Induced-phytoextraction of heavy metals from contaminated soil irrigated by industrial wastewater with Marvel of Peru (*Mirabilis jalapa* L.)

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ABSTRACT

The primary objective of this study was to evaluate the effect of ethylene diamine tetraacetic acid (EDTA) and citric acid (CA) on *Mirabilis jalapa* L. growth and phytoextraction of heavy metals from the multi-metal contaminated soil. The results showed that the application of CA (5 and 8 mmol/kg) and EDTA (5 mmol/kg) increased plant growth, while when the concentration of EDTA was up to 8 mmol/kg, the shoot biomass significantly decreased relative to the control plants ($P < 0.05$); it suffered 48.1%, 53.1%, 58.9%, and 78.2% reduction, respectively, compared to CK, CA-5, CA-8, and EDTA-5. EDTA was more effective than CA at increasing heavy metal uptake in aerial parts of the plants, the shoot concentrations of Cd, Cu, Pb, and Zn increased by 0.55, 3.08, 3.28, and 1.0-fold in the 8 mmol/kg EDTA-treated soils relative to the treatment of 8 mmol/kg CA. The maximum of Cd, Cu, and Pb phytoextraction and remediation factor (RF) were found with 5 mmol/kg EDTA treatment. For Zn**,** 8 mmol/kg EDTA was most efficient in increasing Zn accumulation in aboveground of *M. jalapa.*

Keywords: enhaced-phytoextraction; heavy metal contamination; ethylene diamine tetraacetic acid (EDTA); citric acid (CA); *Mirabilis jalapa* L.

The Shenyang Zhangshi Irrigation Area (SZIA) is a representative area contaminated by heavy metals resulting from sewage irrigation, with an irrigation history of 30 years. The sewage irrigation area covered from 200 ha in 1954 to 2800 ha in 1968 (Sun et al. 2008). Historically, the concentrations of Cd in soils of Sluice Gate III of SZIA were from 2.2 to 6.09 mg/kg in 1977, reached 0.65–9.00 mg/kg in 1989, and then ranged from 2.10 to 4.35 mg/kg in 1996 (Xiong et al. 2004). In the 21st century, Xu et al. (2007) found the concentrations of Cd, Cu, Pb, and Zn in soils that were 1.38–3.88, 15.56–48.19, 9.88–45.2, and 52.7–117.45 mg/kg, respectively. The serious heavy metal contaminated soils in SZIA are of increasing concern because high Cd concentration was detected in rice grain. Cd concentrations in rice grain in Sluice Gate III of SZIA reached 0.06–0.36, 0.08–1.26 and 0.03–2.46 mg/kg, respectively, in 1977, 1986 and 1990 (Xiong et al. 2004). High concentration of Cd in rice posed a potential hazard to human health by way of food chain. Wu et al. (1986) found that Cd contents in

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blood increased from 1.76 μg/L in 1978 to 2.23 μg/L in 1979, and up to 13.26 μg/L in 1982. The surveillances of renal function state in adult women in SZIA were carried out by Xue et al. (2003), the positive rate of low molecular weight protein and the urinary β2-MG and ALP activities in the urine of women in Cd polluted area was significantly higher than those of control, indicating that the renal dysfunction had appeared in the part of women. So, the cleanup of Cd-contaminated soils in SZIA is emergent and imperative.

Phytoremediation is emerging as a new technology that uses plants for cleaning or decreasing the toxicity of soils and waste waters contaminated by heavy metals (McGrath and Zhao 2003). The development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a 'green', sustainable process (Pulfore and Watson 2003, Zhou and Song 2004). However, the remediation efficiency depends on two sticking points: the high concentration of metal in plant, especially in aerial parts, and a relatively large aboveground biomass. Chelate-induced phytoextraction is based on the fact that the application of chelating agents to the soil markedly increases metal accumulated by plants (Garbisu and Alkorta 2001, Alkorta et al. 2004). Synthetic chelators such as ethylene diamine tetraacetic acid (EDTA) and critic acid (CA) were proven to be very effective in enhancing the bioavailability of metals in soils, thus facilitating their plant absorption and translocation (Huang et al. 1998, Lai and Chen 2005, Quartacci et al. 2005, Evangelou et al. 2006).

