Changes in metal mobility assessed by EDTA kinetic extraction in three polluted soils after repeated phytoremediation using a cadmium/zinc hyperaccumulator

Zhu Li [a], Longhua Wu [a, *], Yongming Luo [a, b], Peter Christie [a]

[a] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, China
[b] Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research, Yantai, 264003, China

HIGHLIGHTS

- Repeated phytoextraction redistributed metals from non-labile to labile fractions.
- The hyperaccumulator greatly reduced the mobility in soil of hyperaccumulated metals.
- The mobility of non-hyperaccumulated metals increased by an increase in the labile fractions or desorption rates.

ARTICLE INFO

ABSTRACT

Phytoextraction is one of the most promising technologies for the decontamination of metal-polluted agricultural soils. Effects of repeated phytoextraction by the cadmium (Cd)/zinc (Zn) hyperaccumulator *Sedum plumbizincicola* on metal (Cd, Zn, copper (Cu) and lead (Pb)) mobility were investigated in three contaminated soils with contrasting properties. EDTA kinetic extraction and the two first-order reactions model showed advantages in the assessment of soil metal mobility and clearly discriminated changes in metal fractions induced by phytoextraction. Repeated phytoextraction led to large decreases in readily labile (Q_1^0) and less labile (Q_2^0) fractions of Cd and Zn in all three soils with the sole exception of an increase in the Q_2^0 of Zn in the highly polluted soil. However, Q_1^0 fractions of soil Cu and Pb showed apparent increases with the sole exception of Pb in the acid polluted soil but showed a higher desorption rate constant (k_1). Furthermore, *S. plumbizincicola* decreased the non-labile fraction (Q_3^0) of all metals tested, indicating that the hyperaccumulator can redistribute soil metals from non-labile to labile fractions. This suggests that phytoextraction decreased the mobility of the metals hyperaccumulated by the plant (Cd and Zn) but increased the mobility of the metals not hyperaccumulated (Cu and Pb). Thus, phytoextraction of soils contaminated with mixtures of metals must be performed carefully because of potential increases in the mobility of non-hyperaccumulated metals in the soil and the consequent environmental risks.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Human activities such as mining, wastewater irrigation and application of chemical fertilizers have led to the excessive accumulation of metals in many agricultural soils (Cheng, 2003) and soil metal contamination represents a risk to human and environment health (Liu et al., 2013; Lu et al., 2015). The risk of soil metal pollution is therefore a major concern and methods for remediating polluted soils are of strategic interest. Phytoextraction using hyperaccumulating plant species is regarded as one of most promising technologies for the decontamination of metal-polluted agricultural soils because it is environmentally friendly and is carried out *in situ* at relatively low cost with the topsoil remaining intact (Ali et al., 2013). Soil metal availability or mobility is one of most important factors affecting phytoextraction by influencing plant root metal uptake and also by changing plant metal removal and rhizosphere activities. Studies on changes in soil metals during or after phytoextraction are important in helping to elucidate the

* Corresponding author.
E-mail address: lhwu@issas.ac.cn (L. Wu).

principles of remediation and of risk assessment following remediation.

During phytoextraction hyperaccumulators increase metal mobility by rhizosphere metal activation or decrease soil metal availability and mobility by plant metal removal from the soil. Root exudation of dissolve organic matter (DOM) and acidification of rhizosphere soil increase soil metal mobility and metal uptake by hyperaccumulators (Li et al., 2012). Nickel (Ni) solubility in the rhizosphere increased with the formation of Ni-organic complexes, although the exchangeable (Ni) fraction was reduced by the uptake of the Ni hyperaccumulator *Thlaspi goesingense* (Wenzel et al., 2003). Repeated phytoextraction shows a clear decrease in soil metal availability and Cd and Zn, both in solution and in extractable fractions, showed large decreases after phytoextraction with several croppings of a Cd and Zn hyperaccumulator in our previous studies (Li et al., 2014a, b). However, studies have also indicated that soil metal mobility might be regenerated to some extent by transformation of soil metals between different fractions to reach chemical equilibrium and/or by the rhizosphere effect of the plant (Wenzel et al., 2003; Li et al., 2012). Thus, further investigation is needed to elucidate the release of metals in soils during or after phytoextraction using hyperaccumulator plant species.

Typically, a given hyperaccumulator species extracts a very limited number of metals and the plant may exert different effects on the different metals that it does hyperaccumulate (i.e. those the hyperaccumulator can accumulate in high concentrations in the aerial parts) and those it does not hyperaccumulate (those for which the hyperaccumulator has no strong accumulation capacity). In addition, few soils are typically contaminated with only one type of pollutant and combinations of metals or metals together with organic pollutants are commonly found in contaminated soils (Zhang et al., 2014; Yin et al., 2013). However, studies on changes in soil metals induced by hyperaccumulators have focused on the hyperaccumulated metals without due regard to the remaining metals, especially after long-term phytoextraction. Root exudates of hyperaccumulators such as DOM and organic acids may not have specific roles in the uptake of hyperaccumulated metals and non-hyperaccumulated metals may also complex with DOM or be solubilized by soil acidification. Thus, the mobility or availability of metals that are not accumulated by the hyperaccumulator plant also require investigation for a full understanding of the phytoextraction of metals from contaminated soils.

Chemical extractants have been widely used to assess and estimate soil metal mobility and availability to biota (Tessier et al., 1979; Ure, 1996; Labanowski et al., 2008; Mourier et al., 2011; Sungur et al., 2016). Ethylenediaminetetraacetic acid (EDTA) has a strong capacity to complex with metals and has been used to predict both the maximum labile metal pool in soils (Iqbal et al., 2013) and long-term metal mobility (Labanowski et al., 2008). Kinetic extraction can give improved analysis of metal desorption data when combined with mathematical models such as the two first-order reaction model (TFOR), the two constant rate model, parabolic diffusion models and simple Elovich equations (Fangueiro et al., 2005; Rashti et al., 2014). Previous studies show substantial decreases in the soluble fractions of soil Cd and Zn, extractable fractions or total metal concentrations with repeated phytoremediation using a Cd/Zn hyperaccumulator in soils with combined pollution by several metals (Wu et al., 2012; Li et al., 2014a, b). However, the regeneration of Cd and Zn mobility after cessation of phytoextraction and the mobility of other non-hyperaccumulated metals require further study.

Here, we use EDTA kinetic extraction combined with TFOR model to investigate the mobility of metals in three metal-contaminated soils with different soil properties and pollution loadings subjected to repeated phytoremediation by the Cd/Zn

hyperaccumulator *Sedum plumbizincicola* in previous pot experiments. We discriminate between changes in soil metal distributions induced by the hyperaccumulator and their different effects on the two metals hyperaccumulated by the plant (Cd and Zn) and two other metal contaminants also present in the soils (Cu and Pb) but not hyperaccumulated.

