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Identification and Chemical Enhancement of Two Ornamental Plants for Phytoremediation

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Abstract With an increase in the contamination of urban areas, more and more attention has been paid to the role of ornamental plants in remedying contaminated soils. Thus, cadmium (Cd) tolerance and accumulation characteristics of *Calendula officinalis* and *Althaea rosea* as ornamental plants under the concentration gradient experiment with single Cd, as well as the effect of ethylenegluatarotriacetic acid (EGTA) and sodium dodecyl sulfate (SDS) on their Cd phytoremediation capacity under the chemically enhanced experiment was further investigated. It was showed that they had strong tolerance and accumulation capacity of Cd under single Cd treatments, thus they had great potential to be used for Cd contaminated soil remediation. Furthermore, under chemically enhanced treatments, the great efficiency was found through applying EGTA and SDS, they could not only increase the dry biomass of the plants, but also promote the Cd accumulation in shoots and roots. Particularly, *Althaea rosea* can be regarded as a potential Cd-hyperaccumulator through applying chemical agents. In conclusion, the two ornamental plants are promising to be used for phytoremediation.

Keywords Phytoremediation · Cd · *Calendula officinalis* · *Althaea rosea*

Phytoremediation has received more considerable attention in recent years and it has been shown to be more preponderant than conventional technologies for remedying contaminated soils, which is the use of green plants to remove contaminants from contaminated environment in a cost-effective manner (Salt et al. 1998; Meers et al. 2005; Zhou et al. 2007). Phytoremediation includes degradation remediation, volatilization remediation, stabilization remediation and extraction remediation, the first is mainly used for soils contaminated by organic pollutants (Zhou and Song 2004) and the second is mainly used for soils contaminated by volatile metals (Bañuelos et al. 1997). Whereas, stabilization and extraction remediation are mainly used for soils contaminated by most of heavy metals, the main difference between them is that the former is used by plants that can accumulate high concentration of heavy metals in roots than that in shoots (Vangronsveld et al. 1995) while the latter is contrary (Zhou and Song 2001; Zhou and Wei 2006). In addition, the application of chemically enhanced technology which can facilitate the accumulation of heavy metals and their translocation to the aboveground parts has been shown visible effectiveness (Huang et al. 1997; Blaylock et al.1997).

Up to now, many plants have been found as remediation plants, but there was little report about ornamental plants that can remedy contaminated soils. In fact, ornamental resources are very abundant, and they can indicate and

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monitor atmospheric pollutants (Ma 2003; Liu et al. 2006). Thus, this will provide substantial bases for screening out remediation plants. Especially for 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 resources. According to an elementary experiment from herbaceous ornamentals (Wang 2005), *Calendula officinalis* and *Althaea rosea* had strong Cd tolerance and accumulation capacity compared with other tested ornamentals. Therefore, the aim of this work was: (1) to identify the capacity of the two ornamental plants used for Cd contaminated soil remediation through applying the concentration gradient experiment with single Cd; (2) further to enhance their remediation capacity through applying the chemically enhanced experiment.

Materials and Methods

For the concentration gradient experiment with single Cd, the concentration of Cd (T0–T4) in soil was designed according to the National Soil-Environmental Quality Standard of China (NSEQSC GB15618 1995) (Xia 1996) and the results of our previous experiment using the soil-culture method (Wang 2005). There were five treatments with Cd concentrations of 0, 10, 30, 50, and 100 mg kg^{-1}, respectively. Cd was spiked as $CdCl_2 \cdot 2.5H_2O$. In April of 2005, surface (0–20 cm) soil samples were collected from the Shenyang Station of Experimental Ecology, Chinese Academy of Sciences. The tested soil is meadow burozem which is not contaminated by heavy metals according to the NSEQSC. The soil samples were sieved through a 4.0 mm sieve and filled into plastic pots (diameter = 20 cm, height = 15 cm) by 2.5 kg pot^{-1}, then mixed with $CdCl_2 \cdot 2.5H_2O$ and equilibrated completely for one month. After that, seedlings of *Calendula officinalis* and *Althaea rosea* with one-month-old and similar biomass were transplanted into pots. There were three seedlings in each pot based on the plant size, and all treatments were replicated three times to minimize experimental errors. The experiment was carried out in the outdoor lab of Institute of Applied Ecology, Chinese Academy of Sciences, there was no contamination in the surrounding area. 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 had grown in the contaminated soils for 120 days. For the chemically enhanced treatments, ethylenegluatarotriacetic acid (EGTA) as a synthetic chelator or/and sodium dodecyl sulfate (SDS) as an important anionic surfactant was/were used together with $CdCl_2 \cdot 2.5H_2O$ (Cd = 30 mg kg^{-1}) into the soil. The components and concentrations for the enhanced treatments were listed in Table 1. In April of 2006, the enhanced experiment was carried out and the procedures were the same with the above-mentioned concentration gradient experiment with single Cd.

