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The Influence of Different Growth Stages and Dosage of EDTA on Cd Uptake and Accumulation in Cd-Hyperaccumulator (*Solanum Nigrum* L.)

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Abstract Application of synthetic chelates such as ethylene diamine tetraacetic acid (EDTA) has been proposed as an alternative technology for phytoextraction of contaminated soils. In a pot experiment, the effects of EDTA application at three growing stages on growth and Cd uptake and accumulation of *Solanum nigrum* L. were investigated. The results showed that the 0.1 g/kg EDTA treatment was the most effective treatment, in which the concentrations of Cd in stems and leaves increased significantly compared with the control (Cd only), and the accumulation of Cd in shoots increased by 51.6%, 61.1% and 35.9% at the seedling, flowering and mature stages, respectively. Moreover, at the flowering stage, the height, dry shoot biomass and Cd accumulation in the plants reached the maximum, which were 18.9 cm, 1.8 g/plant and 292.8 μg/pot, respectively. However, higher rate of EDTA (0.5 g/kg) could reduce the plant biomass and the total amount of Cd removed. The results indicated that moderate rate of EDTA applied at the flowering stage would be important to enhance phytoremediation efficiency in practice.

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Cadmium (Cd) is a major anthropogenic pollutant which has been released into the environmental since the industrial revolution (Zhou and Huang 2000), the primary sources of this pollutant are agricultural and industrial practices such as application of pesticides and fertilizers, waste water irrigation, and smelter wastes and residues from the mining and smelting of metalliferous ores (McGrath et al. 2001; Boisson et al. 1999; Zhou and Huang 2000). Scientists and governmental agencies have become concerned over increasing Cd concentration because of its high solubility in water and the fact that Cd is highly toxic to plants, animals and microorganisms (de La Rosa et al. 2004; Prasad 2003). In response to these negative effects, there is a greater emphasis on the problem of Cd pollution with the development of modern industry and agriculture, and there has been ongoing development of a variety of technologies to remediate contaminated soils (Wang et al. 2007; McGrath et al. 2002; McGrath et al. 2006).

However, metal-contaminated soils are notoriously hard to remediate. Cleanup of metal-contaminated soils via conventional engineering methods can be prohibitively expensive (Salt et al. 1995; Zhou and Song 2004). Phytoremediation is emerging as a cost-effective alternative, which has demonstrated that the cost of phytoextraction is only a fraction of that of conventional engineering technologies (Anderson et al. 1999; McGrath et al. 2002). Ideal plants for phytoremediation should possess multiple traits. Specially, they should have a fast growth rate, large biomass and deep roots, and could be easy to harvest, and should tolerate and accumulate a range of heavy metals in their aerial or harvestable parts (Clemens et al. 2002; Hsiao et al. 2007). Unfortunately, no plant that has been described can fulfill all these standards. Therefore, in order to compensate for low metal accumulation by these plants, many researches are being done to increase the availability of heavy metals in soils and enhance the phytoextraction efficiency of potential metal-accumulators (Wu et al. 1999; Wong et al. 2004; Meers et al. 2005; Luo et al. 2005). EDTA is suggested as one of most effective chelating agents for the assistance of phytoextraction, which can increase the metals mobility in soil solid phrase, thus enhance the concentrations of heavy metals in plant shoot tissues (Hong et al. 1999; Wong et al. 2004; Meers et al. 2005).

The plant species used in this study is *Solanum nigrum* L. (black nightshade), which is a newly found Cd-hyperaccumulator (Wei et al. 2005; Sun et al. 2007a, b). The main objective of this research was to evaluate the ability of EDTA to enhance the efficiency of phytoremediation, and to determine the optimal dosage of EDTA added at different growing stages required to increase metals mobilization and accumulation of heavy metals in plant tissues.

