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# Enhancement of Lead Uptake by Hyperaccumulator Plant Species *Sedum alfredii* Hance Using EDTA and IAA

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Lead (Pb) is considered as one of the most frequently encountered heavy metals of environmental concern, causing loss of vegetation, groundwater contamination, and toxicity in plants, animals, and humans (Clistenes et al. 2006). Because of its persistent nature, Pb will continue to be an environmental concern for a long time unless it is removed from the ecosystem.

Conventional cleanup technologies are generally too expensive to be used for restoration of contaminated soils, and are often harmful to the normal properties of soil (Holden 1989). The emerging phytoremediation techniques, with their low cost and environmental friendly nature, have received increasing attention in the last decades (Salt et al. 1998). Over 400 hyperaccumulating plant species from all over the world can accumulate high concentrations of metals from contaminated soils (Baker et al. 2000). However, there are no reliable reports on Pb hyperaccumulating species under natural conditions, presumably since the phytoavailability of Pb is restricted by the strong complexes of Pb within solid soil fractions. To overcome this problem and increase Pb availability to plants, chelators have been used to artificially enhance Pb solubility in soil solution (Kos and Lestan 2004). Many studies have been conducted using synthetic chelators such as EDTA or HEDTA, which are added to Pb contaminated soils to promote metal translocation from roots to shoots (Huang et al. 1997; Vassil et al. 1998). Although several studies have reported elevated metal concentrations in shoots after applying chelators, most of the times it was correlated to a severe decrease in plant biomass. Therefore other amendments have also been used to increase the heavy metal uptake as well as to maintain better plant growth during chelate-assisted phytoextraction technologies. Lopez et al. (2005) studied the combined effects of EDTA and IAA on Pb uptake by *Medicago sativa* (alfalfa) and found that the combination of EDTA and IAA dramatically enhances the Pb uptake from root to shoot as compared to those treated with Pb alone and Pb/EDTA.

*Sedum alfredii* Hance grows in old Pb/Zn-mined areas of southeast China and has been reported to be a Zn/Cd hyperaccumulator (Yang et al. 2002; Yang et al. 2004), and was also proved to be a Pb-accumulating species (He et al. 2002). Previous studies were mainly focused on the Pb accumulation and transportation mechanism in *S. alfredii* (Long et al. 2006; Yang et al. 2006), and less attention was paid to the application of chelate-assisted phytoextraction techniques.

In this study, a promising method was used to investigate the uptake of Pb by two ecotypes of *S. alfredii* growing under hydroponic conditions. The specific objective of this study was to evaluate the effect of indole-3-acetic acid (IAA), a growth-promoting auxin combined with ethylene diamine tetra acetic acid (EDTA), a synthetic chelating agent, to enhance the Pb uptake ability of *S. alfredii* in hydroponic culture.

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**Table 1** Biomass production in hyperaccumulating (HE) and non-hyperaccumulating (NHE) ecotypes of *S. alfredii* under different treatments

| Treatment (μM) | Shoot dry weight (g plant$^{-1}$) | | Root dry weight (g plant$^{-1}$) | | Shoot/Root ratio | |
|---|---|---|---|---|---|---|
| | HE | NHE | HE | NHE | HE | NHE |
| 200 Pb (CK) | 0.846$^{a}$ | 0.482$^{ab}$ | 0.044$^{b}$ | 0.027$^{b}$ | 19.083 | 18.088 |
| 200 Pb + 1 IAA | 0.844$^{a}$ | 0.473$^{ab}$ | 0.051$^{ab}$ | 0.032$^{ab}$ | 16.651 | 14.771 |
| 200 Pb + 10 IAA | 0.868$^{a}$ | 0.517$^{ab}$ | 0.053$^{ab}$ | 0.033$^{ab}$ | 16.371 | 15.827 |
| 200 Pb + 100 IAA | 0.914$^{a}$ | 0.626$^{a}$ | 0.060$^{a}$ | 0.036$^{a}$ | 15.318 | 17.239 |
| 200 Pb + 200 EDTA | 0.883$^{a}$ | 0.462$^{b}$ | 0.044$^{b}$ | 0.024$^{b}$ | 20.068 | 18.973 |
| 200 Pb + 200 EDTA + 1 IAA | 0.887$^{a}$ | 0.549$^{ab}$ | 0.049$^{ab}$ | 0.034$^{ab}$ | 17.973 | 16.317 |
| 200 Pb + 200 EDTA + 10 IAA | 0.960$^{a}$ | 0.573$^{ab}$ | 0.051$^{ab}$ | 0.037$^{a}$ | 18.824 | 15.636 |
| 200 Pb + 200 EDTA + 100 IAA | 0.924$^{a}$ | 0.627$^{a}$ | 0.054$^{ab}$ | 0.037$^{a}$ | 17.012 | 17.109 |

Different letters indicate significant differences ($P < 0.05$) among the treatments and CK within one ecotype

