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Effects of exogenous chelators on phytoavailability and toxicity of Pb in *Zinnia elegans* Jacq.

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Abstract

Chelate-enhanced phytoremediation is considered as an effective method for the extraction of lead (Pb) by plants. However, more detailed studies are needed to evaluate the effect of exogenous chelators on phytoavailability and toxicity of Pb in plants, then to find out the proper applied concentration of chelators to minimize the combined toxicity to the plants and maximize phytoavailable Pb. To clarify these questions, the seed germination test of *Zinnia elegans* Jacq. exposed to solutions containing Pb and four types of chelators including sodium ethylenediamine tetra-acetic acid (Na_2EDTA), oxalic acid, tartaric acid and citric acid was observed. The results showed that the roots and shoots treated with equimolar chelators and Pb were longer than those treated with half and two folds of the molar concentrations of Pb. The growth of seedlings was inhibited by surplus addition of chelators, and the toxicity of complexes was less than that of Pb and chelators. In particular, 2.4 mM EDTA and 1.2 mM oxalic acid significantly ($P < 0.05$) increased Pb uptake when the seeds were treated with 2.4 mM Pb. In the 4.8 mM Pb solution, Pb accumulation in the seedlings was markedly ($P < 0.05$) increased by 4.8 mM EDTA, 2.4 mM tartaric acid, 4.8 mM tartaric acid and 2.4 mM citric acid, and amounted to 6752.4, 6453.8, 6541.4 and 6598.3 $\mu g\, g^{-1}$, respectively. With the superfluous addition of chelators, Pb accumulation in the seedlings decreased in a concentration-dependent manner. When Pb was used at 2.4 mM, an equimolar concentration of EDTA not only increased Pb uptake but also stimulated the seedling growth. Thus, chelating agents in their appropriately concentrations could counteract Pb toxicity, but superfluous chelators resulted in less Pb uptake and growth inhibition of the seedlings.

Keywords: Chelator; Lead; Phytoremediation; Uptake; Germination test

1. Introduction

Lead (Pb) contamination in different ecosystems has run to serious degree due to industrial processes, mining activities, metallic smelting, coal combustion, automotive exhaust fumes, domestic utilization of Pb-based paints, land application of municipal wastewater and sludge, agricultural use of chemical fertilizers and insecticides, as well as waste disposal in landfills [1], which results in many ecological problems and severe damage to biosphere, especially to human health. Therefore, it is an urgent and challenging task to remediate Pb-contaminated sites with appropriate remediation methods.

Unfortunately, traditional techniques of remediation are costly and may cause secondary pollution. Phytoremediation, using green plants to remove, destroy or sequester hazardous substances from environment, is a cost effective and environmentally friendly alternative [2,3]. However, this process sometimes depends upon the bioavailability of toxic metals in the media and the plant capacity to absorb these contaminants [4]. Some researchers have found that chelators can form chemical complexes with heavy metals and improve their solubility, thereby making them more readily available for plant uptake. Therefore, chelator-enhanced phytoremediation is considered as a suitable method for the extraction of Pb by plants [5–7].

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As for auxiliary agents for metal uptake, the most widespread chelating agent is EDTA [4]. Many researches have demonstrated that the ability of EDTA to increase the solubility of Pb is especially strong, and the uptake of Pb by plants is promoted correspondingly [8–13]. Laboratory studies also show that low molecular weight organic acids, such as citric, oxalic, tartaric and malic acids [14,15], can solubilize heavy metals through complexation reactions [16]. They are capable of forming complexes with metals, which can affect their fixation, mobility and availability to plants [5], and can also be used as chelators.

However, the addition of chelating agents may cause side effects, for example, the uncertain toxicity of complexes with heavy metals. Therefore, more detailed studies are needed to separately evaluate the toxic effects of chelators, heavy metals and their complexes, and find out the proper applied concentration of chelators to minimize the combined toxicity to plants and maximize phytoavailable Pb.

In this work, the seed-germination experiment was conducted to clarify how chelators may affect the toxicity and phytoavailability of Pb in seedlings, then to select suitable chelators and appropriate concentration, which can enhance the absorption of Pb and reduce the toxicity. *Zinnia elegans* Jacq. was chosen as the testing plant for the seed-germination experiment. It was demonstrated in our previous study [17] that *Z. elegans* Jacq., a widely used ornament flower, had high tolerant ability to Pb and could be used in phytostabilization, its seeds germinate very quickly and the seedlings grow rapidly.

