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EDTA-enhanced phytoremediation of lead contaminated soil by *Bidens maximowicziana*

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Abstract

Phytoremediation is a potential cleanup technology for the removal of heavy metals from contaminated soils. *Bidens maximowicziana* is a new Pb hyperaccumulator, which not only has remarkable tolerance to Pb but also extraordinary accumulation capacity for Pb. The maximum Pb concentration was 1509.3 mg/kg in roots and 2164.7 mg/kg in overground tissues. The Pb distribution order in the *B. maximowicziana* was: leaf > stem > root. The effect of amendments on phytoremediation was also studied. The mobility of soil Pb and the Pb concentrations in plants were both increased by EDTA application. Compared with CK (control check), EDTA application promoted translocation of Pb to overground parts of the plant. The Pb concentrations in overground parts of plants was increased from 24.23–680.56 mg/kg to 29.07–1905.57 mg/kg. This research demonstrated that *B. maximowicziana* appeared to be suitable for phytoremediation of Pb contaminated soil, especially, combination with EDTA.

Key words: phytoremediation; *Bidens maximowicziana*; lead; amendment; EDTA

Introduction

Lead (Pb) is a major anthropogenic pollutant and has accumulated in different terrestrial and aquatic ecosystems (Verma and Dubey, 2003). Lead presents potential high risks to human health but the clean up of Pb contaminated soil is one of the most difficult tasks for environmental engineering. Some methods, such as immobilisation or extraction by physicochemical techniques, have an adverse effect on soil structure and require engineering costs (Pulford and Watson, 2003). Phytoremediation has been put forward since the late 1980s, to remove heavy metals from contaminated soil by harvesting the plants without damaging the soil. It has attracted much attention because it is environmentally friendly and relatively cheap (Salt *et al*., 1998; McGrath *et al*., 2002).

Pb has limited solubility in soil and is generally not available for plant uptake due to complexation with organic matter, sorption on oxides and clays or precipitation as carbonates, hydroxides and phosphates (Lim *et al*., 2004). So, the two major limitations to Pb phytoremediation are: the low bioavailability in soil and poor translocation from roots to shoots. The potential of adding chelates to Pb-contaminated soil to increase Pb bioavailability and accumulation in plants has also been investigated (Huang *et al*., 1997; Wasay *et al*., 1998). EDTA had been proved to be a strong and relatively biostable chelating agent that has potential for soil remediation applications (Ghestem and Bermond, 1998; Hong *et al*., 1999; Piechalak *et al*., 2003).

Bidens maximowicziana can accumulate Pb which appeared to be a new hyperaccumulator (Nie *et al*., 2004). Additionally, the *B. maximowicziana* has the characteristics of broad ecological amplitude, large biomass, robust roots, which make it easy to be harvested mechanically. Therefore, there is great potential for using *B. maximowicziana* in the remediation of Pb-contaminated soil and it may also provide a new resource for exploring the biochemical mechanisms of Pb hyperaccumulation and detoxification. The objectives of this research were to determine the tolerance and accumulation ability of *B. maximowicziana* to Pb; to explore EDTA and other amendments for enhancing the phytoremediation Pb contaminated soil.

1 Materials and methods

1.1 Soil sampling and characterization

Soil used in this experiment was obtained from the Pb-uncontaminated area in Beijing. Once collected, the soil was air dried at room temperature, followed by screening with a 2-mm sieve. Soil texture, pH, organic matter were determined according to Tan (1995). Total Pb concentration was measured using an atomic fluorescence spectrometer (Z-6100, Hitachi, Japan) after the soil sample (about 0.1 g) was weighed and digested with a mixture of HNO_3-$HClO_4$-H_2SO_4 (Wei, 1990). The characteristics of the soil are listed in Table 1.

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Table 1 Physical and chemical characteristics of the soil

pH (in 0.01 mol/L $CaCl_2$)	Organic matter (wt%)	Soil classification (by USDA)	Background Pb concentration (mg/kg)
6.7	1.14	Sandy-loam (sand 69.5%, clay 18.1%, silt 12.4%)	21.45

1.2 Plant material and pot experiment

B. maximowicziana seeds were collected from the channel beside Qinghe Road of Beijing, China. Seeds were presoaked for 5 min in 100% ethanol and washed with sterilized water. Seeds were sprinkled into a moist soil in a seedbed covered with a plastic cling film to maintain moisture. When the spores germinated and grew into small plants with leaves about 2 cm in height, the plant was then transferred and pre-cultured for one week in a modified Hoagland nutrient solution containing (mmol/L): $Ca(NO_3)_2$ 2.0, K_2SO_4 0.75, KCl 0.1, KH_2PO_4 0.25, $MgSO_4$ 0.65, EDTA-Fe(II) 0.1, H_3BO_3 0.01, $MnSO_4$ 0.001, $ZnSO_4$ 0.001, $CuSO_4$ 0.0001, and $(NH_4)Mo_7O_{24}$ 0.000005. Then the plants were transplanted and cultured in the soil with different Pb concentrations of 0, 100, 200, 400, 800, 1000 and 2000 mg/L (lead nitrate) to test its Pb tolerance and Pb accumulation ability. The plant culture was conducted in a growth chamber with the following conditions: 12 h of light period with a light intensity of 4900 $\mu W/cm^2$, temperature of 26°C/18°C (day/night), and average relative humidity of 60%.