## Materials and Methods

The hyperaccumulating ecotype (HE) of *S. alfredii* was collected from an old Pb/Zn-mined area in Zhejiang province of China, and the non-hyperaccumulating ecotype (NHE) of *S.alfredii* was obtained nfrom a tea garden of Hangzhou in Zhejiang province of China. Healthy and equal-sized shoots of both ecotypes were chosen and grown for two weeks in the Hoagland solution. After pre-culturing for 14 days, the plants were transferred to 2.5 L pots. The composition of nutrient solution was modified; $KH_2PO_4$ concentration was adjusted to 0.025 mM in order to prevent precipitation of Pb, then exposed to the treatments (He et al. 2002). Eight treatments were used: (1) Pb (CK); (2) Pb/1 μM IAA; (3) Pb/10 μM IAA; (4) Pb/100 μM IAA; (5) Pb/EDTA; (6) Pb and EDTA/1 μM IAA; (7) Pb and EDTA/10 μM IAA; (8) Pb and EDTA/100 μM IAA. In each case, EDTA concentrations were 200 μM and source of Pb used was $Pb(NO_3)_2$. All the chemicals were procured in analytical grade from China Chemical Factory, Shanghai, China; IAA and EDTA from Sigma Aldrich, USA (purity ≥ 99%). The experiment was randomly arranged with three replications for each treatment. Plants were grown under glasshouse conditions with natural light, day/night temperature of 25–30°C and day/night humidity of 70–90%. Nutrient solution pH was adjusted daily to 5.5 with 0.1M NaOH or 0.1M HCl. The nutrient solution was continuously aerated and renewed with treatments every four days. The experiment was terminated after 12 days of treatment. At harvest, root morphological parameters such as root length, surface area, diameter, and volume were determined with a root automatism scan apparatus (Min Mac, STD1600$^{+}$), equipped with WinRHIZO software manufactured by Regent Instruments Co. Different plant parts were separated and thoroughly rinsed with de-ionized water, oven-dried at 70°C for 72 h, weighed, ground with stainless steel mill, then passed through 0.1 mm nylon sieves used for Pb analysis. About 0.1 g of the plant sample was digested using the $HNO_3/HClO_4$ digestion method. The digested solutions were washed into 50 ml flasks and made up to volume using de-ionized water. The plant Pb concentrations were determined using ICP-MS (Agilent 7500a) having detection limits of 0.01 ppb to 4.0 ppm for Pb.

Statistical analysis was performed using the SPSS statistical package (version 11.0). All values reported in this work are means of at least three independent replications. Data were tested at significant levels of $P < 0.05$ by one way ANOVA.

## Results and Discussion

After addition of 200 μM Pb, EDTA and IAA had no effects on shoot biomass of both ecotypes of *S. alfredii* (Table 1), which might be due to the treatment time in this experiment not being long enough to cause significant effects on plant biomass. However, it could be noted that the shoot biomass of HE was always higher than that of NHE, implying that HE plants were more tolerant to Pb stress than NHE ones ($P < 0.05$). Our studies are consistent with studies conducted before (He et al. 2002). The heavy metal uptakes are directly affected by root morphology (Marschner 1995). Earlier studies concerning root morphology of *S. alfredii* were mainly focused on the root length (He et al. 2002), but the other parameters of root morphology under the stress of Pb and EDTA are not reported. Results from the present study suggest that the roots of the HE have a greater ability to tolerate and absorb Pb (Fig. 1). Root length of the HE was significantly increased by 22.3% and 32.6% with IAA treatments of 10 and 100 μM, respectively, as compared with CK ($P < 0.05$) (Fig. 1A). As for the NHE, root length increased significantly with the 100 μM IAA treatments, and was always lower than that of HE plants (Fig. 1A). The same trend was observed for the

