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The effectiveness and risk comparison of EDTA with EGTA in enhancing Cd phytoextraction by *Mirabilis jalapa* L.

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Abstract In the previous study, *Mirabilis jalapa* L. had revealed the basic Cd hyperaccumulator characteristics, but the accumulation ability was not as strong as that of other known Cd hyperaccumulators. In order to improve the accumulation ability of this ornamental plant, the chelants were used to activate the Cd in soil. As a substitute, ethylene glycol bis(2-aminoethyl) tetraacetic acid (EGTA) was selected to testify whether it has better effectiveness and can bring lesser metal leaching risk than EDTA. The data showed that the growth of *M. jalapa* was inhibited, while the Cd concentration of the plant was significantly increased under the treatments containing EDTA or EGTA. The Cd translocation ability under the EGTA treatments was higher than that under the EDTA treatments. The available Cd resulted from the application of chelant EGTA to the contaminated soils can be limited to the top 5 cm, while the application of chelant EDTA to the contaminated soils can be limited to the top 10 cm. In a word, EGTA showed better effectiveness than EDTA in enhancing Cd phytoextraction of *M. jalapa*. As an ornamental plant, *M. jalapa* has the potential to be used for phytoextraction of Cd-contaminated soils and it can beautify the environment at the same time.

Keywords Cd · *Mirabilis jalapa* L · Phytoremediation · EDTA · EGTA

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Introduction

Excessive metal concentration in soils poses significant hazard to the environment, including plant, animal, and even human health. For example, the Cd concentration in some seriously contaminated sediments has exceeded 2,000 mg kg^{-1} (Chen et al. 2003). Although a number of techniques have been developed to remove metals from contaminated soils, many sites remain contaminated because economic and environmental costs to clean up those sites with the available technologies are too high (Gunawardana et al. 2010). Phytoremediation has received considerable attention in recent years; it uses green plants to remove pollutants from the environment or to render them harmless (Weiss et al. 2006; Surat et al. 2008). This technique includes phytoextraction which uses plants to remove metals from the soil and concentrate them in the harvestable parts of the plants (Alford et al. 2010).

Up to now, there was no too much information about ornamental plant that has a potential to be used for phytoremediation technology. Once ornamental plants are used for contaminated soil remediation, they may be harvested and then incinerated together to recover the residual heavy metals. Alternatively, they may be sold to households for their ornamental values and then dispersedly discarded after death; thus, the heavy metal contamination in soils will be diffused gradually. Moreover, the species number of ornamental plants is very huge. Thus, it should be a feasible choice to screen out ornamental plants used for heavy metal-contaminated soil remediation (Liu et al. 2006). Especially, if the used ornamental plants can effectively transfer heavy metals from the roots to the aboveground parts which are easy to be harvested, they will be considered more preferentially than other kinds of plants.

Mirabilis jalapa L. is native to the torrid zone of South America. As an annual ornamental plant, it has been commonly cultivated in China as an ornamental plant. The plant is easy to be cultivated, and it grows fast. It can grow as tall as 1 m and has various colors. In the previous experiment (Zhou and Liu 2006), *M. jalapa* had revealed favorable characteristics for Cd hyperaccumulation, viz. (1) showed strong tolerance under high concentration of Cd treatments, (2) the Cd concentration in the shoot was higher than that in the root and also was higher than that in the applied soils, and (3) the shoot Cd concentration was higher than 100 mg kg^{-1} (the critical concentration for Cd hyperaccumulator). However, the Cd accumulation ability of this ornamental plant was not as strong as that of some popularly accepted Cd hyperaccumulators (Leitenmaier and Kupper 2011). Thus, it is necessary to take appropriate measures to increase the phytoremediation efficiency.

