trend from Table 3 showed that these plant species could vegetate within a broad range of Pb concentrations in soil. The total Pb contents in soil of the

Table 1 Properties of the tested soil samples

Soil		pH	Organic matter (g kg^{-1})	CEC (cmol kg^{-1})	Total Pb (mg kg^{-1})
Smeltery soil	Range	5.8–6.7	6.3–18.5	8.2–14.8	1004.0–9385.0
	Mean	6.2	11.6	10.7	3044.0
Clean soil	Range	6.2–6.6	12.1–21.8	9.4–15.7	7.5–18.2
	Mean	6.4	16.5	12.3	12.1

Table 2 Summary of the experimental procedures for the single-step extractions

Type	Extractant/method	Reference
Neutral salts	$Mg(NO_3)_2$ (1 mol L^{-1})	Shao et al. (1994)
	NH_4Cl (1 mol L^{-1})	Xia and Liu (1994)
	$CaCl_2$ (0.1 mol L^{-1})	Houba et al. (1996)
	$MgCl_2$ (1 mol L^{-1})	Navas and Lindhorfer (2003)
Dilute acids	HOAC (0.43 mol L^{-1})	Ure et al. (1993)
	HCl (0.1 mol L^{-1})	Lu et al. (2003)
Chelating reagents	EDTA (0.05 mol L^{-1})	Fangueiro et al. (2002)
	DTPA (0.05 mol L^{-1})	Prokop et al. (2003)

smelter greatly exceeded the ranges which considered toxic to normal plants, so these plants growing in the polluted site exhibited strong metal adaptability. Pb concentrations observed in plant varied from 48.1 to 430.3 mg kg^{-1} for roots, while they varied from 29.5 to 184.8 mg kg^{-1} for overground parts. Pb accumulated by these species was retained in roots except *Abutilon theophrasti*, which demonstrating limited mobility of Pb in these plants. Pb concentration in the overground part of *Conyza canadensis* was lower and it could grow naturally in the contaminated site owing to its metal-resistant characteristics with the help of mycorrhizal fungi. The reason may be that the metal-binding capacity of mycorrhizal fungi was so striking that both the uptake of metals from the soil and their subsequent translocation to *Conyza canadensis* may be effectively restricted when exposed to pollution (Zhou et al. 2003). Pb concentrations in the overground part of *Polygonum lapathifolium* were the highest. The major processes involved in the accumulation of heavy metals from soil to the plants possibly include bioactivation of metals in the rhizosphere through root–microbe interaction. Detoxification of metals by distributing to the apoplasts, e.g., binding to cell walls and chelation of metals in the cytoplasm with various ligands, such as phytochelatins, metallothioneins, metal-binding proteins, and sequestration of metals into the vacuole by tonoplast-located transporters (Qureshi et al. 2005). Pb concentrations in roots and overground parts were not significantly correlated ($R = 0.55$, $p = 0.31$). *Helianthus tuberosus* had the highest contents of Pb in root. The concentration of Pb in root of plants that live in non-polluted environments was less than 5 mg kg^{-1} (Cui et al. 2007). Root Pb concentration was not significantly correlated with Pb content in soil ($R = 0.49$, $p = 0.36$), neither the overground part Pb concentration ($R = 0.48$, $p = 0.02$).

The usefulness of any soil extractants to predict the bioavailability of heavy metals is dependant upon the ability to predict the extent to which plants will accumulate that given metals. Eight reagents classified in three types were listed in Table 2 and were used to evaluate the potential mobility and bioavailability of Pb in the contaminated soils. The metal extraction efficiencies obtained with the single extraction procedures were presented in Table 4. The extractabilities of Pb obtained with chelating reagents were generally higher than dilute acid and neutral salts. The chelating reagents could extract out over 40% of the total Pb. EDTA had a higher chelating capacity than DTPA. This was easily understood because the EDTA method was originally developed for acidic soils (Hammer and Keller 2002). EDTA is a strong chelating reagent and can extract trace elements from soil geochemical phases including labile and nonlabile fractions (Bermond et al.

