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RESEARCH ARTICLE

Leaching characteristics of EDTA-enhanced phytoextraction of Cd and Pb by *Zea mays* L. in different particle-size fractions of soil aggregates exposed to artificial rain

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Abstract Chelator-assisted phytoextraction is an alternative and effective technique for the remediation of heavy metal-contaminated soils, but the potential for heavy metal leaching needs to be assessed. In the present study, a soil column cultivation-leaching experiment was conducted to investigate the Cd and Pb leaching characteristics during assisted phytoextraction of metal-contaminated soils containing different particle-size soil aggregates. The columns were planted with *Zea mays* "Zhengdan 958" seedlings and treated with combined applications of EDTA and simulated rainfall (pH 4.5 or 6.5). The results were as follows: (1) The greatest uptake of Cd and Pb by *Z. mays* was observed after treatment with EDTA (2.5 mmol kg^{-1} soil) and soil aggregates of <1 mm; uptake decreased as the soil aggregate size increased. (2) Simulated rainfall, especially acid rain (pH 4.5), after EDTA applications led to the increasing metal concentrations in the leachate, and EDTA significantly increased the concentrations of both Cd and Pb in the leachate, especially with soil aggregates of <1 mm; metal leachate concentrations decreased as soil particle sizes increased. (3) Concentrations of Cd and Pb decreased with each continuing leachate collection, and data were fit to linear regression models with coefficients of determination (R^2) above 0.90 and 0.87 for Cd and Pb, respectively. The highest total amounts of Cd (22.12%) and Pb (19.29%) were observed in the leachate of soils treated with EDTA and artificial acid rain (pH 4.5) with soil aggregates of <1 mm. The application of EDTA during phytoextraction method increased the leaching risk in the following order: $EDTA_{2.5–1}$ (pH 4.5) > $EDTA_{2.5–1}$ (pH 6.5) > $EDTA_{2.5–2}$ (pH 4.5) > $EDTA_{2.5–4}$ (pH 4.5) > $EDTA_{2.5–2}$ (pH 6.5) > $EDTA_{2.5–4}$ (pH 6.5).

Keywords Heavy metal leaching risk · Phytoextraction · EDTA-enhanced · Soil aggregates · *Zea mays* L. · Cd- and Pb-contaminated soil

Introduction

Heavy metal (HM)-contaminated soil is a serious problem in most countries because of rapid worldwide agricultural and industrial development. Their effects on the environment and human health are potentially toxic; therefore, the remediation of HM-contaminated soil has been one of the most challenging objects of environmental science and engineering. Among the biological, physical, and chemical approaches that can be used to restore HM-contaminated soils, special attention has been given to phytoremediation technologies (especially the phytoextraction method) in view of its cost-effectiveness and environmental friendliness (Vithanage et al. 2012; Bolan et al. 2014). However, although hyperaccumulator plants are ideal for phytoextraction, they usually generate low amounts of biomass and show poor adaptability to contaminated soils. Thus, tolerant plants with high biomass potential would be good candidates for phytoremediation because high biomass production could offset the low HM uptake rate.

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Zea mays has been proposed as a potential specie for phytoextraction of HM-contaminated soil (Komárek et al. 2007; Reinhard et al. 2008; Zhao et al. 2010; Xie et al. 2012). Liu (2011) demonstrated that "Zhengdan 958" has not only high biomass production but also high bioconcentration factors (BCFs) and translocation factors (TFs) for HMs (especially for Cd and Pb). In addition, the distribution of HMs in *Z. mays* is known to be extremely uneven with very little HM content in grain (Smilde et al. 1992). The straw of *Z. mays* can be used as a raw material in the production of fuel ethanol (Corneli et al. 2016). Therefore, the use of *Z. mays* for soil remediation could result in many co-benefits such as grain production, environmental protection, and energy savings.

Most HMs in the soil are in immobile form, however, they can be extracted and utilized by plants upon dissolution (Wang et al. 2009). Therefore, the addition of chelating agents, which can improve the mobility of HMs and increase the absorption by tolerant plants, has also become a hot research topic. Ethylenediaminetetraacetic acid (EDTA), for example, has a good activation effect on a variety of metals (e.g., Cd, Cu, Pb, and Zn), and it can increase the bioavailability of metals to plants by several folds, as shown by several studies whereby the factors of increase amounted to 3.1 (Cd, *Z. mays*), 42 (Cu, *Elsholtzia splendens*), 2.9 (Pb, *Z. mays*), and 2.1 (Cu, *Brassica napus* L.); the corresponding metal mobility in the soil changed by factors of 203, 296, 3.3, and 8, respectively (Luo et al. 2006b; Neugschwandtner et al. 2008; Habiba et al. 2015). However, the low degradation rate of EDTA could increase the potential for HM leaching and groundwater contamination (Hadi et al. 2010).

