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Speciation and bioaccessibility of lead and cadmium in soil treated with metal-enriched Indian mustard leaves

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Abstract

Indian mustard (*Brassica juncea* (L.) Czern.) has shown good potential for the phytoremediation of soil contaminated with heavy metals. However, there is little information about the speciation and bioaccessibility of heavy metals in soil during the decomposition of metal-rich Indian mustard leaves. Incubation experiments (1-, 3-, and 6-month) were carried out in Beijing and Hunan soil with metal-rich Indian mustard leaves addition (1% and 3%) and the effects of mustard leaves addition on the speciation and bioaccessibility of heavy metals were studied. The results showed that the addition of mustard leaves led to significant increases in pH and DOC in the Hunan soil. Both 1% and 3% of mustard leaf amendment caused the percentage of the exchangeable (F1), precipitated with carbonates (F2), bound to Fe/Mn oxides (F3) and bound to organic matter (F4) fractions of Pb and Cd to increase dramatically, while the percentage of the residual fraction (F5) of Cd and Pb significantly dropped in both Beijing and Hunan soils. Mustard leaf addition caused the bioaccessibility of Pb to decrease in the gastric phase, whereas the values increased in the small intestinal phase. The Cd bioaccessibility increased with mustard leaf addition in both the gastric and small intestinal phases. In conclusion, the metal-enriched mustard leaves addition induces Pb and Cd concentrations and their mobility increasing in the Beijing and Hunan soils. Therefore, heavy metal risk in metal-enriched plant leaves should be considered in phytoremediation system in which heavy metal might be brought back to soil and changed over time.

Key words: lead; cadmium; speciation; bioaccessibility; decomposition

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Introduction

Cadmium (Cd) and lead (Pb) contamination of soil is a major environmental problem (Mirsal, 2004). In recent years, phytoremediation is thought to be a low-cost and environmental-friend technology comparing to other soil remediation technologies. Phytoremediation is the use of plants to remove pollutants from the environment or to transform them into harmless species (Baker et al., 1994). There are two main strategies for the phytoremediation of soil heavy metals: using hyperaccumulator plant species or non-hyperaccumulator species with higher yields. The criterion for hyperaccumulation varies for different metals. It was summarized by Baker et al. (1994) as >1000 mg/kg for Pb and > 100 mg/kg for Cd of shoot dry matter. Hyperaccumulators are generally slow-growing with a small biomass. Therefore, some studies have screened fast-growing, high-biomass plant species (non-hyperaccumulator species) for their ability to tolerate and accumulate metals in their aerial tissues (Prasad et al., 2003; Lai et al., 2008). Among these non-hyperaccumulator species, Indian mustard was identified as an important species able to take up and accumulate metals such as Cd and Pb into its above-ground parts (Cui et al., 2004; Haag-Kerwer et al., 1999; Lai et al., 2008).

In phytoremediation system, the senescence and decomposition of metal-enriched plant leaves leads to the accumulation of heavy metals in the topsoil, and the biodegradation of plant residues plays a key role in the cycling of metals in soil (Boucher et al., 2005). It has been shown that incorporation of Zn- and Cd-rich plant residues into soil results in high availability of these metals to plants (Boucher et al., 2005; Perronnet et al., 2000). Therefore, the risks of the heavy metals that were brought into soil by metal-enriched plant residue should be considered. However, it is not clear how to assess the risks of these heavy metals recycling in this system. Chemical speciation is a good indicator and has been used to assess soil metal mobility, availability and toxicity (Christine et al., 2002). The chemical speciation of a heavy metal in soil involves the fractionation of its total content into exchangeable, acid-extractable, reducible, oxidizable and

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residual forms (Christine et al., 2002). The exchangeable and acid-extractable fractions are mobile fractions that are considered to be easily available. The oxidizable and reducible forms will be leached out only under extreme conditions, while the residual fraction is almost inert (Shrivastava and Banerjee, 1998; Wong and Selvam, 2006).

The speciation of heavy metals governs its fate, toxicity, mobility in contaminated soils. To assess the speciation and to accurately determine its impact on human health, metals need to be further characterized under the conditions that are similar to those of the human body. In recent years, *in vitro* bioaccessibility has been thought to be a good method to assess human health risk of soil heavy metals (Ruby et al., 1993). Inadvertent oral ingestion of soil is considered to be an important exposure pathway for heavy metal absorption (Bannon et al., 2009; Dudka and Miller, 1999; Ljung et al., 2006). Total heavy metal content is not a good indicator of heavy metal contamination level with respect to human health risk. Therefore, oral bioaccessibility tests, which measure the solubility of heavy metals in the gastrointestinal compartment, are applicable to the soil ingestion pathway (Ruby et al., 1993). Based on the physiological conditions used in the test and on its ability to predict bioavailability, physiologically based extraction test (PBET) have been used as a practical method to estimate the bioaccessibility of heavy metals in soil (Bosso et al., 2008; Ruby et al., 1993; Sarkar et al., 2007).

However, studies of heavy metal speciation and human health risk assessment of phytoremediation system are limited. Therefore, it is important to estimate the chemical speciation and health risk associated with heavy metals in the phytoremediation system. The objective of this study was to investigate changes in Cd and Pb speciation and bioaccessibility in soil caused by the decomposition of Indian mustard leaves. Possible mechanisms underlying the observed effects were also discussed.

