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# Changes of root morphology and Pb uptake by two species of *Elsholtzia* under Pb toxicity*

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**Abstract:** *Elsholtzia argyi* and *Elsholtzia splendens*, which are Chinese endemic Pb/Zn mined and Cu mined ecotype respectively, were investigated on the aspect of their response to Pb toxicity in the presence or absence of EDTA addition. After 8 d's Pb treatment, root length, root surface area and root volume of *E. splendens* decreased much more than those of *E. argyi*, and reduced considerably with increase of Pb, while no marked change was noted for root average diameter. Compared to *E. argyi*, length of root with diameter ($D$)<0.2 mm was significantly reduced for *E. splendens* as Pb increased. $D$<0.1 mm *E. splendens* root had cross-sectional surface area at Pb≥10 mg/L, while for *E. argyi*, it was at Pb≥25 mg/L. With increase of Pb, DW of *E. splendens* decreased much more than that of *E. argyi*. *E. argyi* exhibited much more tolerance to Pb toxicity than *E. splendens*. Treatment with 100 mg/L Pb plus 50 mmol/L EDTA significantly decreased the length and surface area of $D$≤0.2 mm root, increased the length and surface area of 0.2≤$D$≤0.8 mm root for the case of *E. argyi*, while for *E. splendens*, length and surface area of $D$<0.6 mm root reduced, as compared to 100 mg/L Pb treatment, alone. At 100 mg/L Pb, shoot Pb accumulation in *E. splendens* and *E. argyi* were 27.9 and 89.0 μg/plant DW respectively, and much more Pb was uptaken by the root and translocated to the stem of *E. argyi* as compared to *E. splendens*. Treatment of the plant with 100 mg/L Pb plus 50 mmol/L EDTA increased leaf Pb accumulation from 16.8 to 84.9 g/plant for *E.splendens* and from 18.8 to 52.5 g/plant for *E. argyi*, while both root and stem Pb pronouncedly reduced for both *Elsholtzia* species. The increased translocation of Pb to the leaf of *E. splendens* being than that of *E. argyi* after treatment with 100 mg/L Pb plus 50 mmol/L EDTA should be further investigated.

**Key words:** EDTA, *Elsholtzia*, Pb, Phytoremedation, Root morphology
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## INTRODUCTION

Mining, smelting activities, disposal of municipal sewage and industrial wastes, together with usage of leaded gasoline, lead to widespread Pb pollution. The toxic effects of Pb rests mainly in its ability to react with functional groups such as sulfhydryl, carboxyl and amine, leading to a decrease or loss of activity of many enzymes that are important for cell functions. Pb is a kind of heavy metal with neurovirulent properties that animals and human are very sensitive to it. More than 100 μg/L Pb in children's blood will adversely affect their growth and development, and overdose of Pb may cause kidney problems and high blood pressure in adults. There is considerable interest recently in the use of terrestrial plants as a green biotechnology for remediation of surface soils contaminated by toxic heavy metals. One aspect of phytoremediation is phytoextraction, in which plants extract heavy metals from the soil and concentrate them in their harvestable shoot tissues (Salt *et al.*, 1995). The success of phytoextraction depends upon the identification of suitable plant species that hyperaccumulate heavy metals and produce

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large amounts of biomass. In general, plants, which accumulate more than 1000 mg/kg Pb, are termed as Pb-hyperaccumulators (Baker and Brooks, 1989). An example of such plants is *Thlaspi rotundifoliun*, which reportedly can accumulate shoot Pb concentrations of 130−8200 mg/kg, mean of 1100 mg/kg (Reeves and Brooks, 1983). However, this plant species, like other Pb-hyperaccumulating species reported in the literature, is not suited for phytoextraction of Pb from contaminated soils because of its slow growth rate and small biomass (Scott and David, 1996). *Elsholtzia argyi* was found in an old Pb/Zn mining area in Zhejiang Province of China and can grow up to 160 cm in height with large shoot biomass. The volatile constituents in the flowers of *E. argyi* point to a possible utilization of plant resources after phytoremediation (Peng and Yang, 2005). Because Pb availability in soil is very minimal, chelators such as EDTA or HEDTA (N-(2-hydroxyethyl)-ethylene diaminetriacetic acid) had been used to enhance phytoextraction of Pb from contaminated soil due to the increased $H_2O$-soluble Pb concentrations in soil (Blaylock *et al.*, 1997). For instance, the enhanced shoot Pb concentration from 40 to 10600 mg/kg was observed upon addition of HEDTA one week after transplanting, and Pb translocation rate from root to shoot increased from 0.2 to 1.2 (Huang and Cunningham, 1996). *Elsholtzia splendens*, endemic Cu tolerant and accumulating plant species native to China (Yang *et al*., 1998; Song *et al*., 2004), belongs to the same family as that of *E. argyi*. This study aimed at comparing the root morphological and growth response of *E. argyi* and *E. splendens* under Pb toxicity in the presence or absence of EDTA addition to examine the uptake and translocation of Pb in *E. argyi* and *E. splendens* induced by EDTA addition.