2. Materials and methods

2.1. Soil characterization

Three metal-contaminated soils were collected from the arable layer (top 15 cm) at two field sites. An acid soil collected from Luqiao in Zhejiang Province, east China, and designated 'LQ' is a Typic Fe-leachi-Stagnic Anthrosols that has been polluted by the dismantling of electronic wastes over a period of several decades. The other two soils are calcareous soils that were collected from Zhujiawu in Zhejiang Province, east China. They have been polluted by a copper smelter and the soil type is Fe-accumuli-Stagnic Anthrosols. One of the soils collected near the copper smelter has been subjected to high metal pollution and is referred to as 'ZJW-H'. The other soil is slightly polluted, was sampled further (about 100 m) from the smelter and is labelled 'ZJW-L'. The three soils are polluted with Cd and Zn and also have elevated concentrations of Cu and Pb. All three contaminated soils have been repeatedly phytoextracted using the Cd/Zn hyperaccumulator *Sedum plumbizincicola* with several repeated croppings in glasshouse experiments as reported previously (Wu et al., 2012; Li et al., 2014a, b). Samples of the three soils with or without previous repeated phytoextraction were used to evaluate the changes in the mobility of soil metals induced by *S. plumbizincicola*. Selected chemical properties of the soils with (P) and without (NP) repeated phytoextraction were determined. Soil pH, soil total organic carbon (SOC), soil cation exchange capacity (CEC), and total metal (Cu, Pb, Zn, and Cd) concentrations were determined and the details of the soil analysis have been published previously (Wu et al., 2012; Li et al., 2014a, b). Selected chemical properties of the contaminated soils are listed in Table 1.

2.2. Extraction kinetics using EDTA

EDTA was used to extract soil metals for different time periods from 0 to 24 h to determine the changes in soil metal mobilization between soils with and without phytoremediation. Soil (1 g) was mixed with 20 ml 0.01 M Na_2H_2-EDTA in a 50-ml plastic centrifuge tube and shaken at 40 rpm for different times using an end-over-end shaker (Hualida Model WH-962, Taicang, China). The extraction times were 5, 10, 15, 20, 25, and 30 min and 1, 3, 5, 7, 9, 11, 17, and 24 h. In the case of extraction times <1 h the samples were

Table 1
Chemical properties of the contaminated soils with and without repeated phytoextraction.

Soil		pH	CEC cmol kg^{-1}	SOC g kg^{-1}	Total metal (mg kg^{-1})			
					Cu	Pb	Zn	Cd
LQ	NP	4.56	10.5	21.2	369[*]	52.8[*]	162[*]	7.79[*]
	P	4.65	–	20.0	329	43.6	118	0.51
ZJW-L	NP	7.14[*]	11.2	20.6	175	206	1201[*]	2.28[*]
	P	7.72	–	19.5	166	187	978	1.43
ZJW-H	NP	7.24[*]	10.7	29.1	1012	1343	6191[*]	14.7[*]
	P	6.35	–	28.4	1037	1343	4952	2.92

NP, soil without phytoextraction; P, soil repeatedly phytoextracted with the hyperaccumulator *Sedum plumbizincicola*; SOC, soil total organic carbon; CEC, soil cation exchange capacity; "*" denotes that the value in NP soil is significant different from the value in the corresponding P soil, n = 3.

immediately filtered through a 0.45 μm cellulose membrane. The suspensions of the other samples were firstly centrifuged for 3 min at 3500 rpm before filtration. All extractions were carried out at 14 points in time and each point with three replicates, giving a total of 42 samples of each soil. The pH of the extractant solutions in this study was adjusted to 6.5 to minimize the effects of H$^+$ during extraction as described previously (Fangueiro et al., 2002; Labanowski et al., 2008). Metal concentrations in the filtrates were determined by atomic absorption spectrophotometry (SpectrAA 220FS, Varian, Palo Alto, CA), and the concentrations of some major elements i.e. iron (Fe), aluminum (Al), calcium (Ca) and magnesium (Mg) in the filtrates were also determined using inductively coupled plasma-optical emission spectrometry (Optima 8000, Perkin Elmer, Waltham, MA).

2.3. Theory of the two first-order reactions model for data fitting

Metals in soil bind mostly on the soil solid matrix to different sites such as soil minerals, oxides, and organic matter. The multiple first-order reaction model can reflect the physicochemical relationships between metals and soils and can distinguish types of metal binding sites in soils by different first-order reactions. In the present study the simplified two first-order reactions (TFOR) model was used on the assumptions that the two first-order reactions took place simultaneously and that the rates were independent of each other. The whole reaction can therefore be described by the following equations:

$$\frac{dQ}{dt} = \frac{dQ_1}{dt} + \frac{dQ_2}{dt} = k_1(Q_1^0 - Q_1) + k \qquad (1)$$

For the initial conditions, t = 0, and Q_1 and Q_2 = 0; the above equation can be rearranged as:

$$Q = Q_1^0\left(1 - \exp^{-k_1 t}\right) + Q_2^0\left(1 - \exp^{-k_2 t}\right) \qquad (2)$$

where Q (mg kg^{-1}) is the amount of metal desorbed from the soil at time t, Q_1^0 (mg kg^{-1}) is the amount of metal desorbed from the soil in compartment 1 at equilibrium (t = 24 h) which is defined as the readily extractable metal, and the corresponding rate constant k_1; Q_2^0 is the amount of metal desorbed from the soil in compartment 2 at equilibrium (mg kg^{-1}) which is regarded as the less extractable metal, and the corresponding rate constant k_2. The non-extractable metal (Q_3^0, mg kg^{-1}) can be obtained by the difference between the total metal concentration in the soil (Q_{total}, mg kg^{-1}) and the extracted metal concentrations at equilibrium (Q_1^0 and Q_2^0).

2.4. Statistical analysis

Soil EDTA-extractable metal concentration data are presented as mean ± standard deviation. The amount of metal desorbed from the soil in compartments 1 and 2 (Q_1^0 and Q_2^0) and the corresponding rate constants k_1 and k_2 were gained by fitting extraction data (n = 14) as equation (2) using OriginPro8, and the difference in value between NP soil and the corresponding P soil was distinguished by confidence intervals at a level of 95%. The difference in soil characteristics between NP soil and the corresponding P soil was tested by independent-samples t-test using the SPSS 16.0 software package.