1 Materials and methods

1.1 Plant material

Indian mustard (*Brassica juncea* (L.) Czern.) seeds were sterilized with 10% (W/W) hydrogen peroxide for 10 min followed by thorough washing with deionized water. Then, the seeds were germinated in moist perlite for six days. Seedlings were transferred to a hydroponic culture system where three plants were grown per PVC pot (7.5 cm in diameter and 15 cm in height, thirty containers in all), and the pots contained 500 mL of nutrient solution consisting of the following: 1.3 mmol/L $CaCl_2$; 0.5 mmol/L $MgSO_4 \cdot 7H_2O$; 1.7 mmol/L NH_4NO_3; 4.0 mmol/L KH_2PO_4; 0.7 mmol/L K_2SO_4; 50 μmol/L Fe-EDTA; 2.5 μmol/L $MnSO_4 \cdot H_2O$; 0.5 μmol/L $CuSO_4 \cdot 5H_2O$; 0.5 μmol/L $ZnSO_4 \cdot 7H_2O$; 5.0 μmol/L H_3BO_3; 0.2 μmol/L $Na_2MoO_4 \cdot 7H_2O$; and 0.1 μmol/L $CoSO_4 \cdot 7H_2O$. The pH of the nutrient solution was adjusted to 5.5. Deionized water was used to prepare all the solutions, and the solution was continuously aerated. The experiment was conducted in a greenhouse where the temperature ranged from 18 to 25°C and the relative humidity ranged from 40% to 60%. After seven days, the plants were treated simultaneously with $Cd(NO_3)_2$ (7.5 μmol/L) (Vázquez et al., 2008) and $Pb(NO_3)_2$ (45 μmol/L) to obtain highly polluted Indian mustard leaves. After 30 days of treatment, the leaves of the plant were collected, thoroughly washed with deionized water and then oven-dried at 80°C for 48 hr to determine dry weight. Ground dry leaves were used in the incubation experiment. Moreover, ground dried leaves were acid-digested to assess the total concentrations of Cd and Pb.

Table 1 Physico-chemical properties of the soils

Soil Property	Beijing soil	Hunan soil
pH	7.13	5.58
OM (g/kg)	21.4	23.7
CEC (cmol/kg)	14.23	9.21
Particle size composition (%)		
Clay	18.4	30.7
Silt	72.1	61.5
Sand	9.5	7.8
Total Cd (mg/kg)	0.20	0.30
Total Pb (mg/kg)	28.5	21.2

1.2 Soil characterization

Two surface soils (0–20 cm), a neutral soil (pH 7.13) and an acid soil (pH 5.58), were collected from Qinghe, Beijing City (Beijing soil) and Yueyang, Hunan Province (Hunan soil), respectively. Then, subsamples were air-dried, ground, and sieved by passing through a 2-mm mesh sieve for soil property analysis. The physico-chemical properties of the soils are shown in Table 1.

Soil pH was determined in 0.01 mol/L $CaCl_2$ with a pH meter in a 1:2.5 (W/V) soil to suspension ratio after 1 hr of equilibration. The organic matter (OM) content was determined using the acid dichromate oxidation method described by Bao (2000). The dissolved organic carbon (DOC) was extracted with 0.03 mol/L K_2SO_4 from air-dried soil samples and then centrifuged. The supernatant was filtered through a 0.45-μm membrane, and DOC measured with a TOC analyzer (Phoenix 8000, Tekmar-Dohrmann, USA). The cation exchange capacity (CEC) of the soil was determined using 0.1 mol/L $BaCl_2$ extraction (Hendershot and Duquette, 1986). Soil particle size was analyzed by the traditional pipette and sieve method described by Lu (2000). The total concentrations of Pb and Cd in the soils were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Optima-2000, Perkin-Elmer USA) after being digested with aqua regia (HNO_3:HCl of 1:3, V/V) and $HClO_4$ (1:1, V/V).

1.3 Incubation experiment

The Indian mustard leaves (Pb 1850 mg/kg and Cd 247 mg/kg) were sieved by passing through a 2-mm mesh sieve and then mixed with soil homogeneously at rates of 1 g/100 g soil (1%, i.e., 1.85 mg Pb and 0.247 mg Cd/100 g soil) and 3 g/100 g soil (3%, i.e., 5.55 mg Pb and 0.741 mg Cd /100 g soil) (Boucher et al., 2005; Ohno and Doolan, 2001). Subsamples of 100 g of unamended and amended soil were placed in 250-mL black plastic cups. The unamended subsamples served as the control group. Each treatment had three replicates. All samples were allowed to age for one, three or six months in separate cups (54 in total) in a greenhouse at temperatures ranging from 18 to 25°C. Deionized water was added to the cups to adjust the soil moisture to 15% (W/W) every third day to simulate field-like drying-wetting cycles. At different time intervals (1, 3, and 6 months after the addition of leaves), the soil samples were collected, air-dried, ground and passed successively through a 2-mm mesh sieve and a 0.25-mm mesh sieve. The soil passing through the 2-mm mesh sieve was used to determine the speciation of the heavy metals and the soil properties, and the soil passing through the 0.25-mm mesh sieve was used in the bioaccessibility test.

