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Cadmium tolerance and accumulation of *Althaea rosea* Cav. and its potential as a hyperaccumulator under chemical enhancement

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Abstract The role of ornamental plants has drawn much attention as the urban pollution levels exacerbate. *Althaea rosea* Cav. had showed its strong tolerance and accumulation ability of Cd in our previous work, thus, the effects of ethylenediamine triacetic acid (EDTA), ethylenegluatarotriacetic acid (EGTA) and sodium dodecyl sulfate (SDS) on its Cd phytoremediation capacity were further investigated in this work. It reconfirmed that the species had strong tolerance and accumulation ability of Cd. Particularly, the species can be regarded as a potential Cd-hyperaccumulator through applying chemical agents. However, different chelators and surfactants had great differences in affecting hyperaccumulating characteristics of the species. EGTA and SDS could not only increase the dry biomass of the plants, but also promote Cd accumulation in shoots and roots. On the contrary, EDTA was toxic to the species by restraining the growth of plants, although it could promote Cd accumulation in shoots and roots of the plants to a certain extent. Thus, EGTA and SDS were effective in enhancing phytoremediation with *Althaea rosea* Cav. for Cd contaminated soils, while EDTA is ineffective in this regard.

Keywords Ornamental plant · *Althaea rosea* Cav. · Hyperaccumulator · Chelator · Surfactant · Cadmium

Introduction

The problem of soil contamination which is brought by heavy metals from various human activities is increasingly becoming a global problem (Baker et al. 1994; Cunningham et al. 1995; Ebbs et al. 1997; Lewandowski et al. 2006). For example, in the Zhangshi irrigation area of China, large areas of soils have been contaminated by cadmium (Cd) because irrigation sewage contains large amounts of Cd from industry and mining activities. Heavy metal contamination in soils is one of the major intractable problems, posing significant risks to human health as well as to ecosystems (Zhou and Song 2004; Pendergrass and Butcher 2006). Phytoremediation is the use of green plants to remove pollutants from soils and it has attracted much attention because it is an environmentally friendly and relatively cheap technique (Huang et al. 1997; Pulford and Watson 2003; Chen et al. 2004a, b). This plant-based technique has

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shown significant economic, aesthetic and technical advantages compared with traditional techniques. Selection of plant species for phytoremediation is an ongoing process, up to now, many plants have been found as remediation plants although the known species are limited, there was little report about ornamental plants that can remediate contaminated soils. In fact, ornamental plant resources are very abundant. China is not only one of the countries with the most abundant ornamental species in the world, but also the cradleland of many ornamentals, the so-called "mother country of world gardens". Especially for the urban areas, ornamentals can beautify the environment and also resolve heavy metal pollution at the same time, it will have great and practical significance to screen out remediation plants from ornamental plant resources (Wang and Zhou 2005). Much interest has been focused on hyperaccumulators, the heavy metal concentration in which can reach to more than 100 times than those found in normal plants (Wei and Zhou 2006). According to the results of preliminary hydroponic and soil culture experiments, *Althaea rosea* Cav. had strong tolerance and accumulation ability when it grew in Cd contaminated environment (Liu et al. 2006a). Therefore, the further studies in this work were carried out to investigate whether *Althaea rosea* Cav. has the potential as a Cd-hyperaccumulator applied for remediation of Cd contaminated soils.