Table 1 Components and concentrations of chemically enhanced treatments

Treatment	Cd (mg kg^{-1})	SDS (mmol kg^{-1})	EGTA (mmol kg^{-1})
CK			
C1	30		
S1	30	0.5	
S2	30	1.0	
S3	30	2.0	
E1	30		1.0
E2	30	0.5	1.0
E3	30	1.0	1.0
E4	30	2.0	1.0

Harvested plant samples were mixed for each pot, and then were divided into roots, stems, leaves and inflorescences. They were carefully rinsed with tap water, 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 tissues were ground to powder after their dry weights were weighed. Soil samples were air-dried and ground using a mortar and pestle, and then were sieved through a 0.149 mm mesh (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 concentrations of heavy metals were determined using the atomic absorption spectrophotometer (AAS, Hitachi 180-80) with certified reference materials (bought from an authoritative company in Shijiazhuang, China) for quality assurance purpose. 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%. Data were processed with the Microsoft Excel and SPSS 13.0 software. The values were expressed as mean ± standard deviation (SD) of the three replicates. Data were analyzed by one-way ANOVAs with the Duncan's multiple range tests to separate means. Differences were considered significant at $p < 0.05$.

Results and Discussion

For the concentration gradient experiment with single Cd, *C. officinalis* and *A. rosea* showed high tolerance to Cd without suffering from obvious phytotoxicity during the whole growing period. The plants of *C. officinalis* bloomed while

Table 2 Dry weight in shoots and roots of *Calendula officinalis* and *Althaea rosea* under single Cd treatments (g pot^{-1})

Ornamental	Treatment	Root	Shoot
Calendula officinalis	T0	1.45 ± 0.13ab	6.09 ± 0.32b
	T1	1.43 ± 0.11ab	7.63 ± 0.48a
	T2	1.63 ± 0.07a	7.54 ± 0.48a
	T3	1.29 ± 0.26bc	5.96 ± 0.50b
	T4	1.18 ± 0.030c	6.21 ± 0.12b
Althaea rosea	T0	4.87 ± 0.36c	3.28 ± 0.29bc
	T1	3.83 ± 0.37d	2.74 ± 0.35c
	T2	5.77 ± 0.52ab	4.50 ± 0.36a
	T3	6.34 ± 0.38a	3.82 ± 0.34b
	T4	5.13 ± 0.32bc	3.41 ± 0.10b

Data were expressed as mean ± standard deviation. Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$)

A. rosea did not. There was no obvious difference in appearance between the four Cd treatments (T1–T4) and the control (T0) for *C. officinalis*, and the plant height of *A. rosea* under the four Cd treatments was slightly shorter than that under the control. Dry weight values were also shown in Table 2 to assess the Cd tolerance of the plant. For *C. officinalis*, compared with the control, the shoot dry weight significantly increased by 27% and 26% under T1 and T2, the decrease was not significant under T3. However, the root dry weight significantly decreased by 19% under T4. For *A. rosea*, compared with the control, the shoot dry weight increased under T2, T3, and T4, and the increase was significant under T2. The root dry weight significantly increased by 18% and 30% under T2 and T3, and significantly decreased by 21% under T1. The dry weight of shoots (sum of stem, leaf and inflorescence) will not decrease significantly under high concentration of Cd treatment compared with that under the control if the species has high tolerance to Cd. Therefore, in this work, not only *C. officinalis* and *A. rosea* had high tolerance to Cd contaminated soils but also Cd could facilitate the plant growth in a way.