In fact, the extraction of heavy metals by plants is usually limited by the availability of heavy metals in soils (Stanhope et al. 2000). Methods of increasing the availability of heavy metals in soils and their translocation to the plant shoots are vital to facilitate phytoextraction. The application of chemically enhanced technology which can facilitate the uptake of heavy metals and their translocation to the aboveground parts has shown visible effectiveness (Blaylock et al. 1997; Zhou et al. 2010; Yan and Lo 2012). Thereinto, what was very important was that the ecological risk brought by the leaching through metal-polluted soils should be fully considered. EDTA is a popular reagent for soil remediation applications, but it is also well known that EDTA can bring potential metal leaching risk. In this work, ethylene glycol tetraacetic acid (EGTA) was selected as a substitute to testify whether it has better effectiveness and can bring lesser metal leaching risk than EDTA. Also, sodium dodecyl sulfate (SDS) as an important anionic surfactant was also used together with the synthetic chelators (EDTA, EGTA) to investigate the single and combined effects of them on Cd uptake by this ornamental plant. Especially, the Cd leaching risk through the contaminated soils under the different treatments was also assessed.

Materials and methods

Pot culture experiment

The tested soil in this station is meadow burozem which is not contaminated by heavy metals. Basic physical and chemical properties of the collected soils were analyzed according to the routine analytical methods described by Lu (2004). Data showed that pH, organic matter, total *N*, available *P*, and available *K* in the tested soil was 6.59, 1.19 %, 0.85 g kg^{-1}, 8.96 mg kg^{-1}, and 95.21 mg kg^{-1} respectively. And, the clay, sand, and silt of the tested soil were 22.7 %, 31.9 %, and 45.4 % respectively. In April 2009, surface soil samples (0–20 cm) were collected from the Shenyang Station of Experimental Ecology and then were ground to pass through a 4.00-mm mesh. Soil samples (2.50 kg) were placed in each plastic pot (diameter=20 cm, height=15 cm). According to our previous study and the National Soil Environmental Quality Standard of China (NSEQSC GB 15618, 1995) (Xia 1996), the single Cd pollution treatments and the chemically enhanced treatments including EDTA, EGTA, and SDS were designed as shown in Table 1. The measured soil Cd concentration was 25 mg kg^{-1} in the enhanced treatments. Cd was spiked as $CdCl_2 \cdot 2.5\ H_2O$ in the whole study. Soils were mixed thoroughly with $CdCl_2 \cdot 2.5\ H_2O$ and then were equilibrated for 1 month. After that, uniformly grown 1-month-old seedlings of *M. jalapa* were transplanted into the pots. There were three seedlings in each pot, and all treatments were replicated thrice to minimize experimental errors. As for the enhanced treatments, the dissolved EDTA, EGTA, and SDS were applied to soils 1 month before the plants were harvested. Furthermore, the treatment without Cd, chelators, and surfactant was designed as control (CK). Altogether, there were 39 treatments or pots used in the experiment.

The experiment was carried out in a greenhouse illuminated with a natural sunlight cycle about 16-h day and 8-h night, and there was no contamination in the surrounding areas. The temperature was maintained at 15–28°C during the growth period. Loss of water by evaporation from the pots was compensated by daily addition of tap water (no Cd detected) to sustain 75–

Table 1 Components and concentrations of single Cd pollution and chemically enhanced treatments

Treatment		Cd (mg kg^{-1})	SDS (mmol kg^{-1})	EDTA (mmol kg^{-1})	EGTA (mmol kg^{-1})
Cd+SDS	CK				
	Cd	25			
	Cd+SDS0.5	25	0.5		
	Cd+SDS1.0	25	1.0		
	Cd+SDS2.0	25	2.0		
Cd+EDTA(+SDS)	Cd+EDTA	25		1.0	
	Cd+EDTA+SDS0.5	25	0.5	1.0	
	Cd+EDTA+SDS1.0	25	1.0	1.0	
	Cd+EDTA+SDS2.0	25	2.0	1.0	
Cd+EGTA(+SDS)	Cd+EGTA	25			1.0
	Cd+EGTA+SDS0.5	25	0.5		1.0
	Cd+EGTA+SDS1.0	25	1.0		1.0
	Cd+EGTA+SDS2.0	25	2.0		1.0

85 % of soil water-holding capacity. The plants were harvested after they had grown in the contaminated soils for 5 months.