1.3 Amendment treatments

EDTA was often used as the model chelator to examine enhanced metal mobilization and distribution of heavy metals within the plant tissues, and citric acid had also been found to be one of the most effective chelators to enhance heavy metal bioaccumulation (Huang *et al*., 1997; Wu *et al*., 1999; Zhou *et al*., 2002). To compare the differences of enhancement ability of amendments, even though NaH_2PO_3 maybe not a suitable chelator, they were all selected and tested in this experiment.

The amendment treatments comprised the following steps: (1) control with no amendment (CK); (2) EDTA disodium salt (EDTA); (3) NaH_2PO_3; (4) citric acid. Air-dried soil equivalent to 1.5 kg (oven dry basis) was placed in each pot (lead nitrate concentration was 0, 100, 200, 400, and 800 mg/L, respectively). EDTA, citric acid and NaH_2PO_3 were added to the pots 50 d after sowing and plants were harvested after 62 d. In every case, EDTA, citric acid and NaH_2PO_3 concentrations were 3.148, 3.148, and 4.006 mmol/kg, respectively. Fresh soil were also collected to determine 1 mol/L NH_4NO_3 extractable and H_2O extractable Pb. And there were three replicates of each treatment.

1.4 Plant harvest and analysis

After 62 d cultivating, the plants were harvested and washed with tap-water followed by three rinses with deionized water. Afterwards, plants were separated in roots, stems and leaves, then dried and ground. The ground sample (about 0.1 g) was weighted and digested with a mixture of HNO_3-$HClO_4$-H_2SO_4. The Pb content in roots, stems, and leaves was measured using an atomic fluorescence spectrometer (Z-6100) (Wei, 1990). There were three replicates of the treatment.

2 Results and discussion

2.1 Pb tolerance and Pb accumulation ability of *B. maximowicziana*

Different Pb level (0, 100, 200, 400, 800, 1000 and 2000 mg/L, respectively) was added to soil to test the tolerance of *B. maximowicziana* to Pb. Initially, there were no obvious symptoms of Pb toxicity found in fronds of *B. maximowicziana* even treated with 2000 mg/L Pb. After 60 d growth, the symptom of dark brown coloration at the tips and the margins of pinnae was observed mainly in the bottom senescent fronds when Pb concentration was 2000 mg/L. This toxicity was not so serious and the plants could survive throughout the whole culture period. This demonstrates that *B. maximowicziana* has a strong tolerance to Pb, but that high Pb concentration could also harm growth.

The concentrations of Pb in tissues (leaves, stems and roots) increased progressively with the increase of Pb supply (Table 2). When the soil Pb concentration was 400 and 800 mg/L, compared with control, the Pb concentration in tissues increased dramatically, increase extent reached 90.97% and 79.68%, respectively. But from 1000 to 2000 mg/L, increase extent was only 2.92%. The maximal Pb accumulation concentration reached 1509.3 mg/kg in roots and 2164.7 mg/kg in overground parts (leaves and stems) when soil Pb concentration was 2000 mg/L.

Compared with other plants, *B. maximowicziana* could accumulate greater amount of Pb. For example, when the soil Pb concentration was 400 mg/L, the Pb concentration in shoots of *Aneurolepidium chinense*, *Gnaphalium polycaulon* and *L. Medicogo* sativa were only 73.15, 82.61 and 389.61 mg/kg, respectively, but those in shoots of *B. maximowicziana* reached 617.87 mg/kg high.

Table 2 Concentration of Pb in *B. maximowicziana* (mg/kg (mean±SD))

Soil Pb conc. (mg/L)	0	200	400	800	1000	1500	2000
Root	13.2±5.32	217.35±51.54	381.65±42.97	748.59±74.27	1429.3±61.75	1465.3±83.74	1509.3±98.15
Stem	10.6±2.56	310.24±16.04	592.48±13.45	1064.6±91.32	1756.7±80.28	2016.8±62.66	2075.7±94.7
Leaf	11.2±8.15	336.83±69.72	643.26±97.02	1155.8±83.21	1907.2±103.55	2189.7±120.19	2253.7±100.53
Shoot	10.9±6.23	323.54±42.35	617.87±58.62	1110.2±83.25	1831.9±94.23	2103.3±87.54	2164.7±105.23
TF	0.826	1.49	1.62	1.48	1.28	1.44	1.43

TF: transfer factor.