In order to increase plant remediation efficiency, the solubility process of soil-bound heavy metals can be increasingly promoted by a number of chemical approaches, including the application of chelators associated with phytoremediation (Blaylock et al. 1997; Chen and Cutright 1997; Kayser et al. 2000; Chen et al. 2004a, b). For example, ethylenediamine triacetic acid (EDTA) as a synthetic chelating agent has been shown to be efficient in mobilizing Pb from various soil compartments (Huang et al. 1997; Wu et al. 2003). Whereas EDTA and the formed EDTA-metal complexes are often toxic to the plants and the plants' growth was restrained to a certain extent, this study investigated the efficacy of another synthetic chelator ethylenegluatarotriacetic acid (EGTA) compared with EDTA, which is also capable of forming complexes with metal ions. It is hoped that the application of EGTA can increase not only the biomass but also the Cd accumulation of the species. Furthermore, surfactants can change the characteristics of the interface in a system obviously by their particular hydrophilic and lipophilic traits, so they have been largely used to dispose contaminants that are difficult to dissolve, degrade and utilize in environment (Jiang et al. 2003; Zhou and Song 2004). In this study, sodium dodecyl sulfate (SDS) as an important anionic surfactant was also used together with synthetic chelators to examine their single and joint effects on the extraction of Cd by *Althaea rosea* Cav.

Materials and methods

Pot-culture experiment design

According to our previous study and the National Soil-Environmental Quality Standard of China (GB 15618, 1995; Xia 1996), the applied levels of Cd (spiked as $CdCl_2{\cdot}2.5\ H_2O$) and other chemical agents including EDTA, EGTA and SDS were selected and designed for the pot-culture experiment as Table 1. The tested soil in this study is meadow burozem, the background level of heavy metals in the soil was 0.2, 13.5, 11.3 and 30.3 mg kg^{-1} for Cd, Pb, Cu and Zn respectively, chemical analysis also showed that pH, organic matter, total N, available P and available K in the tested soil was 6.6, 1.2%, 0.9 g kg^{-1}, 8.9 mg kg^{-1} and 95.2 mg kg^{-1} respectively. In April of 2006, surface (0–20 cm) soil samples were collected from the Shenyang Station of Experimental Ecology, Chinese Academy of Sciences. The soil samples were sieved through a 4.0 mm sieve and filled into the plastic pots (diameter = 20 cm, height = 15 cm) by 2.5 kg pot^{-1}, and artificially mixed with $CdCl_2{\cdot}2.5H_2O$, EDTA, EGTA and SDS according to the designed concentrations in Table 1, then equilibrated completely for 1 month. After that, seedlings of *Althaea rosea* Cav. with 1month old and the similar biomass were transplanted into the pots. There were 3 seedlings in each pot, and all treatments were replicated three times to minimize experimental errors. Furthermore, the treatment without addition of Cd, chelators and surfactant was designed as CK. Altogether, there were 75 pots or treatments in the experiment.

The pot-culture experiment was carried out in the outdoor lab of the Institute of Applied Ecology, Chinese Academy of Sciences. There was no metal contamination in surrounding area. The annual accu-

Table 1 Components and concentrations of chemically enhanced treatments

Treatment	Cd (mg kg^{-1})	SDS (mmol kg^{-1})	EDTA (mmol kg^{-1})	EGTA (mmol kg^{-1})
CK				
C1	30			
S11	30	0.5		
S12	30	1.0		
S13	30	2.0		
D11	30		1.0	
D12	30	0.5	1.0	
D13	30	1.0	1.0	
D14	30	2.0	1.0	
G11	30			1.0
G12	30	0.5		1.0
G13	30	1.0		1.0
G14	30	2.0		1.0
C2	100			
S21	100	0.5		
S22	100	1.0		
S23	100	2.0		
D21	100		1.0	
D22	100	0.5	1.0	
D23	100	1.0	1.0	
D24	100	2.0	1.0	
G21	100			1.0
G22	100	0.5		1.0
G23	100	1.0		1.0
G24	100	2.0		1.0

mulative temperature was >10°C and the frostless duration was 127–164 days each year. The plants in pots grew in the soil without fertilizer addition. Loss of water by evaporation from pots was made up daily using tap water (no Cd detected) to sustain 75–85% of soil water-holding capacity. The plants were harvested after they grew in the contaminated soils for 120 days.