Metal determination and data processing

Harvested plant samples were divided into roots, stems, leaves, and inflorescences. The samples were carefully rinsed with tap water then with deionized water. They were dried at 105°C for 20 min then at 70°C in an oven to constant weight. The dried plant samples were ground to powder after their dry weight was weighed. Soil samples from the pot culture experiment were also air-dried and ground using a mortar and pestle and then sieved through a 0.149-mm sieve. The plant and soil samples were digested in a solution containing 3:1 HNO_3/$HClO_4$ solution (Wei and Zhou 2004). The top four soil samples were collected at every 5 cm along 0–20 cm depth; the available soil Cd in every layer was extracted by 1.0 mol L^{-1} CH_3COONH_4 at a soil/solution ratio of 1:10. The concentrations of heavy metals were determined using an atomic absorption spectrophotometer (AAS, Hitachi 180-80 type). The certified reference materials (bought from an authoritative company in Shijiazhuang), citrus leaf material and soil standard sample, were used for quality assurance purposes. Reagent blanks and internal standards were used to ensure accuracy and precision in Cd analysis. Data were statistically processed with the Excel XP, SPSS 13.0. The values were expressed as mean±standard deviation of the three replicates. Data were analyzed by one-way ANOVAs with the Duncan's multiple range tests to separate means.

Results and discussion

Effects of chemical amendments on the growth of *M. jalapa*

During the whole growth period, *M. jalapa* grew under the EGTA treatments showed stronger tolerance than those under the EDTA treatments. In Fig. 1, it was obviously found that the plants were higher and more flourishing under the single EGTA-enhanced treatments (Fig. 1d) than those under the single EDTA-enhanced treatments (Fig. 1c). Also, the same phenomenon was found under the combined EGTA (Fig. 1f) or EDTA (Fig. 1e) and SDS treatments. Compared with CK, the plant biomass decreased significantly ($p<0.05$) under the treatments containing EDTA; this result was similar with what other reports had showed (Rékási and Filep 2012). In fact, most of the reports about EDTA as a soil amendment have showed that EDTA could be phytotoxic to plants. For example, do Nascimento et al. (2006) indicated that the reduction in plant growth by EDTA treatment was probably due to the toxicity of EDTA itself and EDTA–metal complexes. For the single EGTA-enhanced treatment in the study, the shoot and root dry biomass of the plant was increased significantly by 28.5 % and 50.2 % compared with CK ($p<0.05$) (Table 2). The plant biomass