2. Materials and methods

In order to simulate severe Pb-contaminated conditions, Pb concentrations used in this experiment were 800 mg L^{-1} (2.4 mM) and 1600 mg L^{-1} (4.8 mM), which were expressed as Pb^{+} and Pb^{2+}, respectively. Lead was added as $Pb(NO_3)_2$, and the tested chelators were Na_2EDTA (E), tartaric acid (TA), oxalic acid (OA), and citric acid (CA). The molar concentrations of the tested chelators were half, one and two folds of the molar concentrations of Pb, respectively (Table 1). All reagents used in the study were analytical grade and purchased from the Shenyang Second Chemical Factory.

The seeds of *Z. elegans* Jacq., commonly called zinnia, were bought from the Huarong Institute of Horticulture, in Chifeng. Twenty sterilized seeds were scattered on two layers of filter paper in a culture dish. The mixed solution of $Pb(NO_3)_2$ and one of the chelators, at three concentrations each, were supplied to each culture dish. Distilled water treatment was also conducted as control. To investigate the toxic effects of $Pb(NO_3)_2$ and chelators themselves, solutions containing only $Pb(NO_3)_2$ and chelators were also applied to the seeds. All treatments were replicated trebly to minimize experimental errors.

The petri dishes were placed in a climate-controlled chamber (light/dark: 16/8 h, 20/14 °C) for one week. Afterwards, *Z. elegans* Jacq. seedlings were collected from each dish and washed with distilled water prior to use. After measuring the length of roots and shoots, the seedlings were oven-dried at 105 °C for 30 min, then maintained at 70 °C to a constant weight. The dried tissues obtained were weighed and ground into powder for the determination of metal concentration. Metal (Pb) analysis was carried out by acid [87% HNO_3 + 13% $HClO_4$] [18] digestion followed by measurement of total concentrations of all elements of interest, using an atomic absorption spectrophotometer (AAS, Hitachi 180–80). Zinnia seedlings from one petri dish were provided and considered as a single sample, and three replicated experiments were performed. All the data from the experiment were statistically analyzed using the one-way ANOVA procedure of the SPSS 11.0 statistical package.

Table 1
Component and concentration of the mixed solutions treated with chelators and $Pb(NO_3)_2$

Treatment		Concentration		pH
		Pb^{+}	Pb^{2+}	
$Pb(NO_3)_2$		2.4 mM (800 mg L^{-1})	4.8 mM (1600 mg L^{-1})	5.24
Na_2EDTA	E1	1.2 mM		4.25
	E2	2.4 mM	2.4 mM	4.37
	E3	4.8 mM	4.8 mM	4.43
	E4		9.6 mM	4.46
Tartaric acid	TA1	1.2 mM		2.94
	TA2	2.4 mM	2.4 mM	2.66
	TA3	4.8 mM	4.8 mM	2.38
	TA4		9.6 mM	2.15
Oxalic acid	OA1	1.2 mM		2.92
	OA2	2.4 mM	2.4 mM	2.41
	OA3	4.8 mM	4.8 mM	2.24
	OA4		9.6 mM	2.15
Citric acid	CA1	1.2 mM		2.86
	CA2	2.4 mM	2.4 mM	2.56
	CA3	4.8 mM	4.8 mM	2.36
	CA4		9.6 mM	2.16

3. Results

3.1. Chelating effects on Pb phytoavailability

Fig. 1 shows Pb accumulation in the seedlings treated with various chelators in the presence of Pb. In the Pb (Pb^{+}, Pb^{2+}) treatments without chelators, the accumulation of Pb in seedlings amounted to 2313.6 $\mu g\,g^{-1}$ and 5049.5 $\mu g\,g^{-1}$, respectively.

As shown in Fig. 1, in the Pb^{+} treatments, E1, E2, E3, TA1, TA2, OA1 and CA1 increased Pb uptake by zinnia seedlings, especially for E2 (3283.3 $\mu g\,g^{-1}$) and OA1 (3245.1 $\mu g\,g^{-1}$), which were significantly ($P < 0.05$) higher than that in the Pb^{+} treatments without chelators. However, Pb accumulation in the seedlings decreased in a concentration-dependent manner with superfluous addition of chelators, it is maybe a toxic effect by the redundant chelating agents. In the TA3-, OA2-, OA3-, CA2- and CA3-Pb^{+} treatments, Pb accumulation in the seedlings was lower than that in the Pb^{+} control, but no treatment significantly reduced Pb absorption.

