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Transfer of Cd, Pb, and Zn to water spinach from a polluted soil amended with lime and organic materials

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Abstract
Purpose The transfer of heavy metals from soil to crops comprises several steps, including soil-to-root and subsequent root-to-shoot tranfer. The purpose of this study was to investigate the different steps of soil-to-crop transfer of Cd, Pb, and Zn.
Materials and methods This study was carried out with a greenhouse pot experiment using a soil polluted with Cd, Pb, and Zn which was amended with rice straw, pig manure, sheep dung, or peat, with and without lime. Water spinach (*Ipomoea aquatica*) was used as the test crop and was grown after a season of rice cultivation.
Results and discussion The results showed that all the amendments promoted the root-to-shoot transfer of Cd, Pb, and Zn. The soil-to-root transfer factors (TFs) of Pb and Zn tended to increase with increasing available Pb and Zn in the soils, while no clear relationship between the TF of Cd and available soil Cd was observed. The root-to-shoot TF of Cd, Pb, and Zn tended to decrease with increasing available amounts in the soils and were negatively correlated with the concentrations of the metals in the roots (r_{Cd}=0.820, r_{Pb}=0.789, r_{Zn}=0.769).
Conclusions The soil-to-root transfer of Cd, Pb, and Zn was significantly different from the root-to-shoot transfer. The soil-to-root transfer was mainly influenced by the amount of available metal in soil, whereas the root-to-shoot transfer was mainly controlled by the concentrations of the metals in the root.

Keywords Amendment · Heavy metals · Pollution · Soil · Transfer

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1 Introduction

The cadmium and lead content of soil may be harmful to human beings if the metals enter the food chain. Although zinc is an essential nutrient for plants and animals, it is harmful when its concentration is in excess. Therefore, one of the aims of managing agricultural soils polluted by heavy metals is to reduce the bioavailability of the heavy metals and to limit their uptake by crops. Alkaline materials, such as lime and calcium magnesium phosphate, and organic materials, such as composts, peat, and animal manures, are often used as amendments to reduce heavy metal uptake by crops (Goulding and Blake 1998; Gray et al. 2006; Li et al. 2008; Lin and Zhou 2009; Lee et al. 2009; Sato et al. 2010). Their application has become a popular method for remediating heavy-metal-polluted soils because of their low cost and easy acquisition. Alkaline materials are often used for treating acidic heavy-metal-polluted soils. One of the most important mechanisms for reducing the availability and the uptake of harmful metals by crops is the increase in soil pH from a low value to a near neutral value (Amini et al. 2005; Basta et al. 2005; Kukier et al. 2004; Seuntjens et al. 2004). Aerobically composted organic waste residuals are able to strongly bind heavy metals and limit their bioavailability (Smith 2009). Other organic materials such as green manure, animal manure, and peat can also reduce the availability of heavy metals in soils by transforming them from labile fractions to less labile forms (Li et al. 2006; Walker et al. 2003). However, the remediation effects of these amendments are not always positive because their effects on the uptake of heavy metals by crops and the metal translocation within the crops are rather complicated. Although it is often observed that the Cd uptake decreases with increasing soil pH, Bolan et al. (2003) found that Cd uptake by mustard (*Brassica juncea* L.) increased at the maximum level of $Ca(OH)_2$ amendments. Increased availability of heavy metals is also observed by the addition of compost and sewage sludge. For example, Pichtel and Anderson (1997) reported that the concentrations of Zn, Cu, and Ni in the tissues of oats increased when municipal solid waste and sewage sludge composts were added. However, further investigation is necessary to elucidate the mechanisms that determine the effect of such amendments on availability of the heavy metals in the soils, and the transfer of these metals from the soils to the edible parts of crops, in order to control the risks of cultivation of crops on metal contaminated soils.