The ratio between Pb concentration in overground parts and roots, defined as TF (transfer factor) was used to measure the effectiveness of a plant in translocating Pb from root to overground parts. From Table 2, TF value was greater than 1 in all Pb concentration, and TF value reached maximum (1.62) when soil Pb concentration was 400 mg/L. The Pb distribution order in the *B. maximowicziana* was: leaf > stem > root. These may indicate that *B. maximowicziana* may have special Pb transfer mechanism which could transfer absorbed Pb to frond's overground parts.

In general, *B. maximowicziana* could accumulate a large amount of Pb. And the Pb distribution order in the *B. maximowicziana* was: leaf > stem > root. It was suitable for phytoremediation of Pb-contaminated soil.

2.2 Effect of chelator on the plants growth

There were no obvious symptoms of chelator toxicity in *B. maximowicziana* during growth after EDTA, citric acids and NaH_2PO_3 addition into the soil. The influence of different chelator on the biomass of *B. maximowicziana* is shown in Fig.1.

The biomass of *B. maximowicziana* was increased then decreased with the increasement of Pb concentration after adding EDTA, citric acid and NaH_2PO_3, and the biomass of plant get its least value when soil Pb concentration was 800 mg/L. Compared with CK, there was no clear effect of the chelator on growth of plant. It meant that Pb with low concentration could accelerate the growth of *B. maximowicziana*, which was not coincided with other researchers' results, such as the addition of EDTA causes great physiological damage to *B. juncea* (Collins *et al.*, 2002).

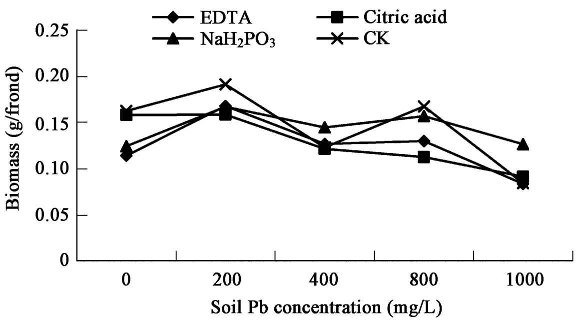

Fig. 1 Influence of chelator on the biomass of *B. maximowicziana*.

2.3 Effects of chelator on mobile fraction of Pb on soil

Pb is readily adsorbed by soil colloids, hence its mobility is always very low, and this is considered to be the main factor restricting the phytoremediation of Pb-contaminated soil. The addition of synthetic chelator could enhance phytoremediation, which significantly increased the Pb mobility and elevated the extractability of Pb in soil. To study the effects of chelator on mobile fraction of Pb on soil, NH_4NO_3 and H_2O were used to extract the Pb in this experiment. The results are shown in Fig.2.

When soil Pb concentration was 800 mg/L, NH_4NO_3 extractable Pb in non amendments soil after plant growth was only 17.29 mg/kg. But when 3.15 mmol/kg of EDTA was added, EDTA addition significantly increased soil NH_4NO_3 extractable Pb to 91.56 mg/kg, almost a six-fold increase compared with the CK. However, soil NH_4NO_3 extractable Pb did not increase remarkablly when citric acid was applied at the same level. On the contrary, NaH_2PO_3 even decreased the concentrations of Pb in the roots and overground parts of *B. maximowicziana*. It was inferred that EDTA application significantly increased the mobility of soil Pb, its ability stronger than citric acid and NaH_2PO_3. The ability order was: EDTA > citric acid > NaH_2PO_3. Similar results were also found when H_2O was used as extractant (Fig.2b).

2.4 Effect of amendments on phytoremediation

Effect of amendments on phytoremediation is showed in Fig.3. EDTA application significantly increased the concentrations of Pb in the roots of *B. maximowicziana*, whereas the citric acid also shows some effect, but it is more less. Pb concentrations in over ground parts was also increased by EDTA, especially at Pb concentrations (1000 mg/L). Although overground parts Pb accumulation was also enhanced by the addition of citric acids, the increases were smaller. Compared with CK, NaH_2PO_3 decreased the