Materials and Methods

Soil samples were collected from an agricultural field in the Shenyang Station of Experimental Ecology, Chinese Academy of Sciences (123°41′ E and 41°31′ N). The parameters of tested soil were listed as follows: soil contained 1.5% organic matter, and pH was 6.56, and CEC was 23.26 g/kg, and total N, P and K were 0.91, 0.40, and 183.00 g/kg, respectively, and the concentrations of Cd, Cu, Zn, and Pb were 0.17, 12.5, 28.1, and 11.1 mg/kg, respectively.

The fresh soil samples were air dried and then passed through a sieve of 4.0 mm. In all treatments, the soil samples were amended at 25 mg/kg Cd with $CdCl_2 \cdot 2.5H_2O$, then incubated for 4 weeks. Three uniform seedlings were selected and transplanted to each pot. Preliminary experiments showed that the 1.0 and 3.0 g/kg EDTA treatments were harmful to the plants, in which leaves presented an extensive chlorosis and necrotic areas, and most of plants died a few days after the EDTA application. Therefore, EDTA levels were decreased to 0.1 and 0.5 g/kg and spiked at 20, 36 and 46 days after transplanting, namely seedling stage, flowering stage and mature stage of. The control treatment was not amended with any EDTA. The pots were placed in individual trays to prevent loss of amendments from leaching, and the soil was irrigated to field capacity on a daily basis. The plants were harvested after 53 days of cultivation.

The plants were immersed in a 0.01 M HCl solution to remove any external Cd and then rinsed with deionized (DI) water (Aldrich et al. 2003). Subsequently, the plant were separated into roots, stems, leaves and seeds/flowers, and dried at 100°C for 10 min, then at 70°C until completely dry in an oven. The plant and soil samples were digested with a solution of 3:1 $HNO_3/HClO_4$ (v/v). The concentrations of heavy metals were determined using a flame atomic absorption spectrophotometry (WFX-120).

All treatments were replicated with three times in the experiment. The means and standard deviations were calculated by Excel. Statistical analysis was carried out by the one-way analysis of variance using SPSS10.0. When significant difference was observed between treatments ($p < 0.05$ or $p < 0.01$), multiple comparisons were made by the LSD test.

Results and Discussion

In the preparative experimental set with higher concentration EDTA (1.0 and 3.0 g/kg) doses, *S. nigrum* presented chlorotic symptoms and showed signs of wilting 2 days after the experiment was initiated. Subsequently, most of them died. Therefore, the EDTA concentrations were lowered to 0.1 and 0.5 g/kg for the next set of experiment. Figure 1 shows influence of EDTA treatments on the height and shoot dry matter yields of *S. nigrum* with 53 days cultivation. As shown in Fig. 1a, the height of plants did not significantly increase with increasing EDTA concentration in soil at the same growing stage. Nevertheless, the 0.1 g/kg EDTA treatment induced a slight increase in the height of plants compared with the control at the three growing stages. Conversely, the 0.5 g/kg EDTA treatment inhibited the height of plants and resulted in a 22%, 6% and 15% of reduction at the seeding, flowering and mature stages, respectively.

Shoot dry biomass of the plants is shown in Fig. 1b. At the seedling and flowering stages, the shoot dry weights were significantly different between the 0.1 and 0.5 g/kg treatment of EDTA ($p < 0.05$). Compared with the control, at the seedling, flowering and mature stages, the level of 0.1 g/kg EDTA enhanced plants growth with 18.4%, 42.1% and 7.9% increase, respectively. However, when the concentration of EDTA was up to 0.5 g/kg, the addition of EDTA was toxic to *S. nigrum*, and the shoot dry weight at the three growing stages suffered a 21.1%, 13.2% and 26.3% reduction, respectively. Specifically, at the flowering stage, aboveground biomass yields began to increase significantly at EDTA level of 0.1 g/kg, reached the maximum of 1.8 g/plant, and showed a 42.1% increase compared with the control. When the concentration of EDTA was 0.5 g/kg, shoot dry biomass was not