Potential leaching risk assessments were speculated adversely, based on the mobilization of HM and the high persistence of EDTA in soil (Luo et al. 2005, 2006a; Wei et al. 2011; Li et al. 2012; Wang and Liu 2014), while only few researchers studied the leaching characteristics by applying the leaching experiment. A leaching column experiment without cultivation conducted by Wu et al. (2004) indicated that rainfall after EDTA addition led to the increasing HM concentrations in the leachate, especially during periods of high rainfall. Grčman et al. (2001) investigated that up to 37.9% and 56.3% of initial total Pb and Cd in the soil were leached from the soil profile, conducting a column cultivation experiment. Kos and Tan (2003) found that 36.2% of the total Pb was leached with the application of EDTA, applying a cultivation-leaching experiment. A cultivation-leaching experiment on vetiver and *Z. mays* by Chen et al. (2007) showed that EDTA had a substantial influence on the downward migration of HMs in the surface soil during large amounts of precipitation. Moreover, it has been shown that the pH of a solution has a large effect on the dissolution of HMs in soil. For example, the adsorbance of Cd in soil doubled with every 0.5 unit increase when the pH ranged from 3.8 to 4.9 (Boekhold et al. 1993). Li et al. (2015) found that 43.2% (Zn), 39.6% (Cu), 38.0% (Pb), and 53.2% (Cd) of metals in soil were leached from the soil under acid rain conditions (no plants were cultivated in that study).

The soil particle-size distribution is an important risk factor for the bioaccessibility and leaching of metals in soil. Cai et al. (2016) revealed that HM concentration distributions in different particle-size fractions. Metal accumulation in soil is known to increase with a decrease in the particle size (Acosta et al. 2009), and the presence of HMs in smaller aggregates can also enhance their mobility in the environment as they co-migrate with colloids. Zheng et al. (2013) found that soil aggregates in the silt size range were the major fraction responsible for Pb adsorption in leafy vegetables. However, research on the leaching characteristics of HM phytoextraction with different soil particle-size fractions is limited.

Given this body of research, it would be prudent to explore the potential for HM leakage in chelant-enhanced phytoremediation systems in different particle-size fractions of soil aggregates, especially when such systems are subjected to acid rain. The test systems employed different particle-size fractions of soil aggregates, and *Z. mays* "Zhengdan 958" seedlings were planted in the soil columns, as the seedling stage exhibits fast growth and high absorption potential for HMs (Zhou 2011); EDTA was selected as the chelating agent. The main goals of this study focus on (1) the phytoextraction potential and translocation ability of *Z. mays* "Zhengdan 958" grown in different particle-size fractions of soil aggregates contaminated with metals, (2) the leaching characteristics of both Cd and Pb in response to changes in the rainfall volume and different particle-size fractions of soil aggregates in the phytoextraction systems, and (3) the maximum leaching risk of Cd and Pb under different particle-size fractions and rainfall pH conditions. Our findings will thus provide theoretical support for the development and application of EDTA-enhanced phytoextraction technology.

Materials and methods

Soil sampling and preparation

The soil samples were collected from the arable layer (0–20 cm, red loam) of a forested land, located near an iron pyrite discharge field in Guangdong Province, China (22° 59′ 25.5″ N, 112° 00′ 40.5″ E). The soil was collected with the permission of the Yunfu Guangye Pyrite Group Co., Ltd. Soil used in our glasshouse experiments was air-dried, ground, and sieved through 1-, 2-, and 4-mm plastic sieves, respectivy, to remove large debris. The data of sand/silt/clay was provided by a soil gravity meter method. The cation exchange capacity (CEC) of the soil was determined using the ammonium acetate

saturation method. The bulk density was detected by the soil bulk density ring method. Several physicochemical characteristics of the soil were measured according to the methods mentioned by Hu et al. (2014), and concentrations of HMs (Cd and Pb) were determined using HNO_3-HF digestion (5:1 by volume; Lu et al. 2015) and were detected by atomic absorption spectrometry (AAS, iCE 3500; Fisher Scientific, Loughborough, UK), and data are shown in Table 1.

Glasshouse experiment

The soils were artificially spiked with solutions of $CdCl_2 \cdot 5/2H_2O$ and $Pb(NO_3)_2$ to bring the final soil concentrations of Cd and Pb to 10.1 and 552 mg kg^{-1}, respectively. The spiked soils were equilibrated for 15 days, during which time they underwent 5 cycles of wetting (70% water holding capacity) and drying (air-drying). In addition, the soils were also fertilized at 0.75 g pot^{-1} ($N/P_2O_5/K_2O$ = 15:15:15) during this time period.

With daily supply of water, saturated seeds of *Z. mays* "Zhengdan 958" (first class seed, with the minimum germination rate of 90%) were placed in a biochemical incubator (LRH-70; Nanbei, Henan, China) at 25 °C for 3 -day germination. One of the germinated seeds was sown in soil subsample (300 g, air-dried) at a depth of ~2–3 cm in each experimental soil column (75 mm in diameter; 130 mm in height). Afterwards, the columns (n = 36) were settled in a glasshouse at Guangzhou University (at a site belonging to the School of Environmental Science and Engineering, Guangzhou University). With the suitable glass house temperatures of 26–37 °C, natural light, and adequate soil moisture, water holding capacity was maintained at 70% by means of inputting deionized water to a constant weight at 8:00 a.m. and 5:00 p.m. each day. Once the seedlings reached the seven-leaf stage (approximately 21 days), six columns were individually treated with one of the following amendments: control, with soil aggregates of <1, <2, and <4 mm (CK_{0-1}, CK_{0-2}, and CK_{0-4}, respectively), and 2.5 mmol kg^{-1} Na_2EDTA salt (EDTA), with soil aggregates of <1, <2, and <4 mm ($EDTA_{2.5-1}$, $EDTA_{2.5-2}$, and $EDTA_{2.5-4}$, respectively).