Table 3 The Pb concentration in soil, overground parts and root parts of plants

Plants	Concentration of Pb (mg kg^{-1})		
	Soil	Overground parts	Root parts
Helianthus tuberosus L.	3044.0 ± 191.2	126.8 ± 25.4	430.3 ± 77.4
Solanum nigrum	7121.3 ± 378.1	90.7 ± 19.1	180.2 ± 34.3
Chenopodium acuminatum	1004.3 ± 121.6	90.5 ± 19.2	203.7 ± 37.7
Polygonum lapathifolium	4828.9 ± 611.1	184.8 ± 31.4	210.4 ± 35.3
Conyza canadensis	2069.8 ± 19.4	29.5 ± 6.1	49.6 ± 10.7
Abutilon theophrasti	1004.3 ± 121.6	72.3 ± 14.7	48.1 ± 10.3

Table 4 Pb contents (mean concentration ± SD, mg kg^{-1}) from the single extraction by different reagents

Extractant	Soil (mg kg^{-1})					
	Helianthus tuberosus	*Solanum nigrum*	*Chenopodium acuminatum*	*Polygonum lapathifolium*	*Conyza canadensis*	*Abutilon theophrasti*
$Mg(NO_3)_2$	43.4 ± 12.7g	101.6 ± 29.8e	14.3 ± 4.2e	68.9 ± 20.2e	29.5 ± 8.6g	14.3 ± 4.2e
NH_4Cl	68.8 ± 6.4e	161.0 ± 14.9f	22.7 ± 2.1e	109.2 ± 10.2g	46.8 ± 4.4e	22.7 ± 2.1f
$CaCl_2$	57.7 ± 5.5f	135.0 ± 15.2f	19.0 ± 2.1f	91.5 ± 10.3g	39.2 ± 4.4f	19.0 ± 2.2f
$MgCl_2$	41.8 ± 5.6g	97.8 ± 13.1e	13.8 ± 1.9f	66.3 ± 8.9f	28.4 ± 3.8g	13.8 ± 1.9e
HOAC	514.6 ± 11.7c	1203.8 ± 27.3c	169.7 ± 3.8c	816.1 ± 18.5c	349.8 ± 7.9c	169.7 ± 3.8c
HCl	111.2 ± 5.9d	260.1 ± 13.9d	36.7 ± 2.0d	176.3 ± 9.4d	75.6 ± 4.0d	36.7 ± 2.0d
EDTA	1410.8 ± 10.6a	3300.4 ± 24.8a	465.3 ± 3.5a	2237.7 ± 16.8a	958.9 ± 7.2a	465.3 ± 3.5a
DTPA	1220.8 ± 17.2b	2855.8 ± 40.3b	402.7 ± 5.7b	1936.2 ± 27.3b	829.8 ± 11.7b	402.7 ± 5.7b

Within columns, values followed by the same letter are not significantly different at $p = 0.05$ (LSR test)

Table 5 The variation of the concentration and percentage of Pb in different fractions

Soil	SE		WSA		OX		OM		RES	
	mg kg^{-1}	%[a]	mg kg^{-1}	%[a]	mg kg^{-1}	%[a]	mg kg^{-1}	%[a]	mg kg^{-1}	%[a]
Helianthus tuberosus	58.1	1.9	110.0	3.6	1042.6	34.3	662.4	21.8	1170.7	38.5
Solanum nigrum	157.4	2.2	286.3	4.0	2102.9	29.5	1581.6	22.2	2993.1	42.0
Chenopodium acuminatum	19.0	1.9	42.8	4.3	271.7	27.1	241.6	24.0	429.4	42.8
Polygonum lapathifolium	58.4	1.2	160.3	3.3	1520.6	31.5	1078.3	22.3	2011.2	41.7
Conyza canadensis	48.4	2.3	64.6	3.1	687.4	33.2	446.7	21.6	822.8	39.8
Abutilon theophrasti	15.8	1.6	48.9	4.9	348.4	34.7	205.2	20.4	386.1	38.4

[a] Percentage of different fractions in the total concents of heavy metals (%)

1998). DTPA extraction method was suitable for neutral and near-alkaline soils with insufficient transition metals (Lindsay and Norvell 1978). When DTPA was applied to acidic soil, the buffering capacity of the soil solution might be exceeded (O'Connor 1988), and considerable amounts of heavy metals occluded by Fe/Mn oxides and carbonates could be released (Hammer and Keller 2002), which were not responsible for plant availability. This was the evidence that DTPA was unsuitable for prediction of bioavailability of heavy metals in acidic soils to plants.