## MATERIALS AND METHODS

Seeds of *E. argyi* and *E. splendens*, collected from plants growing on the Sanmen Pb/Zn mined area and Zhuji Cu mining area of Zhejiang Province, respectively, were surface sterilized, rinsed, and sown in substrate having a combination of perlite+vermiculite in 3:1 ratio and moistened with distilled water. After emergence of seedlings, one-fourth basal nutrient solution was supplied until 6-leaves seedlings appeared, then the uniform plants were selected and transferred to hydroponics culture for another 2 weeks pre-culture in a full-strength aerated nutrient solution (in μmol/L): 2000 $KNO_3$, 50 KCl, 500 $Ca(NO_3)_2{\cdot}4H_2O$, 200 $MgSO_4{\cdot}7H_2O$, 100 $NH_4NO_3$, 10 $KH_2PO_4$, 12 $H_3BO_3$, 2.0 $MnSO_4{\cdot}H_2O$, 0.5 $ZnSO_4{\cdot}7H_2O$, 0.2 $CuSO_4{\cdot}5H_2O$, 0.1 $Na_2MoO_4$, 0.1 $NiSO_4$, 20 Fe-EDTA. Two weeks later, different Pb treatments: 0, 10, 25, 50, 100, 200 mg/L Pb, and 100 mg/L Pb+0.5 mmol/L EDTA (Jarvis and Leung, 2001) were conducted, with Pb supplied as $Pb(NO_3)_2$. The experiment was randomly arranged with each treatment replicated three times. Plants were grown under glasshouse conditions with natural light, day/light temperature of 28/15 °C, and day/light humidity of 70%/90%. The nutrient solution was continuously aerated and renewed after every 4 d, pH was adjusted at 5.5±0.3 daily with 0.1 mol/L HCl or 0.1 mol/L NaOH.

The plants were harvested after 8 d's Pb treatment, some roots of intact plants were rinsed with distilled water, then root length, root surface area, root diameter and root volume were determined by root automatism scan apparatus (MIN MAC, STDI600$^+$), equipped with WinRHIZO software offered by Regent Instruments Company (USA). Other roots were immersed in 0.5 mmol/L $Na_2$EDTA for 30 min to remove putative absorbed $Pb^{2+}$. Root, stem and leaf were separated, washed with distilled water, and oven-dried at 65 °C. Dry weight (DW) of different plant tissues were recorded. Samples of the plant dried materials were ground with a stainless steel mill and passed through a 60-mesh sieve, then ashed at 550 °C for 12 h, and dissolved in 10 ml 1:1 (V:V) $HNO_3$. Lead concentrations in the plant digests were determined by AAS (Shimaduz, AA-6800).

All data were presented as mean values of at least three replicates. SPSS statistical software package (Version 11.0) was used. One-way ANOVA was employed to determine whether the means were significantly different at $P<0.05$.

## RESULTS

### Effects on growth and dry weight of plant

Eight days' Pb treatment caused notable difference between the growth of *E. splendens* and *E. argyi* (Fig.1). For example, dry weight (DW) of both root and shoot of *E. splendens* signifcantly decreased at Pb≥10 mg/L, while that of *E. argyi* were noted at Pb≥25 mg/L, and DW increased with increasing Pb. DW of root, stem and leaf of *E. splendens* decreased faster than that of *E. argyi*. Treatment with 100 mg/L Pb plus 50 mmol/L EDTA pronouncedly reduced DW of root, stem and leaf of both *Elsholtzia* species, as compared to 100 mg/L Pb treatment, alone.