The transfer of heavy metals from the soil to the edible parts of crops depends on the availability of the heavy metals in the soil, on the uptake of the heavy metals by plant roots, and on the translocation of the heavy metals within the plant. Although many studies have been conducted on the uptake and the accumulation of heavy metals by plants, the transfer characteristics of heavy metals within the plant tissues remains unclear. The transfer process involves several steps, such as soil-to-root, root-to-shoot, and shoot-to-grain migration of metals. Many studies measure the overall soil–plant system without considering the soil and the plant separately. The soil-to-root and root-to-shoot transfer of heavy metals differ significantly because different processes and mechanisms are involved (Greger and Löfstedt 2004; Hart et al. 1998a,b; Page et al. 2006; Redjala et al. 2009). An “uptake plateau” phenomenon, whereby the heavy metal uptake by plants does not increase as the heavy metal content of soil increases, has been reported (Dudka et al. 1996; Greger and Löfstedt 2004; Hamon et al. 1999; Krauss et al. 2002; Logan et al. 1997; Perriguey et al. 2008). This phenomenon can be partly induced by chemical and biochemical processes in the soil because such processes control the supply of the heavy metals to roots. The distribution and translocation of the heavy metals adsorbed by the roots to the aboveground parts are mainly controlled by plant physiological processes. Hamon et al. (1999) attributed the plateau response of heavy metals absorbed by plants growing in sewage-sludge-amended soils to plant physiological factors. Greger and Löfstedt (2004) attributed the differences in the wheat grain accumulation of Cd to the variation in the translocation of Cd from the root to the shoot and within the shoot, rather than to Cd uptake by the roots. They suggested that the low Cd-accumulating wheat cultivars have a mechanism to restrict Cd accumulation in the grain. Perriguey et al. (2008) suggested that there is a regulation of root-to-shoot Cd translocation, which could be regarded as a “floodgate” located at the root–stem junction. Recently, Ueno et al. (2010) found that a gene from the low-accumulating cultivar of rice encodes a transporter, which limits the root-to-shoot translocation of Cd by sequestrating Cd into root vacuoles. The transfer factor (also called “uptake factor,” “concentration factor,” or “bioaccumulation factor”) is often used to evaluate the uptake and translocation of soil metals by plants. Distinguishing soil-to-root and root-to-shoot transfer should provide a better understanding of the transfer characteristics of heavy metals from the soil to the edible parts of crops, resulting from the soil amendments.

The main objective of this study is to evaluate the soil-to-root transfer characteristics of Cd, Pb, and Zn, and the root-to-shoot transfer characteristics of water spinach grown on Cd-, Pb-, and Zn-polluted soil amended with lime and organic materials.

2 Materials and methods

2.1 Materials

The soil used for the experiments was collected from the surface layer (0–20 cm) of a field near a smelter in Youxi County, central Fujian Province. The soil samples were dried at room temperature and crushed with a wooden roller to pass a 2-mm sieve. A small portion of the soil was further ground with an agate mortar to pass a 0.149-mm sieve for chemical analyses. The pH and the contents of Cd, Pb, and Zn of the soil are summarized in Table 1. The total contents of Cd, Pb, and Zn of the soil were much higher than the Environmental Quality Standard for Soils (SEPAC 1995). The level of pollution of the soil is high with respect to the Environmental Quality Standard (SEPAC 1995) for acidic (pH≤6.5) agricultural soil (total Cd≤0.3 mg kg^{-1}, total Pb≤250 mg kg^{-1}, total Zn≤200 mg kg^{-1}; see Table 1). The soils were amended with lime, peat, pig manure, sheep dung, and rice straw. The lime was a hydrated lime powder ($Ca(OH)_2$, ≤2 mm) containing 85 % $Ca(OH)_2$. The pig manure and the sheep dung were composted and air-dried. The peat was collected from a field and air-dried. The dried peat, pig manure, and sheep dung were all crushed to pass a sieve of 2 mm. The rice straw was dried at room temperature and ground. The pH and the concentrations of Cd, Pb, and Zn of the amendments are listed in Table 1.

Water spinach (*Ipomoea aquatica*; variety: Jiangxi water spinch), a popular leaf vegetable in southeastern China, was selected and used for the pot experiment.

Table 1 pH and Cd, Pb, and Zn contained in the soil (air-dry weight basis) and amendments (oven-dry weight basis) used in the experiment

	pH	Total Cd mg kg^{-1}	Total Pb mg kg^{-1}	Total Zn mg kg^{-1}
Soil	5.50	13.06	882.32	648.96
Lime	13.00	0.24	26.52	27.16
Rice straw	–	0.41	54.24	40.37
Pig manure	8.26	0.59	295.24	461.74
Sheep dung	8.03	0.75	39.07	20.42
Peat	5.48	1.03	179.74	88.26

2.2 Methods

2.2.1 Pot experiment

Ten soil treatments were tested in the experiment: (1) CK (no amendment), (2) lime (1.33 g kg^{-1} soil), (3) rice straw (20 g kg^{-1} soil), (4) pig manure (20 g kg^{-1} soil), (5) sheep dung (20 g kg^{-1} soil), (6) peat (20 g kg^{-1} soil), (7) lime+rice straw (1.33 g lime+20 g rice straw kg^{-1} soil), (8) lime+pig manure (1.33 g lime+20 g pig manure kg^{-1} soil), (9) lime+sheep dung (1.33 g lime+20 g sheep dung kg^{-1} soil), (10) lime+peat (1.33 g lime+20 g peat kg^{-1} soil). Four replicates were conducted for each type of treatment.

