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Effects of EDTA on Mechanism of Lead Accumulation in *Typha orientalis Presl*

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Abstract The effects of EDTA on the mechanism of accumulation under Pb^{2+} stress in *Typha orientalis Presl* were investigated. The results indicated that addition of the chelator with Pb limited metal phytotoxicity. The mean total chlorophyll concentration and protein content were increased with the addition of EDTA in Pb^{2+} solution. We also demonstrated a significant effect of EDTA on the reduced glutathione (GSH) content which was obviously increased both in leaves and caudices. It was supposed that EDTA elevated the tolerance of *Typha orientalis Presl* under Pb^{2+} stress primarily by increasing the GSH level.

Keywords EDTA · Chlorophyll · Glutathione (GSH) · Pb^{2+}

Lead is one of the most frequently encountered heavy metals in polluted environments. The primary sources of this metal include mining and smelting of metalliferous ores, burning of leaded gasoline, disposal of municipal sewage, and industrial wastes enriched in Pb as well as using of Pb-based paint (Seaward and Richardson 1990). Lead is also one of the most persistent metals with a soil retention time of about 150–5,000 years in the environment (Shaw 1990). Severe Pb contamination may cause a variety of environmental problems, including loss of vegetation, groundwater contamination, and Pb toxicity in plants, animals, and humans (Huang et al. 1997).

Phytoremediation is of growing interest because of its low environmental impact and cost-effectiveness, even if a longer time is required for treatment (Shivendra and Natalie 2002). In addition to natural plant adaptation, the addition of synthetic chelators and soil acidifiers can enhance phytoremediation (Li et al. 2008). Several studies documented the success of pH adjustments for mobilizing metals (Salt et al. 1998). Although soil acidification increased metal mobility, it decreased the microbial activity of the surrounding area (Cornish and Goldberg 1995; Chen 2000). Only the addition of synthetic chelators has been shown to increase both the metal mobility within the soil as well as the uptake through the plant tissue without being irreversibly toxic to microbial activity (Huang et al. 1997). Huang et al. further reported that EDTA was the most efficient in the effectiveness in increasing Pb accumulation in both pea and corn.

The main aim of this research was to investigate the effects of Pb^{2+} on the concentration of total chlorophyll, chlorophyll a (chla), chlorophyll b (chlb) and soluble protein content and reduced glutathione (GSH) concentration, as well as the protective role of EDTA on these parameters in both leaves and caudices of *Typha orientalis Presl*, and to offer referenced evidence for an understanding of the mechanism by which EDTA ameliorated the damage to plants by heavy metals and improved the phytoremediation efficiency.

Materials and Methods

Caudices *Typha orientalis Presl*, obtained from a mine tailings of Yongzhou City, Hunan province in March 2006,

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were rinsed thoroughly first with tap water and then with distilled water, and were then germinated in the dark at 25°C for 7 days. Seedlings 4–5 cm long were transferred in modified Hoagland nutrient solution in greenhouse maintained at 25°C/18°C day/night with 70%–80% humidity and a 14 h photoperiod at approximately 300 μmol m^{-2} s^{-1}. After growth in the nutrient solution for 21 days, Healthy and equal-sized seedlings were chosen and aseptically transferred to plastic pots (500 mL) containing 300 mL of nutrient solution, which was replace twice a week, and were subjected to 0, 0.15, 0.3 mmol/L Pb^{2+} treatment with or without 0.1 or 0.5 mmol/L EDTA. Each pot was incubated 3 plants and each treatment was replicated 3 times. Leaves and caudices were collected after 7 days simultaneous EDTA and Pb^{2+} treatment and washed in 10 mM $CaCl_2$ for 5 min and then in bi-distilled water. The plant material was frozen in liquid nitrogen and stored at −80°C for further analysis or directly used in determination. The experimental design indicating the different concentrations of Pb^{2+} and EDTA used is outlined in Table 1.

Concentrations of chlorophyll a (chla) and chlorophyll b (chlb) and total chlorophyll (total chl = chla + chlb), were calculated using the methodology developed by Arnon (1949). The protein content was determined according to Bradford (1976). The reduced glutathione (GSH) content was assayed according to the methods of the Nanjing Jiancheng Bioengineering Institute (NJBI). The Pb chemical forms were analyzed by sequential extraction procedure (Yang et al. 1995; Antonovillcs et al. 1971). The reagents and order for the sequential extractions were 80% ethanol, deionized water, 1 mol/L NaCl, 2% (v/v) HAc and 0.6 mol/L HCl. Metal concentrations were determined by flame and atomic absorption spectrometer (Perkin Elmer AAnalyst 700).