1.4 Sequential extraction of soil Pb and Cd

Soil subsamples (1 g) passing through the 2-mm mesh sieve were weighed into a 50-mL polyethylene centrifuge tube, and the sequential extraction proposed by Tessier et al. (1979) was conducted to investigate the distribution of Pb and Cd in the samples at room temperature. The order of the extractants used for each of the procedures was as follows: (1) exchangeable: 1 g air-dried sample was extracted with 1.0 mol/L $MgCl_2$ at pH 7 with agitation at 220 r/min for 1 hr at 25°C (fraction 1, F1); (2) precipitated with carbonates: residue from (1) was extracted with 1.0 mol/L NaOAc at pH 5 with agitation at 220 r/min for 5 hr at 25°C (fraction 2, F2); (3) bound to Fe/Mn oxides: residue from (2) was extracted with 0.04 mol/L $NH_2OH{\cdot}HCl$ in 25% HOAc (V/V) for 6 hr at 96°C in a water bath with occasional agitation (fraction 3, F3); (4) bound to organic matter: residue from (3) was extracted with 0.02 mol/L HNO_3 and 30% H_2O_2 at pH 2 for 5 hr at 85°C, and then 3.2 mol/L NH_4OAc in 20% HNO_3 (V/V) was added and agitated for 0.5 hr at 25°C (fraction 4, F4); and (5) residual: residue from (4) was digested by aqua regia and $HClO_4$ (fraction 5, F5).

1.5 Bioaccessibility test

The bioaccessibility of soil Cd and Pb were determined by PBET. The test was adapted from Ruby et al. (1996) with a modification (Rodriguez et al., 1999; Tang et al., 2006). Artificial gastric solution was prepared by adjusting 0.15 mol/L NaCl solution to pH 1.5 with 12 mol/L HCl and then adding 0.5 g citrate, 0.5 g malate, 0.42 mL lactic acid, 0.5 mL acetic acid and 1.25 g pepsin (P7000, Sigma Chemical Co., St. Louis, MO, USA) into each liter solution. Soil (6 g) was added to 600 mL of artificial gastric solution in a 1-L glass reaction vessel which was approximately four-fifths submerged in a temperature-controlled (37°C) water bath. The anaerobic condition of the digestive tract was created by constantly diffusing argon gas at 1 L/min through the solution, and the solution's pH was monitored constantly and adjusted to the selected pH with concentrated HCl or $NaHCO_3$ powder, as necessary, throughout the procedure. After one hour in the gastric phase, the artificial gastric solution was modified to the small intestinal solution by adjusting the pH from 1.5 to 7 with $NaHCO_3$ powder and adding 1.2 g porcine bile extract (B8631, Sigma Chemical Co., USA) and 0.36 g porcine pancreatin (P1500, Sigma Chemical Co., USA) to each reaction vessel. The duration of the small intestinal phase was 4 hr. Constant mixing was performed throughout the procedure using paddle stirrers at a speed of approximately 100 r/min. After each phase, a 10-mL sample of the suspension was collected using a syringe and centrifuged at 4000 r/min for 20 min before filtering the supernatant through a 0.45-μm cellulose-nitrate filter. Soluble Pb and Cd concentrations in the artificial digestive solution were analyzed using ICP-OES. In this experiment, the bioaccessibility was defined as the solubility of soil Pb (or Cd) in the simulated gastric or small intestinal solution divided by the total Pb (or Cd) in the soil. The bioaccessibility (BA, %) of Pb and Cd in the gastric and small intestinal phases were calculated as follows:

$$\mathrm{BA} = C_\mathrm{b}/C_\mathrm{t} \times 100 \tag{1}$$

where, C_b (mg/L) is the bioaccessible concentration of Pb (or Cd) in gastric phase (or small intestinal phase),C_t (mg/L) is the total concentration of soil Pb (or Cd).

1.6 Statistical analysis

Statistical analysis was performed using SPSS for Windows (Ver 11.5; SPSS, Chicago, IL, USA). All data were subjected to ANOVA and subsequently to Duncan's multiple range test.

2 Results and discussion

2.1 Soil pH

During the incubation, soil pH significantly increased with the rate of leaf addition for Hunan soil. The highest increase occurred in the treatment with 3% leaf addition after six months of incubation, where the pH value increased from 5.6 to 6.1. However, for Beijing soil, no significant increase in pH was observed. No consistent change of pH over the 1-, 3- or 6-month incubation time was observed in either of the soils (Fig. 1). Incubation time is an important factor that controls soil pH when plant materials are added (Boucher et al., 2005; Tang et al., 1999). Most studies showed that soil pH increased and reached its highest value during the first day of incubation. Thereafter, soil pH decreased for a period of time (e.g., one month) and then remained essentially unchanged during the incubation (Boucher et al., 2005; Cui et al., 2008; Tang et al., 1999). Plant residues modify soil pH depending on the composition of the organic matter and the soil's physico-chemical properties. The rapid increase in soil pH may be due to H^+ ions adsorbed by organic materials or excess cations rapidly released from the residues reacting with H^+ in the soil (Tang et al., 1999). Vázquez et al. (2008) showed that the pH of the plant residue itself probably contributed to the change in the soil pH, and they