3. Results

3.1. Changes in soil chemical characteristics

Selected chemical characteristics of the soils with (P) and without (NP) repeated phytoextraction are presented in Table 1. Soil pH changes differed in the different soils after repeated phytoextraction. Slight increases in soil pH were observed in both the acid and the slightly polluted calcareous soils but significant decreases in soil pH were found in the highly polluted calcareous soil after repeated phytoextraction, indicating that there was no clear trend of changing soil pH induced by phytoextraction. All contaminated soils showed some changes in total metal concentrations. Total Zn and Cd decreased markedly in all three soils after repeated phytoextraction with S. plumbizincicola, with percentage decreases in Zn of 18.6–26.6% and in Cd of 37.2–93.6%. The decreases in total soil Zn and Cd were consistent with plant Zn and Cd uptake (Wu et al., 2012; Li et al., 2014a, b), which indicates that the soil metals were mostly removed by the hyperaccumulator. However, there was no significant change in soil Cu or Pb, two of the metals not hyperaccumulated by the plant after repeated phytoextraction, with the sole exception of the acid soil (Table 1).

### 3.2. EDTA-extractable soil metals

The increasing cumulative amounts of metals with time in contaminated soils are presented in Figs. 1–3. All metal extractions reached equilibrium after 24 h. The extraction curves for all metals tested were similar in shape, comprising a more rapid region (t < 1) and a slower region over a longer extraction time period (1 < t < 24 h), a result consistent with previous studies (Fangueiro et al., 2005).

In all three soils the EDTA-extractable Cd and Zn concentrations at the more rapid stage and at equilibrium were much lower in phytoremediated soils than in the corresponding non-phytoremediated soils (Fig. 1), indicating that phytoextraction significantly decreased the available and potentially mobile fractions of soil Cd and Zn. Conversely, the amounts of EDTA-extractable Cu and Pb at the more rapid stage (t = 1 h) and at equilibrium (t = 24 h) in the two phytoremediated calcareous soils increased by 7.64–52.9% compared to the non-phytoremediated soils (Fig. 2). The extractable Cu and Pb at the rapid stage in non-phytoremediated acid soil appeared to be lower than in the corresponding phytoremediated acid soil, but not at equilibrium. Taking the first extraction (t = 5 min) for example, the increases in EDTA-extractable Cu and Pb in phytoremediated soil were from 8.19 to 78.4% compared with the non-phytoremediated acid soil. However, Cu and Pb extracted at the equilibrium in the acid soil showed no substantial differences between the phytoremediated and non-phytoremediated soils, indicating that phytoextraction increased the desorption rates of Cu and Pb in the acid soil but exerted no influence on the maximum extractable metals.

For Al and Fe, EDTA-extractable concentrations at the more rapid stage and at equilibrium were much higher in phytoremediated soils compared to the corresponding non-phytoremediated soils (Fig. 3), indicating that the mobility of Fe and Al in all three tested soils increased as phytoextraction proceeded. Conversely, the extractable Ca was lower in phytoremediated soils. For Mg the concentration in early extraction was lower in phytoremediated soils than the corresponding non-phytoremediated soils, but at equilibrium the Mg concentration was higher in phytoremediated soils (LQ and ZJW-L) and no difference was observed from the corresponding non-phytoremediated soil (ZJW-H).

3.3. Fitting of extraction data by the two first-order reactions model

The metal extraction data were fitted using the TFOR model according to equation (2). Soil metals were separated into the readily labile (Q_1^0), less labile (Q_2^0) and non-labile (Q_3^0) fractions.
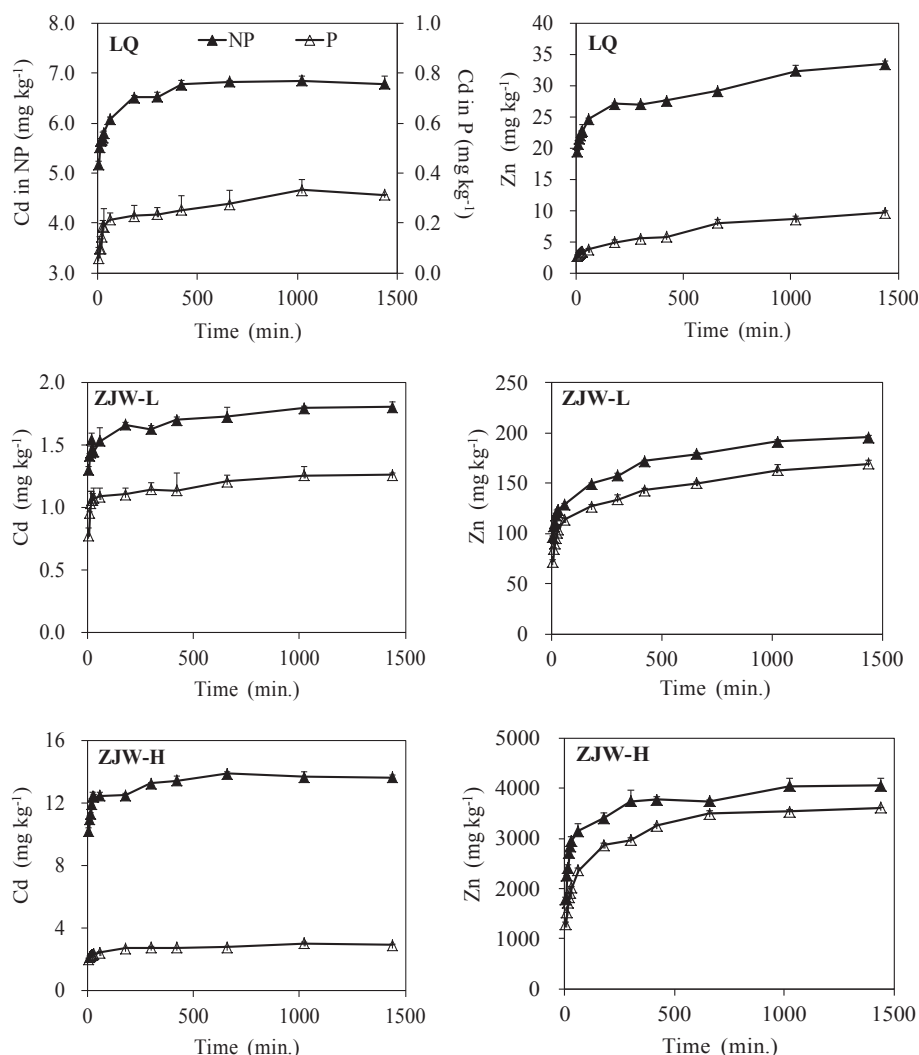
Fig. 1. Dynamic desorption curves of Cd and Zn extracted with EDTA in the contaminated soils with (P) and without (NP) repeated phytoextraction with a Cd/Zn hyperaccumulator; LQ, ZJW-L and ZJW-H are three metal-contaminated soils with different soil properties.