Poly(vinyl chloride) pots with a height of 32 cm and a diameter of 24 cm were used for the experiment. Each pot contained 7.5 kg of air-dried soil. 2.10 of urea, 1.20 g of $NH_4H_2PO_4$, and 2.10 g of K_2SO_4 were applied to each pot (equivalent to 0.15 g N kg^{-1} soil, 0.10 g $P_2O_5 kg^{-1}$ soil, and 0.15 g K_2O kg^{-1} soil). The amendments and the fertilizers were thoroughly mixed with the soil and then placed into the pot. Before growing water spinach, one crop of rice (wetland rice) was grown in the pots because rice–vegetable rotation is a traditional cultivation system in the south of China. The pots were kept submerged during the growth of rice. After the rice harvest (July 26, 2008), the roots of rice were carefully removed from the soil, and fresh soil samples were collected from each pot for measuring pH, organic matter content, cation exchangeable capacity (CEC), and available Cd, Pb, and Zn (Tables 2 and 3). All the pots were kept in the greenhouse until the sowing of water spinach (September 4, 2008). The soils were then mixed again; no further fertilizer or amendment was added before the sowing of water spinach. The seeds of water spinach were germinated in a temperature and humidity-controlled (20–25 °C, 60–70 %) in the dark. The germinated seeds were then transplanted into the pots (12 seeds per pot) and later thinned to eight per pot. The pots were randomly arranged in a greenhouse, and their positions were changed every 2 days. The water spinach grew under greenhouse conditions (average temperature=27.2 °C and mean duration of sunshine=5.3 h). The soil humidity was maintained at 70 % of the maximum field capacity, and the spinach was harvested 26 days after sowing. The pH, organic matter content, and CEC of the soils after rice cultivation were significantly increased by each the amendments (see Table 2), whereas the contents of available Cd, Pb, and Zn of most of the treatments were significantly decreased with respect to the control, non amended soil (see Table 3).

2.2.2 Chemical and physical analyses

The air-dried samples of the soil used in the pot experiment and amendments (<0.149 mm) were digested with HNO_3–HCl–$HClO_4$–HF to determine the total contents of Cd, Pb, and Zn (SEPAC 1995).

Soil samples after water spinach cultivation were collected immediately after harvesting of water spinach for analyzing the available Cd, Pb, and Zn, which were expressed as the sum of water-soluble and exchangeable fractions extracted by a sequential extraction procedure derived from that of Kabala and Singh (2001). The extraction procedure of the water-soluble and exchangeable fractions was as follows:

1. Water-soluble fraction: An appropriate amount of fresh soil (equivalent to 2.0 g air-dried soil) was placed into a 50-mL centrifugation tube and then ultra-pure water was added into the tube at a water-to-soil ratio of 7.5:1. The tube was shaken on a reciprocating shaker at a velocity of 210 rpm for 1 h, centrifuged at 5,000 rpm for 10 min, and then filtered. The filtrate was analyzed for the water-soluble Cd, Pb, and Zn.
2. Exchangeable fraction: The residue resulted from the above step was mixed with 20 mL of NH_4OAc 1 mol L^{-1} (pH 7), shaken for 2 h, centrifuged at

Table 2 Selected properties of the soils after rice and water spinach cultivation

Treatment	After rice cultivation[a]			After water spinach cultivation		
	pH	O.M. (g kg^{-1})	CEC (cmol(+)kg^{-1})	pH	O.M. (g kg^{-1})	CEC (cmol(+)kg^{-1})
Control	5.66h	31.02fe	7.01g	5.17e	25.63e	6.64f
Rice straw	5.74g	36.86b	7.58bc	5.28e	29.54c	7.38cd
Peat	5.62i	32.90cd	7.27ef	5.21e	29.62c	7.16e
Lime	6.22f	31.47de	7.10fg	6.08c	26.30d	7.41cd
Sheep dung	6.34d	36.27b	7.88a	5.88d	33.92a	7.82b
Pig manure	6.93b	40.58a	7.52cd	7.03b	29.70c	8.01a
Lime+rice straw	6.31e	33.60c	7.47cde	6.07c	30.48b	7.50c
Lime+peat	6.43c	31.67de	7.69abc	6.19c	30.06bc	7.33d
Lime+sheep dung	6.91b	35.79b	7.82ab	7.05b	33.82a	7.92ab
Lime+pig manure	7.32a	36.39b	7.28def	7.52a	30.60b	7.47cd

O.M. organic matter content, *CEC* cation exchange capacity

[a]Before water spinach cultivation

Table 3 Available Cd, Pb, and Zn of the soils after rice and water spinach cultivation (mg kg^{-1}, mean±standard deviation)