The main objective of this work was to investigate *Mirabilis jalapa* L. for phytoextraction of heavy metal contaminated soils in SZIA. To this point, chelate-induced phytoextraction study was carried out with EDTA and AC to determine the capacity of *M. jalapa* to remediate Cd, Cu, Pb, and Zn. It could provide a scientific basis for phytoextraction of heavy metal contaminated sites with *M. jalapa*.

MATERIALS AND METHODS

Soil sampling. Soil was collected from the surface layer (0–20 cm) in Sluice Gate III of SZIA, and then air-dried for about 12 days. The soil samples passed through a 2 mm sieve were used for physical and chemical fraction analysis (Bao 2000), those passed through 50 mm sieve were used to determine the concentrations of heavy metals (Sun et al. 2009). The following soil properties were determined: pH 5.78, organic matter (OM) 1.96%, cation exchange capacity (CEC) 16.38 cmol/kg, total N 0.96 mg/kg, available P 16.32 mg/kg, available K 96.83 mg/kg, Cd 3.07 mg/kg, Cu 45.51 mg/kg, Pb 54.66 mg/kg, and Zn 161.37 mg/kg. The soil texture in SZIA is a meadow brown soil and its texture classification is loam.

Pot experiment. Air-dried and homogenized soil equivalent to 2.5 kg (dry basis) was placed in each plant pot (15 cm in height and 20 cm diameter). The treatments comprised the following amendments: (1) control with no amendment (CK); (2) 5 mmol/kg EDTA (EDTA-5); (3) 8 mmol/kg EDTA (EDTA-8); (4) 5 mmol/kg citric acid (CA-5); (5) 8 mmol/kg citric acid (CA-8). Na_2-EDTA and CA were dissolved into 10 ml deionised water, and then applied to soils 20 days after transplanting *M. jalapa*. NH_4NO_3 and K_2HPO_4 were applied as basal fertilizers at the rates of 87.5 mg N/kg, 48.9 mg K/kg, and 19.4 mg P/kg, respectively. Seeds of *M. jalapa* were germinated on a mixture of vermiculite and perlite moistened with deionised water. Twenty days after germination, three uniform seedlings were transplanted into each pot. The tested soil samples were watered to reach 75% of the field water-holding capacity and maintained this humidity by daily watering throughout the cultivation, and a petri dish was placed under each pot to collect potential leachate during the experiment. Plant samples were washed with tap water thoroughly and rinsed with deionised water, and dried at 70°C in an oven until completely dry, then weighed for dry weight (DW).

Plant and soil analysis. Cd, Cu, Pb, and Zn fractionation in the soil was performed using sequential extraction by Tessier et al. (1979). The extraction was carried out progressively on an initial weight of 1.00 g test soil, which was contained in a centrifuge tube (polypropylene, 100 ml) and shaking with variable speed on a reciprocal shaker at 220 strokes/min. The extractant and operationally defined chemical fractions were shown in Table 1. After each successive extraction, separation was done after centrifuging for 30 min. The supernatant was filtered and placed in a tube for measurement.

The plant and soil samples were digested with a solution of HNO_3-$HClO_4$ and HCl-HNO_3-HF-$HClO_4$, respectively. The concentrations of heavy metals were determined by a flame atomic absorption spectrophotometer (WFX-120, Beijing Rayleigh Analytical Instrument Co., Ltd, Beijing, China). The wavelength for Cd determination was 228.8 nm. A certified reference material, bush

Table 1. The sequence extraction processes of heavy metals in soil

Sequence	Speciation	Extractant
Fraction 1	water soluble plus exchangeable (SE)	8 mL of 1.0 mol $MgCl_2$/L at pH 7.0 for 1 h at 25°C
Fraction 2	bound to carbonate or weakly specifically adsorbed (WSA)	8 mL of 1.0 mol NaAc/L adjusted to pH 5.0 with acetic acid for 5.0 h
Fraction 3	bound to Fe-Mn oxides (OX)	20 mL of 0.04 mol/L $NH_2 \cdot HCl$ in 25% (v) acetic acid (pH 2.0) for 6.0 h at 96°C
Fraction 4	bound to organic matter (OM)	3 mL of 30% H_2O_2 and 0.02 HNO_3 (pH 2.0) for 2.0 h at 85 °C, followed by 3 mL 30% (v) H_2O_2 (pH 2.0) for 3.0 h at 85°C and then 5 mL of 3.2 mol/L NH_4Ac in 20% HNO_3 diluted to 20 mL at room temperature for 0.5 h
Fraction 5	residual (RES)	the above four fractions subtracted from the total metal content

leaf material (GBW07603, China), was used to verify the accuracy and precision of the digestion procedure and subsequent analysis.