Table 1 Physicochemical properties of contaminated soil used in the present study

Physicochemical properties	
pH_{water}	4.5
Sand (%) >0.02 mm	51
Silt (%) 0.02–0.002 mm	33
Clay (%) <0.002 mm	16
Texture	Clay loam
Cation exchange capacity (CEC) (cmol kg^{-1})	3.3
OM (g kg^{-1})	15.4
Total N (%)	0.084
Available N (mg kg^{-1})	37.8
Available P (mg kg^{-1})	0.90
Available K (mg kg^{-1})	12.1
Bulk density (g cm^{-3})	1.279
Background total metal concentration (mg kg^{-1})	
Cd	0.12
Pb	52.30

OM organic matter

Leaching experiment

By means of adjusting the pH of the base solution to either 4.5 or 6.5 with a mixture of H_2SO_4, HCl, and HNO_3 ($[SO_4^{2-}]/[Cl^-]/[NO_3^-]$ = 6.1:1:0.7, molar ratio), two rainfall solutions were produced as the artificial rainwater with matching characteristics of rainwater at Yunfu, Guangdong Province (Zheng et al. 2009; Table 2). A leaching experiment was implemented at 2 days following the applications of EDTA of the 36 columns (when maximum-dissolved HMs were present; Zhou et al. 2007). After achieving saturated infiltration, the simulated rainfall of 50 mm (250 mL for the surface area of our experimental columns), which consists of either artificial acid rain (pH 4.5) or non-acid rain (pH 6.5) based on the heavy rain standards that have been promulgated by the China Meteorological Administration (Table 3), leached into the columns. Fifty-milliliter aliquots were used to collect the leachate from each column, and AAS was adopted to detect the HM concentrations in each leachate collection. In order to compare the growth of the *Z. mays* plants, we measured the following morphological characteristics: (a) height (from the soil surface to the top of the plant), (b) stem length (from the soil surface to the bottom of the fourth leaf), (c) stem width (at the second leaf), (d) leaf length (of the fifth leaf), and (e) leaf width (the widest portion of the sixth leaf). Subsequently, aiming to acquire the dry weights (DWs) of both shoots and roots, the plants, which were harvested by the shoots at 0.5 cm above the surface of the soil, were thoroughly washed with deionized water to remove soil particles and dried to constant weight in an oven at 70 ± 2 °C. Besides, after the subsamples of shoot and root samples were digested in a 5:1 (v/v) mixture of concentrated HNO_3 (guaranteed reagent, GR) and H_2O_2 (GR), the

Table 2 Components of the artificial rainwater used to simulate the chemical characteristics of rainwater at Yunfu, Guangdong Province (according to Zheng et al. 2009)

Component	Concentration (mg L^{-1})
NaCl	1.753
$(NH_4)_2SO_4$	2.243
KNO_3	2.141
$MgSO_4 \cdot 7H_2O$	5.792
$CaSO_4 \cdot 2H_2O$	24.480

Table 3 Different treatments used in the leaching experiment

No.	Treatment	Chelator (mmol kg^{-1})	Rainfall pH	Soil particle size (<mm)
1	CK_{0-1} (6.5)	0	6.5	1
2	CK_{0-1} (4.5)	0	4.5	1
3	CK_{0-2} (6.5)	0	6.5	2
4	CK_{0-2} (4.5)	0	4.5	2
5	CK_{0-4} (6.5)	0	6.5	4
6	CK_{0-4} (4.5)	0	4.5	4
7	$EDTA_{2.5-1}$ (6.5)	EDTA, 2.5	6.5	1
8	$EDTA_{2.5-1}$ (4.5)	EDTA, 2.5	4.5	1
9	$EDTA_{2.5-2}$ (6.5)	EDTA, 2.5	6.5	2
10	$EDTA_{2.5-2}$ (4.5)	EDTA, 2.5	4.5	2
11	$EDTA_{2.5-4}$ (6.5)	EDTA, 2.5	6.5	4
12	$EDTA_{2.5-4}$ (4.5)	EDTA, 2.5	4.5	4

Each treatment was performed in triplicate

elemental analyses of the digestion solutions were carried out using AAS. Lastly, the BCFs, HM uptakes, and TFs of the *Z. mays* plants were described in the "Data processing and statistical methods" section.