Most of the fitting coefficients of correlation R^2 values were close to 1, indicating the goodness of fit of the data using this model (Tables 2 and 3). The desorption rate constants of readily labile metal, k_1, were two or three orders of magnitude higher than those of less labile ones, k_2, in all soils, suggesting that the model distinguished the two metal fractions with different binding forces to the soil solid matrix.

Turning to Cd and Zn, the two metals hyperaccumulated by *S. plumbizincicola*, almost all values of Q_1^0 and Q_2^0 in non-phytoremediated soil were much higher than in the corresponding phytoremediated soil, indicating that both the readily labile and less labile fractions of Cd and Zn were removed by the hyperaccumulator. However, it was found that the value of Q_2^0 for Zn in the highly polluted phytoremediated calcareous soil was higher than in the non-phytoremediated soil. This may have resulted from metal transfer from the non-labile fraction as indicated by the large decreases in Q_3^0. However, in the case of Cu and Pb, the metals not hyperaccumulated by *S. plumbizincicola*, there was a clear increase in the value of Q_1^0 in the calcareous soils following phytoextraction, with increases of 35.1 and 11.6% for Cu and 33.9 and 6.26% for Pb compared with the non-phytoremediated polluted calcareous soils (Table 3). The labile Cu and Pb (Q_1^0) in the acid soil showed no

apparent increase after repeated phytoextraction, but higher k_1 values were also found after phytoremediation, with increases in k_1 for Cu and Pb of 25 and 109%, respectively. In the case of the non-labile fractions (Q_3^0) there were apparent declines in all tested metals following phytoremediation but not in Cu or Pb in the acid soil.

Metal distribution patterns differed greatly depending on soil and metal (Fig. 4). Cadmium in all three non-phytoremediated soils was mainly distributed in the readily labile fraction (Q_1^0), >60% of total concentration, and >80% of total Zn was present in the non-labile fraction in the acid and slightly polluted soils. In contrast, Cu and Pb had relative even distributions in the three fractions in all three soils. Repeated phytoextraction had apparent effects on the distribution of the metals studied in all three soils. Compared to non-phytoremediated acid soil, the percentages of labile fractions to total Cd and Zn decreased, and of non-labile fractions increased in the phytoremediated acid soil. In the calcareous soils the percentages of Cd and Zn showed a decline in Q_3^0 and an increase in the labile fraction ($Q_1^0 + Q_2^0$) after phytoremediation. Copper and Pb were distributed more in the labile fractions and less in the non-labile fractions in all soils after repeated phytoextraction with the sole exception of Pb in the acid soil.
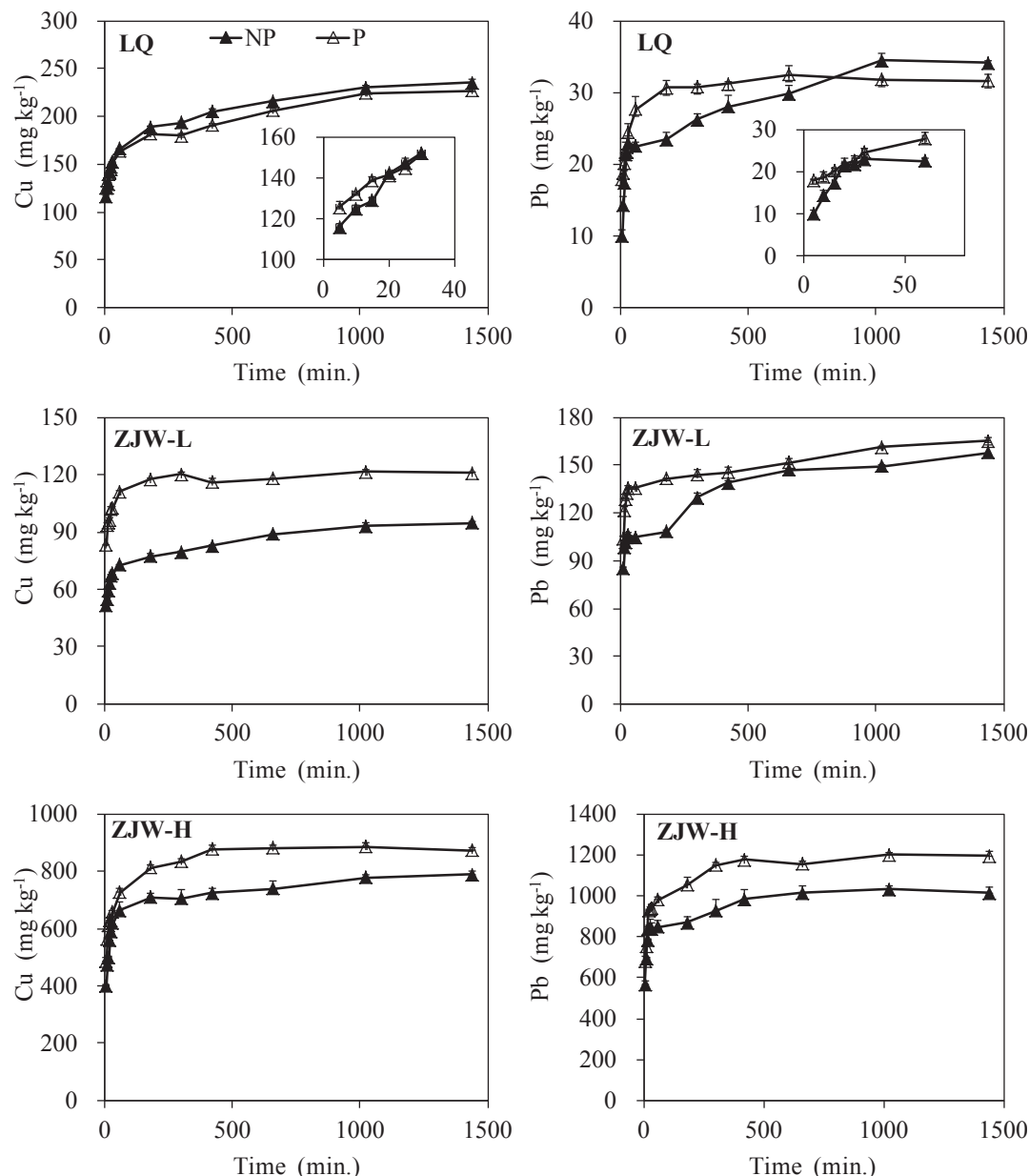
Fig. 2. Dynamic desorption curves of Cu and Pb extracted with EDTA in the contaminated soils with (P) and without (NP) repeated phytoextraction with a Cd/Zn hyperaccumulator; LQ, ZJW-L and ZJW-H are three metal contaminated soils with different soil properties.