Statistical analysis. All treatments were replicated three times in the experiments. The means and standard deviations (*SD*) were calculated by the Microsoft Office Excel 2003. One-way analysis of variance was carried out with SPSS10.0. When a significant ($P < 0.05$ or $P < 0.01$) difference was observed between treatments, multiple comparisons were made by the *LSD* test.

RESULTS AND DISCUSSION

Effect of EDTA and CA on heavy metal fractionation. The concentrations of Cd, Cu, Pb, and Zn in soils of SZIA were 3.07 ± 0.28, 45.51 ± 3.78, 54.66 ± 2.76, and 161.37 ± 6.19 mg/kg, respectively. Compared with Environmental quality standard for soils (GB 15618-1995), which are considerably higher than the natural background value of soils in China, showing about 15.35, 1.30, 1.56, and 1.61 time, respectively. Especially, Cd content was 3 times more than the China Environmental Quality Standard (Grade III) for Soils (GB 15618-1995). The chemical fractionation of heavy metals in soils is given in Figure 1. Due to relatively low pH, the most fractions of Cd were predominantly bound to SE (46.2%), followed by RES (35.0%), WSA (9.2%), OM (9.2%), and OX (7.3%), suggesting a high mobility of Cd in the soils and which may become available to plant, while the majority of Cu, Pb, and Zn were associated with residual fraction, which were 74.5%, 51.9%, 52.5%, respectively. After applying EDTA and CA, the exchangeable fractions of Cd, Cu, Pb, and Zn significantly enhanced compared with the control, the portion of

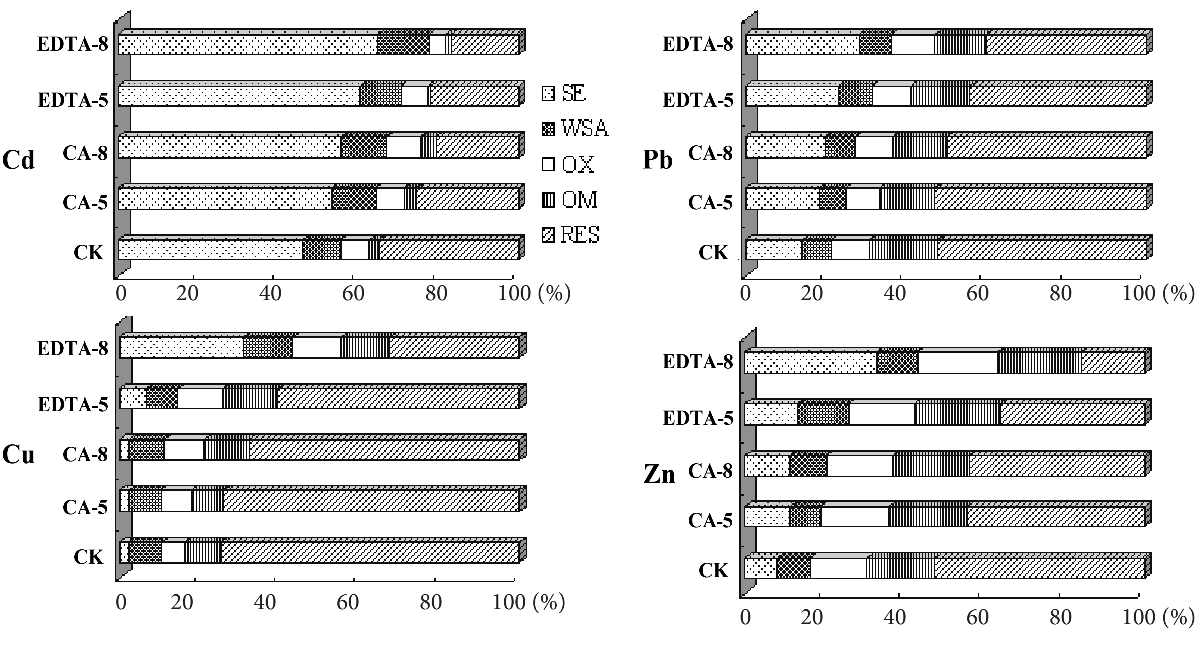

Figure 1. Heavy metal distribution into separate fractions in soils

SE increased from 0.15 to 0.40 times, from 0.07 to 13.45 times, from 0.29 to 1.0 times, and from 0.35 to 2.98 times, respectively. However, Cu, Pb, and Zn showed similar results where major portion of these metals were bound with non-mobile fractions (WSA + OM + OX + RES).