Data processing and statistical methods

The soil metal concentrations, BCF values, and uptake amounts were used to assess the plant phytoextraction capacity for HMs, and TFs were used to demonstrate the transferring ability of HMs (Cd and Pb in the present study) from roots to shoots. The BCF, uptake, and TF were calculated by the following equations:

$$\mathrm{BCF} = C_{\mathrm{shoot}}/C_{\mathrm{soil}} \quad (1)$$

$$\mathrm{Uptake} = C_{\mathrm{shoot}} \times \mathrm{DW}_{\mathrm{shoot}} \quad (2)$$

$$\mathrm{TF} = C_{\mathrm{shoot}}/C_{\mathrm{root}} \quad (3)$$

where C_{shoot} is the concentration of Cd (or Pb) in plant dry aboveground biomass (mg kg^{-1}, DW), which was determined as described in the "Leaching experiment" section; C_{soil} is the concentration of Cd (or Pb) in the soil (mg kg^{-1}, DW); $\mathrm{DW}_{\mathrm{shoot}}$ is the aboveground biomass (g, DW); and C_{root} is the concentration of Cd (or Pb) in belowground biomass (mg kg^{-1}, DW).

Statistical analyses were performed by using the Origin software package (version 8.5; OriginLab, Northampton, MA, USA). Data are presented as mean values ± standard deviations (SDs) of replicates. For a more thorough analysis of the results, the means were also compared by using ANOVA prior to Tukey's test with a 95% confidence level ($P < 0.05$). Linear regression equations and coefficients of determination (R^2) were also used in the analyses.

Results and discussion

Effects of HMs and EDTA on *Z. mays* growth

The *Z. mays* plants displayed no visible toxic symptoms from the HM-contaminated soil during germination and seedling growth stage, and at 2 days following the addition of EDTA, *Z. mays* still failed to exhibit any visible symptoms, which indicates that *Z. mays* "Zhengdan 958" was tolerant to the concentrations of both HMs (Cd and Pb) and EDTA used in the present study. Luo et al. (2005) reported that the addition of 5 mmol kg^{-1} EDTA significantly affected the growth of *Z. mays*. Therefore, it is important to emphasize that EDTA-enhanced phytoextraction should only be used under conditions where no poisoning symptoms of the *Z. mays* plants occur, and this can be achieved with the minor concentrations of EDTA (2.5 mmol kg^{-1}) applied in this study. Moreover, different soil particle sizes also did not show significant effects ($P > 0.05$; Fig. 1) on the plant growth parameters of *Z. mays*, including the heights (Fig. 1a), stem lengths (Fig. 1b), stem diameters (Fig. 1c), leaf lengths (Fig. 1d), leaf widths (Fig. 1e), shoot DWs (Fig. 1f), root DWs (Fig. 1g), and total DWs (Fig. 1h).

Phytoextraction and translocation of HMs by *Z. mays*

The presence of EDTA significantly increased the plants' capacity for Cd and Pb phytoextraction ($P < 0.05$; Table 4; Cd and Pb concentrations in shoots), and the values were 1.59-, 1.33-, and 1.59-fold higher than those of the control (CK, without the application of EDTA) for Cd and 1.69-, 1.54-, and 1.24-fold higher than those of the control for Pb with soil particle-size fractions of <1, <2, and <4 mm, respectively. Although the HM concentrations decreased with the increasing soil particle size, no significant differences ($P > 0.05$; Table 4) were detected among the three treatments, both in the absence of EDTA and with the application of EDTA. Moreover, EDTA enhanced the translocation of HMs from roots to shoots, of which the TF values were 4.15-, 2.56-, and 2.83-fold that of the control (CK) for Cd and 3.19-, 2.24-, and 1.68-fold that of the control for Pb with soil particle-size fractions of <1, <2, and <4 mm, respectively. As it is well known, metal accumulation in soils tends to decrease with increases in the soil particle size (Acosta et al. 2009), and similar results were found in the present study; thus, it may be inferred that the presence of HMs in smaller aggregates can also enhance the mobility of metals in the environment as they will co-migrate with colloids. In addition, the bioavailability of HMs can be enhanced upon application of EDTA, especially in small particle size, and these metals can then be absorbed easily by *Z. mays* plants. Zheng et al. (2013) found that soil aggregates in the silt size range were the