Treatment	After rice cultivation[a]			After water spinach cultivation		
	$CaCl_2$-extractable Cd	DTPA-extractable Pb	DTPA-extractable Zn	$CaCl_2$-extractable Cd	DTPA-extractable Pb	DTPA-extractable Zn
Control	3.42±0.12b	44.44±0.73c	26.14±0.50b	6.91±0.60a	72.96±3.88a	54.01±6.29b
Rice straw	3.99±0.04a	59.94±2.62a	38.65±0.07a	5.29±0.31b	77.98±4.77a	61.28±3.21a
Peat	3.24±0.16bc	41.56±1.09cd	27.00±0.30b	4.43±0.04c	72.38±5.31a	48.86±1.87c
Lime	3.05±0.02c	38.69±1.89d	21.76±0.26cd	4.70±0.34c	47.38±2.38c	26.15±0.63e
Sheep dung	1.56±0.01f	30.22±1.01f	20.15±0.69d	2.69±0.12d	53.89±3.47b	33.67±0.43d
Pig manure	1.14±0.11g	31.26±0.12f	21.65±0.05cd	2.01±0.20e	25.66±0.56e	15.16±1.08fg
Lime+ice straw	2.24±0.19d	51.20±2.21b	27.91±1.92b	3.03±0.23d	48.90±3.23bc	27.48±2.46e
Lime+peat	2.39±0.04d	34.98±0.59e	23.32±1.14c	2.88±0.22d	45.01±3.77c	24.31±2.17e
Lime+sheep dung	1.89±0.09e	35.48±0.03e	21.59±0.22cd	1.84±0.16e	35.81±0.77d	18.13±0.40f
Lime+pig manure	0.95±0.06g	23.32±0.04g	16.48±0.64e	1.31±0.15f	18.77±0.89f	13.42±0.25g

[a] Before water spinach cultivation

5,000 rpm for 10 min, and then filtered. The filtrate was placed in a 50-mL volumetric flask, while the residue was washed with ultra-pure water, centrifuged, and filtered. The resulting filtrate was placed in the same volumetric flask and ultra-pure water was added up to the fixed volume of the flask.

The weights (FW) of the shoots of water spinach (biomass) were measured. The plants were then washed with a 0.2 % HCl solution, followed by tap water and ultra-pure water washing. The washed samples were homogenized in a stainless steel mill. The slurry of the sample was then digested with HNO_3 and $HClO_4$ for the determination of Cd, Pb, and Zn.

The concentrations of Cd and Pb in the digested or extracted solutions were measured with a graphite furnace atomic absorption spectrometer with Zeeman background correction (Varian GTA120/AA240Z, USA), while those of Zn were measured with a flame atomic absorption spectrometer (WFX-130, Beijing, China). Calibration standards for the Cd, Pb, and Zn determination were prepared by appropriate dilution of a stock solution of 1,000 mg kg^{-1} of Cd, Pb or Zn provided by China National Center for Standard Materials. A soil sample (GBW-ESS3) with certified concentrations of Cd (0.044±0.014 μg g^{-1}), Zn (89.3±4.0 μg g^{-1}), and Pb (33.3±1.3 μg g^{-1}), and a plant sample (GBW07605, tea leaf) with certified concentrations of Cd (0.057±0.010 μg g^{-1}), Zn (26.3±2.0 μg g^{-1}), and Pb (4.4±0.3 μg g^{-1}) provided by the China National Center for Standard Materials were used for quality assurance. The recoveries of Cd, Pb, and Zn of the reference soil sample were 89–109 %, 97–114 %, and 93–101 %, respectively. The recoveries of Cd, Pb, and Zn of the reference plant sample were 90–116 %, 99–109 %, and 91–117 %, respectively.

The soil pH was measured by the potentiometric method with the ratio of soil to water being 1:2.5. The organic matter content (OM) was determined by the $K_2Cr_2O_7$ oxidation method (SSSC 1999), and the cation exchange capacity (CEC) was determined by the ammonium acetate method (SSSC 1999). The free iron in the soil was extracted with dithionite–citrate–bicarbonate (0.3 mol L^{-1} citrate, 1 mol L^{-1} bicarbonate; the DCB method) and determined by flame atomic absorption spectrometry (SSSC 1999). The particle size distribution was measured by sedimentation applying Stokes' Law (SSSC 1999).

The soil-to-root transfer factor relative to the availability of the metal in soil was calculated as follows (Luo et al. 2010):

$TF_{soil\text{-}root}$=the concentration of the heavy metal in the root (mg kg^{-1}, FW)/the concentration of the available metal in soil (mg kg^{-1}).

The root-to-shoot transfer factor relative to the metal concentration in the root was calculated as follows:

$TF_{root\text{-}shoot}$=the concentration of the heavy metal in the edible parts (mg kg^{-1}, FW)/the concentration of the metal in the root (mg kg^{-1}, FW).

2.2.3 Statistical analysis

Correlations and regressions were carried out using SPSS Statistics17.0 or Microsoft Excel 2003.

3 Results and discussions

3.1 Effects of the amendments on the availability of Cd, Pb, and Zn in the soil

All the amendments significantly reduced the available Cd of the soils, compared with the control (see Table 3). The most effective amendments to reduce Cd availability were pig manure with and without lime, lime and sheep dung.

Three main soil parameters, namely, pH, organic matter content, and CEC, were significantly and negatively correlated with available Cd. The correlation coefficients (r) were -0.869^{**}, -0.735^{**}, and -0.805^{**} (n=10), respectively, indicating that the increase in soil pH, organic matter, and CEC induced by the addition of the amendments caused a decrease in Cd availability.