In the Pb^{2+} treatments, E2, E3, TA2, TA3, TA4 and CA2 increased Pb accumulation in the seedlings, especially in E3, TA2, TA3 and CA2, which were significantly ($P < 0.05$) higher

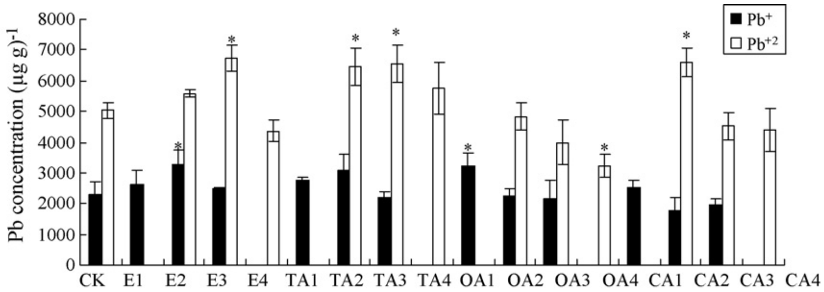

Fig. 1. Pb concentrations in zinnia seedlings treated with different chelators. CK: control without chelators; labels for identities and concentrations of chelators are explained in Section 2; Pb^{+} and Pb^{2+} mean treatments containing 2.4 mM and 4.8 mM of Pb, respectively. Significant differences between the control and each treatment are indicated by one asterisk (*) for $P < 0.05$. Error bars represent standard deviation values from triplicate cultures.

than the Pb^{2+} control and amounted to 6752.4, 6453.8, 6541.4 and 6598.3 μg g^{-1}, respectively. On the other hand, more chelators resulted in less Pb uptake in the seedlings. The E4-, OA2-, OA3-, OA4-, CA3- and CA4-Pb^{2+} treatments showed the opposite effects, however, only the OA4 treatment significantly decreased the Pb content in the seedlings.

3.2. *Chelating effects on Pb toxicity*

3.2.1. *Root length*

The length of zinnia roots responded differently to the combined toxicity of various chelators with and without Pb (Fig. 2). In the Pb- (no lead) control, the roots were about 4.2 cm long, but the roots were only 1.6 and 1.1 cm long in the Pb^{+} and Pb^{2+} control, respectively. The results indicated that the high concentration of Pb in solution severely inhibited the development of seedling roots. As shown in Fig. 2, the root growth was improved in the E1, E2 and TA1-only treatments, moreover, the root length was significantly ($P < 0.01$) longer than that in the control in E1-only treatment, whereas adding more chelator, stronger toxicity was exhibited. In the E3, E4, TA3, TA4, OA2, OA3, OA4 and CA4 treatments, chelators themselves obviously inhibited the growth of zinnia roots, which means that excessive chelators would affect the root elongation and were toxic to seedling growth.

In the Pb^{+} treatments, E2 ($P < 0.01$), OA2 ($P < 0.05$) and OA3 ($P < 0.05$) significantly improved root growth, and the roots were 2.8, 2.2 and 2.1 cm long, respectively, indicating their considerable protection against the toxicity of Pb to root development. The results illustrated that the presence of organic chelators with the proper dose could lessen the toxicity of Pb. In the E1- ($P < 0.01$) and TA1-Pb^{+} ($P < 0.05$) treatments, the roots were significantly shorter comparing with the Pb^{+} control, they were 0.6, 1.1 and 1.0 cm long, respectively, and the plant tissues were soft and limp.

In treatments with chelating agents and Pb^{2+}, most roots were significantly shorter than the Pb^{2+}-only treatment ($P < 0.01$). The E3, TA2, TA3, TA4 and OA4 treatments had no significant effects on root growth, but roots were still slightly shorter than the control. Therefore, the exogenous chelating agents appeared to hinder root elongation, and enhanced the toxicity of solution when Pb is present in especially high concentrations.

The proportion between chelators and Pb is an important factor that can affect root elongation and the combined toxicity to seedling growth. In the treatments with equimolar chelators and Pb, the roots were longer than those in the half and two folds of the molar concentrations of Pb treatments.

3.2.2. *Shoot length*

As shown in Fig. 3, effects of various chelators and Pb on the shoot length were similar to the root length; however, shoots were not as sensitive as roots in response to the toxicity of Pb. Taking the Pb^{+} and Pb^{2+} treatments without chelators for instance, the root length decreased to 38 and 26% of the control, but the shoot length only decreased to 83 and 76%, respectively. The results indicated that the high concentration of Pb also inhibited shoot growth, but slighter than root growth. As shown in Fig. 3, the shoot length was all longer than the control in EDTA-only treatments, especially for the E1, E2-only treatments, which improved the shoot elongation significantly ($P < 0.01$). The CA-