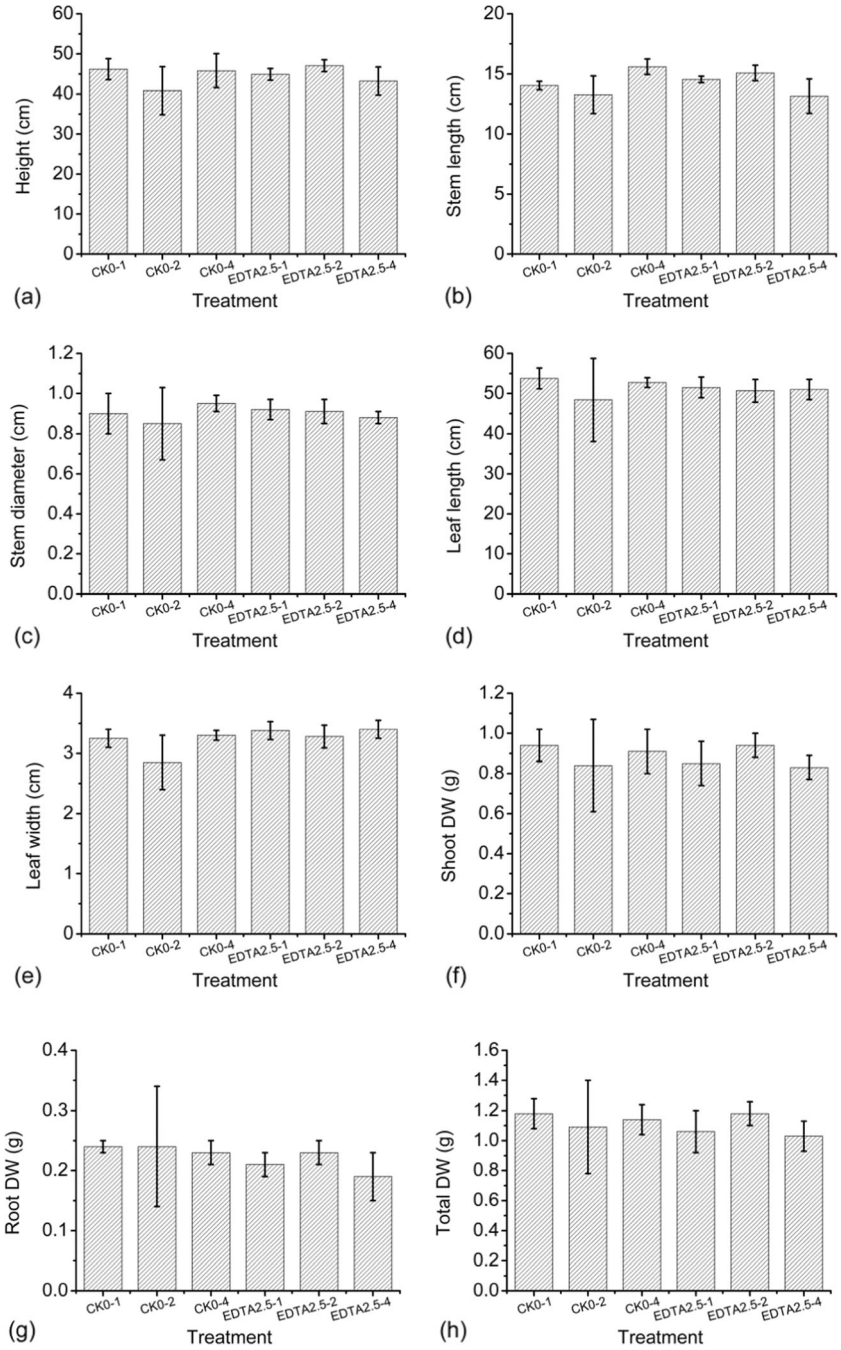

Fig. 1 Physiological indices of glasshouse grown *Zea mays* seedlings in response to treatment with EDTA (2.5 mmol kg^{-1} soil) and different soil particle-size fractions (<1, 2, and 4 mm). **a** Height. **b** Stem length. **c** Stem diameter. **d** Leaf length. **e** Leaf width. **f** Shoot DW. **g** Root DW. **h** Total DW. *Bars* indicate means ± SD ($n = 6$). *CK* control with no EDTA, *DW* dry weight of the plant tissues

major fraction responsible for Pb adsorption in leafy vegetables, and this was consistent with the results of this study.

HM concentrations in the plant shoots are used to evaluate phytoextraction efficiency. Therefore, plant species that have high biomass may be the best candidates for phytoextraction; ideal plants can tolerate and bioaccumulate several or most of the HMs in soils. In addition, *Z. mays* is a good candidate for remediating slightly HM-contaminated soils (especially soils containing Cd, Zn, Pb, and Ni). Similar to HM concentrations in shoots of *Z. mays*, EDTA increased the uptake of Cd and Pb in this study. Moreover, *Z. mays* "Zhengdan 958" exhibits high biomass as well as high bioconcentration and translocation factors for Cd and Pb (Liu 2011). In order to achieve economically feasible for use in phytoextraction technology, it is necessary for plants (DW) to accumulate more than 1% of the initial HM amounts of the soils into the shoots (aboveground; Huang et al. 1997; Luo et al. 2005). In the present study, the highest uptake factors for Cd (1.47%) and Pb (0.14%) were obtained in the presence of EDTA with soil particle sizes of <1 mm, and uptakes decreased as the soil particle sizes increased. Similar results were obtained in our other experiments. Therefore, the uptake of HMs (Cd and Pb) by *Z. mays* seedlings did not meet

Table 4 Heavy metal concentrations, bioconcentration factors (BCFs), uptake, and translocation factors (TFs) in *Zea mays* seedlings planted in columns with 300 g contaminated soil

Treatment[a]	Shoot concentrations (mg kg^{-1} plant tissue)		BCF[b]		Uptake (mg shoot^{-1})		TF[b]	
	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb
CK_{0-1}	32.92 ± 8.16	162.47 ± 29.17	3.29	0.32	0.03	0.15	0.72	0.21
CK_{0-2}	33.71 ± 7.19	149.12 ± 23.79	3.37	0.30	0.03	0.13	0.61	0.21
CK_{0-4}	23.6 ± 6.06	134.52 ± 5.15	2.36	0.27	0.02	0.12	0.35	0.19
$EDTA_{2.5-1}$	52.5 ± 9.55	273.93 ± 67.6	5.25	0.55	0.04	0.23	2.99	0.67
$EDTA_{2.5-2}$	44.91 ± 8.98	229.37 ± 37.66	4.49	0.46	0.04	0.22	1.56	0.47
$EDTA_{2.5-4}$	37.51 ± 7.14	166.52 ± 28.88	3.75	0.33	0.03	0.14	0.99	0.32