Available Pb was significantly reduced by all the amendments except rice straw and peat (see Table 3). The pig manure, lime, and sheep dung, and lime and pig manure were also more effective than the other amendments in lowering the Pb availability (see Table 3). The pH was the only soil parameter that significantly and negatively correlated with the available Pb ($r=-0.977^{**}$, n=10), suggesting that the pH was the most important factor in decreasing the availability of Pb.

All the amendments except rice straw significantly decreased the Zn availability in the soil (see Table 3). As with Cd and Pb, the pig manure with and without lime and sheep dung were more effective than the others in reducing Zn availability. The pH and CEC were significantly and negatively correlated with available Zn, the correlation coefficients (r) being -0.921^{**} and -0.653^{*} (n=10), respectively.

3.2 Transfer of Cd, Pb, and Zn from soil to root

The concentrations of Cd, Pb, and Zn in the roots were significantly reduced by the addition of the amendments (see Table 4), and significantly and positively correlated with the contents of available Cd, available Pb and available Zn ($r_{Cd}=0.859^{**}$, $r_{Pb}=0.891^{**}$, $r_{Zn}=0.8292^{**}$, n=10). The concentrations of Pb and Zn in the roots grown in soil treated with rice straw and peat were lower than those of the control and higher than those of the other amendments (see Table 4). This indicates that the two organic materials posed certain capacity to inhibit the uptake of Pb and Zn by the roots, while this was significantly weaker than the other amendments. The concentrations of Pb and Zn in the shoots of rice straw and peat treatments were higher than the control and the other amendments (see Table 4) showing clearly that the two organic materials promoted the transfer of Pb and Zn from the roots to the shoots. These positive correlations indicated that metal uptake by water spinach root was mainly controlled by chemical availability. The average contents of the available Cd, Pb, and Zn in the amended soils were 45.3, 64.8, and 55.2 % of those of the control, respectively, whereas their concentrations in the spinach root were 45.2, 26.7, and 26.2 %, respectively. This indicates that the amendments were even more effective in suppressing the uptake of Pb and Zn by the root than in reducing their availability in the soil. The amendments efficiencies were almost the same in terms of reducing the available Cd and inhibiting its uptake by the root.

The soil-to-root TF values of Cd varied from 2.46 (rice straw) to 5.87 (lime+rice straw, see Table 5). The lime and rice straw treatment led to the highest soil-to-root TF value, while the treatment using only rice straw gave the lowest TF value. The lime, sheep dung, lime and rice straw, and lime and pig manure increased the soil-to-root transfer of Cd, whereas rice straw, pig manure, lime and peat, and lime and sheep dung tended to decrease it (see Table 5).

The soil-to-root TF values of Pb varied between 2.03 (CK) and 0.51 (pig manure) (see Table 5). The soil-to-root TF values of Pb tended to increase with increasing Pb availability, although they were not significantly correlated (see Fig. 1b). Sheep dung, with and without lime, and pig manure were the most effective amendments in limiting the soil-to-root transfer of Pb (see Table 5).

The soil-to-root TF values of Zn varied between 5.34 (CK) and 1.65 (pig manure) (see Table 5). Although they were not significantly correlated, the soil-to-root TF values of Zn tended to increase with increasing Zn availability (see

Table 4 The concentration of Cd, Pb, and Zn in different parts of water spinach (fresh weight basis, mean±standard deviation)

Treatment	Biomass of shoot (g pot^{-1}, FW)	Root (mg kg^{-1})			Shoot (mg kg^{-1})		
		Cd	Pb	Zn	Cd	Pb	Zn
Control	2.99±0.14e	25.10±1.35a	147.91±3.76a	288.34±10.78a	5.04±0.72ab	12.19±1.05b	60.00±5.15c
Rice straw	3.88±0.11e	13.02±0.32de	76.88±3.01c	142.32±3.56c	3.43±0.14e	15.78±3.35a	75.81±2.90a
Peat	3.56±0.08e	14.05±0.30d	86.66±3.97e	155.80±2.34b	4.96±0.30ab	14.77±0.21a	69.00±2.28b
Lime	15.12±0.56d	19.41±1.59b	45.58±7.20d	99.99±16.47d	5.09±0.46ab	6.26±1.15cd	38.08±1.56d
Sheep dung	15.57±0.96d	12.08±0.45e	29.95±4.13b	71.03±4.65e	4.10±0.06cd	7.74±0.88c	40.83±1.35d
Pig manure	27.42±1.79b	5.54±0.96g	13.17±0.62g	24.95±2.08h	2.20±0.32f	5.15±1.68de	18.01±1.10g
Lime+rice straw	26.97±0.80b	17.76±0.93c	29.52±0.73e	64.42±4.84e	4.45±0.30bc	6.00±0.75cd	27.20±2.39f
Lime+peat	14.63±0.70d	8.36±0.32f	29.62±0.77e	51.23±2.83f	5.28±0.22a	8.14±1.03c	32.10±2.82e
Lime+sheep dung	20.01±1.78c	6.55±0.18g	20.90±1.83f	32.02±0.75gh	3.54±0.37de	6.69±0.86cd	17.03±1.46g
Lime+pig manure	31.23±0.30a	5.39±1.21g	22.84±0.54f	37.31±2.61g	3.41±0.45e	3.55±0.34e	17.55±0.86g