The experiments were performed in triplicate and the results were the mean ± SD. Analysis of the significance of difference between control and each treatment was performed using SPSS statistical software (SPSS, Chicago, IL, USA).

Table 1 Experimental design using different concentrations of Pb^{2+} (mmol/L) and EDTA (mmol/L)

Treatment	Pb^{2+}	EDTA
1	0	0
2	0	0.1
3	0	0.5
4	0.15	0
5	0.15	0.1
6	0.15	0.5
7	0.30	0
8	0.30	0.1
9	0.30	0.5

Results and Discussion

The changes of chla and chlb and total chlorophyll are shown in Fig. 1. No negative effect of EDTA presence on the content of chlorophyll was observed. The chlorophyll content in plants grown in a medium with the addition of 0.1 and 0.5 mmol/L EDTA were at the level similar with the plants grown in nutrient solution. With increasing Pb^{2+} concentrations, the content of chla and chlb and total chl all decreased, except in plants treated simultaneously with Pb^{2+} and EDTA. Compared with control, EDTA increased chlorophyll level in leaves, the difference between control and those treated with 0.1 and 0.5 mmol/L EDTA both reaching statistical significance ($p < 0.05$), whereas the chlorophyll content declined quite significantly ($p < 0.01$) following treatment with only Pb^{2+}. The ratio of chla to chlb was increased from 3.626 to 3.921 in control, however, the ratio dropped from 2.940 to 2.412 with the addition of 0.1 mmol/L EDTA, and from 3.032 to 2.312 with the addition of 0.5 mmol/L EDTA (Table 2).

From Fig. 2a, it can be seen that the protein content in both leaves and caudices decreased with an increase in Pb^{2+} in control plants. However, the protein concentration in both leaves and caudices was increased after addition 0.1 or 0.5 mmol/L EDTA and this increase was similar. But EDTA enhanced the protein content greatly in plants grown in only nutrient solution and was much more obvious in caudices following the application of 0.5 mmol/L EDTA. The difference reached statistical significance ($p < 0.05$).

As shown in Fig. 2b, in leaves and caudices of *Typha orientalis Presl* treated only with EDTA the level of GSH remained at the same level as in the plants grown only in nutrient solution and GSH content was positively correlated with the Pb^{2+} and EDTA concentrations. The GSH content increased with increasing concentration of Pb^{2+}, which was much more obvious in caudices than in leaves of control plants and was markedly higher in caudices than in leaves regardless of whether plants were control plants or subjected to EDTA treatment. Compared with control, EDTA could induce a dose-dependent decrease in GSH content both in leaves and caudices, the difference between control plants and those treated with 0.1 or 0.5 mmol/L EDTA reaching statistical significance ($p < 0.05$).

Particular species, and even plant varieties differed as to the tolerance to the harmful effect of heavy metals and their ability to accumulate trace metals (Kabata-Pendias and Pendias 1999). Plant characteristics change according to the environmental conditions and there were several possible areas through which lead could penetrate plants. However, it was commonly that roots were the main pathway through which trace metal ions penetrated plants. It was determined that in plants metal uptake at first

