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RESEARCH ARTICLE

Leaching variations of heavy metals in chelator-assisted phytoextraction by *Zea mays* L. exposed to acid rainfall

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Abstract Chelant-enhanced phytoextraction method has been put forward as an effective soil remediation method, whereas the heavy metal leaching could not be ignored. In this study, a cropping-leaching experiment, using soil columns, was applied to study the metal leaching variations during assisted phytoextraction of Cd- and Pb-polluted soils, using seedlings of *Zea mays*, applying three different chelators (EDTA, EDDS, and rhamnolipid), and artificial rainfall (acid rainfall or normal rainfall). It showed that artificial rainfall, especially artificial acid rain, after chelator application led to the increase of heavy metals in the leaching solution. EDTA increased both Cd and Pb concentrations in the leaching solution, obviously, whereas EDDS and rhamnolipid increased Cd concentration but not Pb. The amount of Cd and Pb decreased as the leaching solution increased, the patterns as well matched LRMs (linear regression models), with *R*-square (R^2) higher than 90 and 82% for Cd and Pb, respectively. The maximum cumulative Cd and Pb in the leaching solutions were 18.44 and 16.68%, respectively, which was amended by EDTA and acid rainwater (pH 4.5), and followed by EDDS (pH 4.5), EDDS (pH 6.5), rhamnolipid (0.5 g kg^{-1} soil, pH 4.5), and rhamnolipid (pH 6.5).

Keywords Leaching variation · Phytoextraction method · *Zea mays* L. · EDTA · EDDS · Rhamnolipid

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Introduction

A critical concern of heavy metal (HM)-contaminated soil has been proposed all over the world, owing to rapid agricultural and industrial development and as HMs were poisonous and could exist in soil environment persistently, which may be more challenging for contaminated soil remediation. In recent decades, the cost-effectiveness and eco-friendliness of phytoremediation, particularly of phytoextraction, have drawn more attention than other physical, chemical, and biological approaches for restoring HM-polluted soils (Vithanage et al. 2012; Bolan et al. 2014). Nevertheless, though hyperaccumulators are effective for extracting HMs, they have low biomass and poor adaptability as usual, and as a result, tolerant plants with higher biomass may represent promising alternatives for phytoremediation, since their higher biomass can offset their low phytoextraction rate.

To date, *Z. mays* has been widely investigated as a tolerant plant species (Akhtar et al. 2017; Rizwan et al. 2016a; Rehman et al. 2016; Shafigh et al. 2016) for phytoextraction technologies. Liu (2011), for example, studied HM extraction by 29 *Z. mays* cultivars and found that *Z. mays* "Zhengdan 958" had high bioconcentrations and translocation factors for Cd and Pb, with higher biomass than other varieties. Moreover, metal distribution in *Z. mays* plants is extremely uneven, with very low metal accumulation in grain, and *Z. mays* stalk could be applied as a starting stuff in ethanol fuel production (Rizwan et al. 2016a; Lou et al. 2005). Thus, the application of *Z. mays* plants for soil restoration has multiple functions, including cropping and environmental conservation, as well as energy regeneration. Moreover, most metals are immobile in soil environment, which could be absorbed as well as used by plants in dissolved form (Wang et al. 2009). Thus, chelators could promote the movement of HMs (from the soil surface to soil solution) and the increase of HM extraction efficiency by tolerant plants has also gained attention recently.

Chelators mostly applied in soil remediation were EDTA, EDDS, and NTA (Alkorta et al. 2004; Evangelou et al. 2007). Ethylenediaminetetraacetic acid (EDTA), for instance, is effective for mobilizing a number of HMs, such as Cd, Pb, Zn, and Cu. Some studies have indicated that EDTA could increase the extraction efficiency of Cd, Cu, and Pb in *Lolium perenne*, *Brassica napus*, and *Z. mays* by factors of 3.44, 42, and 2.9 and solubilized HMs in the soil solution were 1.85-, 296-, and 8-factor that of control, respectively (Habiba et al. 2015; Lambrechts et al. 2011; Neugschwandtner et al. 2008;). In addition, ethylenediamine-*N*,*N*′-disuccinic acid (EDDS) has been proposed as a promising biodegrable chelator for enhancing phytoextraction (Luo et al. 2006; Meers et al. 2008). It has been found that, after application of EDDS, HM migration from soil profile to groundwater presented an environmental risk, according to some physicochemical properties (pH, metal solution, et al.) of the contaminated soils (Hu et al. 2007). Among all the chelators studied, rhamnolipid, a non-ionic biosurfactant that exhibits solubilization capacity, low toxicity, thermostability, and degradability, which could activate HMs in soils by decreasing the surface tension, and enhancing plant root permeability to chelators (Shi et al. 2015; Lu and Feng, 2009; Chen et al. 2008), is also widely applied in soil remediation research (Zhang 2016; Zheng et al. 2012; Jia et al. 2009; Asha et al. 2007; Chen et al., 2004a), but has rarely been applied in the phytoextraction method. However, the application of chelators could increase the leaching risk of HM to deeper soil and even to groundwater.