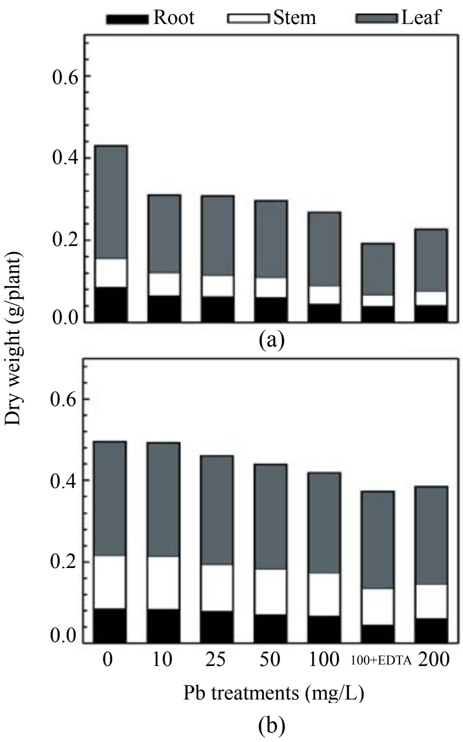


**Fig.1 Dry weight of both *Elsholtzia* species exposed to Pb with and without 50 mmol/L EDTA additon for 8 d's plant growth. Data are means of three replications, and bars depict Standard error (SE) (a) *E. splendens*; (b) *E. argyi***

## Effects on root morphology of plant

Pb>10 mg/L significantly decreased root elongation of *E. splendens*, while for *E. argyi*, it was noted at Pb>50 mg/L. And the root length of *E. splendens* decreased faster than that of *E. argyi* (Fig.2a). The length of root with diameter ($D$)≤0.6 mm for both *Elsholtzia* was the main root length for all the Pb treatments, accounting for more than 90 % of the total (Table 1). Pb treatment affected mostly the length of 0.2≤$D$≤0.6 mm root for the case of *E. argyi*, while for *E. splendens*, it was the length of 0≤$D$≤0.6 mm root. The length of $D$≤0.2 mm root of *E. splendens* decreased rapidly, while that of *E. argyi* decreased slightly, when the plants were exposed to Pb for 8 d. Treatment with 100 mg/L Pb plus 50 mmol/L EDTA significantly decreased the length of $D$≤0.2 mm root and increased the length of 0.2≤$D$≤0.8 mm root of *E. argyi*, while for *E. splendens*, length of $D$<0.6 mm root reduced, as compared to 100 mg/L Pb treatment, alone.

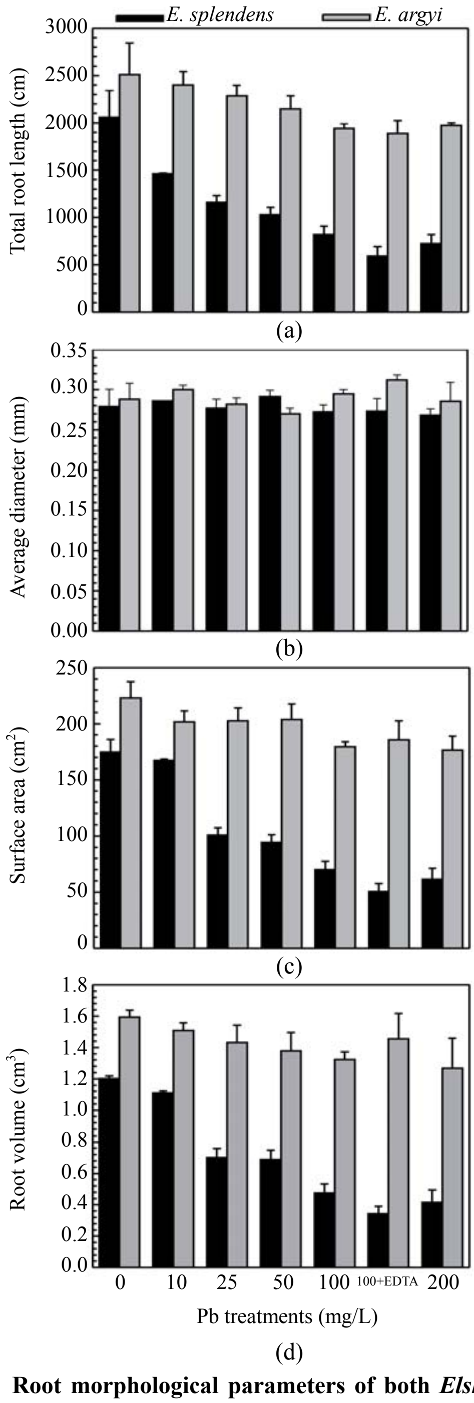


**Fig.2 Root morphological parameters of both *Elsholtzia* species exposed to Pb with and without 50 mmol/L EDTA addition for 8 d's plant growth. Data are means of three replications, and bars depict SE (a) Total root length; (b) Average diameter; (c) Surface area; (d) Root volume**