Numerous studies revealed that applying synthetic chelating agents have potential to remobilize metals and to form strong soluble complexes (Chen and Cutright 2001, Lai and Chen 2005, Neugschwandtner et al. 2008). Komárek et al. (2008) found that the addition of 9 mmol/kg EDTA resulted in 100, 188, 121, and 61-fold increase of water-soluble Cd, Cu, Pb and Zn concentrations, respectively, compared to the control. Cr had the greatest increase as its mobile fraction was raised by approximately 40-fold in HEDTA-treated soil and 60-fold in EDTA amended soil (Chen and Cutright 2001). The application of EDTA and CA can chelate and mobilize the heavy metals in soils; it is potential for decontamination or phytoremediation of metal polluted soils.

Effects of EDTA and CA on plant growth. Enhancing metal accumulation in existing high yielding plants without diminishing their yield is the most feasible strategy in the development of phytoremediation (Evangelou et al. 2007). The dry mass yields of *M. jalapa* are shown in Figure 2. It revealed that the dry biomass of roots, stems, leaves and shoots were as a whole in the order of EDTA-5 > CA-8 > CA5 > CK > EDTA-8. Obviously, the application of CA-5, CA-8 and EDTA-5 could enhance the plant growth, and all the parts of the plants obtained certain degree of increase; whereas there were no significant differences on the root, stem, leaves, shoots biomass between the treatments of CA (5 and 8 mmol/kg) and the control ($P > 0.05$). For the addition of EDTA, low concentration of 5 mmol/kg EDTA markedly promoted plant growth relative to other treatments (CK, CA-5, CA-8 and EDTA-5), the dry biomass of roots, stems, leaves and shoots caused 0.51, 2.11, 1.10, and 1.39-fold increase, respectively, compared with those in the corresponding control plants, reaching the maximum of 2.59, 1.14, 1.93, and 3.07 g/pot, respectively. However, when 8 mmol/kg EDTA was spiked to the soils, the plants exhibited visual symptoms of metal toxicity and appeared to be chlorotic and necrotic 2 days after the experiment was initiated. The growth of plants severely retarded and the biomass of all parts of the plant significantly decreased in comparison to other treatments (CK, CA-5, CA-8 and EDTA-8) ($P < 0.01$).

Although chemically enhanced phytoextraction was proposed as an effective approach for cleanup of heavy metal contaminated soils using plants, chelate-metal complexes can be toxic to plants by severely decreasing aerial part biomass (Kulli et al. 1999, Luo et al. 2005, Evangelou et al. 2006). Pot experiments showed that the treatments with 5 mmol/kg EDTA and EDDS significantly affected corn and bean growth, the plants showed strongly chlorotic and necrotic symptoms, and root growth was severely impaired 14 days after the application of chelates (Luo et al. 2005). According to Chen and Cutright (2001), the severe reduction in growth was attributed to the combination of heavy metal concentration and chelator (metal-chelator compounds), and synthetic chelator phytotoxicity had a significantly adverse effect on plant growth. In