Assessments of potential leaching risk were widely investigated, based on speciation analysis of HMs (Wu et al. 2004; Wang and Liu 2014; Wei et al. 2011), as well as leaching experiments with no cultivation (no plants were cultivated in columns; Gabos et al. 2009), whereas only few experiments were conducted upon cultivation (Lu et al. 2017; Chen et al., 2004b; Thayalakumaran et al. 2003; Grčman et al., 2003). Grčman et al. (2001) showed that up to 37.9% (Pb) and 56.3% (Cd) were percolated after applying EDTA, in the cropping-column experiment. As was shown by Chen et al. (2004a), EDTA exerted a persisting effect on HM leaching, upon conducting a cultivation-leaching experiment. Lu et al. (2017) reported that 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 southern part of the Yangtze River in China is the main HM-contaminated area and also the main area in which acid rain is distributed (Liu et al. 2011), especially in Guangdong Province. According to the environmental report of Guangdong Province in 2016, acid rain (pH < 5.6) existed in 81.8% of the total cities. 45.6% of which were polluted by acid rainfall. And, the minimum pH value of acid rain in the Guangdong area was 4.38, which was lower than 4.5 (Qin et al. 2006). Some studies have also indicated that pH could largely affect the dissolved ability of metals in the soil profile. Boekhold et al. (1993) demonstrated that soil adsorptive capacity for Cd was twice as before when pH increased in 0.5 unit, ranging from 3.8 to 4.9. Li et al. (2015) found that up to 38.0 and 53.2% of Pb and Cd, respectively, were percolated from the soil profile when exposed to acid rain with no cultivation. Researches on rainfall pH based on cultivation-leaching have rarely been found. Thus, experiments on leaching characteristics of phytoextraction exposed to acid rainfall will be of great significance.

Accordingly, the present study investigated the potential HM leaching risk of chelator-assisted phytoextraction systems, especially when these systems are exposed to acid rainfall, using *Z. mays* Zhengdan 958 seedlings, and EDTA, EDDS, and rhamnolipid were used as chelants. The main goals of this study were aimed at (1) determining the botanical extraction and translocation efficiency of *Z. mays* Zhengdan 958 with the application of different chelators, (2) leaching variations of Cd and Pb resulting from the combined effects of rainfall volume, pH, and the addition of different chelators, and (3) the largest risk of both Cd and Pb leached with the addition of different chelators, rainfall pH, and volume. Our findings provide theoretical support to the development and use of chelator-enhanced phytoextraction within acid rain-distributed areas.

Materials and methods

Preparations for soil

The experimental soils came from a forested site near an iron pyrite discharge field in Guangdong Province, located in the southern part of China (22°59′25.5″N, 112°00′40.5″E); some of the top 20-cm soils were collected. The soil sample was dried and sieved through a 2-mm plastic sieve in preparation of glasshouse experiments. The soil was red loam, and the pH was determined as 4.5 (in deionized water, *w*/*v* = 1:1). The physicochemical properties like sand/silt/clay, cation exchange capacity (CEC), bulk density, and field water capacity were determined (Lu et al. 2017). Elemental analysis of the subsample soils were conducted using HNO_3-HF digestion system (Lu et al. 2017) concentrations of HMs in each sample were measured by AAS (atomic absorption spectrometry, iCE 3500; Fisher Scientific, Loughborough, UK). Some other basic physicochemical properties were determined as mentioned in Hu et al. (2014) and Lu (1999).

The subsample soil was a clay loam with sand (> 0.02 mm), silt (0.02–0.002 mm), and clay (< 0.002 mm) of 51, 33, and 16%, respectively. It has a moderate OM (organic matter, 15.4 g kg^{-1}) and a low CEC (3.3 cmol kg^{-1}), as well as a high bulk density of 1.279 g cm^{-3}. The total N was 0.084%. The determinations of available N, P, and K were 37.8, 0.90, and 12.1 mg kg^{-1}, respectively. Concentrations of Cd and Pb were low (0.12 and 52.30 mg kg^{-1}, respectively).