Table 5 Transfer factors (TF) of Cd, Pb, and Zn between different compartments (mean±standard deviation)

Treatment	TF (soil-to-root)			TF (root-to-shoot)		
	Cd	Pb	Zn	Cd	Pb	Zn
Control	3.63±0.20cd	2.03±0.05a	5.34±0.20a	0.20±0.02e	0.08±0.01f	0.21±0.01g
Rice straw	2.46±0.06e	0.99±0.04c	2.32±0.06de	0.26±0.01de	0.20±0.04cde	0.53±0.01cd
Peat	3.17±0.07de	1.20±0.05b	3.19±0.05c	0.35±0.02c	0.17±0.01def	0.44±0.01def
Lime	4.13±0.34bc	0.96±0.15c	3.82±0.05b	0.26±0.03de	0.14±0.0003ef	0.38±0.05f
Sheep dung	4.49±0.17b	0.56±0.08d	2.11±0.14ef	0.34±0.02c	0.26±0.01bcd	0.57±0.05bc
Pig manure	2.75±0.48de	0.51±0.02d	1.65±0.14f	0.40±0.02c	0.39±0.11a	0.72±0.08a
Lime+rice straw	5.87±0.31a	0.60±0.01d	2.34±0.18de	0.25±0.01ad	0.20±0.02cde	0.42±0.01def
Lime+peat	2.90±0.11de	0.66±0.02d	2.11±0.12ef	0.63±0.02a	0.27±0.03bc	0.63±0.02b
Lime+sheep dung	3.56±0.10cd	0.58±0.05d	1.77±0.04ef	0.54±0.07b	0.32±0.01b	0.53±0.03bd
Lime+pig manure	4.11±0.92bc	1.22±0.03b	2.78±0.19d	0.63±0.10a	0.16±0.01ef	0.47±0.01de

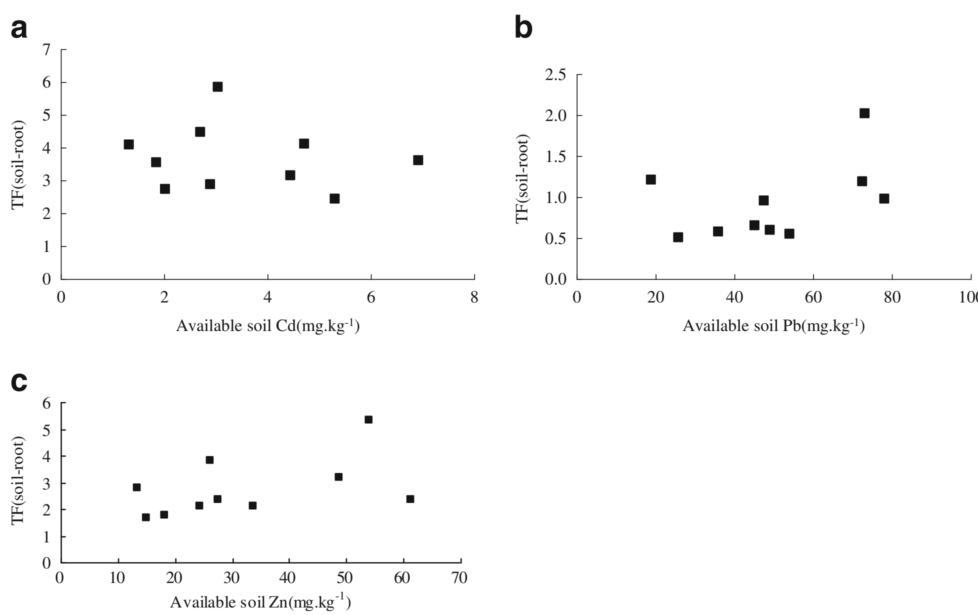
Fig. 1c). All the amendments decreased the soil-to-root transfer of Zn, and this was most marked for pig manure plus lime and sheep dung (see Table 5).