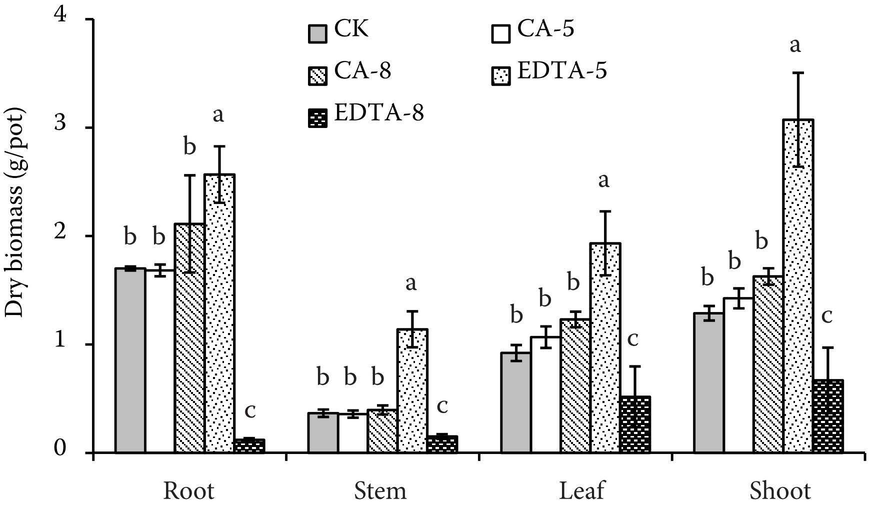

Figure 2. Dry biomass yields of *M. jalapa* following the addition of EDTA and CA. Same letters are not significantly different at $P = 0.05$ ($n = 3$) between the same tissue of different treatments according to the *LSD* test. The statistical analysis was a one-way ANOVA

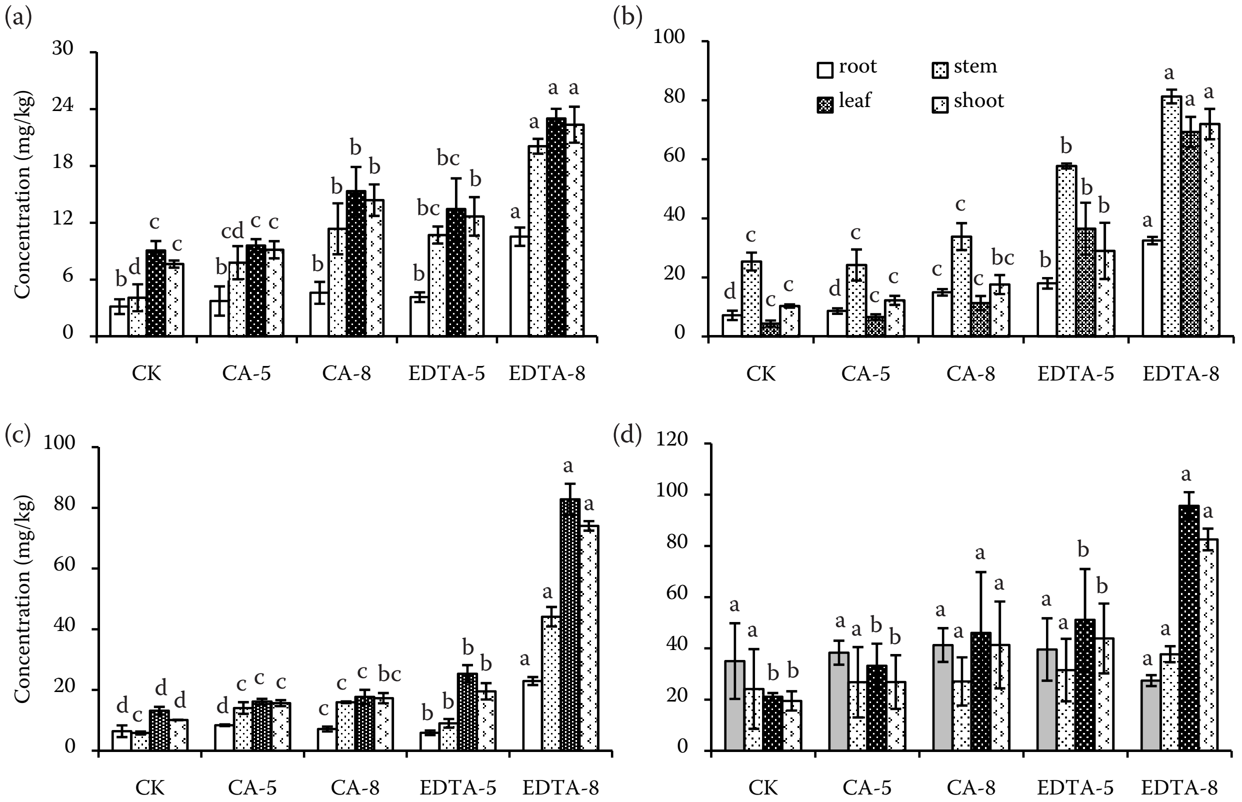

Figure 3. Influence of application of chelates on heavy metal uptake of Cd (a), Cu (b), Pb (c), and Zn (d) in *M. jalapa*. Same letters are not significantly different at $P = 0.05$ ($n = 3$) between the same tissue of different treatments according to the *LSD* test. The statistical analysis was a one-way ANOVA

comparison with these studies, *M. jalapa* had high tolerant capability to the toxic of metal-chelator complex and the concentration of 5 mg/kg EDTA was the optimal application mode.