**Table 1 Root length of different diameter for both *Elsholtzia* species exposed to Pb treatments (cm)**

| Treatment (μmol/L) | | 0.0<*D*≤0.2 | 0.2<*D*≤0.4 | 0.4<*D*≤0.6 | 0.6<*D*≤0.8 | 0.8<*D*≤1.0 | 1.00<*D* |
|---|---|---|---|---|---|---|---|
| *E. argyi* | CK | 1419.75±132.55 | 935.58±97.30 | 228.73±7.25 | 77.07±4.93 | 36.99±0.73 | 41.14±4.74 |
| | 10 | 1068.45±160.26 | 811.16±32.43 | 227.56±16.36 | 78.59±5.56 | 35.09±4.47 | 48.04±12.89 |
| | 25 | 792.74±75.08 | 550.95±60.52 | 149.30±48.63 | 48.48±10.36 | 22.99±5.37 | 34.91±8.46 |
| | 50 | 1220.83±76.21 | 764.02±45.60 | 170.16±28.67 | 61.86±5.58 | 30.17±3.94 | 41.71±5.36 |
| | 100 | 1092.90±48.32 | 577.53±64.59 | 141.01±4.48 | 56.81±3.31 | 29.36±0.65 | 51.43±1.52 |
| | 100+EDTA | 744.23±48.55 | 790.39±55.50 | 218.29±25.17 | 64.96±11.92 | 28.93±6.37 | 40.16±8.35 |
| | 200 | 1195.30±65.33 | 662.39±69.79 | 150.71±30.43 | 70.52±8.32 | 35.80±4.67 | 60.14±1.17 |
| *E. splendens* | CK | 1337.03±93.23 | 555.12±35.34 | 232.11±10.05 | 63.11±1.21 | 28.79±3.63 | 26.30±5.83 |
| | 10 | 765.82±17.22 | 362.34±11.04 | 121.40±1.28 | 87.43±1.40 | 35.14±1.28 | 88.91±0.50 |
| | 25 | 804.59±25.57 | 247.28±12.62 | 74.52±12.32 | 36.06±8.06 | 11.90±2.60 | 20.26±6.54 |
| | 50 | 555.54±34.07 | 275.36±57.88 | 77.74±15.26 | 35.90±8.70 | 11.48±2.09 | 16.38±2.79 |
| | 100 | 528.85±30.52 | 204.28±24.26 | 46.38±12.05 | 20.75±6.22 | 6.23±2.22 | 13.42±3.66 |
| | 100+EDTA | 336.19±9.06 | 100.61±1.39 | 24.00±3.65 | 14.84±4.51 | 5.79±1.70 | 13.13±3.59 |
| | 200 | 466.61±76.29 | 183.12±47.72 | 37.86±7.36 | 19.60±6.51 | 6.22±2.30 | 10.15±6.26 |

Note: *D* indicates root diameter (mm)

Root surface area of the plant directly affects ions in the soil solution uptaken by plant root. Pb≥25 mg/L significantly reduced root surface area of *E. splendens*, while for *E. argyi*, the reduction occurred at Pb≥100 mg/L. And root surface area of *E. splendens* decreased faster than that of *E. argyi* as Pb increased. Treatment with 100 mg/L Pb plus 50 mmol/L EDTA slightly decreased the root surface area of both *Elsholtzia* species, as compared to the treatment with 100 mg/L Pb, alone. Pb≥10 mg/L treatment significantly decreased the surface area of *D*≤1.0 mm root for the case of *E. splendens*, while for *E. argyi*, decrease was found at Pb≥25 mg/L (Fig.2b). Surface area of *D*>1.0 mm root for the case of *E. splendens* reduced with increasing Pb, and decreased slightly for the case of *E. argyi* (Table 2). Treatment with 100 mg/L Pb plus 50 mmol/L EDTA significantly decreased surface area of *D*≤0.2 mm root but increased surface area of 0.2≤*D*≤0.8 mm root for the case of *E. argyi*, while for *E. splendens*, surface area of *D*<0.6 mm root reduced, as compared to 100 mg/L Pb treatment, alone (Table 2).

Slightly changed root average diameter of both *Elsholtzia* species was noted for all the Pb treatments. As compared to 100 mg/L Pb treatment, treatment with 100 mg/L Pb plus 50 mmol/L EDTA slightly changed root average diameter of *E. splendens*, but markedly increased root average diameter of *E. argyi* (Fig.2c).

Root volume is an important parameter for assessing the root physiological function. Pb≥10 mg/L significantly reduced root volume of *E. splendens*. With increasing Pb, root volume of *E. splendens*. was reduced much fast than that of *E. argyi*, which slightly changed with increasing Pb. Treatment with 100 mg/L Pb plus 50 mmol/L EDTA reduced root volume of *E. splendens*, while increased root volume of *E. argyi*, as compared to 100 mg/L Pb treatment, alone (Fig.2d).