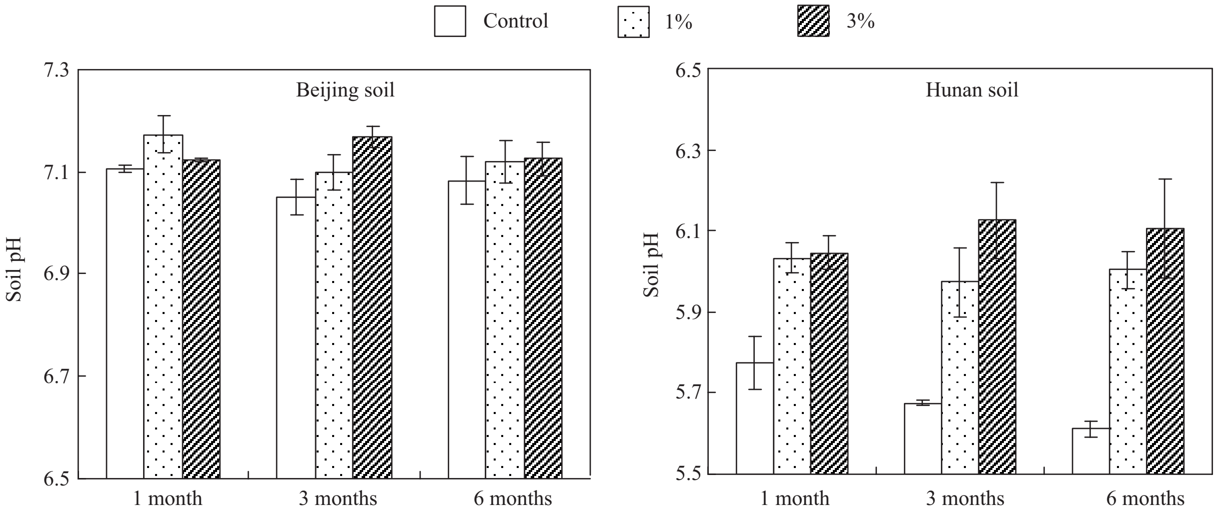

Fig. 1 Soil pH changes over time with or without the addition of metal-rich Indian mustard leaves.

estimated that the pH value of the plant residues should be about 6. Therefore, cations may be released from plant residues during microbial degradation and increase the pH value of acidic soil (Vázquez et al., 2008). Because the pH of neutral and alkaline soils is higher than that of plant residues, it may decrease during plant residue degradation (Vázquez et al., 2008). Similarly, in the present study, the pH value of Hunan soil (pH 5.58) increased, and the results agreed with the work of Vázquez et al. (2008). However, for Beijing soil (pH 7.13), the results of soil pH did not agree with the work of Vázquez et al. (2008). Tang et al. (1999) reported that addition of clover roots to Kojonup soil (pH 5.3) slightly increased soil pH, whereas addition of the same material to Moora soil (pH 5.5) decreased soil pH. Therefore, this and previous study (Tang et al., 1999) together show that change in soil pH during the incubation time is controlled by many factors.

2.2 Soil DOC

Compared with the control soil, the addition of mustard leaves significantly increased soil DOC, and the greatest DOC increase was at six months, ranging from 105.2 to 177.0 mg/kg for Beijing soil and from 126.7 to 196.8 mg/kg for Hunan soil. However, no significant change was observed among the 1-, 3- and 6-month incubation time in either soil (Fig. 2). In general, DOC concentrations in soil drastically decline in the first few days after plant residue addition and then remain constant (Boucher et al., 2005; Ohno and Doolan, 2001; Tang et al., 1999). This plant residue decomposition pattern has been interpreted as indicating the presence of labile and recalcitrant carbon fractions, and the decomposition has been divided into two phases. The initial rapid phase corresponds to the decomposition of various organic acids, and it increases DOC levels in soil after plant material addition. The slower and more constant second phase is attributable to the decomposition of cell walls and structural components as well as the decomposition of secondary, more stabilized products of the initial phase (Ohno and Doolan, 2001). In this study, plant decomposition was likely at the second phase after one month, explaining why no significant changes were observed among the 1-, 3- and 6-month incubation time. In addition to organic matter degradation, increased pH can also lead to higher DOC concentrations during plant residue treatments. Increased pH promotes the dissolution and desorption of organic matter and therefore