Glasshouse experiment

The subsample soils were factitiously polluted by Cd (10.1 mg kg^{-1} of soil) as $CdCl_2·5/2H_2O$ (salt solution) and Pb (552 mg kg^{-1} of soil) as $Pb(NO_3)_2$. Moreover, soil samples were fertilized as well, according to the first-class standard of the Second National Soil Fertility Grading Recommendation (0.75 g per pot, N:P_2O_5:K_2O = 15:15:15; Lu et al. 2017). After having added the HMs and manure, the contaminated soils underwent five cycles of wetting by adding deionized water (with a water-holding capacity (WHC) of approximately 70%) and drying, for 15 days, using the methods of Blaylock et al. (1993).

Seeds of Zhengdan 958 (kernel rate ≥ 89.5%, germination rate ≥ 90%) were saturated and germinated at a temperature of 25 °C for 3 days, with the addition of a proper amount of water each day. Individually germinated seeds were sown in air-dried soil (300 g) in experimental columns (75-mm diameter × 130-mm height) at a depth of ~ 2–3 cm. All the columns were placed (n = 24) in a glasshouse, located in Guangzhou University, which were prepared for the HM leaching experiment in the section "Leaching experiment". In order to maintain an approximately 70% WHC, deionized water was added to keep the weight constant twice a day (8:00 a.m. and 5:30 p.m.). All columns were settled under natural light, at a temperature ranging from 26 to 37 °C. When the *Z. mays* plants grew to the seven-leaf stage (21 days), six columns were treated with one of the following, respectively, control, with no amendment (CK_0); 2.5 mmol Na_2EDTA salt per·kilogram of soil ($EDTA_{2.5}$); 2.5 mmol EDDS per kilogram of soil ($EDDS_{2.5}$); or 0.5 g rhamnolipid·per kilogram of soil ($rhamnolipid_{0.5}$), using a fully randomized design.

Leaching experiment

Artificial rainwater was produced to match the chemical properties of rainwater at Yunfu City, China (Table 1), according to (Lu et al. 2017). In order to adjust the rainwater pH to either 4.5 or 6.5, a mixed solution of HCl, H_2SO_4, and HNO_3 ($[Cl^-]:[SO_4^{2-}]:[NO_3^-]$ = 1:6.1:0.7, molar ratio) was added to the base solution produced in Table 1. At 2 days following the chelator treatments to the 24 columns (Zhou et al. 2007), a leaching experiment was performed, using saturated infiltration with a volume of 250 mL (50 mm; heavy rain standard, China Meteorological Administration) of either acid rainwater or normal rainwater (pH 4.5 or 6.5) (Table 2). The leachate from each column was collected in 50-mL volumes and the concentration of HMs in each sample was detected using atomic absorption spectrometry (iCE 3500; Fisher Scientific, Loughborough, UK). We measured various morphological characteristics of the *Z. mays* plants, including height of the plant, length and width of the stem, and length and width of the leaf, which represented as mentioned in Lu et al. (2017). Whereafter, the shoots over the soil surface were reaped, and the dry weights (DWs) of both the shoots and roots were obtained after a thorough wash, using deionized water, and dried to constant weight at 70 ± 2 °C. In addition, plants were digested by HNO_3 (guaranteed reagent, GR)-H_2O_2 (GR) system, and elemental analysis of the solutions was conducted using atomic absorption spectrometry. And, the BCF, HM uptake, and TF values of the plants were calculated, as described in the section "Data processing and statistical analysis."

Table 1 Chemical properties of rainfall solutions at Yunfu City, located in Guangdong Province (Lu et al. 2016)

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

Data processing and statistical analysis

Plant phytoextraction of metals was usually assessed by concentration, bioconcentration factor (BCF), and uptake, and translocated factor (TF) was used to define the transferring ability of HMs. The factor of BCF was determined as C_{shoot} (HM concentration of shoots, mg kg^{-1}) divided C_{soil} (HM concentration in the soils, mg kg^{-1}); factor of uptake was decided as the multiplication of C_{shoot} and DW_{shoot} (dry weight of shoots, g); factor of TF was calculated as C_{shoot} divided C_{root} (HM concentration of roots, mg kg^{-1}).

Data were analyzed by the Origin software package (version 8.5; OriginLab, Northampton, MA, USA) and showed by means±SD (standard deviation) of triplicates. In order to give a thorough analysis, the mean values were analyzed by ANOVA prior to Tukey's test with a 95% confidence level ($P < 0.05$) as well. And, regression analyses were presented as the linear regression equation as well as coefficients of determination (R^2).