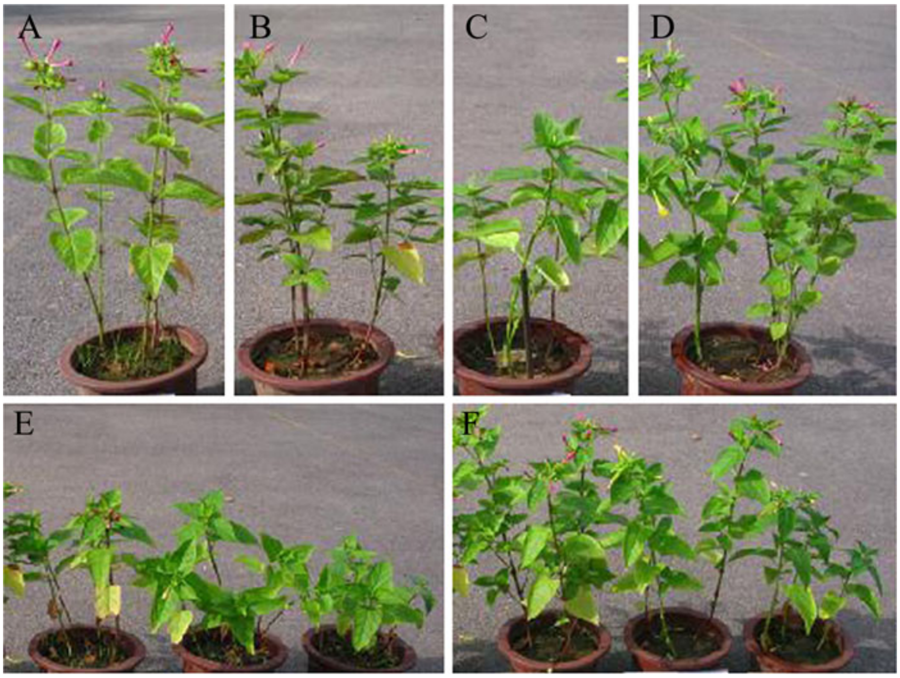

Fig. 1 Effect of EDTA and EGTA on *Mirabilis jalapa* L. growth. **a** CK. **b** Cd. **c** Cd+EDTA. **d** Cd+EGTA. **e** Cd+EDTA+SDS0.5/1.0/2.0(*left*/*middle*/*right*). **f** Cd+EGTA+SDS0.5/1.0/2.0(*left*/*middle*/*right*)

was lesser under the combined surfactant and chelator-enhanced treatments than those under the single chelator enhanced treatments, especially for the treatments containing two higher concentrations of SDS ($p<0.05$). This maybe because the combined application of chemical amendments had taken more serious toxicity to the plants than the single chemical amendment. For the combined SDS and chelator-enhanced treatments, the 0.5-mmol kg$-^{1}$ SDS treatments showed better effectiveness than the other two SDS treatments in the study, while Chen et al. (2004) showed that 1.0-mmol kg$-^{1}$ SDS treatments was the right concentration when SDS was used together with EDTA or EGTA.

Table 2 Dry biomass of the plant under the different treatments

Treatment		Shoot biomass (g pot^{-1})	Root biomass (g pot^{-1})
Cd+SDS	CK	6.64±0.76d	3.98±0.32b
	Cd	7.73±0.75b	4.11±0.51b
	Cd+SDS0.5	7.20±1.28c	4.00±0.39b
	Cd+SDS1.0	5.42±0.69e	3.54±0.31bc
	Cd+SDS2.0	7.32±0.99bc	4.09±0.55b
Cd+EDTA(+SDS)	Cd+EDTA	4.96±0.68ef	3.19±0.36c
	Cd+EDTA+SDS0.5	4.22±0.50fg	3.08±0.25c
	Cd+EDTA+SDS1.0	2.83±0.40h	1.32±0.15e
	Cd+EDTA+SDS2.0	4.06±0.55g	2.88±0.28d
Cd+EGTA(+SDS)	Cd+EGTA	8.53±0.95a	5.98±0.54a
	Cd+EGTA+SDS0.5	6.66±0.81d	3.95±0.41b
	Cd+EGTA+SDS1.0	4.61±0.57f	3.09±0.35c
	Cd+EGTA+SDS2.0	3.22±0.35h	2.32±0.30de

The values in the same column followed by the same letters are not significantly different, whereas by the different letters are significantly different at $p<0.05$