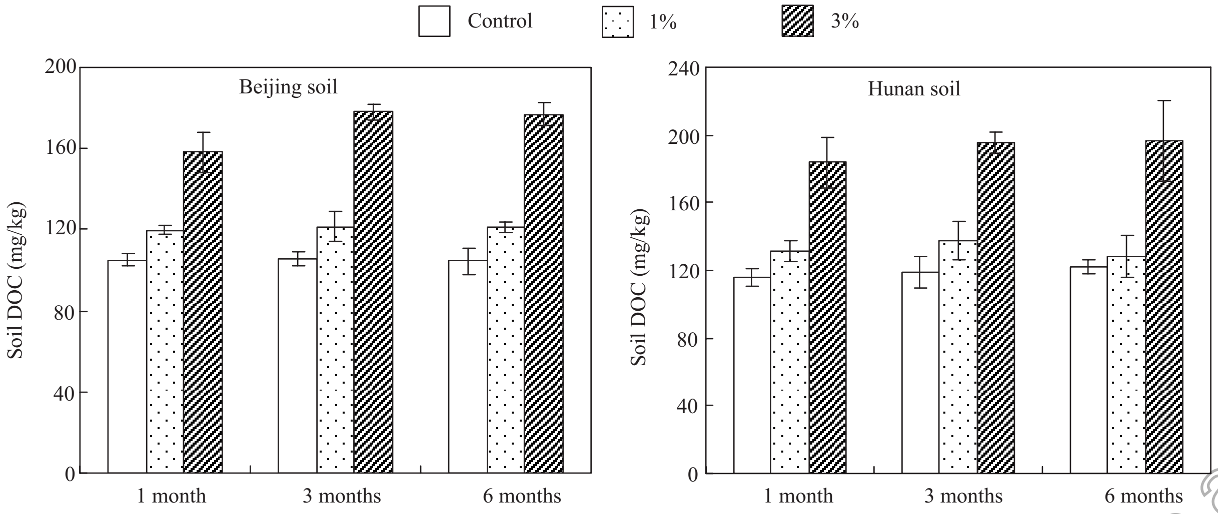

Fig. 2 Soil DOC changes over time with or without the addition of metal-rich Indian mustard leaves.

results in higher DOC concentrations (DeWit et al., 2007; Weng et al., 2002). In this study, plant residue addition increased soil DOC levels in Hunan soil. This may be attributable to the higher pH levels associated with plant residue addition.

2.3 Speciation of soil Pb and Cd

Mustard leaf amendment resulted in significant increases in Pb and Cd concentrations for all five soil fractions in both soils (Fig. 3). Without the addition of mustard leaves, the most abundant fraction was the residual (F5), for example, up to 76% and 67% for Pb and 62% and 60% for Cd after 6-month incubation in Beijing and Hunan soils, respectively (Fig. 3). Without the mustard leaf amendment, the order of the contribution of different fractions of Pb to the total metal content was F5 > F3 > F4 > F2 > F1 for both soils (Fig. 3). However, the order of the values for Cd were F5 > F1 ≈ F2 ≈ F3 > F4 and F5 > F1 > F3 > F2 > F4 for Beijing soil and Hunan soil, respectively. Mustard leaf addition caused the percentage of the F1, F2, F3 and F4 Pb and Cd species to increase dramatically, while the percentage of the F5 Cd and Pb species significantly dropped in both soils. For example, after 6-month incubation, the F5 Pb species decreased from 73% to 54% in Beijing soil and from 67% to 54% in Hunan soil with 3% mustard leaf addition. The relative abundance of the different Pb fractions was F5 > F3 > F4 > F2 > F1 in both soils (Fig. 3). For the Cd fractions, after 6-month incubation, the Cd fraction (F5) decreased from 62% to 36% in Beijing soil and from 60% to 32% in Hunan soil with 3% mustard leaf addition. The relative abundance of the different Cd fractions was F5 > F1 > F3 > F4 > F2 in both soils (Fig. 3). Mustard leaf addition caused the F2 and F4 Pb species to significantly decline with increasing incubation time ($p < 0.001$) (Fig. 3), whereas the F5 Pb species significantly increased ($p < 0.001$). The F1 and F4 Cd species significantly decreased ($p < 0.01$) and the F2 and F5 Cd species significantly increased ($p < 0.01$) (Fig. 3).

Many previous studies have characterized Cd and Pb fractions in soil using various sequential extraction procedures (Banat et al., 2007; Christine et al., 2002; Kim and Owens, 2009). The most abundant metal fraction was variable. For example, the most abundant Cd species was oxide-bound in 35 soil samples from the Czech Republic and carbonate-bound in some agricultural soils from the Jordan valley (Banat et al., 2007; Száková et al., 1999). Pb was mainly found in the exchangeable fraction in forest soil, whereas it was predominantly associated with the Fe/Mn-oxide fraction in tilled soil (Ettler et al., 2005). The exchangeable (F1) and acid-extractable fractions (F2) are considered to be easily soluble and available. Soil pH has