For the two plants, the Cd concentration in shoots and roots both increased with the increasing Cd concentration in soil (Fig. 1). All the Cd accumulation in shoots was less than that in roots for *C. officinalis*, while it was contrary for *A. rosea* except for T4 treatment. In Fig. 1a, the Cd concentration in roots and shoots was as high as 1,084 mg kg^{-1} and 284 mg kg^{-1} under T4. Furthermore, there was no obvious symptom of suffering from phytotoxicity. The results implied that *C. officinalis* might grow normally and accumulate higher content of Cd in more heavily Cd contaminated soils. In Fig. 1b, *A. rosea* showed high capacity of transferring Cd from the roots to the shoots under T1, T2 and T3, this is a characteristic of hyperaccumulators. Among the three treatments which the Cd concentration in shoots was higher than that in roots (T1–T3), the maximum Cd concentration in shoots did not reach 100 mg kg^{-1} which is the critical concentration of Cd-hyperaccumulators. However, the Cd accumulation capacity of the plants can be increased by chemically enhanced treatments (Blaylock et al. 1997; Satos et al. 2006) in the further experiment. In addition, the total Cd content (the product of Cd concentration and dry weight) in each part of *C. officinalis* and *A. rosea* under single Cd treatments were shown in Fig. 2, and it increased with the increasing Cd concentration in soil. For *C. officinalis*, the maximum total Cd content accumulated by the shoots was up to 1.76 mg pot^{-1} while 1.28 mg pot^{-1} in roots under T4, when the total Cd content accumulated by the shoots exceeded that by the roots (Fig. 2a). Among the three tissues of the shoot, leaf was most effective in accumulating Cd for *C. officinalis*. Although *C. officinalis* could not be used as hyperaccumulators, the species could grow normally and considerable heavy metals could be extracted when the plants were harvested, especially for the shoots which were easier to be harvested, therefore, the species could remedy Cd contaminated soils to a certain extent and beautify the environment at the same time. For *A. rosea*, with the increase of Cd concentration in soil, the increase of the total Cd content in the roots was more obvious than that in the stems and leaves (Fig. 2b). Based on the above-mentioned results of the single Cd treatments, it was concluded that *C. officinalis* and *A. rosea* had great potential in phytoremediation according to their high tolerance and Cd accumulation capacity.

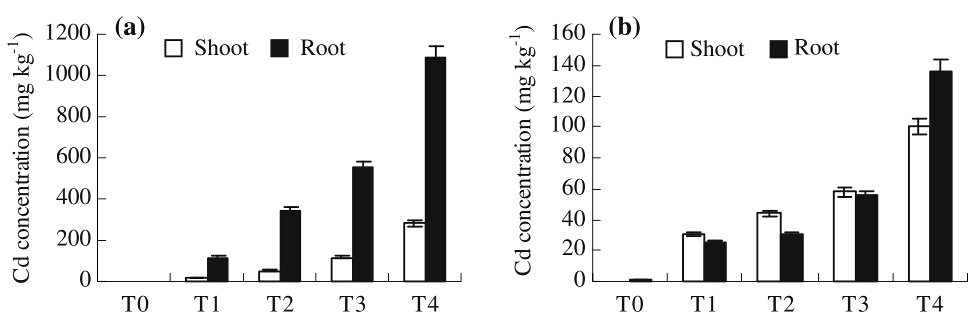


Fig. 1 Cd accumulation in shoots and roots of *Calendula officinalis* (**a**) and *Althaea rosea* (**b**) under single Cd treatments

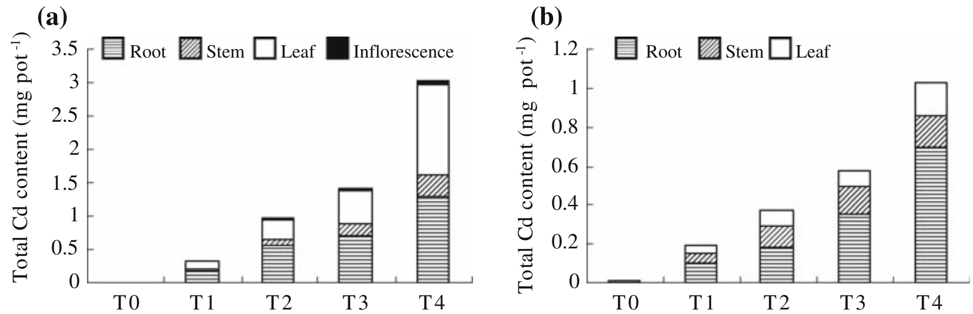


Fig. 2 Total Cd content in each part of *Calendula officinalis* (**a**) and *Althaea rosea* (**b**) under single Cd treatments