Metal determination and data processing

Harvested plant samples were divided into roots, stems and leaves. They were carefully rinsed with tap water and then with deionized water. The samples were oven-dried at 105°C for 20 min and then at 70°C to constant weight. The dried plant samples were ground to powder after their dry biomass was weighed. Soil samples from the pot-culture experiment were air-dried and ground using a mortar and pestle, and then were sieved through a 0.149 mm sieve (Wei and Zhou 2004). The plant and soil samples were digested in an acid solution [conc. HNO_3 + conc. $HClO_4$ (3:1, *v*/*v*)] (Wang and Zhou 2003). The available Cd was extracted by 1.0 mol l^{-1} CH_3COONH_4 at the ratio of 1:10 (soil: solution). The concentrations of heavy metals were determined using the atomic absorption spectrophotometer (AAS, Hitachi 180-80, made in Japan) with certified reference materials (bought from an authoritative company in Shijiazhuang, China) for the quality assurance purposes. The determining wavelength for Cd is 228.8 nm. The limit of detection for Cd is 0.005 mg l^{-1}. The recovery rates for Cd in all samples were within 92.01 ± 8.63%. The data were processed using the Excel XP, SPSS13.0. The values were expressed as mean ± standard deviation (SD) of the three replicates.

Results

Cd tolerance of *Althaea rosea* Cav.

During the whole growing period, *Althaea rosea* Cav. could basically display its high tolerance to the toxicity of Cd. When the concentration of Cd in soil was 30 mg kg^{-1}, the plants did not suffer from obvious phytotoxicity except for those grown under D11-D14 treatments (Fig. 1a). The growth of plants was stunted due to the use of EDTA. Therefore, EDTA was toxic to *Althaea rosea* Cav.. This phenomenon was similar to a report by Ozturk et al. (2003). Compared with C1 treatment (with only 30 mg kg^{-1} Cd and without application of chelator or surfactant), the dry biomass of the plants in S11-S13 and G11-G14 treatments increased, which implied that the application of SDS or EGTA could facilitate the growth of *Althaea rosea* Cav.. Especially for the G11 treatment, the dry biomass of the plants increased from 5.2 (C1) to 8.6 g pot^{-1}, what was interesting was that the plants under G11 treatment bloomed while others including CK (without Cd, chelators, and surfactant) did not. Therefore, EGTA could improve phytoremediation efficiency through facilitating the growth of the plants. No matter D12-D14 or G12-G14 treatments, the addition of SDS decreased the dry biomass of *Althaea rosea* Cav. compared with that under D11 or G11. However, there was no obvious effect from adding SDS concentration to the dry biomass. It is likely that SDS concentration could not affect the growth of *Althaea rosea* Cav. obviously when the Cd concentration in soil was at the level of 30 mg kg^{-1}.

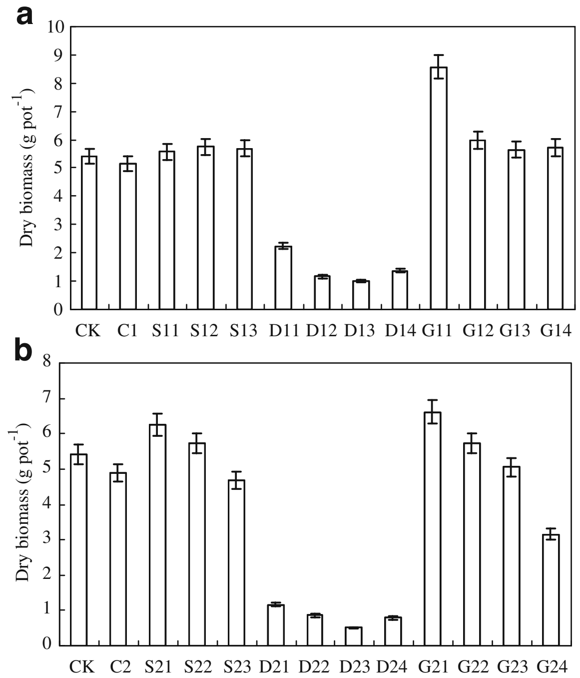

Fig. 1 The comparison of dry biomass of shoots when soil Cd was 30 (**a**) or 100 (**b**) mg kg^{-1}