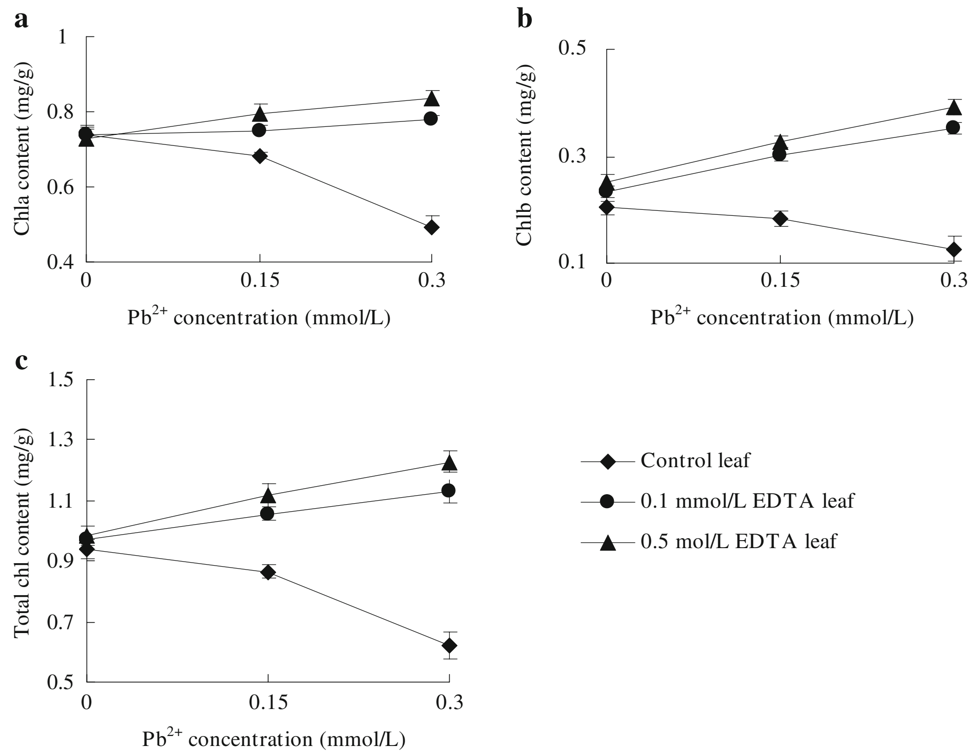

Fig. 1 Effects of EDTA on the concentrations of **a** chla, **b** chlb and **c** total chl in leaves of *Typha orientalis Presl* under Pb^{2+} stress. FW, fresh weight

Table 2 Mean ratios of chla to chlb concentration in all treatments

Treatment	0	0.15 mmol/L Pb^{2+}	0.3 mmol/L Pb^{2+}
0	3.626	3.727	3.921
0.1 mmol/L EDTA	2.940	2.479	2.412
0.5 mmol/L EDTA	3.032	2.436	2.312

stopped on root surface and then a portion of ions which penetrated roots was bound in cell walls and the rest was accumulated in the intercellular space (Malone et al. 1974; Wierzbicka 1987). Our earlier study has also shown that most of Pb uptake was accumulated in the roots.

EDTA application made it possible to raise the level of metal translocation to aboveground and Pb accumulation (Huang et al. 1997). It was demonstrated that Pb was taken up form the medium or the soil in the form of EDTA complexes and in this form it entered the plant more easily (Vassil et al. 1998; Wu et al. 1999).

Total chlorophyll concentration was a unifying parameter for indicating the effect of specific interventions. However, it was important to record changes in the two components of chlorophyll, chlorophyll a (chla) and chlorophyll b (chlb) and especially their ratio. This was due to the fact that heavy metals could affect each component at different level creating changes in some part of plants physiology and not in others. Figure 1 suggested that the chlorophyll concentration increased with addition of 0.1 or 0.5 mmol/L EDTA. In Fig. 1c the mean total chlorophyll concentration was reduced from 0.939 mg/kg fresh weight to 0.62 mg/kg f.w. when treated with only Pb. This could be an indication of some inhibition of growth. However, the chlorophyll concentration was increased after adding EDTA.

According to Gadallah (1994); Drazkiewicz (1994); Abdel-Basset et al. (1995); Sharma and Gaur (1995) and Ewais (1997), changes in the concentration of chl a and b and particularly changes in their ratio were an equal important parameter, which should always been taken under consideration when estimating the effect of an environmental parameter in plants. Many studies agreed that heavy metals accumulation, responsible for the reduction of total chlorophyll concentration, had a similar negative affect in the ratio of chla to chlb. This occurs due to a faster hydrolysis ratio of chla compared with chl b when plants are under stress (Schoch and Brown 1987; Drazkiewicz 1994; Abdel-Basset et al. 1995). Table 2 presents the mean ratios of chla to chlb in all treatments. Additionally monitoring total chl concentration and chla to chlb ratio can be used as an early warning system for the toxic effect of metals accumulation in plants.

Soluble protein content was another parameter for indicating phytotoxicity. In control, the mean protein content was reduced from 46.581 mg/kg fresh weight to 36.351 mg/kg f.w. in leaves, a probable evidence of inhibition. In caudices, it was reduced from 38.922 mg/kg f.w. to 32.636 mg/kg f.w.. Whereas, the soluble protein content