4. Discussion

When metals enter the soil they will be fixed by the soil solid phase and their mobility will thus decrease. The metals will reach a quasi-equilibrium state between the soil solid phase and the soil solution as reactions occur over the long term. Soil metal mobility is controlled not only by the metal in the soil solution but also by the processes governing the release of metal from the soil solid phase to the solution (McGregor et al., 1998). Kinetic extraction provides good evaluation of soil metal mobility (Labanowski et al., 2008).

In the present study EDTA kinetic extraction and TFOR models were used and soil metals were distributed into three fractions with different abilities to exhibit labile behavior, and the corresponding desorption rate constants were also obtained (Tables 2 and 3). After repeated phytoextraction with *S. plumbizincicola* there were large decreases in soil readily labile (Q_1^0) and/or less

labile (Q_2^0) Cd and Zn in all three soils examined, and the corresponding desorption rate constants also decreased under certain conditions, for example Cd in the acid soil or the slightly contaminated calcareous soil (Table 2). This indicates that repeated phytoextraction with *S. plumbizincicola* decreased the mobility of accumulated metals by reducing the amount of desorbed metal and/or the desorption rates. However, *S. plumbizincicola* induced an increase in the mobility of the non-hyperaccumulated metals Cu and Pb. For example, soil labile Cu and Pb (EDTA-extractable) increased in the highly polluted calcareous soil. Although the labile Cu and Pb showed no significant changes with repeated phytoextraction of the acid soil, the amounts released and desorption rates at early extraction stages increased compared to the corresponding non-phytoremediated acid soil (Fig. 2 and Table 3). In addition, the non-labile fractions (Q_3^0) of hyperaccumulated metals in all three soils and the non-hyperaccumulated metals in the slightly and
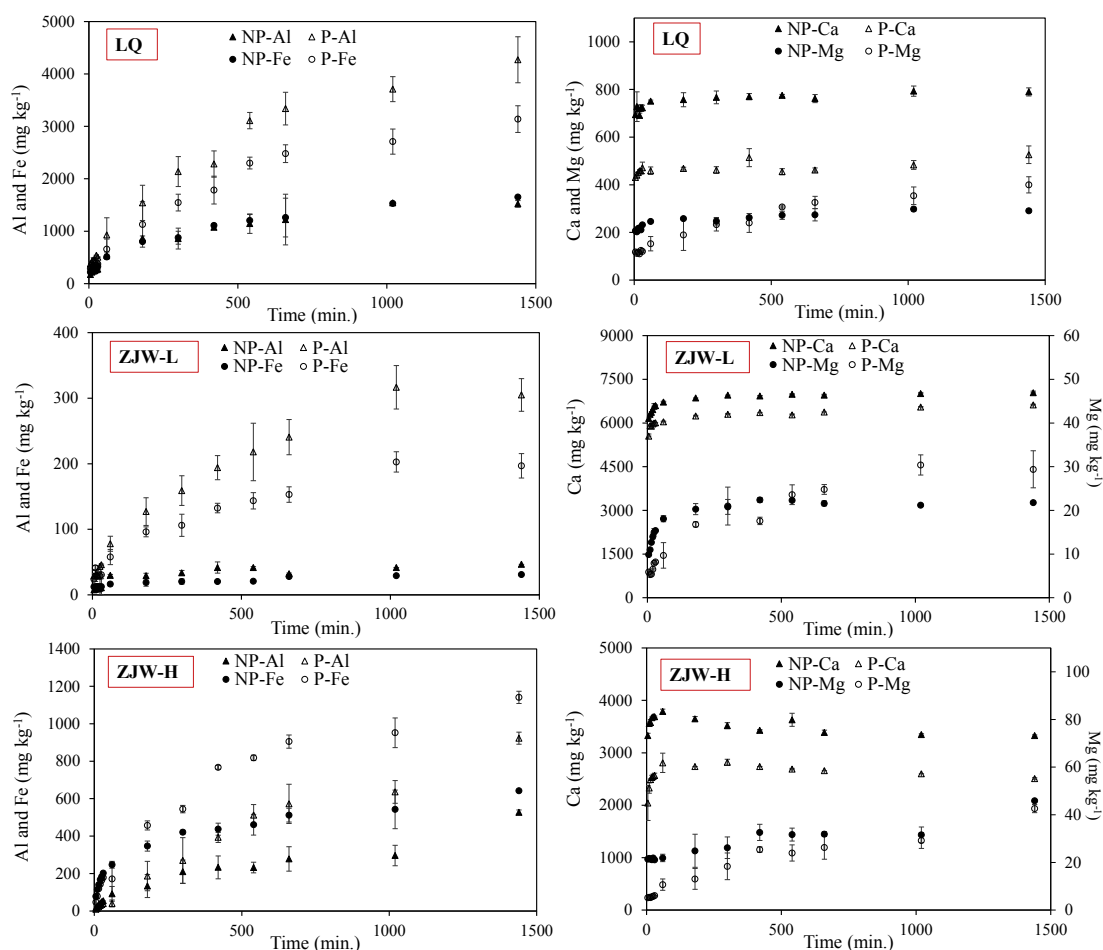
Fig. 3. Dynamic desorption curves of Al, Fe, Ca and Mg extracted with EDTA in the contaminated soils with (P) and without (NP) repeated phytoextraction with a Cd/Zn hyper-accumulator; LQ, ZJW-L and ZJW-H are three metal contaminated soils with different soil properties.

Table 2
Fitting results of the two first-order reactions model for cadmium (Cd) and zinc (Zn) extracted by EDTA in the contaminated soils with and without repeated phytoextraction.