When the concentration of Cd in soil was 100 mg kg^{-1}, the dry biomass of the plants under D21-D24 treatments decreased compared with that under other treatments (Fig. 1b). This phenomenon further testified the toxicity of EDTA to the growth of the plants. Compared with C2 treatment (with only 100 mg kg^{-1} Cd and without application of chelators or surfactant), the dry biomass of the plants in S21-S22 and G21-G23 treatments increased by 28.2%, 17.6%, 35.8%, 17.9% and 4.6% respectively. Among them, G21 treatment (EGTA = 1.0 mmol kg^{-1}) produced the most promoting effect. Under D22-D24 and G22-G24 treatments, the dry biomass of plants decreased with the application of SDS compared with that under D21 and G21 treatments. Moreover, the dry biomass of the plants decreased with the increase of SDS concentration under S21-S23 and G22-G24 treatments. Therefore, the increase of applied SDS concentration could restrain the growth of *Althaea rosea* Cav. when the Cd concentration in soil was at the level of 100 mg kg^{-1}.

Cd accumulation of *Althaea rosea* Cav.

Compared with C1, the shoot Cd concentration in *Althaea rosea* Cav. increased under all chemically enhanced treatments when the Cd concentration in soil was 30 mg kg^{-1} (Fig. 2a). In other words, SDS, EDTA and EGTA could facilitate the Cd accumulation in *Althaea rosea* Cav. to a certain extent. The shoot Cd concentration under S11-S13, D12-D14 and G12-G14 treatments also increased with the increase of SDS concentration, and the maximum shoot Cd concentration was 2.1, 2.6, and 3.5 times as much as that under C1 treatment, respectively. The Cd concentration in plants under G11 treatment was higher than that under D11 treatment, implying that EGTA was more effective than EDTA in enhancing Cd accumulation of the plants when soil Cd was 30 mg kg^{-1}. The root Cd concentration under most of the chemically enhanced treatments also increased compared with C1. Under D12-D14 and G12-G14 treatments, the root Cd concentration increased with the increase of SDS concentration, and reached the maximum value when the applied SDS concentration was 2.0 mmol kg^{-1}. The maximal root Cd concentration under G14 treatment was 2.0 times as much as that under C1. There was no obvious difference in the root Cd concentration under S11-S13 treatments. Under all the chemically enhanced treatments when soil Cd was 30 mg kg^{-1}, the Cd concentration in

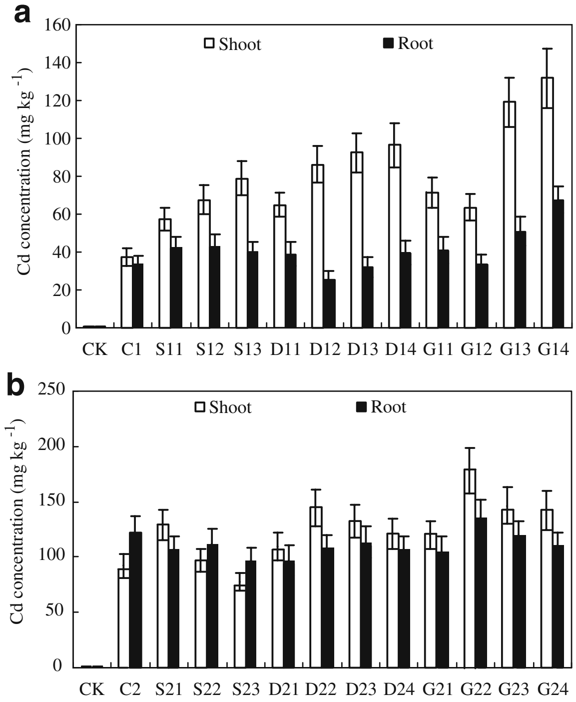

Fig. 2 Cd accumulation in shoots and roots when soil Cd was 30 (**a**) or 100 (**b**) mg kg^{-1}