Table 3 Dry weight in shoots and roots of *Calendula officinalis* and *Althaea rosea* under chemically enhanced treatments (g pot^{-1})

Ornamental	Treatment	Root	Shoot
Calendula officinalis	CK	1.40 ± 0.17b	7.21 ± 0.28e
	C1	1.52 ± 0.16ab	8.76 ± 0.92d
	S1	1.43 ± 0.13b	11.24 ± 0.89b
	S2	1.48 ± 0.13ab	10.18 ± 0.94bcd
	S3	1.51 ± 0.16ab	10.38 ± 0.85bc
	E1	1.72 ± 0.17a	12.97 ± 0.59a
	E2	1.24 ± 0.11bc	9.61 ± 0.81cd
	E3	1.00 ± 0.12c	9.71 ± 0.98cd
	E4	0.99 ± 0.18c	9.87 ± 0.70bcd
Althaea rosea	CK	5.26 ± 0.35ab	5.41 ± 0.45b
	C1	5.49 ± 0.34a	5.17 ± 0.46b
	S1	2.42 ± 0.38e	5.57 ± 0.53b
	S2	2.82 ± 0.51de	5.75 ± 0.43b
	S3	3.05 ± 0.39cde	5.69 ± 0.33b
	E1	2.96 ± 0.31cde	8.58 ± 0.52a
	E2	3.39 ± 0.26cd	5.98 ± 0.51b
	E3	4.67 ± 0.45b	5.64 ± 0.39b
	E4	3.54 ± 0.33c	5.72 ± 0.46b

Data were expressed as mean ± standard deviation. Data in the same column followed by the same letter are not significantly different, whereas with different letters data are significantly different ($p < 0.05$)

For the chemically enhanced treatments, the dry weight in shoots and roots of the two plants was shown in Table 3. For *C. officinalis*, the dry weight in shoots under all treatments increased significantly compared with CK (without application of Cd, SDS and EGTA), for example, the maximum shoot dry weight increased by 80% under E1 when single EGTA was used to enhance remediation efficiency. For the three SDS enhanced treatments (S1–S3), the shoot dry weight increased by 56% at most under S1 when SDS was used at minimal concentration. For the three joint SDS and EGTA treatments (E2–E4), the addition of SDS decreased the shoot dry weight compared with the single EGTA enhanced treatment (E1). The dry weight in roots increased under most of the treatments except the joint SDS and EGTA treatments (E2–E4), but the difference was significant only under E3 and E4 treatments. For *A. rosea*, the dry weight in shoots under all the enhanced treatments (S1–S3 and E1–E4) increased and the difference was only significant under E1, when the dry weight increased by 59% compared with CK. Especially, the plants bloomed under E1 while others including CK did not, this indicated that the application of EGTA could accelerate the growth of *A. rosea*. The growth of roots was restrained under all enhanced treatments. In a word, the application of SDS and EGTA to Cd contaminated soils could facilitate the increase of shoot dry weight for both *C. officinalis* and *A. rosea*, especially when 1.0 mmol kg^{-1} EGTA was singly used to enhance remediation efficiency.

The Cd accumulaton in plants also increased under chemically enhanced treatments (Fig. 3). For *C. officinalis*, the maximum Cd concentration in shoots and roots were both observed under E4, which was 1.68 times and 1.50 times as much as that under C1, respectively (Fig. 3a). There was a sharp decrease of the Cd concentration in shoots and roots under E3 when 1.0 mmol kg^{-1} EGTA and