Effects of SDS, EDTA, and EGTA on the Cd accumulation of *M. jalapa*

For the shoot Cd concentration, the Cd concentration increased significantly under the treatments containing EDTA or EGTA ($p<0.05$) and most of them had exceeded 100 mg kg^{-1} (the critical concentration of a Cd hyperaccumulator) (Table 3). This phenomenon showed that the application of the dissolved chelators to Cd-contaminated soils could obviously increase the shoot Cd concentration of *M. jalapa*. However, this phenomenon was not accordant with that under the single SDS-enhanced treatment. For the treatments containing EDTA and EGTA, the maximal shoot Cd concentration was 200.89 (Cd+EDTA+SDS2.0) and 253.87 mg kg^{-1} (Cd+EGTA+SDS2.0), respectively, while the shoot Cd concentration under the single Cd pollution treatments (without chemical amendments) was 47.91 mg kg^{-1}. For the treatments containing EDTA or EGTA, the stress induced to the plants appears to disrupt cell membranes and increase the flow of the soluble pool through passive transport (Van Engelen et al. 2007). Although the enhanced shoot Cd concentration was observed, the difference was not as obvious as that of what some other reports had showed. The maximal shoot Cd concentration was 5.3 times as much as that under the single Cd pollution treatments (without chemical amendment) in the study, while some reports showed that the heavy metal concentration of the plant could be increased to 10 or more than ten times by addition of amendments to soils (Mihucz et al. 2012). The efficiency of the enhanced treatment is different from the type and concentration of heavy metal and chelator, the plant species, and other environmental factors. Smolińska and Król (2012) showed that the mobility of mercury in contaminated soil was dependent on both mercury and chelator concentration; also, there were many factors that could influence the mobility of mercury in natural soil environment. The mobility of heavy metal usually is dependent on the physical and chemical properties of soil (soil water, sorption, redox conditions, mechanical, and chemical properties of soil), as well as the concentration of pollution and atmospheric precipitations. Up to now, there was very little data available regarding the effects of the chemical amendments on ornamental plants. It could also be found in Table 3 that the root Cd concentration was obviously lesser than that in the shoot for *M. jalapa*. In addition, there was no obvious rule about the SDS concentration on the root Cd accumulation of the plant.

Effects of SDS, EDTA, and EGTA on the Cd translocation and enrichment ability of *M. jalapa*

An important characteristic of hyperaccumulators is that the heavy metal concentration in shoots should be higher than that in roots, which means the plant has the strong ability of transferring heavy metals from roots to shoots. The concentration ratio of shoots to roots is designed as translocation factor (TF) (Dahmani-Muller et al. 2000). Another important characteristic of hyperaccumulators is

Table 3 Cd concentration of the plant under the different treatments (mg kg^{-1})

Treatment		Shoot Cd concentration	Root Cd concentration
Cd+SDS	CK	1.34±0.20h	0.96±0.11g
	Cd	47.97±6.89f	13.09±1.91de
	Cd+SDS0.5	42.88±5.61fg	16.99±2.03bc
	Cd+SDS1.0	36.90±3.56g	15.68±2.11c
	Cd+SDS2.0	42.75±4.56fg	17.93±2.19b
Cd+EDTA(+SDS)	Cd+EDTA	99.90±10.11d	11.84±1.51e
	Cd+EDTA+SDS0.5	124.12±13.09c	13.72±1.40de
	Cd+EDTA+SDS1.0	240.76±22.78a	23.44±2.50a
	Cd+EDTA+SDS2.0	200.89±19.05b	21.67±2.24a
Cd+EGTA(+SDS)	Cd+EGTA	84.55±9.08e	7.72±0.91f
	Cd+EGTA+SDS0.5	116.50±12.00c	16.88±1.91bc
	Cd+EGTA+SDS1.0	202.53±21.01b	12.29±1.30e
	Cd+EGTA+SDS2.0	253.87±26.09a	16.13±1.71bc

that the heavy metal concentration in shoots should be higher than that in soils; the concentration ratio of shoots to soils is designed as enrichment factor (EF) (Wei et al. 2005). If the TF value of Cd is lesser than 1, it maybe because the internal Cd translocation from the roots towards the shoots is restricted. In fact, the Cd translocation ability relates with not only the Cd mobilization in roots but also with the cell compartments of Cd in shoots (Verkleij et al. 2009). For most of the plants that could accumulate high concentrations of heavy metal, the heavy metal was mainly stored in their roots. However, in our study, Cd was effectively transported to the shoots of *M. jalapa* as an ornamental. According to Fig. 2, the TF values were higher than 1.0 for all the treatments, which undoubtedly showed the strong Cd translocation ability of *M. jalapa*; this was an important characteristic for the phytoextraction plant. As an ornamental plant, the Cd translocation from the shoots to the roots was an especially uncommon ability. There was little report that the ornamental plant could translocate Cd from soils as effectively as *M. jalapa*. As ornamental plants, *Calendula Officinals* L. and *Althaea rosea* Cav. had strong Cd tolerance and could accumulate high concentration of Cd; however, the Cd concentration in shoots was lower than that in roots (Liu et al. 2009, 2010). Wang et al. (2012b) showed that *Chlorophytum comosum* had tremendous application value in the treatment of Cd-contaminated soils for the advantage of high tolerance, high accumulation, and high ornamental value; however, the Cd translocation factor of this ornamental plant was less than 1 under each Cd treatment. The TF values of *M. jalapa* ranged from 2 to 16; it increased significantly ($p<0.05$) under the treatments containing EDTA or EGTA compared with the single Cd pollution treatments (without chemical amendments), i.e. the accumulated Cd was effectively translocated from the roots to the stems and the leaves. As for the enrichment ability of *M. jalapa*,