Results and discussion

Influence of HMs and chelators on growth of *Z. mays*

The seedling plants have not exhibited any obvious poisoning features during the growing stage, and at 2 days after the chelator application (EDTA, EDDS, and rhamnolipid), *Z. mays* still exhibited no poisoning features, indicating that *Z. mays* Zhengdan 958 could bear both HM concentrations as well as chelators applied during this experiment, that homologous regulations have also been indicated during our other experiments (data not shown). However, it was reported that excess Cd is toxic and may decrease growth and biomass of some plants and especially maize (Metwali et al. 2013; Rizwan et al. 2012, Rizwan et al. 2016b; Anjum et al. 2015, 2016; Farooq et al. 2016), which was related to the HM concentration in the soil of their experiments. In addition, it was found that the application of EDTA and EDDS in a high concentration like 5 mmol kg^{-1} obviously influenced growth of *Z. mays* (Luo et al. 2005). Shi et al. (2009) reported a significantly decreased biomass in plants treated with 1 g kg^{-1} rhamnolipid. Therefore, we hypothesized that chelator-assisted phytoextraction should be treated under the condition of not poisonous to the plants and minor concentrations of the chelators were set in this study. In the present study, the physiological indices were slightly higher with application of EDTA, while no significance was found among the four treatments ($P > 0.05$; Fig. 1).

Phytoremediation efficiency and translocation abilities of metals by *Z. mays*

Phytoextraction effects and translocation factors were not only the reflection of remediation abilities of plants, but also the reflection of HM leaching risk when exposed to rainfall. Data are shown in Table 3.

Applying EDTA obviously enhanced Pb phytoextraction (Pb concentration in shoots), which was 1.54-fold of control (CK, without adding a chelator; $P < 0.05$; Table 3), whereas the other two chelators did not. EDDS addition to the soil significantly increased Cd phytoextraction, which was 1.42-fold of control ($P < 0.05$; Table 3). Similar to the HM phytoextraction, EDTA and EDDS increased Pb and Cd uptake, respectively, that were 1.69- and 1.41-fold of control ($P < 0.05$; Table 3). In addition, EDTA could significantly strengthen Cd and Pb to be translocated from the roots towards the aboveground part, which was 2.34- and 2.24-fold

Table 2 Different treatments of the leaching experiment

No.	Treatment	Chelator		Rainfall pH
		Name	Concentration (mmol kg^{-1})	
1	CK_0 (6.5)	–	0	6.5
2	CK_0 (4.5)	–	0	4.5
3	$EDTA_{2.5}$ (6.5)	EDTA	2.5	6.5
4	$EDTA_{2.5}$ (4.5)	EDTA	2.5	4.5
5	$EDDS_{2.5}$ (6.5)	EDDS	2.5	6.5
6	$EDDS_{2.5}$ (4.5)	EDDS	2.5	4.5
7	$Rhamnolipid_{0.5}$ (6.5)	Rhamnolipid	0.5 g kg^{-1}	6.5
8	$Rhamnolipid_{0.5}$ (4.5)	Rhamnolipid	0.5 g kg^{-1}	4.5