Values are means ± SD (n = 6)

[a] CK_{0-1}, control with no chelator and in which the soil particle-size fraction was <1 mm; CK_{0-2}, control with no chelator and in which the soil particle-size fraction was <2 mm; CK_{0-4}, control with no chelator and in which the soil particle-size fraction was <4 mm; $EDTA_{2.5-1}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle-size fraction was <1 mm; $EDTA_{2.5-2}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle-size fraction was <2 mm; $EDTA_{2.5-4}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle-size fraction was <4 mm

[b] No unit of measure; please refer to the "Data processing and statistical methods" section for formulas for these variables

the needs in practical phytoextraction applications involving combined Cd- and Pb-contaminated soil in the present study. In order to attain total cleanup of Cd- and Pb-contaminated soil in the present study, 711 crops would be required to remediate the total amount of Pb (552 mg kg^{-1} soil). In contrast to Pb, only 68 crop cycles would be required to extract the total amount of Cd (10.1 mg kg^{-1} soil).

Cumulative HMs in the leaching experiment

In the absence of EDTA (CK), the total amount of Cd in the leachate was 0.20–0.83 and 0.23–0.94 mg L^{-1} under simulated normal rain and acid rain conditions, respectively; likewise, the total amount of Pb in the leachate was 0.02–0.13 and 0.02–0.16 mg L^{-1} under simulated normal rain and acid rain conditions, respectively. These findings indicate that only a small amount of the HMs was leached from the soil by the rainfall, which may be attributed to the low solubility and bioavailability of toxic metals, and in regard to Pb, its strong association with organic matter, Fe-Mn oxides, and clays in most soils may have also played a role (Chen et al. 2004; Neugschwandtner et al. 2008).

When soil columns were exposed to artificial normal rain treatments (pH 6.5), the cumulative Cd leached from EDTA-treated soils with different soil particle-size fractions of <1, <2, and <4 mm were 5.33-, 4.60-, and 3.82-fold higher than that from the control ($P < 0.05$; Fig. 2a), and this accounted for 20.99, 15.19, and 13.91% of the initial HMs in the soil, respectively; the total amounts of Pb in the leachate were 1423-, 2231-, and 680-fold higher than that of the control ($P < 0.05$; Fig. 2b), and this accounted for 17.18, 13.47, and 12.32% of the initial HMs in the soil, respectively. For further comparisons, higher total amounts of Cd and Pb were detected in the treatments with small soil particle sizes of <1 mm, and the amounts decreased with the increases in particle size. Similar results were detected for the HM leaching when the columns were exposed to artificial acid rain (pH 4.5), whereas the total Cd and Pb leaching in the two rainfall treatments regardless of EDTA treatment showed no significant differences, which could indicate that the soil pH (4.5) was similar to acid rain (pH 4.5), and thus, the rain did not further activate Cd and Pb into the solution. Importantly, the time span of the simulated precipitation in the present study was likely too short to affect the soil pH owing to the soil's high buffering capacity (Wu et al. 2004).

As expected, the greatest amounts of HM leaching appeared in treatments with EDTA and artificial acid rain at soil-size fractions of <1 mm, and the corresponding levels were 22.12 and 19.29% of the total Cd and Pb in the soil, respectively, among the six treatments. However, Chen et al. (2004) found that the application of 5 mmol EDTA kg^{-1} soil led to 3.5 and 20.6% of the total amounts of Cd and Pb leaching from soil columns. The difference in the values could have been caused by different soil types, plant species, soil aggregates, and combined influences of other HMs. Moreover, some studies have found that Pb extraction is dependent on the quantity of EDTA, and most Pb is extracted when EDTA/Pb stoichiometric ratios are greater than 10. Therefore, the increased amount of Cd and Pb leached from EDTA-treated soil could potentially increase the HM leaching to deeper soil and even to groundwater, especially in soils with smaller soil particle sizes. The leaching risk of EDTA-enhanced phytoextraction of HMs with different soil particle sizes in the present experiment was found to decrease in the following order: $EDTA_{2.5-1}$ (pH 4.5) > $EDTA_{2.5-1}$ (pH 6.5) > $EDTA_{2.5-2}$ (pH 4.5) > $EDTA_{2.5-4}$ (pH 4.5) > $EDTA_{2.5-2}$ (pH 6.5) > $EDTA_{2.5-4}$ (pH 6.5).