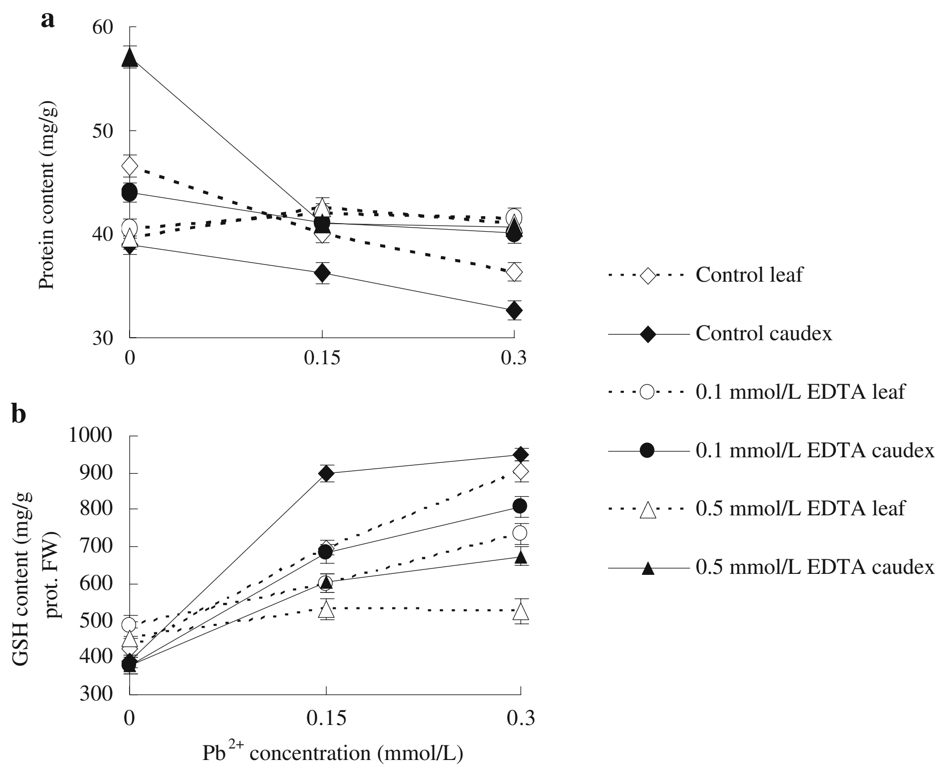

Fig. 2 Effects of EDTA on the contents of **a** soluble protein and **b** reduced glutathione (GSH) in *Typha orientalis Presl* seedlings under Pb^{2+} stress. FW, fresh weight

was significantly increased when 0.1 and 0.5 mmol/L EDTA were applied.

In our experiments the application of EDTA at the concentration of 0.1 or 0.5 mmol/L together with Pb ions into medium resulted in a significant limitation of the metal phytotoxic effect. This concerned inhibition of root elongation growth, root coloring, and the appearance of mucus. There were no literature data on EDTA effect on the metabolism of thiol compounds in treated plants. Based on the presented results it could be said that the level of glutathione changes depend on the intensity of a stress factor. Glutathione synthesized intensely in leaves was most probably transported to the roots and it was used as a metal chelator (Mehra and Mulchandani 1995; Piechalak et al. 2000), a substrate for the synthesis of phytochelatins and as an antioxidant (Noctor et al. 1998). A steady increase in glutathione level was observed after cultivation of *Typha orientalis Presl* with the addition of $Pb(NO_3)$ and EDTA. % and % more GSH were determined in caudices and leaves of plants incubated with 0.15 mmol/L Pb and 0.1 or 0.5 mmol/L EDTA respectively. 0.3 mmol/L Pb were % and % more GSH respectively.

The highest tolerance of *Typha orientalis Presl* cultivated with the addition of Pb-EDTA could be associated with the increased capacity of the GSH de-toxic system. It was highly probable that the higher amount of glutathione contributed to lower Pb phytotoxicity, which increased chlorophyll content and protein content in comparison with plants cultivated only with lead nitrate (Figs. 1, 2). De-toxic system was also connected with the emission of exudates into the rhizosphere, binding metals in cell walls, chelating ion of metals by organic acids or free aminoacids and many other mechanisms, with which plants defend themselves against phytotoxicity of heavy metals.

The toxic effect of heavy metals had an essential significance not only for plants but also for the existence of the whole ecosystem. Plants growing in a contaminated environment showed disturbances of cell metabolism, growth reduction, lower biomass production etc. simultaneously, these plants had also a number of various defense mechanisms enabling them to adapt to increasing environmental pollution. Plant adaptive abilities were the subject of intense research aimed at finding applications for such plants in phytoremediation.

In the present study, applying EDTA obviously elevated chlorophyll and protein content, especially GSH level, in both leaves and caudices. In conclusion, EDTA could effectively improve the tolerance of *Typha orientalis Presl* to Pb^{2+} stress by elevating antioxidant GSH levels.

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