Effects of EDTA and CA on the plant metal concentration. As depicted in Figure 3, when growing in unamended soils, the distribution of Cd, Cu, Pb and Zn in *M. jalapa* were in the sequence order of stem > shoot > leaf > root, stem > shoot > root > leaf, leaf > shoot > root > stem, and root > stem > leaf > shoot, respectively. Compared with the control group, EDTA and CA application to soils enhanced metal uptake and accumulation in all parts of the plants.

As shown in Figure 3a, the effectiveness of chelate-induced experiments on Cd absorption in the plants were EDTA-8 > CA-8 > EDTA-5 > CA-5 > CK. The treatment of EDTA-8 was the most effective at enhancing Cd concentration in the plants, the concentrations of Cd accumulated in all parts of *M. jalapa* were significantly increased relative to other treatments (CK, CA-8, EDTA-5 and EDTA-8) ($P < 0.05$), the Cd contents in roots, stems, leaves and shoots reached the maximum of 10.5, 20.1, 23.0, and 22.4 mg/kg, respectively.

The Cu concentrations in all parts of *M. jalapa* followed the order: EDTA-8 > EDTA-5 > CA-8 > CA-5 > CK (Figure 3b). Compared with the control, the Cu concentrations in aerial parts of the plants did not significantly increased at the treatments of CA-5 and CA-8, but the addition of EDTA (5 and 8 mmol/kg) resulted in marked increase in Cu contents than other treatments (CK, CA-5 and CA-8). The Cu concentrations in root, stems, leaves and shoots were 2.51, 2.27, 8.30, and 2.81-fold, respectively, under the EDTA of 5 mmol/kg higher than those of the control group, and for EDTA-8, which reached 4.53, 3.20, 15.73, and 6.96-fold, respectively.

Pb concentrations in all parts of *M. jalapa* are presented in Figure 3c. The addition of EDTA and CA could increase Pb concentration in aerial parts of the plants, whereas, under different CA treatments induced experiments, only the shoot Pb content at CA-8 significantly enhanced in comparison with the control. For EDTA treatments (5 and 8 mg/kg), the concentrations of Pb in roots, stems, leaves and shoots significantly increased compared with the treatments of CK, CA-5 and CA-8 ($P < 0.05$). Furthermore, higher concentration of EDTA (8 mmol/kg) was more effective in increasing Pb concentration than EDTA-5; root, stem, leaf and shoot concentrations of Pb were significantly higher than those the treatments of EDTA-5.