The soil used for this experiment was heavily polluted with Cd, Pb, and Zn, and toxic effects on the plants seem likely. One of the possible reasons for that the soil-to-root TF values of Pb and Zn increased with higher availabilities of the metals is their toxic effects to the root. The damage of the metals produced to the membrane of root cells may result in enhanced metal absorption. Conversely, the amendments not only lowered the levels of the available Cd, Pb, and Zn in the soils but also promoted the growth of the crop by alleviating the toxic effects of the metals, which produced larger biomass (see Table 4). There were no significant correlations between the shoot biomass and the soil-to-root transfer factors, indicating that the enhancement of growth produced by the amendments did not determine the observed differences in root-to-shoot transfer. We did not observe the "plateau" phenomenon cited above. Many researchers observed the aforementioned "plateau" phenomenon during heavy metals absorption by plants (Chaney and Ryan 1993; Hamon et al. 1999; McBride 1995).

3.3 Transfer of Cd, Pb, and Zn from root to shoot

With some exceptions, the concentrations of the metals in the shoots were significantly reduced by most of the amendments (see Table 4). The addition of lime, peat, lime and rice straw, and lime and peat did not significantly lower the Cd

Fig. 1 Relationships between the soil-to-root transfer factors of Cd, Pb, and Zn and their availability in soil



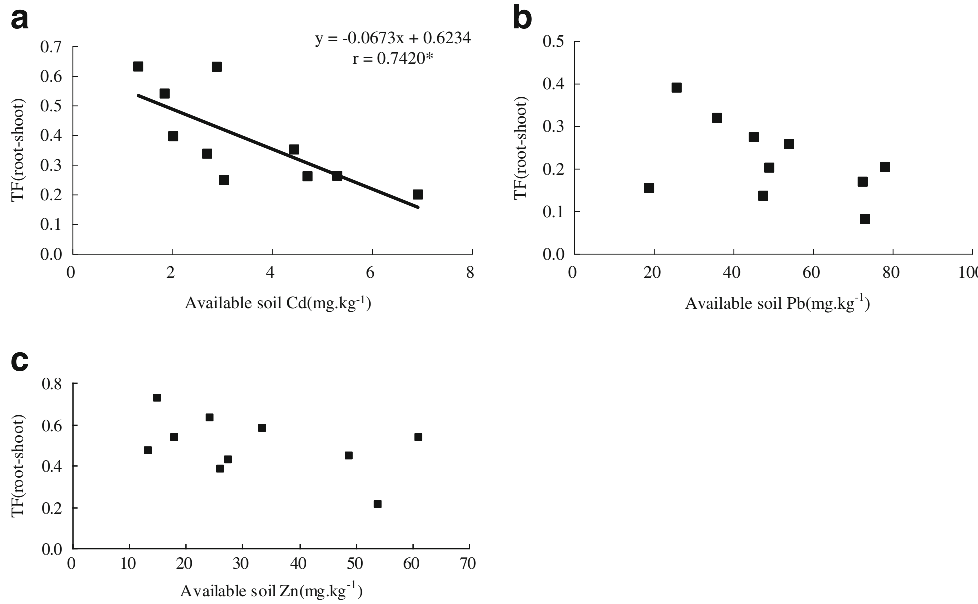


Fig. 2 Relationships between root-to-shoot transfer factors of Cd, Pb, and Zn and their available content

concentration in the shoot although they significantly reduced the Cd concentrations in the root (see Table 4). Peat and rice straw significantly increased the concentrations of Pb and Zn in the shoot, whereas the other amendments significantly reduced the concentrations of Pb and Zn in the shoot. These results were in agreement with some findings showing that a high organic matter content in the soil or the addition of organic amendments to the soil sometimes enhanced accumulation of heavy metals in the edible parts of crops (Murray et al. 2011; Lei et al. 2011). The concentrations of Cd, Pb, and Zn in the shoots were all significantly and positively correlated with their concentrations in the roots (r_{Cd}=0.6889*, r_{Pb}=0.8216**, r_{Zn}=0.7771**, n=10). Similarly, the concentrations of Pb and Zn in the shoots were significantly and positively correlated with the available Pb and Zn in the soil (r_{Pb}=0.9259**, r_{Zn}=0.9724**, n=10), whereas the Cd concentration of shoots did not significantly correlate with the available Cd. These results suggest that both the available amounts in the soil and the concentrations in the root of Pb and Zn were important for their accumulation in the shoot, while the available Cd seems to be less important than the level of Cd in roots for shoot accumulation.

All the amendments significantly promoted the root-to-shoot transfer of the three metals (see Table 5). For some of