shoots was higher than that in roots, which was an important characteristic of a Cd-hyperaccumulator. Especially for the G13 and G14 treatments in which EGTA and SDS were jointly used, the shoot Cd concentration was 119.1 and 131.9 mg kg^{-1}, and the root Cd concentration was 50.9 and 67.5 mg kg^{-1}. It was thus clear that the shoot Cd concentration had reached 100 mg kg^{-1} which is the critical concentration of a Cd-hyperaccumulator. In combination with the results of tolerance analysis, *Althaea rosea* Cav. can be thus regarded as a potential Cd-hyperaccumulator by chemical enhancement.

When soil Cd was 100 mg kg^{-1}, compared with C2, the shoot Cd concentration under most of the chemically enhanced treatments increased except for S23 treatment (Fig. 2b). However, the shoot Cd concentration under S21-S23, D22-D24 and G22-G24 treatments decreased with the increase of SDS concentration, it reached the maximum value when SDS was 0.5 mmol kg^{-1}. In particular, the shoot Cd concentration under S21, D22 and G22 treatments was 1.4, 1.6 and 1.9 times as much as that under C2, respectively. The Cd concentration in plants under G21 was also higher than that under D21, this phenomenon further testified that the application of EGTA was more effective than EDTA in enhancing Cd accumulation. Under C2 treatment, the Cd concentration in shoots was lower than that in roots and was also lower than 100 mg kg^{-1}. However, under all the chemically enhanced treatments except for S22 and S23 treatments, the Cd concentration in shoots was higher than that in roots and exceeded 100 mg kg^{-1} when soil Cd was 100 mg kg^{-1}. Compared with C2, the root Cd concentration decreased under most of the chemically enhanced treatments, implying that more Cd was translocated to shoots successfully through chemical enhancements. According to these accumulation characteristics, the species can be regarded as a potential Cd-hyperaccumulator by chemical enhancements. For instance, the Cd concentration in shoots and roots under G22 treatment was up to 178.5 and 135.6 mg kg^{-1}, respectively.

Available Cd in soil

According to Fig. 3, the available Cd concentration in soil increased with the increase of SDS concentration under S11-S13, D12-G14 and G12-G14 treatments when soil Cd was 30 mg kg^{-1}. On the contrary, the available Cd concentration in soil decreased with the increase of SDS concentration under S21-S23, D22-G24 and G22-G24 treatments when soil Cd was 100 mg kg^{-1}. In Fig. 3a, under the joint SDS and EDTA treatments (D12-D14), the addition of 2.0 mmol kg^{-1} SDS (D14) increased available Cd concentration compared with the only application of EDTA (D11); and under the joint SDS and EGTA treatments (G12-G14), the addition of 1.0 and 2.0 mmol kg^{-1} SDS (G13 and G14) increased available Cd concentration compared with the only application of EGTA (G11). In Fig. 3b, the addition of SDS (D22-D24, G22-G24) decreased available Cd concentration compared with the only application of EDTA (D21) or EGTA (G21).

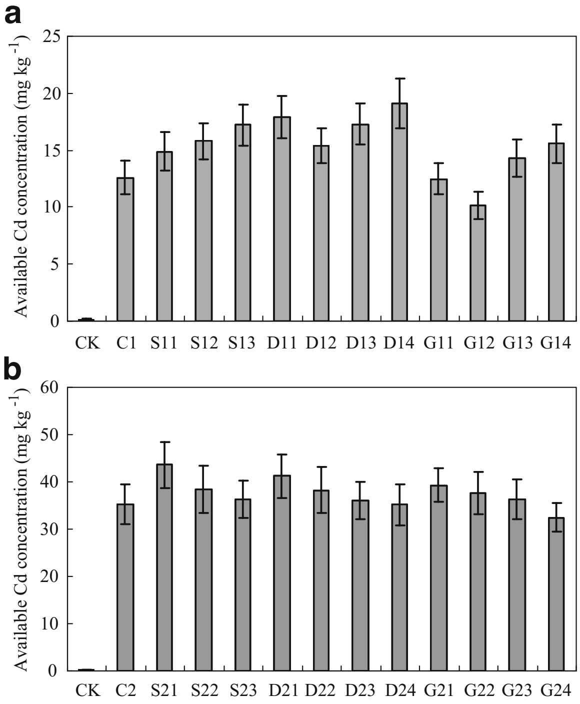

Fig. 3 Available Cd concentration in soils when soil Cd was 30 (**a**) or 100 (**b**) mg kg^{-1}