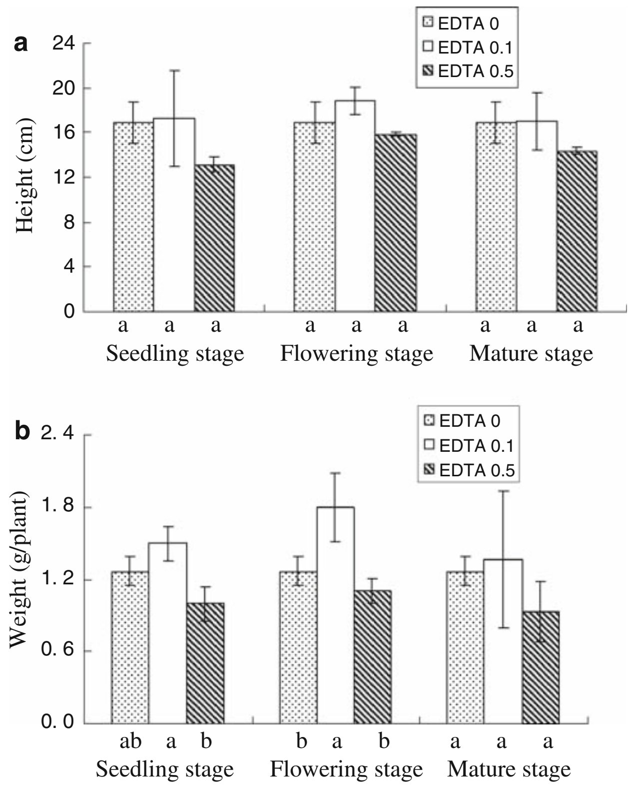

Fig. 1 Influence of EDTA application at three growing stages on growth of *S. nigrum* **a** height (cm), and **b** shoot dry biomass (g/plant)

significantly different from that of the control, but it remarkably decreased compared with that of EDTA level of 0.1 g/kg with a 24% reduction. The results illuminated that moderate dosage of EDTA could enhance plants growth, and it would be helpful for *S. nigrum* to remediate metal-contaminated soils because improvement of plant growth under stressed conditions is critical to the optimum performance of phytoremediation (Belimov et al. 2005).

Much literature demonstrated that addition of synthetic chelators had a significantly adverse effect on plant growth (Chen and Cutright 2001; Grčman et al. 2001; Turgut et al. 2004). The severe reduction in growth was attributed to the combination of heavy metal concentration and chelator addition that exceed the capacity of plants to activated defense systems. Pot experiments showed that NTA and citrate at 10 mmol/kg caused severe toxicity to *B. juncea* seedlings and their death 2 days after chelators amendment, Indian mustard shoot dry weights suffered significant reductions following NTA application (Quartacci et al. 2006). Plant dry matter yield was also significantly affected by the application of chelating agents. Blaylock et al. (1997) demonstrated that plant grown in untreated or the 0.1 mmol/kg treated soil produced nearly twice the biomass of the plants receiving the 10 mmol/kg chelate application. Wang et al. (2006) reported that spiking EDTA adversely affected plant growth, and shoot and root dry biomass significantly decreased compared with the control and reduced with increasing concentration of EDTA in soil. Luo et al. (2006a, b) found the 5 mmol/kg treatments of EDTA and EDDS significantly depressed the growth of *Zea mays* L. and *Phaseolus vulgaris* L. And EDDS appeared to be more toxic to plants than EDTA, as shown by a significantly lower biomass following the addition of EDDS. Interestingly, plants at the combined treatments of EDTA and EDDS exhibited a slight decrease in biomass compared with those at a 5 mmol/kg EDTA treatment. Among the combined treatments of EDTA and EDDS at the ratios of 1:1, 1:2 and 2:1 and the treatment of EDDS alone, there were no significant differences in dry mass yields.

The Cd concentrations in all parts of *S. nigrum* exposed to the single treatment of Cd (CK) and combinations of Cd and EDTA are shown in Table 1. As seen in the table, the contents of Cd in plants were in the order of leave > stem > root > seed, and shoot > root, and the Cd contents in stems, leaves and shoots were more than 100 mg/kg, the threshold level for a Cd-hyperaccumulator (Baker 1981; Baker and Brooks 1989), which was similar to the reports by Wei et al. (2005) and Sun et al. (2007a, b).