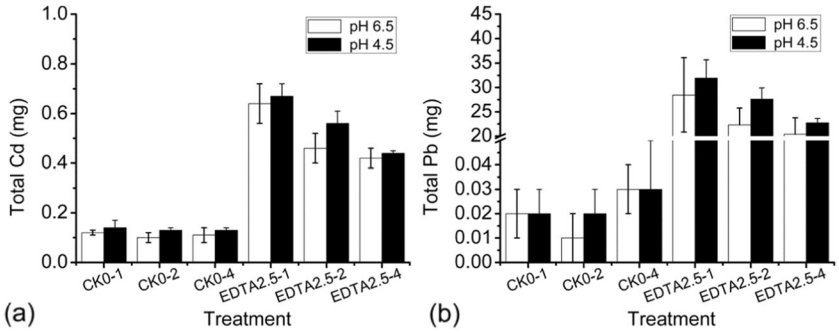

Fig. 2 Total Cd (**a**) and Pb (**b**) in the leachate (250 mL) of EDTA-treated soil columns with soil particle sizes of <1, <2, and <4 mm. *Bars* indicate means ± SD ($n = 3$). $CK_{0–1}$, control with no chelator and in which the soil particle size was <1 mm; $CK_{0–2}$, control with no chelator and in which the soil particle size was <2 mm; $CK_{0–4}$, control with no chelator and in which the soil particle size was <4 mm; $EDTA_{2.5–1}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle size was <1 mm; $EDTA_{2.5–2}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle size was <2 mm; $EDTA_{2.5–4}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle size was <4 mm

HM leaching patterns in the leaching experiment

The Cd and Pb concentrations decreased with every continuing leachate collection (Fig. 3), and the data could fit to linear regression models (Table 5) well, with coefficients of determination (R^2) above 0.90 for Cd and 0.87 for Pb. The maximum concentrations of both Cd and Pb appeared in the first 50 mL of leachate, and then, the amount of HMs decreased as the rainfall volumes increased (Fig. 3), which may have been associated with the reduction of EDTA as leaching proceeded. Similar to the cumulative HMs, concentrations of Cd and Pb in the leachate collections were higher in treatments with smaller soil particle sizes of <1 mm (Fig. 3a, d), and leaching decreased as the soil aggregates increased.

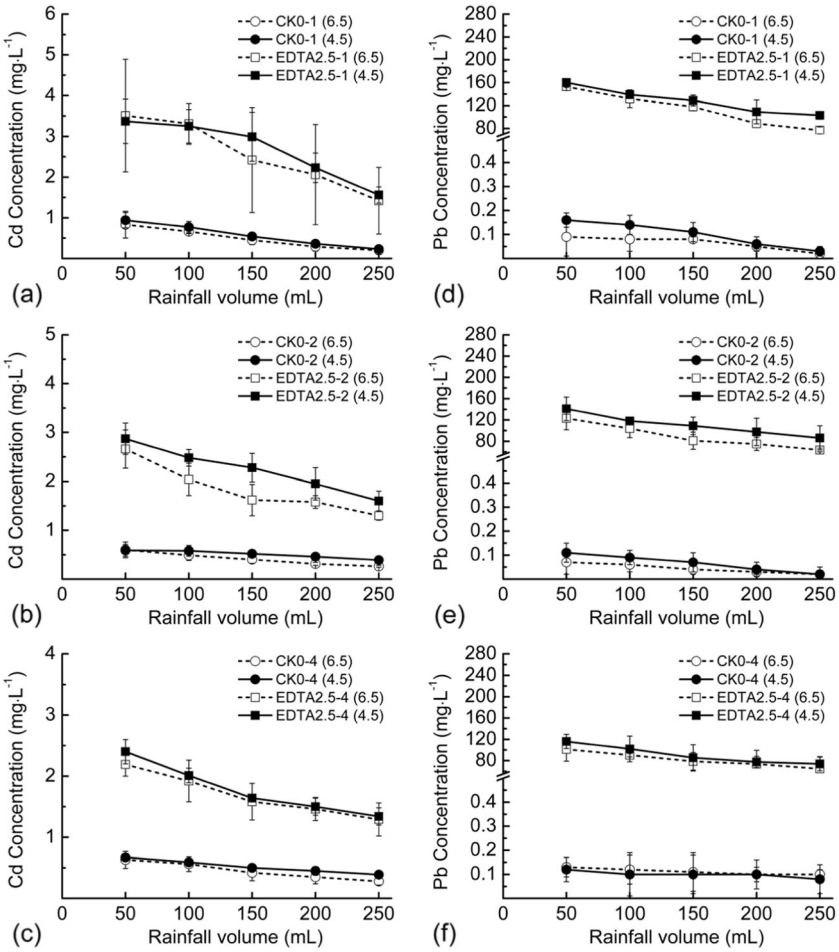

Fig. 3 Cd (**a**–**c**) and Pb (**d**–**f**) concentrations in the successive leachates of EDTA-treated soil columns with soil particle-size fractions of <1, <2, and <4 mm. *Symbols* indicate means ± SD ($n = 3$). $CK_{0–1}$, control with no chelator and in which the soil particle-size fraction was <1 mm; $CK_{0–2}$, control with no chelator and in which the soil particle-size fraction was <2 mm; $CK_{0–4}$, control with no chelator and in which the soil particle-size fraction was <4 mm; $EDTA_{2.5–1}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle-size fraction was <1 mm; $EDTA_{2.5–2}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle-size fraction was <2 mm; $EDTA_{2.5–4}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle-size fraction was <4 mm. *Values in parentheses* (i.e., 6.5 or 4.5) indicate the rainfall pH