Discussion

It has been reported that some ornamental plants can indicate and monitor atmospheric pollutants (Ma 2003). Especially, ornamental plants can imbibe CO_2 where the population density is high, release plenty of O_2 to keep air fresh by photosynthesis, also are termed “air vitamins” due to their flourishing branches and leaves (Liu et al. 2006b). Ornamental plants are mostly applied in urban areas such as parks, two sides of the roads, and other sites where population density is high, therefore, the application of ornamental plants to contaminated soil remediation will have wider prospects and bring significant economic and social benefits.

Similarly with other kinds of plants, ornamental plants can absorb pollutants such as heavy metals from soil and transfer some of them to shoots, so the content of heavy metals in soil can be decreased. Ornamental plants have a strong ability of absorbing sunlight, water and nutrition, this can facilitate the plants' growth and promote metal accumulation, and potentially provide the possibility of hyperaccumulating heavy metals. In addition, an important characteristic as a hyperaccumulator is the translocation ability of a plant. Usually, the translocation factor (TF) can indicate the ability of metal-transferring from roots to shoots of a plant and can be defined as follows (Madrid et al. 2003; Dickinson and Pulford 2005):

$$\mathrm{TF} = M_2/M_1 \tag{1}$$

Where M_2 is the concentration of heavy metals in shoots; M_1 is the concentration of heavy metals in roots. If TF > 1.0, it shows that the accumulation of heavy metals in the shoots is higher than that in the roots. Moreover, the higher the TF value is, the stronger the phytoextraction ability is. In Fig. 4a, compared with C1, all TF values increased and were higher than 1.0, this phenomenon implied that the translocation ability of *Althaea rosea* Cav. was strengthened to some extent by chemically enhanced treatment when soil Cd was 30 mg kg^{-1}. With an increase in the SDS concentration, the TF values increased under single SDS treatments (S11-S13), decreased under joint EDTA and SDS treatments (D12-D14), and increased at first and then decreased under joint EGTA and SDS treatments (G12-G14). Under single EDTA or EGTA treatment (D11 or G11), there was no obvious difference in promoting Cd translocation. However, the TF values were higher under joint EDTA and SDS treatments (D12-D14) than that under joint EGTA and SDS treatments (G12-G14). Compared with C2, all TF values increased and were higher than 1.0 except for S22 and S23 when soil Cd was 100 mg kg^{-1} (Fig. 4b). With the increase of the SDS concentration, the TF values decreased under single SDS treatments (S21-S23). The addition of SDS decreased TF values under D22-D24 and G22-G24 treatments compared with that under single EDTA and EGTA treatments (D21 and G21). No matter 30 or 100 mg kg^{-1} Cd contaminated soil, the maximum TF value was observed under D12 and D22 treatments (EDTA = 1.0 and SDS = 0.5 mmol kg^{-1}). In a word, EDTA, EGTA and SDS were effective in enhancing Cd translocation from the roots to shoots of *Althaea rosea* Cav.