			Q_1^0 (mg kg^{-1})	k_1 (min^{-1})	Q_2^0 (mg kg^{-1})	k_2 (e−3 min^{-1})	Q_3^0 (mg kg^{-1})	R^2
Cd	LQ	NP	5.49 ± 0.05*	0.54 ± 0.04*	1.30 ± 0.05*	8.12 ± 1.17*	1.00	0.99
		P	0.21 ± 0.02	0.06 ± 0.01	0.11 ± 0.04	1.18 ± 1.16	0.19	0.96
	ZJW-L	NP	1.45 ± 0.02*	0.43 ± 0.05*	0.34 ± 0.04	3.08 ± 0.97	0.49	0.94
		P	1.06 ± 0.01	0.25 ± 0.01	0.25 ± 0.03	1.27 ± 0.38	0	0.99
	ZJW-H	NP	11.7 ± 0.2*	0.38 ± 0.05	2.00 ± 0.30*	4.60 ± 2.30*	0.85	0.91
		P	2.12 ± 0.04	0.49 ± 0.07	0.68 ± 0.04	10.0 ± 2.36	0.05	0.97
Zn	LQ	NP	22.0 ± 0.5*	0.39 ± 0.07	12.1 ± 1.6*	1.74 ± 0.56	128	0.95
		P	3.09 ± 0.14	0.39 ± 0.14	7.83 ± 0.68	1.29 ± 0.24	103	0.98
	ZJW-L	NP	114 ± 3*	0.34 ± 0.05	87.2 ± 4.9	2.54 ± 0.41	1000	0.98
		P	96.8 ± 2.8	0.23 ± 0.03	71.7 ± 4.7	2.50 ± 0.48	810	0.98
	ZJW-H	NP	2705 ± 105*	0.18 ± 0.02	1265 ± 116*	4.85 ± 1.34	2220	0.97
		P	1747 ± 77	0.21 ± 0.03	1808 ± 89	4.67 ± 0.71	1397	0.98

"*" denotes that the value in NP soil is significantly different from the value in the corresponding P soil distinguished by confidence intervals at a level of 95%; Q_3^0 has been evaluated by subtracting Q_1^0 and Q_2^0 from the total metal amount per kg of soil; 14 samples (n = 14) were used for the fitting, with three replicates for each mean value.

highly contaminated calcareous soils also showed marked decreases after repeated phytoextraction (Tables 2 and 3). Although the magnitudes of decrease in non-labile metals were much higher for the hyperaccumulated than the non-hyperaccumulated metal during repeated phytoextraction, the results clearly suggest that growing *S. plumbizincicola* in metal contaminated soils can transfer non-labile fractions into the labile fraction in the case of all four metals tested. The above results also suggest that, technically speaking, EDTA kinetic extraction and TFOR models confer

advantages in the assessment of soil metal mobility and clearly discriminate among changes in metal fractions during the phytoextraction process.

Changes in metal mobility during phytoextraction are related to plant metal uptake and interactions between the soil and plant roots. Firstly, plant metal uptake decreases soil metal mobility by removing the readily mobile metal from the soil. Secondly, biochemical processes in the rhizosphere can decrease or increase soil metal mobility depending on plant and metal species.

Table 3
Fitting results of the two first-order reactions model for copper (Cu) and lead (Pb) extracted by EDTA in the contaminated soils with and without repeated phytoextraction.

Element	Soil		Q_1^0 (mg kg^{-1})	k_1 (min^{-1})	Q_2^0 (mg kg^{-1})	k_2 (e-3 min^{-1})	Q_3^0 (mg kg^{-1})	R^2
Cu	LQ	NP	137 ± 5	0.32 ± 0.06	94.1 ± 7.1	3.50 ± 0.79	138	0.96
		P	140 ± 4	0.40 ± 0.09	89.1 ± 8.6	2.36 ± 0.64	140	0.95
	ZJW-L	NP	63.5 ± 1.9*	0.28 ± 0.04*	31.4 ± 3.4	2.63 ± 0.82*	159	0.96
		P	85.8 ± 3.1	0.53 ± 0.09	33.3 ± 3.1	23.4 ± 4.38	135	0.98
	ZJW-H	NP	517 ± 46*	0.24 ± 0.03	230 ± 13	13.2 ± 3.29	299	0.94
		P	577 ± 13	0.33 ± 0.03	299 ± 13	9.32 ± 1.41	161	0.99
Pb	LQ	NP	22.0 ± 0.8*	0.11 ± 0.01	17.2 ± 4.3	0.10 ± 0.04*	13.6	0.98
		P	15.8 ± 0.6	0.23 ± 0.08	15.7 ± 0.6	2.37 ± 0.23	21.3	0.99
	ZJW-L	NP	98.6 ± 1.1*	0.38 ± 0.03	57.5 ± 1.5*	4.04 ± 0.34*	144	0.99
		P	132 ± 2	0.29 ± 0.02	38.0 ± 4.1	1.82 ± 0.51	130	0.97
	ZJW-H	NP	814 ± 14	0.22 ± 0.02	221 ± 22*	2.70 ± 0.80*	308	0.98
		P	865 ± 28	0.26 ± 0.03	324 ± 30	6.14 ± 1.93	154	0.96

"*" denotes that the value in NP soil is significantly different from the value in the corresponding P soil distinguished by confidence intervals at a level of 95%; Q_3^0 has been evaluated by subtracting Q_1^0 and Q_2^0 from the total metal amount per kg of soil; 14 samples (n = 14) were used for the fitting, and there were three replicates for each mean value.
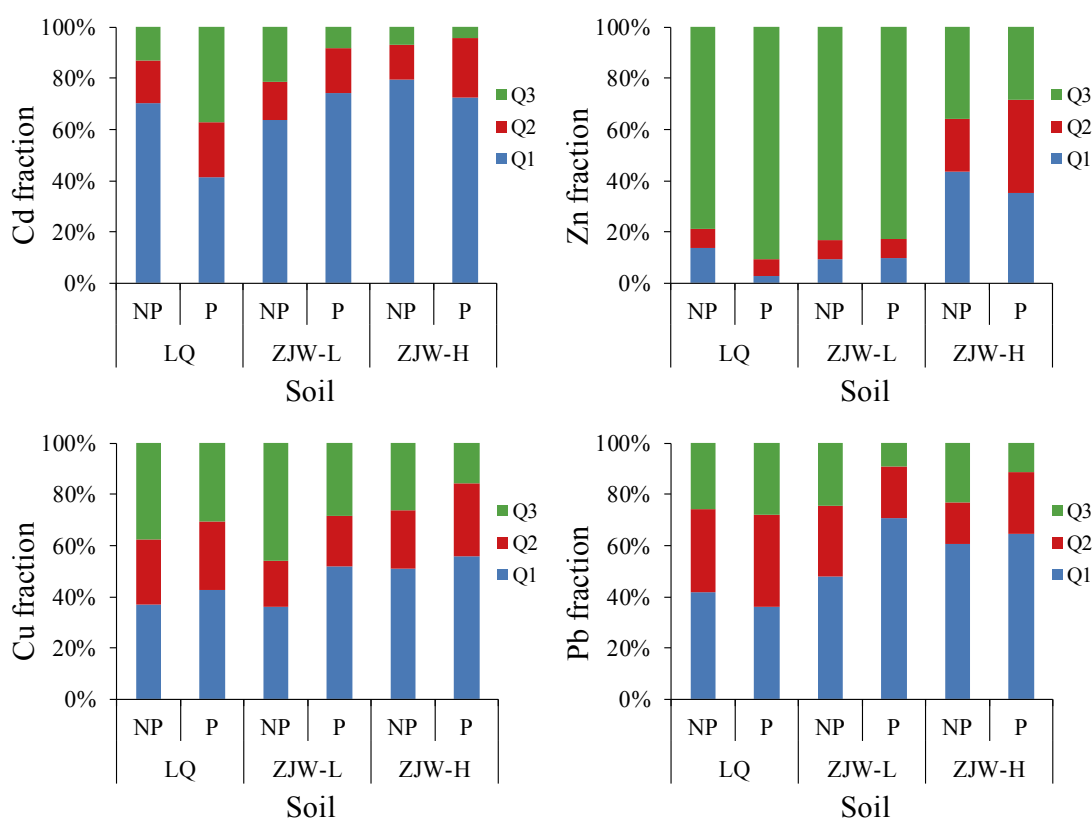
Fig. 4. Distribution of metals (Cd, Zn, Cu and Pb) between three fractions in the contaminated soils with (P) and without (NP) repeated phytoextraction with a Cd/Zn hyperaccumulator; LQ, ZJW-L and ZJW-H are three metal contaminated soils with different soil properties.