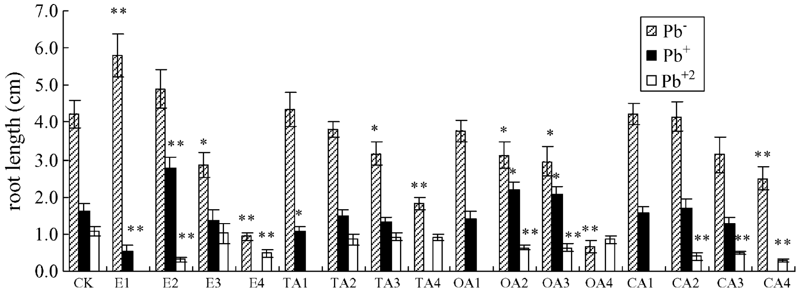

Fig. 2. Root length of zinnia seedlings germinated in each treatment. CK: control (without added chelators, with or without Pb); Pb-: no added lead treatment; other abbreviations are as in Fig. 1. Symbols “*” and “**” indicate the significant difference between the control and corresponding treatments with chelators, at $P < 0.05$ and $P < 0.01$, respectively. Error bars represent standard deviation values from triplicate cultures.

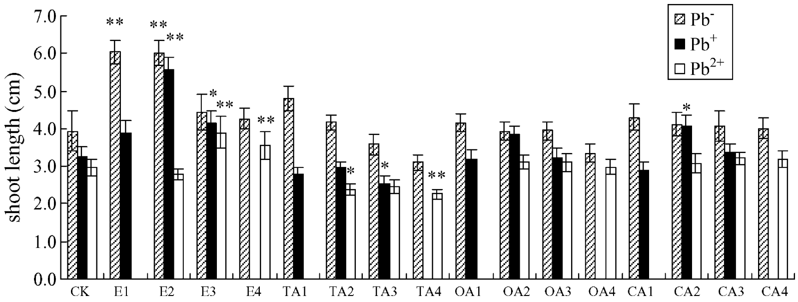

Fig. 3. Shoot length of zinnia seedlings germinated in each treatment. Abbreviations are as in Fig. 2. Symbols "*" and "**" indicate the significant difference between the control and corresponding treatments with chelators, at $P < 0.05$ and $P < 0.01$, respectively. Error bars represent standard deviation values from triplicate cultures.

only treatments slightly increased the growth of shoots. Only the TA3, TA4 and OA4 treatments decreased the shoot length. Shoot growth was inhibited by overfull addition of chelators, but was not as obvious as root growth.

In the Pb^+ treatments, EDTA, OA2, CA2 and CA3 improved shoot growth. The shoots treated with E2 ($P < 0.01$), E3 ($P < 0.05$) and CA2 ($P < 0.05$) were 5.6, 4.1 and 4.1 cm long respectively, which were significantly longer than the control, indicating their significant protection against the toxicity of Pb to shoot development. In the TA3 and Pb^+ treatments, the shoots (2.5 cm long) were significantly ($P < 0.05$) shorter than those in the Pb^+ control.

In the Pb^{2+} and chelating treatments, the shoots were significantly longer than the Pb^{2+} control in E3 and E4 treatments ($P < 0.01$), but significantly shorter than those in the TA2 ($P < 0.05$) and TA4 ($P < 0.01$) treatments. As for the OA and CA treatments with Pb, this had no significant effects on shoot elongation (Fig. 3), and the shoot length was almost the same as in the Pb^{2+}-only treatment.

The proportion between chelators and Pb is also a main factor that affects shoot elongation and seedling growth. In the treatments with equimolar chelating agents and Pb, the shoots were longer than those in the half and two folds of the molar concentrations of Pb treatments. Chelators complexed proportion with Pb caused the discrepant results. Similarly, chelating agents appear to hinder zinnia shoot growth in higher concentrations, but mitigate Pb toxicity when present in appropriate concentrations.

4. Discussion

EDTA is effective in enhancing the mobility of Pb through the plant and increasing the translocation of Pb from roots to leaves. As shown in Fig. 1, the E2-Pb^+ and E3-Pb^{2+} treatments significantly increased Pb uptake by zinnia seedlings. Our results demonstrated that EDTA could enhance Pb absorption by the seedlings in both lower and higher contents of Pb. Haag-Kerwer et al. [19] reported that about 80% of the total metal was solubilized and available for phytoremediation when EDTA was applied. López et al. [4] found that the addition of EDTA at equimolar concentration to the hydroponics medium containing Pb increased Pb translocation to leaves by about 300%. Khodadoust et al. [20] also confirmed that EDTA and organic acids were effective in removing Pb.