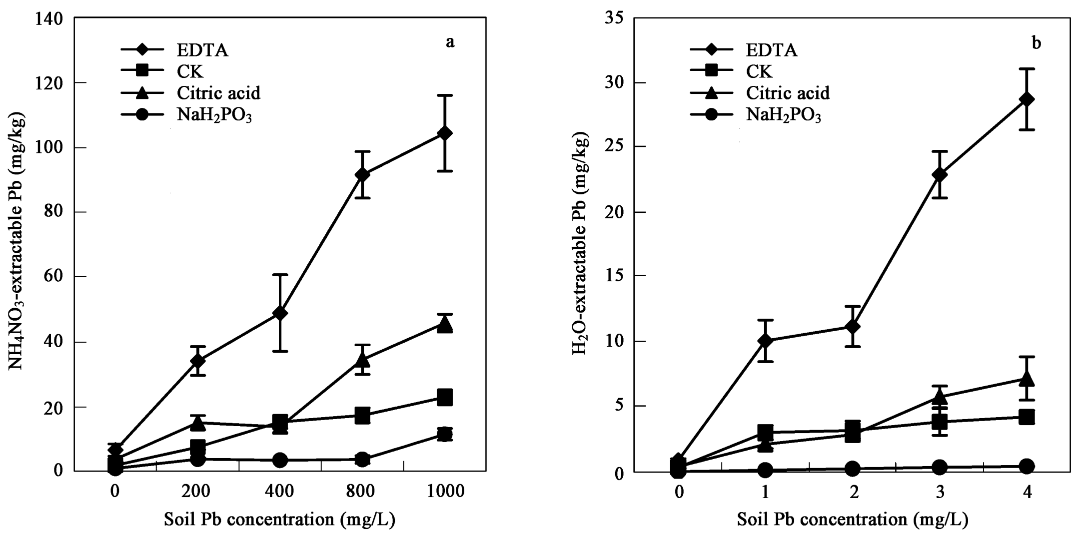

Fig. 2 Amount of NH_4NO_3-extractable Pb (a) and H_2O-extractable Pb.

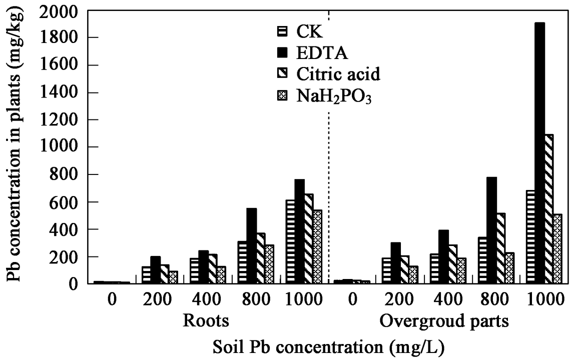

Fig. 3 Pb concentrations in roots and overground parts of *B. maximowicziana*.

concentrations of Pb in the roots and overground parts of *B. maximowicziana*. It could be concluded that the mobility of soil Pb and the Pb concentrations in plants were both increased significantly by EDTA application.

Additionally, Pb was also promoted by EDTA to translocate from roots to overground parts. Compared with CK, the Pb concentrations in overground parts of *B. maximowicziana* increased from 24.23–680.56 mg/kg to 29.07–1905.57 mg/kg by applying EDTA at soil Pb concentration (200–1000 mg/L). The reason may be assumed that P-type ATPases are responsible for the translocation of both necessary (e.g., Cu^{2+}, Zn^{2+}, Mn^{2+}) and nonessential metals (e.g., Cd^{2+}, Pb^{2+}, Hg^{2+}) through the biological membranes (Ghestem and Bermond, 1998; Rensing *et al.*, 1998; Williams *et al.*, 2000). EDTA may induce the activation of the ATPases in the plasma membrane producing changes on transport of ions through the membrane. And possibly EDTA also regulates a protein membrane which is related to Pb transport function. Thus, Pb can easily be translocated from roots to overground parts through prevention of cell wall retention.

Above all, the addition of EDTA significantly enhanced the Pb concentration in tissues of B. *maximowicziana*. And a lot of Pb could also be promoted by EDTA to translocate from roots to overground parts. Thus, EDTA-enhanced phytoremediation might be an adequate technique for the Pb-contaminated soil remediation.

3 Conclusions

The *B. maximowicziana* had a strong tolerance and accumulation ability to Pb. The Pb distribution order in the *B. maximowicziana* was: leaf > stem > root. EDTA amendment is more effective in assisting phytoremediation of Pd from contaminated soil than citric acid and NaH_2PO_3 amendment. The mobility of soil Pb and the Pb concentrations in plants were both increased significantly by EDTA application. EDTA could greatly promote Pb to translocate from roots to overground parts.

Using of such chelators and the potential of such a process to enhance the mobility of metals in soil also caused the related risk of leaching to ground water. EDTA tends to form metal complexes with high stability constants. Metal-EDTA complexes were leached into the soil pore water and were found to be relatively biologically stable even under conditions favorable for biodegradation (Hong *et al.*, 1999). Although EDTA can facilitate metal transport from contaminated soil to plant, it may also cause damage to plant and increase soil acidity.

In conclusion, *B. maximowicziana* may be suitable for the remediation of Pb contaminated soil without the application of high amounts of EDTA, thereby reducing the environmental risk from persistent metal-chelate complexes and it may provide a new resource for exploring the biochemical mechanisms of Pb hyperaccumulation and detoxification.

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