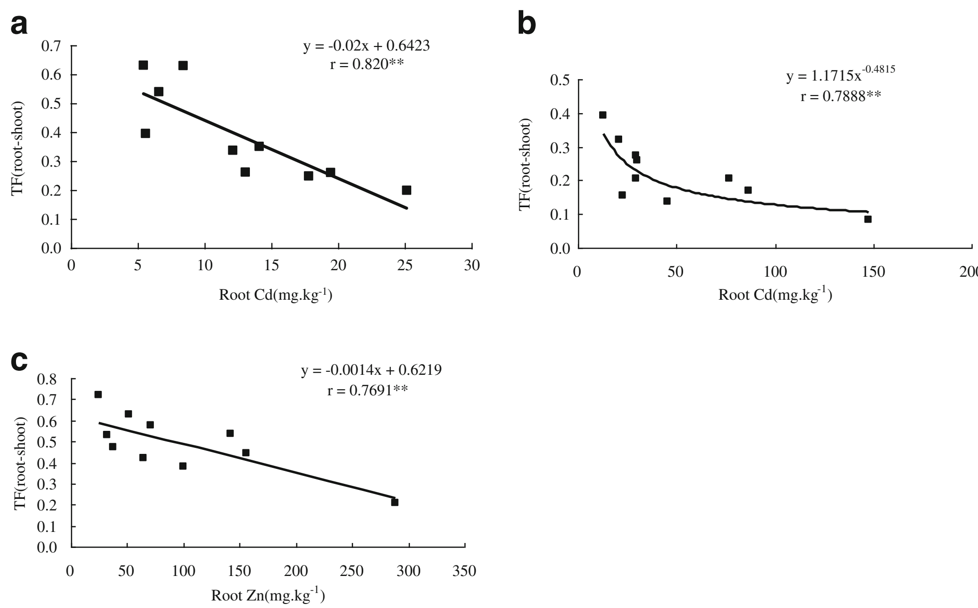


Fig. 3 Relationships between root-to-shoot transfer factors and the concentrations of Cd, Pb, and Zn in the roots

the treatments, this behavior was in agreement with the findings of Gigliotti et al. (1996), who reported that the plant-soil transfer coefficients of Cu, Zn, and Pb were generally greater in the plots treated with urban waste compost than in the untreated plots. The root-to-shoot TF values of Cd correlated significantly and negatively with the available Cd in soil (see Fig. 2a), while the root-to-shoot TF values of Pb and Zn tended to decrease with increasing the available Pb and Zn, without significant correlations (see Fig. 2b, c). These relationships were in agreement with the findings of Wang et al. (2006), who found that soil-to-plant TF values of Cd and Pb decreased with decreasing availability for six vegetables. For all three metals, the root-to-shoot TF values correlated significantly and negatively with their concentrations in the roots (see Fig. 3a–c), indicating that the concentrations in the root were more important than those in soil in affecting the root-to-shoot transfer. There were no significant correlations between the shoot biomasses and the root-to-shoot transfer factors, suggesting that the biomass did not determine the transfer of the metals from the root to the shoot. There are clearly two distinct phases for the metal transfer from the soil to the edible part of water spinach, i.e., the soil-to-root and the root-to-shoot transfer phases, respectively. As discussed above, the uptake of Cd, Pb, and Zn from the soil by the root depends largely on their availability while the root-to-shoot transfer of the metals seems to depend mainly on the concentrations of the metals in the roots. Greger and Löfstedt (2004) state that the latter process is controlled by plant physiological factors rather than the soil factors. Hart et al. (2006) and Perriguey et al. (2008) suggested that for Cd translocation within a plant there is a "floodgate" at the root-stem junction regulating the transfer of Cd from the root to the shoot. When the total root influx is higher than the maximum flux allowed by the "floodgate", Cd remains in the roots and its shoot-to-root ratio decreases. Belleghem et al. (2007) found that phosphorus association, phytochelatin sequestration, granular deposits in the vacuole and in the cytoplasm in the root, and the re-translocation from the shoot were all responsible for restraining the transfer of Cd from the root to the shoot of *Arabidopsis thaliana*. Ueno et al. (2010) further reported that a transporter encoded by a gene from a low-accumulating cultivar of rice functioned as a "firewall" that sequestrated Cd into root vacuoles and limited its translocation from the root to the shoot. The growth status of the plant induced by the nutrient supply, the toxic effects of contaminants, and other environmental conditions also influence the translocation of heavy metals within the plant (Greger et al. 1991). In this study, the application of the amendments improved the growth of water spinach (see Table 4). However, no significant correlation was found between the shoot biomass and the transfer factors. There may exist several possible reasons for the inverse relationships between the root-to-shoot TF values and the concentrations of Cd, Pb, and Zn in the roots. Therefore, further studies are needed in order to clarify the exact reasons and mechanisms.

4 Conclusions

This study revealed that (1) the addition of lime and organic amendments not only reduced the availability of Pb and Zn in the soil but also limited their transfer from the soil to the root of water spinach; (2) the root-to-shoot transfer factors of Cd, Pb, and Zn showed inverse relationships with their concentrations in the roots; and (3) the amendments enhanced the root-to-shoot translocation of the metals. These observations indicated a distinct difference between the soil-to-root and the root-to-shoot transfers, as a direct result of the addition of lime and other organic amendments. The difference was mainly attributed to the plant physiological factors rather than to the chemical characteristics of the soil. On the basis of these results, it is important to develop strategies that can restrict the transfer of heavy metals from the root to the shoot when we use various amendments to treat agricultural soils polluted with such metals.

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