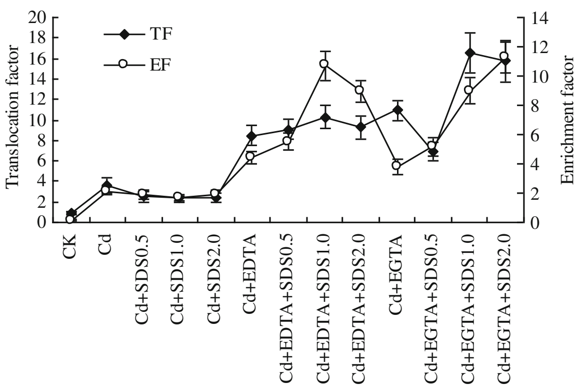

Fig. 2 Cd translocation factor and enrichment factor of *Miribilis jalapa* L. under the different treatments. Data are means ±standard deviation (indicated with *error bars*, n=3)

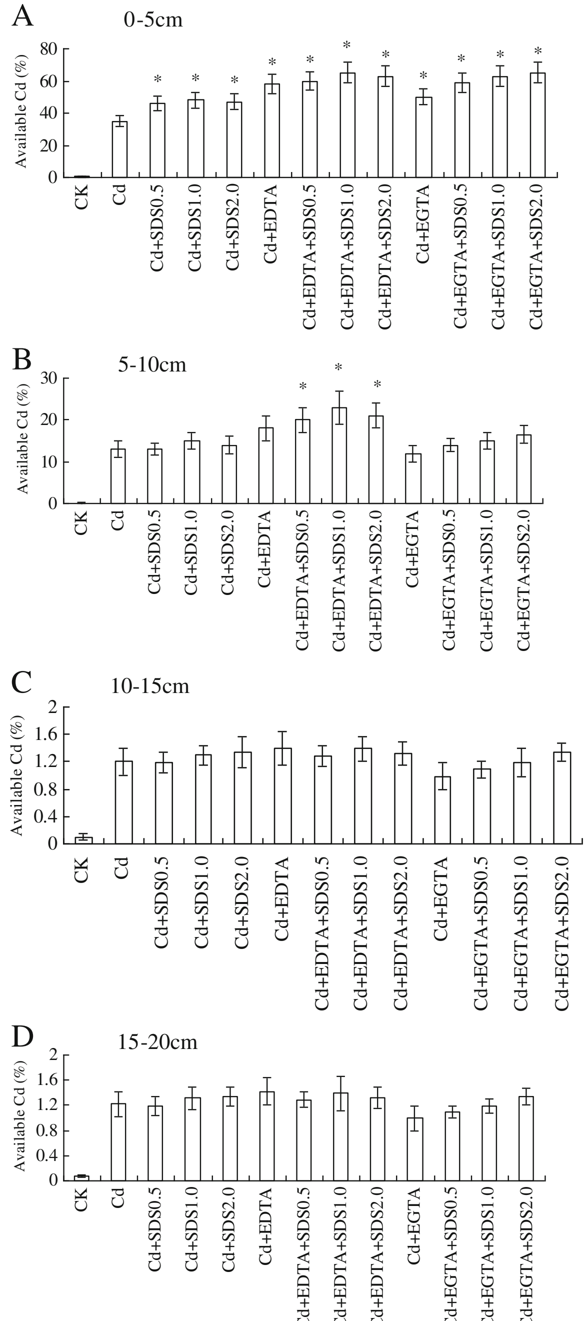

Fig. 3 Available Cd concentration in the different soil layers. Data are means±standard deviation (indicated with *error bars*, n=3). Data were analyzed by one-way ANOVAs with the Duncan's multiple range tests (*$p<0.05$)