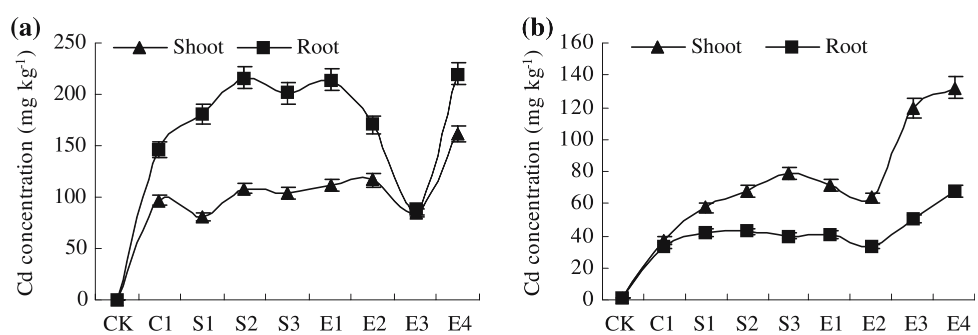
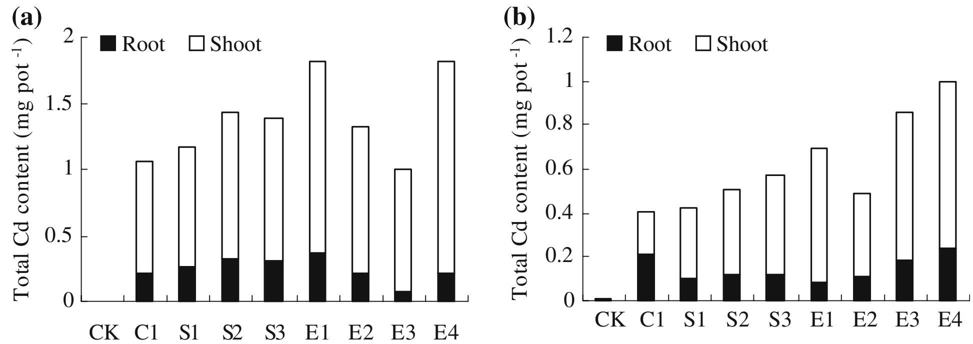


Fig. 3 Cd accumulation in shoots and roots of *Calendula officinalis* (**a**) and *Althaea rosea* (**b**) under chemically enhanced treatments

Fig. 4 Total Cd content in shoots and roots of *Calendula officinalis* (**a**) and *Althaea rosea* (**b**) under chemically enhanced treatments



SDS were jointly used. Perhaps an antagonistic effect took place between them. For the single SDS treatments (S1–S3), the maximum Cd concentration in shoots and roots was observed under S2, when SDS was used as a concentration of 1.0 mmol kg^{-1}. For *A. rosea*, the Cd concentration in shoots and roots increased under all chemically enhanced treatments, especially for the E3 and E4 treatments. The Cd concentration in shoots was 119 and 132 mg kg^{-1}, respectively, which had exceeded the critical content of Cd-hyperaccumulators (Fig. 3b). Furthermore, the Cd concentration in shoots was higher than that in roots for all the treatments. In other words, *A. rosea* showed characteristics as a Cd-hyperaccumualtor under chemically enhanced treatments. As for the effect of SDS concentration on the Cd accumulation in shoots, with the increase of SDS concentration the Cd concentration increased for S1–S3 (single SDS enhanced treatments) and E2–E4 (joint SDS and EGTA treatments). The total Cd accumulation in plants was shown in Fig. 4, it was obvious that the Cd content in shoots was higher than that in roots under all enhanced treatments, i.e., most of Cd was accumulated by the shoots which was easier to be harvested, this phenomenon showed that the application of SDS and EGTA was effective in enhancing Cd accumulation. For *C. officinalis*, compared with C1, the total Cd accumulation in plants increased under all enhanced treatments except for E3, and the maximum total Cd content was observed under E1 and it increased by 72% (Fig. 4a). For *A. rosea*, compared with C1, the total Cd accumulation in plants increased under all enhanced treatments except for E2, and the maximum total Cd content was observed under E4 and it was 2.5 times as much as that under C1 (Fig. 4b). In addition, for *C. officinalis* and *A. rosea*, the maximum Cd-removing ratio in shoots (total Cd content ratio of shoots to soil) was both observed under E4, about 1.77 and 2.36 times as much as that under C1, respectively. Based on the above-mentioned results of chemically enhanced treatments, the great efficiency was found with the application of SDS and EGTA, because they could not only increase the dry weight but also promote Cd accumulation of the plants. Particularly, *Althaea rosea* can be regarded as a potential Cd-hyperaccumulator through applying chemical agents.

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