Treatments were performed with three replicates

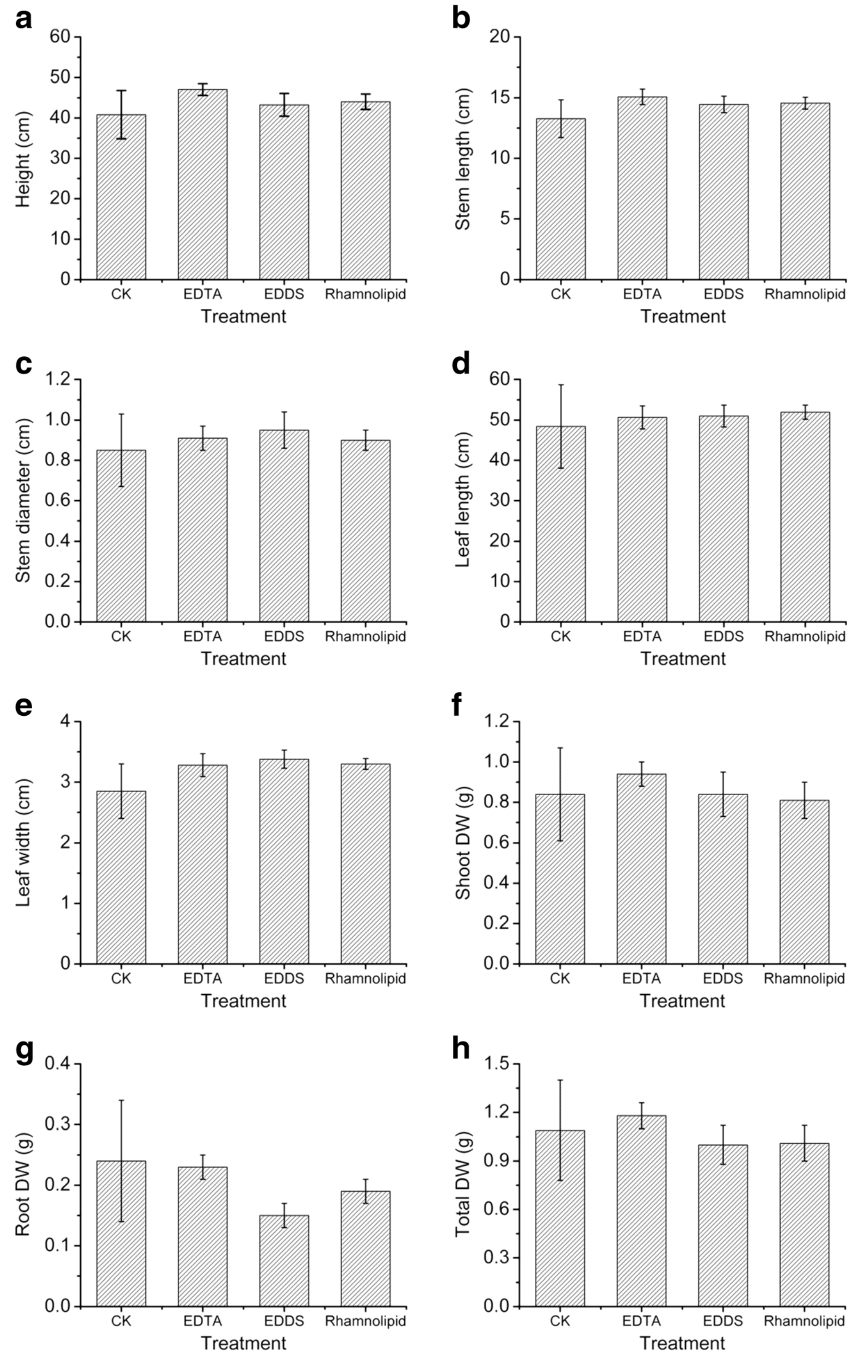

Fig. 1 Morphological characteristics of glasshouse-grown seedlings of *Zea mays* plants, by applying EDTA (2.5 mmol kg^{-1} soil), EDDS (2.5 mmol kg^{-1} soil), or rhamnolipid (0.5 g kg^{-1} soil). Height (**a**), stem length (**b**), stem diameter (**c**), leaf length (**d**), leaf width (**e**), shoot DW (**f**), root DW (**g**), and total DW (**h**). Bars represented means ± SD (with six replicates). CK, without the application of chelators. No significance was found among the four treatments ($P > 0.05$)

of control ($P < 0.05$; Table 3), whereas the other two chelators could not.

Similar to most studies, EDTA significantly mobilized Pb in the soil profile, since EDTA has a strong chemical combination with Pb (logKs = 18.04), whereas EDDS possesses a relatively low combination with Pb (logKs = 12.7; Tandy et al. 2004). A related study by Shi et al. (2009) also demonstrated that neither Cd nor Pb concentrations are significantly increased in the underground part of ryegrass when rhamnolipid and EDDS are applied separately, and similarly, Jorden et al. (2002) found that rhamnolipid was not effective for increasing Pb in *Z. mays* shoots, although chelant labeled by radio was detected in the shoot.

In addition, phytoextraction efficiency is related to both HM concentration and DW of shoot. Therefore, the best candidates for phytoextraction are plant species which exhibit high biomass which allows them to bear and extract some or most of metals. *Z. mays* has been demonstrated as an effective plant for restoring moderately HM-contaminated soils (Akhtar et al. 2017; Rizwan et al. 2016; Rehman et al. 2016; Shafigh et al. 2016). According to a research by Liu (2011), *Z. mays* Zhengdan 958 owns some characteristics such as high

Table 3 Cd and Pb concentrations, bioconcentration factor (BCF), uptake, and translocated factor (TF) of *Z. mays* Zhengdan 958 during the glasshouse experiment

Treatment[a]	Shoot concentrations[b]		BCF[c]		Uptake[d]		TF[c]	
	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb
CK	33.7 ± 7.19	149 ± 23.79	3.34	0.30	0.029	0.13	0.61	0.21
EDTA	40.29 ± 8.78	229 ± 37.66*	3.99	0.46*	0.038	0.22*	1.43*	0.47*
EDDS	47.8 ± 5.56*	158 ± 27.18	4.73*	0.32	0.041*	0.13	0.69	0.20
Rhamnolipid	40.7 ± 8.75	178 ± 41.19	4.03	0.36	0.033	0.15	0.71	0.20