the EF values under all the treatments were higher than 1.0 and increased significantly ($p<0.05$) under the treatments containing EDTA or EGTA. The maximal TF and EF values were found under the Cd+EGTA+SDS2.0 treatment, and they were 4.4 and 5.3 times as much as that under the single Cd pollution treatments (without chemical amendments), respectively. In the study, the application of EGTA showed better effectiveness than EDTA in enhancing Cd translocation as the TF value under the single EGTA-enhanced treatment was obviously higher than that under the single EDTA-enhanced treatment ($p<0.05$).

Effects of SDS, EDTA, and EGTA on the available Cd in the different soil layers

The use of chemical agents as soil amendments can bring potential dangers to the environment, and it is necessary to evaluate the risk before the practical application (Jean-Soro et al. 2012). It is extremely difficult to predict whether or not chemically induced phytoextraction will be a good option. The available Cd concentration in soil could provide basic data for evaluating the risk. In Fig. 3a, the increase of the available Cd concentration was significantly obvious under all the enhanced treatments compared with that under the single Cd pollution treatments (without chemical amendments) ($p<0.05$). The results indicated that the application of the dissolved chelators and/or surfactant could significantly increase the availability of Cd in the top 5 cm soil layer. It was reported by Zheng et al. (2009) that the content of water soluble and exchangeable Cd was increased by 19.3 % similar to 22.4 % induced by 6 mmol kg^{-1} EDTA and EGTA, and the bioavailability of Cd was improved although they did not show the layered available Cd in soil. In Fig. 3b, for the available Cd concentration in the 5–10 cm soil layer, the available Cd concentration was only increased significantly under the combined EDTA and SDS-enhanced treatments ($p<0.05$), and there was no obvious difference under the treatments containing EGTA compared with that under the single Cd pollution treatments. In this way, the application of EGTA was safer than EDTA when they were used in soils to enhance Cd uptake by *M. jalapa* because the Cd mobility in soils under the EGTA-enhanced treatments was not as high as that under the EDTA treatments. In Fig. 3c, d, the available Cd concentration decreased and there was no obvious difference compared with that under the single Cd pollution treatments (without chemical amendments). That was to say, the chemical amendments did not affect the soil Cd mobility in 5–10 cm layer in the study. Wang et al. (2012a) showed that the solubilized metals resulted from the application of chelant EDDS to the contaminated soils can be limited to the top 25 cm, while in the study, the application of chelant EGTA to contaminated soils can be limited to the top 5 cm. In addition, the soil Cd mobility and plant Cd uptake were not positively correlated in the study. For example, the available Cd concentration under the combined EDTA- and SDS-enhanced treatments (Cd+EDTA+SDS2.0) was higher than that under the combined EGTA- and SDS-enhanced treatments (Cd+EGTA+SDS2.0), while the reverse was found for the Cd concentration in the shoots.

Efficiency of the enhanced treatments on the Cd phytoextraction of *M. jalapa*

The final purpose of phytoremediation is to acquire as much heavy metals as the remediation plants can and so