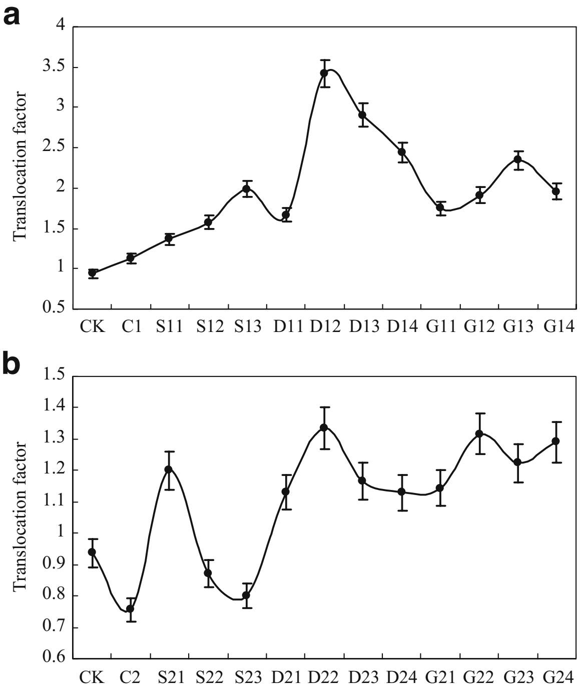

Fig. 4 Translocation factor trendline of Cd uptake by *Althaea rosea* Cav. when soil Cd was 30 (**a**) or 100 (**b**) mg kg^{-1}

As Huang et al. (1997) showed, a chelator has a strong ability of chelating various metals and also increasing the bioavailability of the metals in soil. In soil, the applied chelator acts first to combine the soluble metals in the soil solution. As the free metal activity decreases, dissolution of bound metal ions begins to compensate for the shift in equilibrium, thus, a great amount of metals is set free from soil matrix. This process can facilitate the bioavailability of heavy metals and then increase the effectiveness of phytoremediation (Schmidt 2003). Some investigation results have been reported about chelators that can facilitate heavy metal accumulation, for example, EDTA has been used in different situations in phytoremediation to enhance the extraction of heavy metals by plants from soil (Wu et al. 1999; García et al. 2004). Ideal remediation plants should be fast-growing, tolerant to grow in contaminated soils, have a well-established agronomic system and, finally, should produce a large biomass while accumulating high concentration of heavy metals in their shoots (Satos et al. 2006). According to the results of tolerance analysis in this work, it was shown that the dry biomass of *Althaea rosea* Cav. decreased greatly under EDTA treatments whether for 30 or for 100 mg kg^{-1} Cd contaminated soils (D11-D14, D21-D24). Therefore, EDTA was toxic to the growth of the plants and could restrain the growth badly although it could facilitate Cd uptake under the experimental conditions. The final purpose of phytoremediation is to acquire as much heavy metals as remediation plants can, especially for the shoots which are easier to be harvested (Berndes et al. 2004). In Fig. 5, the total Cd content in shoots (the product of the Cd concentration and the dry biomass in shoots) of *Althaea rosea* Cav. was evaluated to investigate the efficiency of each chemically enhanced treatment. It was obviously shown that there was a sharp decrease in total Cd content under D11-D14 and D21-D24 treatments. Therefore, EDTA was not effective in extracting much Cd from soils to plants. On the contrary, the total Cd content was increased by the application of EGTA. When soil Cd was 30 mg kg^{-1} (Fig. 5a), the total Cd in shoots accumulated by the species was up to 0.6 mg pot^{-1} under G11 treatment. Correspondingly, the total Cd accumulated by the species under C1 treatment was only 0.2 mg pot^{-1}. Higher Cd content under G12-G14 treatments was observed with the increase of SDS concentration. The maximum total Cd content was 0.8 mg pot^{-1} under G14 when joint EGTA and SDS were used. It is likely that there was the synergistic effect between EGTA and SDS when they were used together in 30 mg kg^{-1} Cd contaminated soils. In addition, single SDS treatments (S11-S13) also showed higher promoting effectiveness in Cd accumulation compared with C1, moreover, Cd accumulation by the species increased with the increase of SDS concentration. It can be inferred that the total Cd content in the species could be further increase through increasing the applied SDS concentration, because the dry biomass of the plants increased when SDS was used at the maximal concentration in this work and the plants did not suffer from phytotoxicity. In Fig. 5b, when soil Cd was 100 mg kg^{-1}, the lower total Cd content was observed with the increase of SDS concentration under S21-S23 and G22-G24 treatments. This phenomenon was contrary with that when soil Cd was 30 mg kg^{-1}. It is likely that the high concentration of Cd in soil was the main reason to restrain the function of SDS. Therefore, the effect of chelators and/or surfactant on Cd accumulation by plants was closely related with the heavy metal concentration in soil. There were different responses of the plants to low or high soil Cd concentration. The total Cd content in *Althaea rosea* Cav. was up to 0.8 mg pot^{-1} under G21 treatment, correspondingly, it was only 0.5 mg pot^{-1} under C2 treatment. In particular, the maximum Cd content was 1.0 mg pot^{-1} under G22 treatment when joint EGTA and SDS were applied to Cd contaminated soils. Under single SDS treatments, the maximum Cd content accumulated by the species was 0.8 mg pot^{-1} when the applied SDS concentration was 0.5 mmol kg^{-1}.