Acidification of rhizosphere soils and release of organic materials by roots or soil rhizosphere organisms to increase element availability is one of the most important accumulation mechanisms for some hyperaccumulators (Wenzel et al., 2003; Li et al., 2012; Liu et al., 2017). However, plants may also secrete specific organic compounds to complex with metals and to prevent the accumulation of specific metals (Dong et al., 2007). In hyperaccumulators the overall result of plant uptake and rhizosphere reactions will reduce the mobility of hyperaccumulated metals in the soil for a long time. After repeated phytoextraction by the hyperaccumulator *S. plumbizincicola* there were large declines in the mobility of soil Cd and Zn and this effect was independent of soil properties (Fig. 1).

However, the non-hyperaccumulated metals behave differently and repeated phytoextraction with *S. plumbizincicola* increased the mobility of the non-hyperaccumulated Cu and Pb. The hyperaccumulator has a weak ability to take up non-hyperaccumulated metals from contaminated soils and the changes in mobility depend mainly on biochemical reactions in the rhizosphere. Rhizosphere soil acidification might be one of the important accumulation mechanisms to some hyperaccumulators (Bernal et al., 1994; Li et al., 2011), and low soil pH will also increase the mobility of non-hyperaccumulated metals. Decreasing soil pH may partly explain the increased Cu and Pb mobility in the highly polluted calcareous soil, but not in the other two soils which had higher soil pH values after repeated phytoextraction (Table 1). Some exudates, notably low-molecular-weight organic acids, can

increase the mobility of non-hyperaccumulated metals by forming soluble complexes (Lu et al., 2007; Wang and Mulligan, 2013). The root exudates of hyperaccumulators can also enhance the dissolution of iron minerals to release the binding of metals (Liu et al., 2017). Moreover, through the action of soil organisms in the rhizosphere the binding of metals to Fe/Mn oxides may be released with a reduction in Fe/Mn (Weiss et al., 2004). The explanation may be further supported by our results showing much higher extractable Fe and Al in phytoremediated soils than the corresponding non-phytoremediated soils (Fig. 3). In addition, metals may be redistributed with the decomposition of binding phase soil organic materials (Schroth et al., 2008). When the hyperaccumulator did not take up enough soil activated metals (non-hyperaccumulated metals) their mobility would be enhanced. This is supported by the fast that the hyperaccumulator showed high t uptake of Ca and Mg (essential major elements for plants), but the rhizosphere action usually resulted in a weak mobilization such as root exudate complexation, thus the mobility was reduced by phytoextraction (Fig. 3). The low uptake by the hyperaccumulator and strong mobilization by rhizosphere activities might be the explanation for the increased mobility of Cu and Pb after repeated phytoextraction with *S. plumbizincicola* in the present study.

Our results suggest that phytoextraction of metal-contaminated soils with *S. plumbizincicola* can definitely decrease the mobility of soil Cd and/or Zn but also increase the mobility of other metals present in contaminated soils such as Cu and Pb. Thus, phytoextraction with the hyperaccumulator decreased the environmental risk from the hyperaccumulated metals but increased the risk from non-hyperaccumulated metals in the soil. The resulting increase in the mobility of non-hyperaccumulated metals may even induce toxicity to the hyperaccumulator and thereby decrease the efficiency of phytoextraction (Li et al., 2013). Therefore, when phytoextraction of metals in soils polluted with mixtures of metals is conducted using hyperaccumulators the potential effects of increasing mobility of non-hyperaccumulated metals must be taken into consideration.

5. Conclusions

Repeated phytoextraction of metal-contaminated soils using the Cd/Zn hyperaccumulator *S. plumbizincicola* changed soil metal mobility and distribution, and this was discriminated by combining EDTA kinetic extraction with TFOR models. The soil mobility of the metals Cd and Zn which are hyperaccumulated by *S. plumbizincicola* was reduced by decreases in the labile fractions and/or the corresponding desorption rates, but in contrast the mobility of the non-hyperaccumulated metals Cu and Pb increased in the soils after repeated phytoextraction. This indicates that on one hand the environmental risk from hyperaccumulated metals can be reduced by the hyperaccumulator but on the other hand there can be negative effects due to the increased mobility of non-hyperaccumulated metals during phytoextraction. Thus, when soils with multiple metal contamination are remediated, intercropping with two or more hyperaccumulators with the capacity to hyperaccumulate different metals might be an effective measure to solve this problem. In addition, the non-labile fractions of metals can also be transferred to labile fractions by the repeated cropping of hyperaccumulators irrespective of which metals are present in the contaminated soils.

References

Ali, H., Khan, E., Sajad, M.A., 2013. Phytoremediation of heavy metals-Concepts and applications. Chemosphere 91, 869—881.

Bernal, M.P., McGrath, S.P., Miller, A.J., Baker, A.J.M., 1994. Comparison of the chemical changes in the rhizosphere of the nickel hyperaccumulator *Alyssum-murale* with the non-accumulator *Raphanus-sativus*. Plant Soil 164, 251—259.

Cheng, S.P., 2003. Heavy metal pollution in China: origin, pattern and control. Environ. Sci. Pollut. Res. 10, 192—198.