As shown in Tables 1 and 2, the addition of EDTA significantly enhanced Cd accumulation and translocation of plants. At the 0.1 and 0.5 g/kg treatments of EDTA, the concentrations of Cd in stems, leaves and shoots showed 17.1%–33.6%, 28.5%–63.3% and 1.6%–28% increase relative to the control at the seedling, flowering and mature stages, respectively. And at the seedling and flowering stages, the contents of Cd in stems and leaves increased markedly under 0.1 and 0.5 g/kg EDTA treatments compared with the control. However, higher EDTA level (0.5 g/kg) restricted Cd uptake and accumulation while comparing with those under the 0.1 g/kg treatment of EDTA at the seedling and mature stages. As a result, the concentrations of Cd in the aboveground parts of plants showed a certain extent reduction. But at the flowering stage, the concentrations of Cd in stems and shoots showed negligible increase.

Table 2 shows that EDTA treatments showed more highly efficient uptake and accumulation of Cd than the control. The BFs and TFs were higher than those of the control plants and more than 1.0, reaching 5.8–7.4, 1.6–2.1, respectively. These ratios suggest that the mechanisms of metal tolerance in these populations and the high metal translocation from the roots to the shoots are vital characteristics for plants to be used in phytoextraction techniques (Baker 1981; Zhou and Song 2004). Among the treatments of EDTA, the 0.1 g/kg EDTA treatment had the best performance in enhancing Cd uptake and accumulation, and the BF and TF values were higher than those at the control and 0.5 g/kg EDTA treatment.

Table 1 Concentrations of Cd in the tissues of *S. nigrum* at three growing stages under different EDTA treatments (mg/kg)

	Treatments of EDTA (g/kg)	Root	Stem	Leaf	Seed/flower	Shoot
Ck	0	109.2 ± 10.7	132.2 ± 55.2	191.0 ± 19.8	84.7 ± 11.5	143.5 ± 13.6
Seedling stage	0.1	86.5 ± 1.9	176.6 ± 9.2	311.9 ± 19.9	56.8 ± 10.1	183.8 ± 10.7
	0.5	92.2 ± 3.2	170.6 ± 43.9	261.5 ± 5.0	40.5 ± 14.0	145.9 ± 12.9
Flowering stage	0.1	81.6 ± 6.4	156.4 ± 34.5	293.9 ± 12.1	62.9 ± 17.0	162.7 ± 12.7
	0.5	88.7 ± 14.1	162.0 ± 38.8	292.0 ± 26.4	54.3 ± 12.7	165.9 ± 25.3
Mature stage	0.1	101.6 ± 35.0	154.8 ± 46.6	279.3 ± 94.9	76.9 ± 12.5	180.7 ± 43.1
	0.5	110.6 ± 15.5	159.0 ± 14.9	245.4 ± 69.6	73.2 ± 16.0	148.7 ± 11.9

Table 2 BF, TF and extraction ratio of Cd in *S. nigrum*

	Treatments of EDTA(g/kg)	Bioaccumulation factors (BFs)	Translocation factors (TFs)	Cd extraction ratio (%)
Ck	0	5.7	1.3	0.29
Seedling stage	0.1	7.4	2.1	0.44
	0.5	5.8	1.6	0.23
Flowering stage	0.1	6.5	2.0	0.46
	0.5	6.6	1.9	0.19
Mature stage	0.1	7.2	1.8	0.40
	0.5	5.9	1.3	0.22