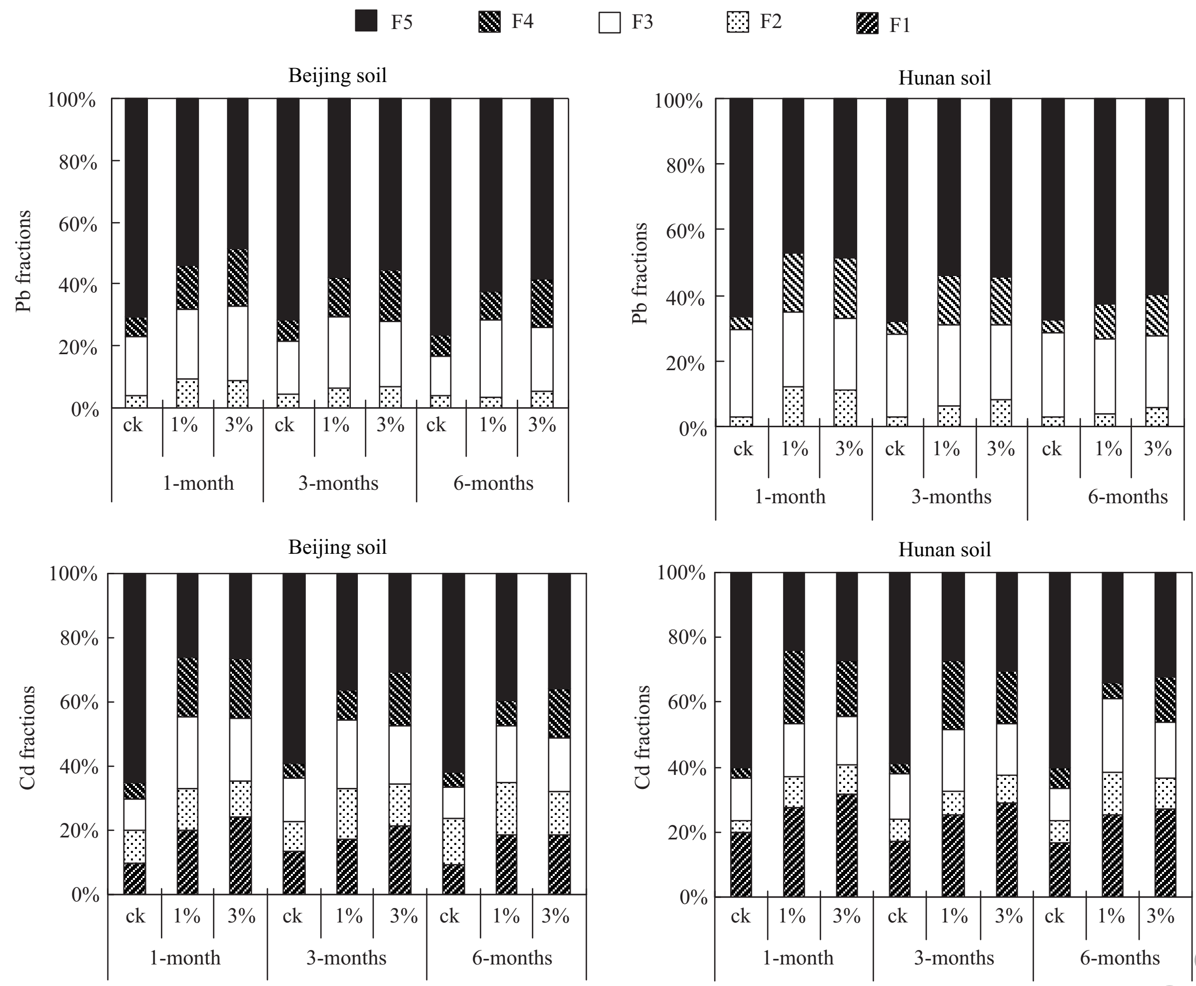

Fig. 3 Distribution of lead fractions and cadmium fractions in soils based on a sequential extraction procedure. ck: control.

a direct impact on the mobility of metals as it affects their solubility and their capacity to form chelates in soil (Wu et al., 2006). The concentrations of cations in soil solution usually increase greatly in a low pH environment. Tang et al. (2006, 2008) reported that the exchangeable fractions of Cd and Pb in acid soils were higher than those in higher pH soils, whereas the carbonate-bound fractions of Cd and Pb in higher pH soils were higher than those of lower pH soils. The concentration of $(NH_4)_2SO_4$-extractable Cd was higher in acid soil than in neutral soil when metal-loaded lupine roots were added to the soils (Vázquez et al., 2008). Similarly, in the present study, the percentage of the F1 species was higher in Hunan soil (pH 5.58) than in Beijing soil (pH 7.13), and the percentage of the F2 species was lower in Hunan soil than in Beijing soil. However, a difference between Beijing soil and Hunan soil regarding the F1 and F2 Pb species was not observed. With metal-enriched plant leaf addition, the concentrations of the F1 and F2 Cd species were significantly affected by the soil pH; however, the concentrations of the F1 and F2 Pb species were not. Therefore, the variations in soil Cd and Pb fractionation may be attributable to differences in the origin (native or polluted) of the Cd and Pb, the pollution source and soil properties (soil pH, organic matter, clay mineralogy).

Different soil amendment experiments have used different heavy metal-containing materials, which induce different heavy-metal fractionation patterns during incubation (Boucher et al., 2005; Tang et al., 2006, 2008). With metal salt addition, decreases in exchangeable Pb and Cd (F1) occurred faster in strongly acidic soils (within one week) than in higher pH soils (within two weeks) (Tang et al., 2006, 2008). With metal-loaded lupine root addition, the Cd content of extracted soil solution was found to be lowest at 7 and 14 days, and it then increased after 30 and 60 days. However, unamended and amended metal-free lupine root released no significant amounts of Cd in extracted soil solutions during the incubation (Boucher et al., 2005). In the present study, no significant change of Pb and Cd fractions was observed over time in the treatment without plant leaf addition. However, the treatment with the amended metal-free plant leaves was not conducted in the present study. Therefore, limited information is available for the effect of metal-free plant leaves on the metal release. With sewage sludge compost addition, Brazauskiene et al. (2008) found that more than 50% of the Pb fraction was F5, and Pb tended to form immobile forms (F5) during the incubation time (50 days). However, the amount of exchangeable Pb (F1) increased over the course of the experiment. The reason for the initial immobilization of Pb by the sewage sludge compost application may be the precipitation of insoluble salts such as phosphates, but this effect decreased during the incubation, probably due to increased solubility. In this study, decreases in the exchangeable Pb and Cd (F1) fractions were observed in both soils following Indian mustard leaf addition. In the phytoremediation system, the exchangeable of Pb and Cd is considered as the important fraction readily available for uptake by plants. The present results showed that the bioavailability of Pb and Cd in the plant residues decreased over the incubation. Therefore, decrease in bioavailability of Pb and Cd in the plant residues should be considered in the process of phytoremediation. No significant difference in extractable metals was observed between Beijing soil and Hunan soil. Some studies have shown that increased proportions of extractable metals may be due to reductions in pH and/or increases in soluble organic carbon (Alloway, 1995; Boucher et al., 2005). However, in the present study, no declines in pH or increases in DOC were observed during the incubation time. Therefore, the effects of plant leaf addition on Cd and Pb speciation in soil depend not only on soil pH and soluble organic carbon, but also on the plant material and other soil properties such as texture and moisture.