Table 5 Regression analysis of heavy metal concentrations in the successive leachates obtained during the leaching experiment

Treatment[a]	Linear regression equations and coefficients of determination (R^2)[b]	
	Cd	Pb
CK_{0-1} (6.5)	$Y = -0.0033X + 0.9807$, $R^2 = 0.9835$	$Y = -0.0003X + 0.1145$, $R^2 = 0.9042$
CK_{0-1} (4.5)	$Y = -0.0036X + 1.114$, $R^2 = 0.9924$	$Y = -0.0007X + 0.2006$, $R^2 = 0.9698$
CK_{0-2} (6.5)	$Y = -0.0018X + 0.675$, $R^2 = 0.9853$	$Y = -0.0003X + 0.0875$, $R^2 = 0.9876$
CK_{0-2} (4.5)	$Y = -0.0011X + 0.6697$, $R^2 = 0.9563$	$Y = -0.0004X + 0.1323$, $R^2 = 0.995$
CK_{0-4} (6.5)	$Y = -0.0018X + 0.7227$, $R^2 = 0.9799$	$Y = -0.0002X + 0.1355$, $R^2 = 0.9747$
CK_{0-4} (4.5)	$Y = -0.0014X + 0.7314$, $R^2 = 0.9873$	$Y = -0.0002X + 0.1265$, $R^2 = 0.8761$
$EDTA_{2.5-1}$ (6.5)	$Y = -0.0109X + 4.1745$, $R^2 = 0.972$	$Y = -0.3922X + 172.65$, $R^2 = 0.9845$
$EDTA_{2.5-1}$ (4.5)	$Y = -0.0093X + 4.0697$, $R^2 = 0.9157$	$Y = -0.2879X + 170.95$, $R^2 = 0.9743$
$EDTA_{2.5-2}$ (6.5)	$Y = -0.0064X + 2.7947$, $R^2 = 0.9006$	$Y = -0.2939X + 133.31$, $R^2 = 0.9562$
$EDTA_{2.5-2}$ (4.5)	$Y = -0.0061X + 3.1544$, $R^2 = 0.9923$	$Y = -0.2623X + 149.82$, $R^2 = 0.9683$
$EDTA_{2.5-4}$ (6.5)	$Y = -0.0045X + 2.3607$, $R^2 = 0.9666$	$Y = -0.1771X + 108.15$, $R^2 = 0.9842$
$EDTA_{2.5-4}$ (4.5)	$Y = -0.0052X + 2.5603$, $R^2 = 0.9487$	$Y = -0.2169X + 123.45$, $R^2 = 0.9453$

[a] CK_{0-1}, control with no chelator and in which the soil particle-size fraction was <1 mm; CK_{0-2}, control with no chelator and in which the soil particle-size fraction was <2 mm; CK_{0-4}, control with no chelator and in which the soil particle-size fraction was <4 mm; $EDTA_{2.5-1}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle-size fraction was <1 mm; $EDTA_{2.5-2}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle-size fraction was <2 mm; $EDTA_{2.5-4}$, 2.5 mmol EDTA kg^{-1} soil in which the soil particle-size fraction was <4 mm; values in parentheses (i.e., 6.5 or 4.5) indicate the rainfall pH

[b] Y, heavy metal (HM) concentration (mg L^{-1}) in the leachate; X, amount of rainfall (mL)

Similarly, Hu et al. (2014) demonstrated that the peak concentration of Cd was found at a cumulative volume of leachate of ~70 mL with the application of 5 mM EDTA-treated soil. However, the maximum concentration of Pb occurred at a cumulative volume of ~300–350 mL (Chen et al. 2004; Hu et al. 2014), which could be attributed to the different leaching modes between these two experiments. In the present study, Cd and Pb were activated for 2 days to reach the maximum soluble HM concentrations according to Zhou et al. (2007). Therefore, we deduced that the maximum HM concentrations appeared at the first collection of leachate and decreased as the rainfall volume increased. Moreover, if the rainfall was heavy enough to saturate the contaminated soil, the linear regressions (Table 5) could potentially be used in forecasting the leaching patterns of HMs (especially for Cd and Pb) over short periods.

Finally, our analysis of leachate patterns also confirmed that EDTA significantly increased the concentrations of both Cd and Pb in the leachate, especially in treatments with soil particle sizes of <1 mm.

Conclusions

The present study demonstrated the effects of EDTA, artificial rainfall, and different particle-size fractions of soil aggregates on the phytoextraction efficiency, uptake, and the potential leaching characteristics of Cd and Pb by *Z. mays* plants. The results indicated that different particle sizes of soil aggregates showed significant effects, and the phytoextraction efficiency, uptake, and leaching risk were increased as the soil particle size decreased, based on the application of EDTA and artificial rainfall. The maximum phytoextraction efficiency, uptake, and HM leaching appeared in the treatment of application of EDTA and artificial acid rainfall in the soil particle size of <1 mm. The results, therefore, emphasize the importance of soil particle size of soil aggregates for the assessment of the HM leaching, and a standard particle size should be unified in the future phytoextraction research and application.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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