Vassil et al. (1998) speculated that at a threshold concentration, synthetic chelators including EDTA destroy the physiological barrier (s) in root that normally functions to control uptake and translocation of solutes. The plasma membrane surrounding root cells is thought to play a major role in forming this barrier. Therefore, synthetic chelators may induce metal-chelator uptake and accumulation. Chelators have been shown to desorb heavy metals from the soil matrix into the soil solution and make large amounts of heavy metals more phytoavailable. Subsequently, it facilitates Pb transport into xylem, and increases Pb translocation from roots to shoots (Huang et al. 1997). Chen and Cutright (2001) found that EDTA at a rate of 0.5 g/kg significantly increased the shoot concentrations of Cd and Ni from 34 and 15 to 115 and 117 mg/kg, respectively. The total phytoextraction of Cu, Pb, and Zn in the *Chrysanthemum coronarium* L. shoots at the 7th day after chelator application reached 590, 390, and 590 μg/plant, which were 5.2, 38, and 3.5 time of the level in the control group, respectively. Much literature has reported that chelators such as HEDTA and EDTA may enhance the shoot concentration of Pb by more than 100-fold (Huang and Cunningham 1996; Blaylock et al. 1997).

The large amounts of heavy metals accumulated in the shoots of a hyperaccumulator were favorable to shift out metals form soil by harvesting the aboveground parts and to reach the aim of ecological remediation of contaminated soils by heavy metals (Zhou and Song 2004; McGrath et al. 2006). The dominating Cd uptake by *S. nigrum* was in the shoots, up to 86.3%–94.1% of the whole plant (Fig. 2). In

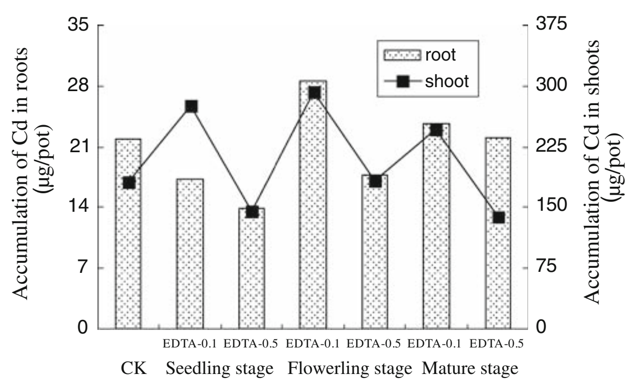

Fig. 2 Accumulation of Cd in *S. nigrum* at three growing stages under different EDTA treatments

this study, EDTA at the 0.1 g/kg rate appeared to be the best treatments, with Cd accumulation in shoot of 275.7, 292.8 and 247.0 μg/pot and highest Cd removal efficiency of 0.44%, 0.46% and 0.40% at the three growing stages, respectively. Conversely, at a higher EDTA treatment (0.5 g/kg), the accumulation and extraction ratios of Cd were lower than those both at the control and 0.1 g/kg EDTA treatment. This was possibly attributed to the severe biomass reduction. Hence, Chen and Cutright (2001) noted that if phytoremediation enhancement with chelators is going to succeed, a strategy that may protect plant biomass from heavy loss is necessary. The enhancement of high tissues concentration as a result of chemical amendment might not necessarily produce high removal efficiency for the target metal contaminant since biomass change is another determining factor (Chen and Cutright 2001; Jiang et al. 2003).

Conclusion

In this study, the application of EDTA had a positive effect on Cd bioavailability and enhanced the Cd uptake. EDTA at the 0.1 g/kg rate appeared to be the best addition and resulted in an enhancement from 1.0 to 1.1-fold in height and from 1.1 to 1.4-fold in shoots dry biomass of plants compared with the control, and it facilitated Cd accumulation in the shoots of plants with a 51.7%, 61.1% and 35.9% increase at the seeding, flowering and mature stages, respectively. Especially, at the flowering stage, the maximum of height, shoot biomass and Cd accumulation of plants were obtained. However, higher EDTA treatment (0.5 g/kg) resulted in severe loss of plant height and biomass. As a result, the accumulation of Cd in shoots was reduced by 19.7% and 23.6% compared with the control at the seedling and mature stages. In summary, the appropriate dosage of EDTA was utilized at the flowering stage of *Solanum nigrum* L. would be more suitable for in situ phytoextraction of Cd-contaminated soils.

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