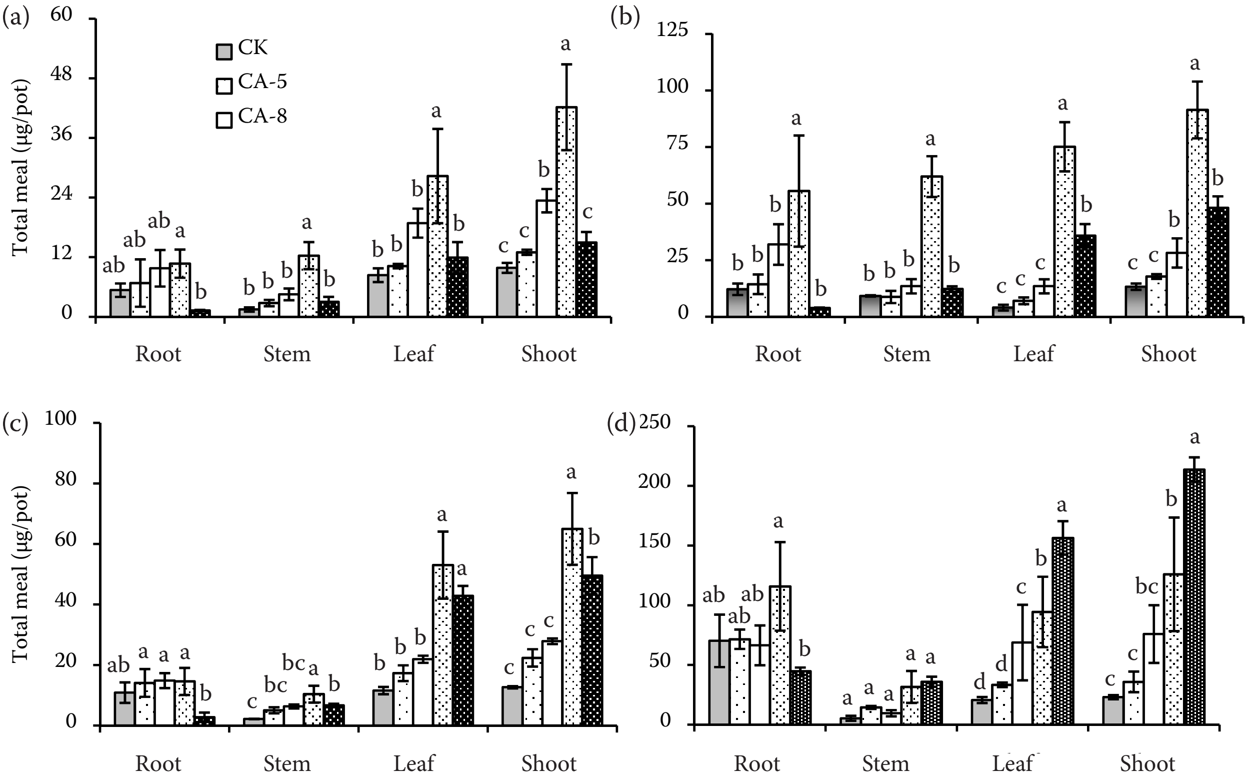

Figure 4. Effects of EDTA and CA on the total metal accumulation in *M. jalapa*, (a) Cd, (b) Cu, (c) Pb, and (d) Zn. Same letters are not significantly different at $P = 0.05$ ($n = 3$) between the same tissue of different treatments according to the *LSD* test. The statistical analysis was a one-way ANOVA

Zn concentrations in *M. jalapa* after application of chemical agents are summarized in Figure 3d. Although higher concentrations of Zn in the plants were observed under the chelate-induced experiments, there were not significantly different at the Zn contents in all parts of the plants among the treatments of CK, CA-5, CA-8 and EDTA-5. The Zn concentrations in leaves and shoots after application of EDTA-8 were similar to Cd, Cu and Pb; the addition of EDTA-8 could observably increased Zn accumulation in leaves and shoots compared with other treatments (CK, CA-5, CA-8 and EDTA-5), and especially the leaf concentration of Zn resulted in 4.54, 2.88, 2.08, and 1.87-fold higher than that CK, CA-5, CA-8 and EDTA-5, respectively.

Effects of EDTA and CA amendments on heavy metal accumulation. Total metal phytoextraction by *M. jalapa* is shown in Figure 4. When no chelates were added to the soil, the metal accumulation in the plants were shoot > leaf > root > stem for Cd, shoot > root > stem > leaf for Cu, shoot > leaf > root > stem for Pb, and root > shoot > leaf > stem for Zn. The effectiveness at increasing Cd, Cu and Pb accumulation in shoot of the plants were EDTA-5 > CA-8 > EDTA-8 > CA-5 > CK, EDTA-5 > EDTA-8 > CA-8 > CA-5 > CK, EDTA-5 > EDTA-8 > CA-8 > CA-5 > CK, and EDTA-8 > EDTA-5 > CA-8 > CA-5 > CK. Among the treatments of EDTA (5 and 8 mmol/kg) and CA (5 and 8 mmol/kg), the total metal accumulated in aerial parts of plants obtained a different grade of increase relative to the control. However, under the concentrations of 5 and 8 mg/kg, the metal enhancement of Cd, Cu, Pb and Zn in most parts of plants was not significant compared with the control. After the application of 5 mg/kg EDTA, the stem, leaf and shoot metal accumulation were 8.33, 3.38, and 3.28-fold, respectively, for Cd, 6.71, 18.34, and 6.86-fold, respectively, for Cu, and 4.73, 4.58, and 5.12, respectively, for Pb higher than those in control plants. However, the increase of metal translocation into the plants after EDTA-8 application was not high enough to compensate for the drastic reduction in biomass compared to EDTA-5, the total Cd, Cu and Pb accumulation in all parts of plants significantly decreased. For Zn accumulation, EDTA-8 was most efficient in increasing Zn accumulation in aboveground parts of *M. jalapa*, the total Zn accumulation in leaves and shoots were significantly higher than those in other treatments (CK, CA-5, CA-8 and EDTA-5), increasing by 658.0%, 368.5%, 127.5%, and 65.7% for leaves, respectively, and by 829.0%, 496.2%, 181.6%, and 69.7% for shoots, respectively.