Values represent means ± SD (with six replicates)

*Significant $P < 0.05$

[a] CK, without applying chelant; EDTA, 2.5 mmol EDTA·kg^{-1} soil; EDDS, 2.5 mmol EDDS·kg^{-1} soil; rhamnolipid, 0.5 g rhamnolipid·kg^{-1} soil

[b] mg kg^{-1} dry matter of plant

[c] No unit of measure; the section "Data processing and statistical analysis" has given the formulas of these variables

[d] mg shoot^{-1}

biomass and high BCF and TF for several metals, especially Cd and Pb. Once more than 1% of the initial amount of HMs was bioaccumulated by the plant shoots, which was assessed as an economical phytoremediation plant species (Luo et al. 2005), and during this study, the maximum phytoextraction efficiency (uptake) for Cd and Pb was 1.34 and 0.08%, respectively, after applying EDDS and EDTA. Based on the economic consideration, metal uptake of *Z. mays* seedling could be better to meet the criteria of Cd in the present study. After a proper calculation, we inferred that 762 and 75 crop cycles were needed for extracting the initial Pb and Cd, respectively.

Amounts of HMs during the leaching experiment

When no chelating agent was added (CK), the cumulative Cd in the leaching solution was only 3.4 and 4.2% of the initial concentration of the soil, under artificial rain and acid rain, respectively, and only 0.01 and 0.07% of the initial amount was found for Pb, indicating that very small proportions of the HMs were removed by rainfall. This observation can be ascribed to the low dissolved ability of HMs, and especially Pb, owing to its strong combination of some components such as organic matter, Fe-Mn oxides, and clays (Chen et al., 2004a; McBride 1994). For further comparison, the cumulative Cd and Pb in the leaching solution with acid rainfall (pH 4.5) were 1.24- and 7.00-fold that of normal rainfall (pH 6.5), which indicated that bioavailability of HMs could be affected by rainfall pH.

Under the non-acid rainfall treatment (pH 6.5), the total amount of Cd and Pb in the leaching solution by EDTA amendment presented 4.5- and 2013-fold higher than the control ($P < 0.05$; Fig. 2) and accounted for 15.20 and 13.47% of the initial soil profile, respectively. In addition, the cumulative Cd leached from EDDS- and rhamnolipid-treated soils were 2.0- and 1.9-fold greater than the control ($P < 0.05$; Fig. 2a). However, EDDS and rhamnolipid had less of an effect on Pb leaching (Fig. 2b), which could be related to the strong chelating ability of EDTA for cationic HMs (Hu et al. 2014), especially Pb (Tandy et al. 2004). After applying EDTA, some of the metal-EDTA complexes might have been released from the soil, allowing them to be leached from the soil through soil solution (Jelusic et al. 2013). However, Wen et al. (2009) found that only a small portion of EDTA was degraded after 3 weeks, whereas up to 36% of rhamnolipid was degraded, and Tandy et al. (2006) reported that EDDS degrades after only 7–11 days in soil, with a calculated half-life of 4.18–5.60 days. Moreover, complex stabilities between chelators and HMs could also affect the leaching amounts of HMs, of which the constant of EDTA-Cd (logKs = 16.46; Liang et al. 2015) was higher than that of EDDS-Cd (logKs = 10.8; Luo and Xin, 2008; Liang et al. 2015) and rhamnolipid-Cd (logKs = 6.89; Francisco et al. 2001); and that of EDTA-Pb (logKs = 18.04; Tandy et al. 2004) was higher than that of EDDS-Pb (logKs = 12.7; Luo and Xin, 2008) and rhamnolipid-Pb (logKs = 8.58; Ochoa-Loza et al. 2001).

The cumulative Cd and Pb leached with acid rainfall (pH 4.5) were 1.22- and 1.24-fold that of normal rainfall (pH 6.5), with the application of EDTA ($P < 0.05$; Fig. 2). Results appearing in Pb leaching with the application of acid rainfall (pH 4.5) from EDDS- and rhamnolipid-treated soils were 1.25- and 1.37-fold that of the non-acid rainfall treatments (Fig. 2), whereas no significant difference was observed for Cd leaching ($P > 0.05$; Fig. 2). For further inference, acidic effect may be another main factor influencing the complexing power of EDTA and HMs. As the minimum pH of EDTA-HMs (Cd and Pb) complexation were approximately 3.3 and 4.0, respectively, and soil pH was 4.5, thus, EDTA-HM complexation in the present study increased as the rainfall pH decreased.