Dong, J., Mao, W.H., Zhang, G.P., Wu, F.B., Cai, Y., 2007. Root excretion and plant tolerance to cadmium toxicity - a review. Plant Soil Environ. 53, 193—200.

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 extraction: search for optimal experimental conditions. Anal. Chim. Acta 459, 245—256.

Fangueiro, D., Bermond, A., Santos, E., Carapuca, H., Duarte, A., 2005. Kinetic approach to heavy metal mobilization assessment in sediments: choose of kinetic equations and models to achieve maximum information. Talanta 66, 844—857.

Iqbal, M., Bermond, A., Lamy, I., 2013. Impact of Miscanthus cultivation on trace metal availability in contaminated agricultural soils: complementary insights from kinetic extraction and physical fractionation. Chemosphere 91, 287—294.

Labanowski, J., Monna, F., Bermond, A., Cambier, P., Fernandez, C., Lamy, I., van Oort, F., 2008. Kinetic extractions to assess mobilization of Zn, Pb, Cu, and Cd in a metal-contaminated soil: EDTA vs. citrate. Environ. Pollut. 152, 693—701.

Li, T.Q., Di, Z.Z., Islam, E., Jiang, H., Yang, X.E., 2011. Rhizosphere characteristics of zinc hyperaccumulator *Sedum alfredii* involved in zinc accumulation. J. Hazard. Mater 185, 818—823.

Li, T.Q., Xu, Z.H., Han, X., Yang, X.E., Sparks, D.L., 2012. Characterization of dissolved organic matter in the rhizosphere of hyperaccumulator *Sedum alfredii* and its effect on the mobility of zinc. Chemosphere 88, 570—576.

Li, Z., Wu, L.H., Hu, P.J., Luo, Y.M., Christie, P., 2013. Copper changes the yield and cadmium/zinc accumulation and cellular distribution in the cadmium/zinc hyperaccumulator *Sedum plumbizincicola*. J. Hazard. Mater 261, 332—341.

Li, Z., Wu, L.H., Hu, P.J., Luo, Y.M., Zhang, H., Christie, P., 2014a. Repeated phytoextraction of four metal-contaminated soils using the cadmium/zinc hyperaccumulator *Sedum plumbizincicola*. Environ. Pollut. 189, 176—183.

Li, Z., Wu, L.H., Luo, Y.M., Christie, P., 2014b. Dynamics of plant metal uptake and metal changes in whole soil and soil particle fractions during repeated phytoextraction. Plant Soil 374, 857—869.

Liu, X.M., Song, Q.J., Tang, Y., Li, W.L., Xu, J.M., Wu, J.J., Wang, F., Brookes, P.C., 2013. Human health risk assessment of heavy metals in soil-vegetable system: a multi-medium analysis. Sci. Total Environ. 463, 530—540.

Liu, X., Fu, J.W., Da, Silva, E., Shi, X.X., Cao, Y., Rathinasabapathi, B., Chen, Y.S., Ma, L.Q., 2017. Microbial siderophores and root exudates enhanced goethite dissolution and Fe/As uptake by As-hyperaccumulator *Pteris vittata*. Environ. Pollut. 223, 230—237.

Lu, H.L., Yan, C.L., Liu, J.C., 2007. Low-molecular-weight organic acids exuded by Mangrove (*Kandelia candel* (L.) Druce) roots and their effect on cadmium species change in the rhizosphere. Environ. Exp. Bot. 61, 159—166.

Lu, Y.L., Song, S., Wang, R.S., Liu, Z.Y., Meng, J., Sweetman, A.J., Jenkins, A., Ferrier, R.C., Li, H., Luo, W., Wang, T.Y., 2015. Impacts of soil and water pollution on food safety and health risks in China. Environ. Int. 77, 5—15.

McGregor, R.G., Blowes, D.W., Jambor, J.L., Robertson, W.D., 1998. The solid-phase controls on the mobility of heavy metals at the copper cliff tailings area, Sudbury, Ontario, Canada. J. Contam. Hydrol. 33, 247—271.

Mourier, B., Fritsch, C., Dhivert, E., Gimbert, F., Coeurdassier, M., Pauget, B., de Vaufleury, A., Scheifler, R., 2011. Chemical extractions and predicted free ion activities fail to estimate metal transfer from soil to field land snails. Chemosphere 85, 1057—1065.

Rashti, M.R., Esfandbod, M., Adhami, E., Srivastava, P., 2014. Cadmium desorption behaviour in selected sub-tropical soils: effects of soil properties. J. Geochem. Explor 144, 230—236.

Schroth, A.W., Bostick, B.C., Kaste, J.M., Friedland, A.J., 2008. Lead sequestration and species redistribution during soil organic matter decomposition. Environ. Sci. Technol. 42, 3627—3633.

Sungur, A., Soylak, M., Ozcan, H., 2016. Chemical fractionation, mobility and environmental impacts of heavy metals in greenhouse soils from Canakkale. Turk. Environ. Earth Sci. 75, 334.

Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51, 844—851.

Ure, A.M., 1996. Single extraction schemes for soil analysis and related applications. Sci. Total Environ. 178, 3—10.

Wang, S.L., Mulligan, C.N., 2013. Effects of three low-molecular-weight organic acids (LMWOAs) and pH on the mobilization of arsenic and heavy metals (Cu, Pb, and Zn) from mine tailings. Environ. Geochem. Health 35, 111—118.

Weiss, J.V., Emerson, D., Megonigal, J.P., 2004. Geochemical control of microbial Fe(III) reduction potential in wetlands: comparison of the rhizosphere to non-rhizosphere soil. FEMS Microbiol. Ecol. 48, 89—100.

Wenzel, W.W., Bunkowski, M., Puschenreiter, M., Horak, O., 2003. Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil. Environ. Pollut. 123, 131—138.

Wu, L.H., Li, Z., Han, C.L., Liu, L., Teng, Y., Sun, X.H., Pan, C., Huang, Y.J., Luo, Y.M., Christie, P., 2012. Phytoremediation of soil contaminated with cadmium, copper

and polychlorinated biphenyls. Int. J. Phytoremediat 14, 570–584.

Yin, R.J., Wang, D.Q., Deng, H.G., Shi, R.H., Chen, Z.L., 2013. Heavy metal contamination and assessment of roadside and foliar dust along the outer-ring highway of Shanghai, China. J. Environ. Qual. 42, 1724–1732.

Zhang, Q., Ye, J.J., Chen, J.Y., Xu, H.J., Wang, C., Zhao, M.R., 2014. Risk assessment of polychlorinated biphenyls and heavy metals in soils of an abandoned e-waste site in China. Environ. Pollut. 185, 258–265.