2.4 Bioaccessibility of soil Pb and Cd

When no mustard leaves were added, the bioaccessibility of neither Pb nor Cd changed significantly during the incubation in both soils in either the gastric or the small intestinal phase. The bioaccessibility of both Pb and Cd in both the gastric and small intestinal phases was higher for Hunan soil than that for Beijing soil (Fig. 4). When the mustard leaves were added, the bioaccessibility of Pb and Cd decreased with increasing incubation time in both soils in both the gastric and small intestinal phases. In both soils, the bioaccessibility of Pb in the gastric phase decreased with mustard leaf addition, whereas at the neutral pH (7.0) of the small intestinal phase, the bioaccessibility of Pb increased with mustard leaf addition (Fig. 4). For example, after six month of incubation, the Pb bioaccessibility was 44% and 64%, and 35% and 22% in the gastric and small intestinal phase with (3% addition) and without mustard leaf addition in Hunan soil, respectively (Fig. 4). Cd bioaccessibility increased with mustard leaf addition in the gastric and small intestinal phase for both soils. For example, in Beijing soil, the bioaccessibility of Cd increased from 32% to 55% in the gastric phase and from 20% to 43% in the small intestinal phase after one month of incubation with 3% mustard leaf addition (Fig. 4).

Values of soil Pb and Cd bioaccessibility that are higher in the gastric phase than in the small intestinal phase have been reported by many studies (Schroder et al., 2003; Yang et al., 2003). The simulated parameters, such as gastric and small intestinal pH and chemistry, soil-to-solution ratio, gastric mixing and gastric emptying rates could affect the bioaccessibility values (Lamb et al., 2009; Morrison and Gulson, 2007; Ruby et al., 1996; Schroder et al., 2003). The bioaccessibility of Pb and Cd in relation to pH in the soil and the gastric phase was discussed previously. Ruby et al. (1996) found that the amount of bioaccessible Pb decreased 60% when the gastric pH was raised from 1.3 to 2.5. Yang et al. (2003) also found a decrease in Pb bioaccessibility (81% to 11%) when the pH was raised from 1.5 to 4.0 in a simple simulated gastric solution. The bioaccessibility of Cd in acidic soil was higher than that in neutral soil in both the gastric and small intestinal phases (Tang et al., 2006). In the present study, the average of bioaccessibility of Pb and Cd in the gastric and small