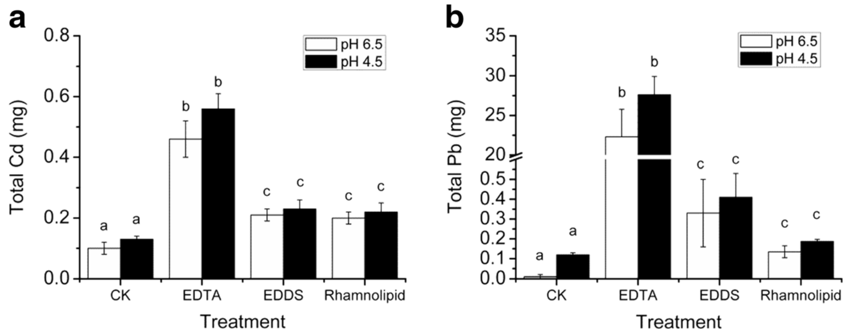

Fig. 2 Cumulative Cd (**a**) and Pb (**b**) leached from soil profile treated with EDTA, EDDS, and rhamnolipid. Bars represented means ± SD (with three replicates). CK, without applying chelators. EDTA, 2.5 mmol EDTA kg^{-1} soil; EDDS, 2.5 mmol EDDS kg^{-1} soil; rhamnolipid, 0.5 g rhamnolipid kg^{-1} soil. The small letters above the bars in each figure were used to express the significant differences between the chelator-treated HMs leaching amounts and CK (control group without adding chelators). Bars with the same small letters represent no significant differences ($P > 0.05$) and with different small letters represent significant differences ($P < 0.05$)

As expected, the maximum amounts of metals were leached when soils were treated with EDTA and simulated acid rainfall, which were 18.44 and 16.68% of the initial Cd and Pb levels, respectively. In contrast, Chen et al. (2004b) indicated that more than 3.0 and 20% of the initial Cd and Pb in the leaching solution after applying 5 mmol EDTA kg^{-1} soil, with *Z. mays* planted in yellow brown soils. The discrepancy could be attributed to the differences in the soil, plant species, and combined influences of other metals. In addition, some studies have demonstrated that resulted from the stoichiometric ratio of EDTA and Pb, which is greater than 10, Pb could be extracted from soil profile in the largest quantity (Kim et al. 2003). Therefore, the increased level of HMs in the leachate which was treated by EDTA may as well lead the HMs down to the deeper part of the soil and the groundwater, whereas EDDS and rhamnolipid pose little risk of HM leaching to groundwater. The leaching risk of chelator-assisted phytoextraction of metals during this study follows the order: EDTA (pH 4.5) > EDTA (pH 6.5) > EDDS (pH 4.5) > EDDS (pH 6.5) > rhamnolipid (pH 4.5) > rhamnolipid (pH 6.5).

HM variations leached from the soil columns

The Cd and Pb amounts descended as the successive leaching solution percolated (Fig. 3), and the patterns adequately matched the linear regression, with *R*-square (R^2) above 0.90 for Cd and above 0.82 for Pb. The peak concentration of both HMs was observed in the leaching solution of the first 10 mm (50 mL), and descended with rainfall volume increase (Fig. 3) that might be related to the leaching of complexes of chelator-HMs.

Similar to our findings, it was found that the highest Cd concentration in the leachate treated by 5 mM EDTA appeared in the first 70-mL rainfall volume (Hu et al. 2014). However, maximum Pb concentration appeared in the volume of approximately 300–350 mL (Chen et al., 2004a); that might be related to the differentia of leaching patterns. In addition, Zhou et al. (2007) indicated that soluble HMs reached their maximum concentrations at 2 days after applying 5 mM EDTA or EDDS. In this study, HMs have also been activated for 2 days. Thus, it was inferred that the maximum amount of HMs presented in the previous 50 mL leaching solution, then dropped with the rainwater volume increase. In addition, in the saturated HM-polluted soil profile, the linear regressions of the leaching patterns shown in Fig. 3 might be better to forecast the leaching variations in a short time.

Accordingly, leaching variations in this experiment also indicated that the application of EDTA distinctly enhanced the amounts of Cd (Fig. 3a) and Pb (Fig. 3d) in the leaching solution and that EDDS and rhamnolipid significantly increased the concentration of Cd (Fig. 3b, c) but not Pb (Fig. 3e, f).

Conclusions

During this study, which is based on a cultivation-leaching experiment, the effect of EDTA, EDDS, and rhamnolipid on HM phytoextraction by *Z. mays* was demonstrated and the possible leaching variations of Cd and Pb under artificial rainfall (especially acid rain) were investigated.