In the CA2-Pb^{2+} treatments, Pb accumulation in the seedlings was significantly higher than that in each own control. Similarly, Khodadoust et al. [20] found that 1 M citric acid was effective for the removal of Pb. Vandenhove and Hees [21] reported that the addition of citric acid (25 mmol kg^{-1}) one week before the harvest increased U uptake up to 500 folds.

On the other hand, Meers et al. [22,23] reported that citric acid induced no significant effects on heavy metal uptake. Similar results were found in the CA2-, CA3-Pb^+ and CA3-, CA4-Pb^{2+} treatments. Although Pb accumulation in seedlings was slightly lower than that in the control, no treatment reached a significant degree. The results indicated that citric acid could improve the absorption of Pb, but higher concentrations of citric acid result in less Pb uptake in the seedlings.

In view of the effects of the four chelators on phytoavailability and toxicity of Pb, EDTA was more effective and less toxic. EDTA and TA exhibited strong ability on enhancing Pb uptake by seedlings. Considering the length of roots and shoots, the toxicity of Pb treated by EDTA was less than that by TA, CA and OA. Sun et al. [24] found that biomass increased more or less by proper application of organic chelators, and EDTA was the most effective of organic chelators. Piechalak et al. [25] reported that the addition of EDTA eliminated the inhibition of root elongation, lowered browning of roots and resulted in a growing number of side roots.

Toxicity and bioavailability are generally used synonymously when one speaks of heavy metal species [26]. However, they are not always equivalent since different plants, pollutant types and levels will make different metal uptake. Treatment with E2 and Pb^+ not only enhanced the phytoavailability of Pb, but also reduced its toxicity to roots, which is giving an evidence for the inconsistency between toxicity and phytoavailability. The result was similar to that of Inaba and Takenaka [26] who found that lettuce treated with Cu and dissolved soil extract solution accumulated much more Cu than the Cu-only treated seedlings, and the visible effects of Cu were less severe.

The proportion between chelators and Pb is an important factor affecting the combined toxicity to the growth of seedlings due to their different complexed proportion with Pb. The entirely complexed proportions between Pb and E, TA, CA, OA were 1:1, 1:1, 1:1 and 3:2, respectively. In the treatments with equimolar chelators and Pb, the roots were longer than those in the half and two folds of the molar concentrations of Pb treatments. Taking the EDTA-Pb^+ treatments for instance, the solution was composed of complex and surplus Pb ions in the E1 treatment. EDTA and Pb were completely complexed in the E2 treatment. In the E3 treatment, the solution was composed of complex and surplus EDTA. The roots and shoots in E2 were longer than those in E1 and E3, so the toxicity of the entirely complexed treatments was less than that of superfluous Pb or chelators treatments. The results indicate that the toxicity of the complexes is less than that of Pb and the chelators, and superfluous Pb or chelators can inhibit the growth of the seedlings. The similar phenomena were reported for organic ligands of Ni, Al and Cd, the free metal ions were more toxic compared with organically complexed molecules [5–7].

As shown in Figs. 2 and 3, the roots were more sensitive than the shoots to the toxicity of the treatments. Jarvis and Leung [27] found that Pb supplied in the unchelated form accumulated predominantly in root tissues, and Pb chelated with either HEDTA or EDTA was transported principally to the needles in *Pinus radiata*, which probably is the reason why the roots exhibited stronger inhibition than the shoots.

5. Conclusions

The primary objectives of this work were to evaluate the effect of exogenous chelators on toxicity and phytoavailability of Pb in seedlings, and then to select suitable chelators and appropriate concentration, which can enhance the absorption of Pb and reduce the toxicity to plants. Based on the results mentioned above, E2 and OA1 significantly increased Pb uptake in the Pb^{+} treatments. In the Pb^{2+} treatments, E3, TA2, TA3 and CA2 significantly increased Pb accumulation in the seedlings. With the superfluous addition of chelators, the content of Pb in the seedlings decreased in a concentration-dependent manner. As for the combined toxicity of Pb and chelators, in the treatments with equimolar chelators and Pb, the roots and shoots were longer than those in the half and two folds of the molar concentrations of Pb treatments. Chelating agents appeared to counteract the toxicity of Pb when presented in appropriate concentrations, but superfluous chelators hindered zinnia growth and resulted in less Pb uptake in the seedlings. In conclusion, 2.4 mM EDTA was the optimal choice in the Pb^{+} treatments, which not only increased the uptake of Pb, but also stimulated the growth of the seedlings. Chelators could improve Pb accumulation in the Pb^{2+} treatments but had no effects on reducing the toxicity of Pb. In application, we should control the proportion between chelators and Pb, improve the phytoremediation efficiency and reduce the possible toxicity. Moreover, the optimal time of application should be evaluated seriously in future.

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