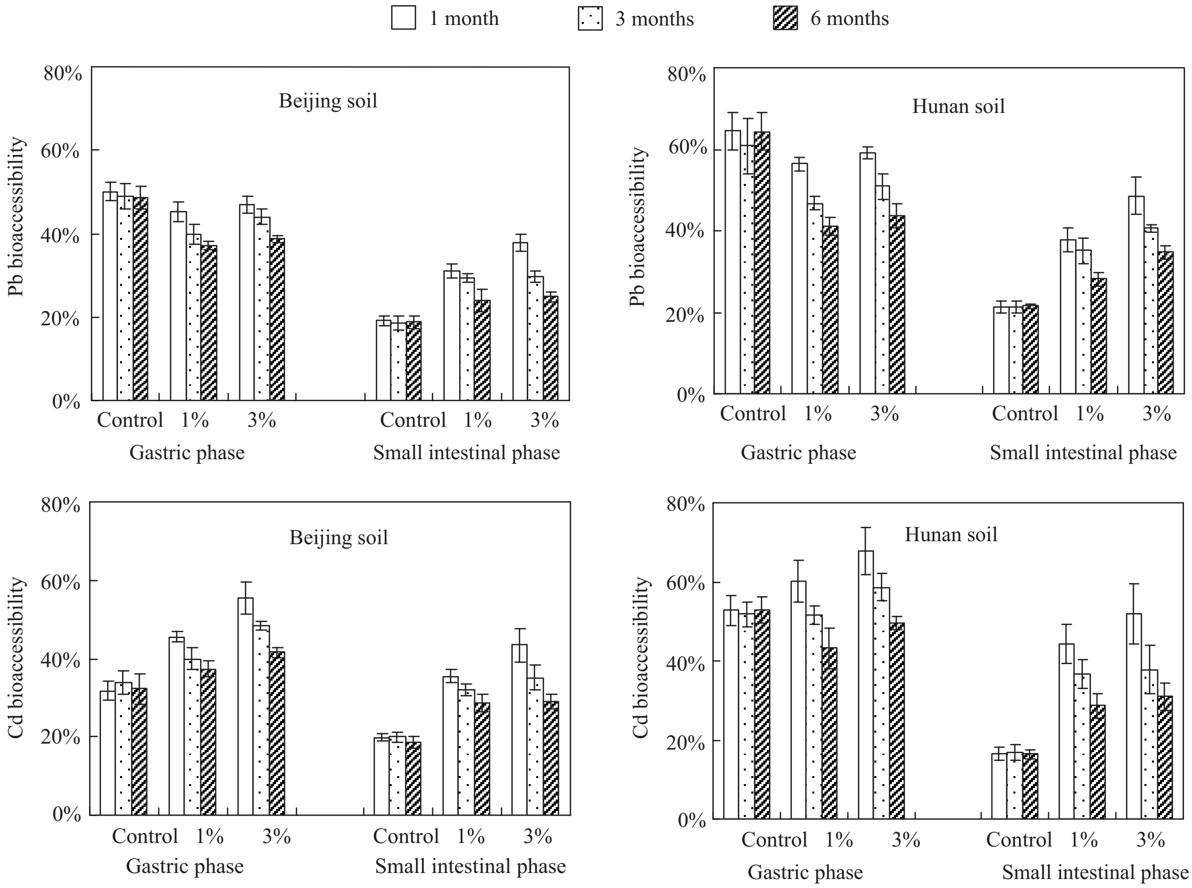

Fig. 4 Soil Pb and Cd bioaccessibility changes over time with or without the addition of metal-rich Indian mustard leaves.

intestinal phase for Hunan soil was higher than that for Beijing soil. This result may be attributable to the lower pH of Hunan soil. Soil Pb and Cd bioaccessibility is also controlled by the food in the simulated solution (Marschner et al., 2006; Schroder et al., 2003). The addition of dough was found to decrease the bioaccessibility of Cd, possibly by the formation of Cd-phytate complexes (Schroder et al., 2003). Furthermore, the addition of powdered milk decreased Pb bioaccessibility in soil, possibly via the formation of soluble Pb complexes with various milk constituents (Marschner et al., 2006). The bioaccessibility of metal in food was also reported. Intawongse and Dean (2008) showed that the Pb bioaccessibility in the gastric phase was lower than that in the small intestinal phase for selected vegetables, and the bioaccessibility of Pb was 11%–26%, 14%–27%, 11%–21% and 7%–23% in the gastric phase, and 29%–50%, 20%–30%, 28%–46% and 33%–61% in the small intestinal phase in lettuce, spinach, carrot and radish, respectively. However, similar results were not found for Cd in these vegetables. In the present study, the soil matrix in the simulated gastric and small intestinal solution was a mixture of soil and mustard leaves. This mixture therefore induces a simulated condition different from other bioaccessibility tests that only use soil or food in the simulated solution. Soil organic matter has been shown to tightly bind Pb and could also play a vital role in determining lead speciation in soils. The low bioaccessibility of Pb in some soils could perhaps be explained by its strong pH-dependence and by adsorption to organic substances. Mercier et al. (2002) reported that Pb bioaccessibility was 2.5 times higher in soil with high soil organic matter (SOM), and that high SOM can prevent dissolution of soil-bound Pb by gastric acid. In the present study, mustard leaf addition caused the total organic matter to increase in the simulated gastric and small intestinal phases. The bioaccessibility of Pb increased in the small intestinal phase with mustard leaf addition whereas the bioaccessibility of Pb in the gastric phase did not significantly change. The bioaccessibility of Cd in both the gastric and small intestinal phases increased with mustard leaf addition, showing that even high OM cannot prevent the dissolution of soil-bound Cd by gastric acid.

The bioaccessibility of Cd and Pb decreased (1–2 weeks) and then remained steady in some artificially polluted soils for both the gastric and small intestinal phases (Tang et al., 2006, 2008). With mustard leaf addition, the concentrations of Cd and Pb increased in both soils in the present experiment. The bioaccessibility of Cd and Pb kept decreasing during the six-month incubation time, showing that heavy-metal release from the mustard leaves (the process of mineralization of the plant leaves) may be slower than that of heavy metal salts in the simulated gastric and small intestinal phases.

The heavy metal fractions govern its solubility behavior and influence its availability for organism absorption. Bioaccessibility is often used to estimate the available metals for absorption into the human body. Therefore, some studies about the relationship between the heavy metal fractions and bioaccessibility have revealed the possible fractions of heavy metals available for the human body absorption (Ruby et al., 1999; Tang et al., 2006, 2008). For example, Tang et al. (2006, 2008) found that some Pb and Cd fractions (water soluble and exchangeable) were significantly and positive correlated with the metals bioaccessibility in both the gastric and small intestinal phases. However, in the present study, no positively correlation was found between the Pb (or Cd) fractions with the metal bioaccessibility in both the gastric and small intestinal phases. Therefore, the important reason was the different heavy metal-containing materials which were used in the different soil amendments experiments.

3 Conclusions

The addition of Indian mustard leaves led the soil pH and DOC to increase significantly in Hunan soil. For Beijing soil, only DOC increased significantly. When mustard leaves were added, the percentages of the F1, F2, F3 and F4 fractions of Pb and Cd increased dramatically, while the percentage of the F5 fraction of Cd and Pb dropped in both soils. The bioaccessibility of Pb decreased with mustard leaf addition in the gastric phase, but increased in the small intestinal phase. By contrast, the bioaccessibility of Cd increased with mustard leaf addition in both the gastric and small intestinal phases. Therefore, in phytoremediation system, the heavy metal risk in metal-enriched plant leaves should be taken into account when the leaves were brought back to soil.

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