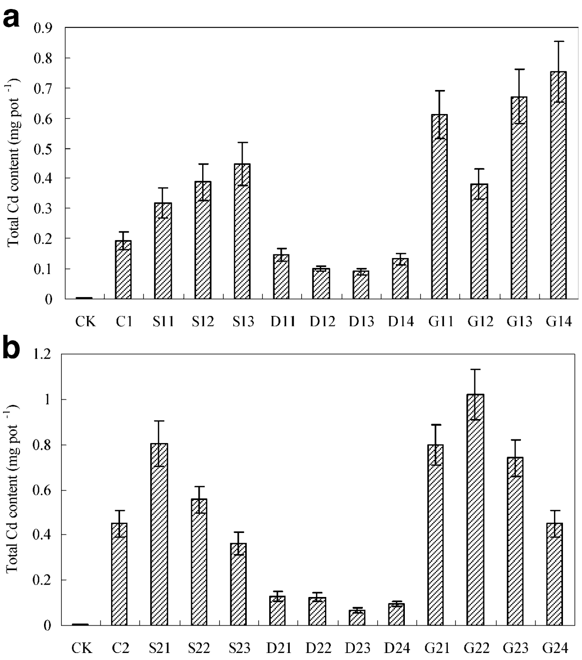

Fig. 5 Total Cd content in shoots accumulated by *Althaea rosea* Cav. when soil Cd was 30 (**a**) or 100 (**b**) mg kg^{-1}

Conclusions

Under experimental conditions, *Althaea rosea* Cav. exhibited its strong tolerance to the toxicity of soil Cd and had high Cd accumulation ability when it grew in Cd contaminated soils. However, different chelators and surfactants had a great difference in influencing hyperaccumulating characteristics of the species. Under all chemically enhanced treatments, the maximum dry biomass of the plants was increased by 70% (G11) and 40% (G21) respectively. Similarly, single SDS treatments could also increase the dry biomass of the plants. In particular, under joint EGTA and SDS treatments, the maximal Cd accumulation in shoots and roots was up to 131.9 and 67.5 mg kg^{-1} when soil Cd was 30 mg kg^{-1}, and 178.5 and 135.6 mg kg^{-1} when soil Cd was 100 mg kg^{-1}, respectively. Thus, *Althaea rosea* Cav. can be regarded as a potential Cd-hyperaccumulator by chemical enhancement.

Basically, EGTA and SDS were effective in enhancing phytoremediation of *Althaea rosea* Cav. for Cd contaminated soils. On the contrary, EDTA was toxic to the species by restraining the growth of the plants, although Cd accumulation in shoots and roots increased to a certain extent compared with C1 and C2, so EDTA was not effective in enhancing phytoremediation of *Althaea rosea* Cav. for Cd contaminated soils. Because *Althaea rosea* Cav. can remedy Cd contaminated soils and beautify environment at the same time especially in urban areas, there was great potential and value in application of the species to the remediation of Cd contaminated soils.

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