Applying EDTA and EDDS obviously enhances Pb and Cd phytoextraction, bioconcentration, and uptake, respectively. And, EDTA addition could strengthen Cd and Pb translocation from roots to shoots. Chelator-enhanced phytoextraction under the rainwater treatment resulted in a high concentration of Cd and Pb leached through the soil profile. HM concentration in the leaching solution dropped as the rainwater volume increased, and the leaching style could fit to linear regressions, with *R*-square (R^2) higher than 0.90 for Cd and 0.82 for Pb, respectively. The maximum cumulative Cd and Pb in the leaching solution were 18.44 and 16.68%, respectively, treated with EDTA and acid rainfall. Use of chelators during

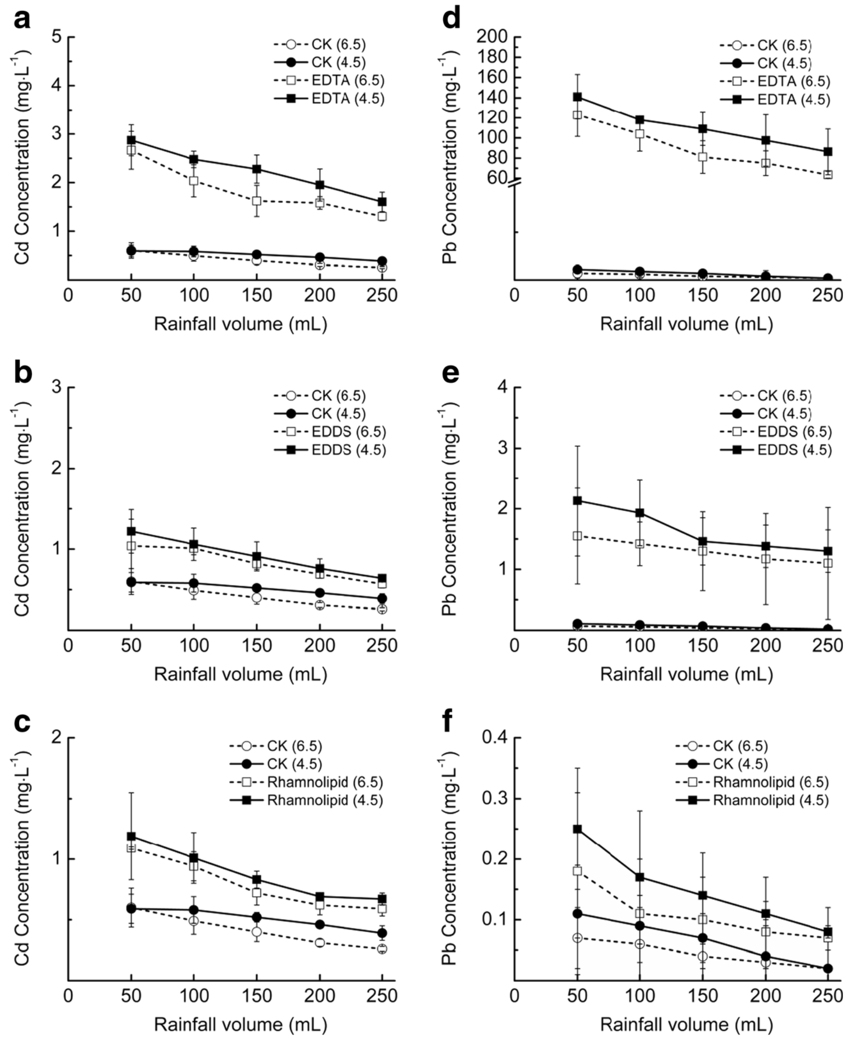

Fig. 3 Concentrations of Cd (**a**, **b**, **c**) and Pb (**d**, **e**, **f**) in the cumulative leachates of EDTA-, EDDS-, and rhamnolipid-treated soil columns. Symbols represent means ± SD (with triplicates). CK, without applying chelants; EDTA, 2.5 mmol EDTA kg^{-1} soil; EDDS, 2.5 mmol EDDS kg^{-1} soil; rhamnolipid, 0.5 g rhamnolipid kg^{-1} soil. Figures of 6.5 and 4.5 in the parentheses demonstrate the pH of the rainwater used in the present study. Patterns adequately matched the linear regression, with *R*-square (R^2) above 0.90 for Cd and above 0.82 for Pb (data not shown)

phytoextraction increased the risk of HM leaching in the following order: EDTA (4.5) > EDTA (6.5) > EDDS (4.5) > EDDS (6.5) > rhamnolipid (4.5) > rhamnolipid (6.5).

Leaching risk during chelator-assisted phytoextraction is complex, which is related to plant species, growing stages, soil types, HM contamination, and fertilization in soil. The relationships among these vital factors need to be further studied.

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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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