The percentage extractable by dilute acid was lower than that extracted by chelating reagent and higher than that extracted by neutral salts. While the Pb concentration in soil samples extracted by dilute acid was close to the concentrations in root and overground parts of *Helianthus tuberosus*, *Chenopodium acuminatum*, *Polygonum lapathifolium* and *Abutilon theophrasti*. HOAC had higher extracting efficiency than HCl. The neutral salts had different extraction efficiencies for Pb. The concentrations of Pb in root and overground parts of *Solanum nigrum* and *Conyza Canadensis* were close to those from the neutral salts. Neutral salts extraction methods were suitable for exchangeable metals (Hammer and Keller 2002). Neutral salts would extract exchangeable metals mainly because its ionic strength is similar to that of soil solution and it does not affect the equilibrium between soil solution and soil solid (Gupta and Aten 1993). Although easily exchangeable metals can be extracted with neutral salts, neutral salts exerted a weak competition for the adsorption sites of organic matter and oxide surfaces (Novozamsky et al. 1993). Hammer and Keller (2002) also suggested that plants seemed to take up heavy metals from pools rather than solely from neutral salts-extractable one. Overall, the suitability of neutral salts extraction methods were restricted to different types of soils (Stewart et al. 2003). Results also indicated NH_4Cl had the highest extractability among the neutral salts. This could be due to the possible reaction of these elements with NH_3 and to the higher salt concentration of the NH_4Cl solution (Pueyo et al. 2004).

Within columns, values followed by the same letter are not significantly different at $p = 0.05$ (LSR test).

In sequential extraction, Pb was strongly retained in the residual fraction in all the samples. As shown in Table 5, the fractions of Pb were mainly bound to the residual fraction with 38.4% to 42.8% for the soil samples. These fractions of heavy metals are contained in the crystal lattices of minerals with strong bonds and consequently they will not be released into the environment. Compared with the residual fraction, much lower proportions of Pb were extracted in the exchangeable and carbonate fractions, which was 1.2% to 2.3% and 3.1% to 4.9% of total Pb concentration. Processes of metal mobilization–immobilization are affected by plenty of soil properties. The free ion activity in the soil is largely determined by metal bonding to the solid phase. Solid organic matter, dissolved organic matter, clay and ion hydroxides could have a significant impact on the metal activity. In this study, a higher proportion (27.1%–34.7%) of Pb was associated with the oxide fraction and only in the case of a change in the redox conditions towards reductive would it be released from oxides. In this fraction, Pb has the more stable bonds and can be more difficultly released into the environment. The Pb bound to the carbonate fraction would be released if conditions become acidic. This could be the supplement for the available Pb equilibrium in soil solution.

The distribution of heavy metal speciation in the polluted soil samples was different from that in the clean soil samples (Zhou and Song 2004). The total and extractable Pb concentrations in polluted soil samples are generally higher than in the clean soils (Table 1). External sources were the main contributors to the increasing Pb. According to its total content in the study area, Pb is danger in the contaminated site corresponding with its source and speciation distribution. The most mobile fraction of Pb in the contaminated area could transfer to the groundwater or the plants and endanger public safety.

Acknowledgments The author acknowledges the financial support from the High Technology Research and Development Program (863) of China (No. 2007AA06A405); the National Key Basic Research Program (973) of China (No. 2004CB418506); the open foundation of Ministry of Education Key Laboratory of Environmental Remediation and Ecological Health, College of Natural Resources and Environmental Science, Zhejiang University (No. EREH 0713); and the open foundation of Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences.

References

Bermond A, Yousfi I, Ghestem JP (1998) Kinetic approach to the chemical speciation of trace metals in soils. Analyst 123:785–789. doi:10.1039/a707776i

Chen S, Zhou QX, Sun LN, Sun TH, Chao L (2007) Speciation of cadmium, lead in soils as affected by metal loading quantity and aging time. Bull Environ Contam Toxicol 79:184–187 doi: 10.1007/s00128-007-9154-2

Cui S, Zhou QX, Chao L (2007) Potential hyperaccumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China. Environ Geol 51:1043–1048 doi: 10.1007/s00254-006-0373-3