Table 2. The bioaccumulation factor (BF), transfer factor (TF) and remediation factor (RF) (%) of heavy metals in *M. jalapa*

Treatment (mg/kg)	Cd			Cu			Pb			Zn		
	BF	TF	RF	BF	TF	RF	BF	TF	RF	BF	TF	RF
CK	2.71	2.43	0.14	1.08	1.44	0.01	0.19	1.58	0.01	0.12	0.56	0.01
CA-5	2.78	2.45	0.16	1.18	1.69	0.01	0.28	1.87	0.02	0.16	0.70	0.01
CA-8	4.47	3.12	0.29	2.16	1.18	0.02	0.32	2.43	0.02	0.25	1.0	0.02
EDTA-5	4.49	3.06	0.60	2.71	1.61	0.08	0.37	3.32	0.05	0.27	1.11	0.03
EDTA-8	7.09	2.12	0.19	5.20	2.21	0.04	1.33	3.23	0.04	0.55	3.01	0.06

Phytoextraction efficiency. The phytoextraction efficiency of plants relies on the concentration of heavy metals accumulated in the dry biomass of shoots and the biomass yield of the plants (Neugschwandtner et al. 2008, Komárek et al. 2008, Sun et al. 2009). The remediation factor (RF) is defined as the ratio of an element accumulation in shoots to that in soil, which is calculated as follows:

$$\text{RF (\%)} = \frac{M_{\text{shoot}} \times W_{\text{shoot}}}{M_{\text{soil}} \times W_{\text{soil}}} \times 100\%$$

Where: M_{shoot} is the metal concentration in shoots of the plants (mg/kg); W_{shoot} is the plant dry aboveground biomass (gRF (%) is the metal content in soil (mg/kg) and W_{soil} is the amount of soil in the pot (g).

The RF reflects the amount of metal extracted by plants from the soil, therefore it indicates phytoextraction efficiency under chelate-induced experiments. The calculated RFs for different amendments are given in Table 2, which suggests that the application of chemical agents enhanced phytoextraction of heavy metals from soil. Higher Cd RFs were obtained from the treatments of CA-5, CA-8, EDTA-5 and EDTA-8, which were 0.16%, 0.29%, 0.60%, and 0.19%, respectively. And even the control variant gave a relatively high Cd RF value (0.14%) as well. The RFs of Cd, Cu, Pb, and Zn at the treatments of EDTA were higher than those at the addition of CA, showing EDTA was more efficient than CA at increasing these metals absorption by plants. The treatment of EDTA-5 obtained the highest RF values of Cd, Cu, and Pb, while the application of EDTA-8 was the most effective at translating Zn from soil to plants.

The bioaccumulation factor (BF = $C_{\text{shoots}}/C_{\text{roots}}$; where C_{shoots} is metal concentration in shoots and C_{roots} is metal concentration in roots) and transfer factor (TF = $C_{\text{shoots}}/C_{\text{soil}}$; where C_{shoots} is metal concentration in shoots and C_{soil} is metal concentration in soil) are usually calculated to evaluate plants for phytoextraction purpose in the effectiveness of the harvestable aerial parts of plants in metal accumulation and translocation (Zhou and Song 2004, Sun et al. 2008, Sun et al. 2009). Table 1 shows the BF and TF values in the plants, applying CA (5 and 8 mmol/kg) and EDTA (5 and 8 mmol/kg) apparently enhance Cd, Cu, Pb and Zn uptake and translocation, the BFs and TFs increased after the application of CA and EDTA, the effectiveness of metal absorption by plants was Cd > Cu > Pb > Zn and EDTA-8 > EDTA-5 > CA-8 > CA-5. Especially for Cd and Cu, the BF and TF values in all treatments were higher than 1.0, the extent of metal translocation to the shoots is an important factor for the choice of a phytoextraction strategy (Marques et al. 2007), so it indicated *M. jalapa* had high capability of Cd and Cu uptake and accumulation. Therefore, chemical induction of decontamination of heavy metal polluted soil using *M. jalapa* would be potential and feasible.

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