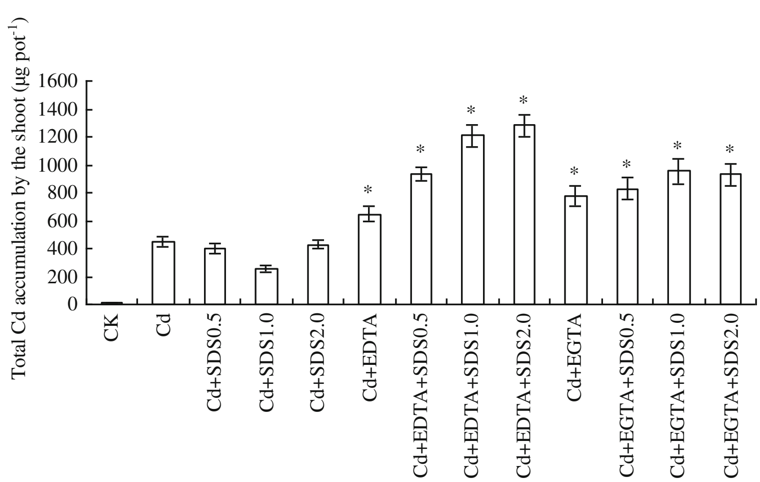

Fig. 4 Total Cd accumulation by the shoot under the different treatments. Data are means±standard deviation (indicated with *error bars*, $n=3$). Data were analyzed by one-way ANOVAs with the Duncan's multiple range tests (*$p<0.05$)

to alleviate the degree of contamination. The aboveground part, which is easier to be harvested is especially an important tissue to accumulate heavy metals. In this study, the total Cd accumulation (the product of the Cd concentration and the dry biomass of the plant) in the different parts of the shoots was evaluated (Fig. 4), and it increased significantly under the treatments containing EDTA or EGTA ($p<0.05$) compared with the single Cd pollution treatments. For the treatments containing EDTA and EGTA, the maximal total Cd accumulation in shoots was observed under Cd+EDTA+SDS2.0 (1,283 μg pot^{-1}) and Cd+EGTA+SDS1.0 (953 μg pot^{-1}) treatments, which were 2.88 and 2.14 times as much as that under the single Cd pollution treatments, respectively. The single SDS-enhanced treatments could not enhance the shoot Cd extraction in the study. There are few reports about the comparison of EDTA with EGTA in enhancing Cd phytoextraction of the plants. A report showed that 4.0 mmol kg^{-1} EGTA could maximize Cd absorption by carrot and 6.0 mmol kg^{-1} EDTA by potherb mustard, which were 1.96- and 2.9-fold of the controls, respectively (Zheng et al. 2009). That was to say, the enhanced phytoextraction efficiency differed from the species and the dose of the applied chelators. In addition, the total Cd accumulation under the single EGTA treatment was higher than that under the single EDTA treatment ($p<0.05$); therefore, as a chelator, EGTA was more effective than EDTA in enhancing Cd contaminated soils for *M. jalapa*. *Mirabilis jalapa* L. has always played an important role in urban construction and family life. In particular, with a gradual increase in spending by consumer, the commercial value of the ornamental plant is increasing. However, the Cd accumulated in shoots of *M. jalapa* was not very sufficient for an effective phytoremediation within an acceptable period. In the future, measures to improve plant Cd concentration should be the major tasks. Based on the data derived from the study about Cd phytoextraction, it could be anticipated that *M. jalapa* has the potential to be used for remediating polluted soils distributed in crowded urban or mining areas.

Conclusions

Firstly, *M. jalapa* showed stronger Cd tolerance under the EGTA-enhanced treatments than that under the EDTA-enhanced treatments. Secondly, the Cd translocation factors of *M. jalapa* under the most of the EGTA-enhanced treatments were higher than those under the EDTA-enhanced treatments. Thirdly, the application of EGTA in soil was safer than EDTA to enhance Cd uptake for *M. jalapa* in the study because the available Cd resulted from the application of chelant EGTA to the contaminated soils can be limited to the top 5 cm, while the application of chelant EDTA to the contaminated soils can be limited to the top 10 cm. Fourthly, the total shoot Cd under the single EGTA treatment was higher than that under the single EDTA treatment. In a word, EGTA was more effective than EDTA in enhancing Cd uptake for *M. jalapa*. However, further studies such as molecular ecology measures may be required to fully evaluate the residual effects of chelators applied in the contaminated soils. In light of our results, as an ornamental plant, *M. jalapa* is a useful material for phytoremediation of Cd-contaminated soils.

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