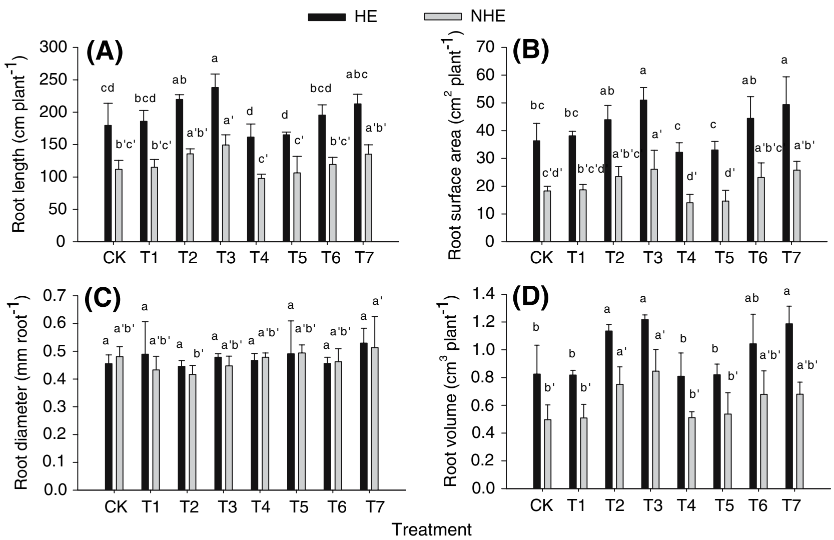


**Fig. 1** Changes in selected root morphological parameters of both ecotypes of *S. alfredii* treated for 12 days. CK, T1, T2, T3, T4, T5, T6 and T7 represent the treatment of Pb (200 μM), Pb (200 μM) + IAA (1 μM), Pb (200 μM) + IAA (10 μM), Pb (200 μM) + IAA (100 μM), Pb (200 μM) + EDTA (200 μM), Pb (200 μM) + EDTA (200 μM) + IAA (1 μM), Pb (200 μM) + EDTA (200 μM) + IAA (10 μM) and Pb (200 μM) + EDTA (200 μM) + IAA (100 μM), respectively. Values are means ± SD ($n$ = 4). Different letters indicate significant differences ($P$ < 0.05) among the treatment and CK.

surface area and volume since in plants treated with 10 or 100 μM IAA, the root morphological parameters increased significantly compared with CK regardless of EDTA addition (Figs. 1B, 1D). However, for root diameter, treatments had no significant effect in either ecotypes of *S.alfredii* as compared to CK ($P$ > 0.05) (Fig. 1D).

Pb uptake in shoots and roots of both ecotypes of *S. alfredii* plants exposed to the different Pb treatments as well as combinations of IAA and EDTA are shown in Table 2. After treatment with 200 μM Pb/100 μM IAA, Pb accumulation in the shoots of both ecotypes of *S. alfredii* increased significantly compared with those treated with Pb alone, but had no effects on the root accumulation (Table 2). It was noted that Pb accumulation in the shoots of HE was always higher than those of NHE in both CK and treatment, which implies that HE plant had stronger Pb-accumulating ability than NHE. It is well established that EDTA acts as a chelating agent that has proven to assist in Pb transportation from roots to shoots (Lopez et al. 2005; Meers et al. 2005; Turgut et al. 2005). In this study, the addition of EDTA in equimolar concentration increased Pb translocation to shoots by 87.1% ($P$ < 0.05). However, IAA and EDTA produced a synergistic effect by dramatically increasing Pb translocation from roots to shoots. The treatment EDTA/10 or 100 μM IAA increased the Pb accumulation in shoots by 149.2% and 243.7% compared with those treated with Pb alone, and by 33.2% and 83.7% compared with Pb/EDTA treatment, respectively. It is well known, that IAA induces the activation of the ATPases in the plasma membrane, producing changes in ion transport

**Table 2** Pb uptake in shoots and roots of both ecotypes of *S. alfredii* under different treatments

| Treatment (μM) | Shoot uptake (μg plant$^{-1}$) | | Root uptake (μg plant$^{-1}$) | |
|---|---|---|---|---|
| | HE | NHE | HE | NHE |
| 200 Pb (CK) | 0.313$^{e}$ | 0.083$^{e}$ | 0.366$^{abc}$ | 0.229$^{ab}$ |
| 200 Pb + 1 IAA | 0.333$^{e}$ | 0.085*e* | 0.391$^{ab}$ | 0.257$^{ab}$ |
| 200 Pb + 10 IAA | 0.389$^{de}$ | 0.137$^{de}$ | 0.374$^{abc}$ | 0.281$^{ab}$ |
| 200 Pb + 100 IAA | 0.498$^{cd}$ | 0.184$^{cd}$ | 0.469$^{a}$ | 0.297$^{a}$ |
| 200 Pb + 200 EDTA | 0.585$^{c}$ | 0.210$^{cd}$ | 0.217$^{d}$ | 0.207$^{b}$ |
| 200 Pb + 200 EDTA + 1 IAA | 0.541$^{cd}$ | 0.254$^{bc}$ | 0.262$^{bcd}$ | 0.263$^{ab}$ |
| 200 Pb + 200 EDTA + 10 IAA | 0.779$^{b}$ | 0.291$^{ab}$ | 0.245$^{cd}$ | 0.277$^{ab}$ |
| 200 Pb + 200 EDTA + 100 IAA | 1.075$^{a}$ | 0.351$^{a}$ | 0.263$^{bcd}$ | 0.286$^{a}$ |

Different letters indicate significant differences ($P$ < 0.05) among the treatments and CK within one ecotype through the membrane, and maybe plants are able to accumulate more cations by active transport in membranes with added IAA. Lopez et al. (2005) reported that the combination of 100 μM IAA/200 μM EDTA by *Medicago sativa* (alfalfa) increased the Pb accumulation in leaves by about 2800% and by about 600%, as compared to Pb content in leaves of plants exposed to Pb alone and those cultivated with Pb/EDTA, respectively. However, in the present study, synergistic effect by the combination of EDTA and IAA is not strong as compared with prior reports, which maybe due to the different uptake ability between two kinds of species under the treatment.

In this study, our results show that there is a synergistic effect produced by IAA in combination with EDTA on the Pb uptake by *Sedum alfredii* Hance. This method could be considered as a feasible technique for the cleanup of Pb contaminated sites. Further experiments are required in order to gain insights into the mechanisms involved in Pb uptake using plant hormones.

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