Fangueiro D, Bermond A, Santos E, Carapuca H, Duarte A (2002) Heavy metal mobility assessment in sediments based on a kinetic approach of the EDTA extractions: search for optimal experimental conditions. Anal Chim Acta 459:245–256. doi:10.1016/S0003-2670(02)00134-4

Guo GL, Zhou QX, Koval PV, Belogolova GA (2006) Speciation distribution of Cd, Pb, Cu, and Zn in contaminated Phaeozem in north-east China using single and sequential extraction procedures. Aust J Soil Res 44:135–142. doi:10.1071/SR05093

Gupta SK, Aten C (1993) Comparison and evaluation of extraction media and their suitability in a simple model to predict the biological relevance of heavy metal concentrations in contaminated soils. Int J Environ Anal Chem 51:25–46. doi:10.1080/03067319308027609

Hammer D, Keller C (2002) Changes in the rhizosphere of metal accumulating plants evidenced by chemical extractants. J Environ Qual 31:1561–1569

Houba VJG, Lexmond TM, Novozamsky JJ, Van der Lee JJ (1996) State of the art and future developments in soil analysis for bioavailability assessment. Sci Total Environ 178:21–28. doi: 10.1016/0048-9697(95)04793-X

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J 42:421–428

Lu AX, Zhang SZ, Shan XQ, Wang SX, Wang ZW (2003) Application of microwave extraction for the evaluation of bioavailability of rare earth element in soils. Chemosphere 54:54–63

Maiz I, Arambarri I, Garcia R, Millan E (2000) Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. Environ Pollut 110:3–9. doi: 10.1016/S0269-7491(99)00287-0

Navas A, Lindhorfer H (2003) Geochemical speciation of heavy metals in semiarid soils of the central Ebro Valley (Spain). Environ Int 29:61–68. doi:10.1016/S0160-4120(02)00146-0

Novozamsky I, Lexmond THM, Houba VJG (1993) A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. Int J Environ Anal Chem 51:47–58. doi: 10.1080/03067319308027610

O'Connor GA (1988) Use and misuse of the DTPA soil test. J Environ Qual 17:715–718

Prokop Z, Cupr P, Zlevorova-Z V, Komarek J, Dusek L, Holoubek I (2003) Mobility, bioavailability, and toxic effects of cadmium in soil samples. Environ Res 91:119–126. doi:10.1016/S0013-9351(02)00012-9

Pueyo M, Lopex-S JF, Rauret G (2004) Assessment of $CaCl_2$, $NaNO_3$ and NH_4NO_3 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils. Anal Chim Acta 504:217–226. doi:10.1016/j.aca.2003.10.047

Qureshi MI, Israr M, Abdin MZ, Iqbal M (2005) Responses of Artemisia annua L to lead and salt-induced oxidative stress. Environ Exp Bot 53:185–193. doi:10.1016/j.envexpbot.2004.03.014

Shao XH, Xing GX, Hou WH (1994) Research and application of sequential extraction in identifying metal speciation in soils (in Chinese). Prog Soil Sci 22:40–46

Stewart MA, Jardine PM, Barnett MO, Mehlhorn TL, Hyder LK, Mckay LD (2003) Influence of soil geochemical and physical properties on the sorption and bioaccessibility of chromium (III). J Environ Qual 3:129–137

Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51:844–851. doi:10.1021/ac50043a017

Ure AM, Quevauviller P, Muntau H, Griepink B (1993) Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of the European Communities. J Environ Anal Chem 51:135–151. doi: 10.1080/03067319308027619

Wei SH, Zhou QX, Wang X (2005) Identification of weed plants excluding the uptake of heavy metals. Environ Int 31:829–834. doi:10.1016/j.envint.2005.05.045

Xia SY, Liu Q (1994) The extraction of different speciation of lead and sampling depth in soil (in Chinese). Environ Pollut Control 16:27–29

Zhou QX (2003) Interaction between heavy metals and nitrogen fertilizers applied in soil-vegetable systems. Bull Environ Contam Toxicol 71:338–344. doi:10.1007/s00128-003-0169-z

Zhou QX, Rainbow PS, Smith BD (2003) Comparative study of the tolerance and accumulation of the trace metals zinc, copper and cadmium in three populations of the polychaete *Nereis diversicolor*. J Marine Biol Assoc UK 83:65–72

Zhou QX, Song YF (2004) Principles and method of treating contaminated soils (in Chinese). Science Press, Beijing, China