**Effects on Pb concentration and accumulation in plant**

Increasing Pb concentration in roots, stems and leaves of both *E. splendens* and *E. argyi* increased accumulation in the plant. More Pb in root and stem of *E. argyi* was noted than in *E. splendens* after Pb≥100 mg/L treatment for 8 d. As compared to 100 mg/L Pb, treatment with 100 mg/L Pb plus 50 mmol/L EDTA significantly decreased root Pb, but markedly increased leaf Pb of both *E. splendens* and *E. argyi*, slightly reduced stem Pb of *E. splendens* and significantly reduced stem Pb of *E. argyi*, with leaf Pb of *E. splendens* increased much more than that in leaf of *E. argyi*, as compared to 100 mg/L Pb treatment, alone (Fig.3).

Pb accumulation in both *Elsholtzia* species was in the order of root>>stem>leaf, and it considerably increased as increasing Pb. After Pb≥50 mg/L treatment for 8 d, more Pb accumulated in the stem and root of *E. argyi* as compared to *E. splendens*. As compared to 100 mg/L Pb treatment, root and stem Pb accumulation decreased considerably, but leaf Pb accumulation increased significantly in both *Elshotzia* species, and leaf Pb was increased much more in *E. splendens* than in *E. argyi*, after treatment with 100 mg/L Pb plus 50 mmol/L EDTA (Fig.4).

## DISCUSSION AND CONCLUSION

Lead is well known to be extremely toxic harmful to plant growth and human health through the food chain. Lead concentration in common plants does not

**Table 2 Root surface areas of different diameter for both *Elsholtzia* species exposed to Pb treatments ($cm^2$)**

| | Treatment (μmol/L) | 0.0<*D*≤0.2 | 0.2<*D*≤0.4 | 0.4<*D*≤0.6 | 0.6<*D*≤0.8 | 0.8<*D*≤1.0 | 1.00<*D* |
|---|---|---|---|---|---|---|---|
| *E. argyi* | CK | 41.26±10.79 | 78.24±10.23 | 38.00±5.63 | 16.86±1.22 | 10.58±0.79 | 21.31±3.05 |
| | 10 | 33.70±6.45 | 74.82±5.55 | 33.72±1.98 | 16.19±1.22 | 9.20±1.20 | 20.50±4.26 |
| | 25 | 29.26±5.83 | 51.51±4.30 | 23.09±3.46 | 10.21±1.84 | 6.03±1.30 | 15.09±2.10 |
| | 50 | 47.86±6.20 | 72.45±9.87 | 26.82±4.35 | 13.92±1.82 | 8.91±1.54 | 19.93±3.17 |
| | 100 | 36.03±4.24 | 60.39±7.93 | 24.36±6.15 | 12.98±1.43 | 8.14±0.63 | 23.55±4.90 |
| | 100+EDTA | 25.80±2.08 | 76.61±5.09 | 32.34±3.89 | 13.70±2.53 | 7.84±1.70 | 19.26±4.80 |
| | 200 | 47.37±3.25 | 59.09±5.12 | 23.82±3.83 | 15.23±1.62 | 10.44±1.43 | 32.65±5.51 |
| *E. splendens* | CK | 47.75±3.79 | 51.93±2.81 | 34.45±1.37 | 13.29±0.27 | 7.81±1.02 | 10.61±2.63 |
| | 10 | 29.01±2.28 | 37.05±1.12 | 19.34±1.05 | 19.36±0.56 | 9.80±0.32 | 9.82±0.02 |
| | 25 | 30.43±4.06 | 25.28±2.65 | 11.87±3.58 | 7.99±1.82 | 3.32±0.73 | 8.34±2.89 |
| | 50 | 20.63±1.34 | 28.62±1.79 | 12.39±2.43 | 7.90±1.93 | 3.20±0.58 | 7.16±1.30 |
| | 100 | 19.78±3.11 | 20.73±4.60 | 7.39±1.92 | 4.59±1.39 | 1.74±0.62 | 6.49±1.70 |
| | 100+EDTA | 12.42±0.39 | 10.15±0.02 | 3.83±0.58 | 3.29±1.01 | 1.61±0.47 | 5.82±1.37 |
| | 200 | 17.46±4.52 | 18.20±1.17 | 6.03±1.42 | 4.32±0.64 | 1.73±0.26 | 4.59±1.56 |

Note: *D* indicates root diameter (mm)

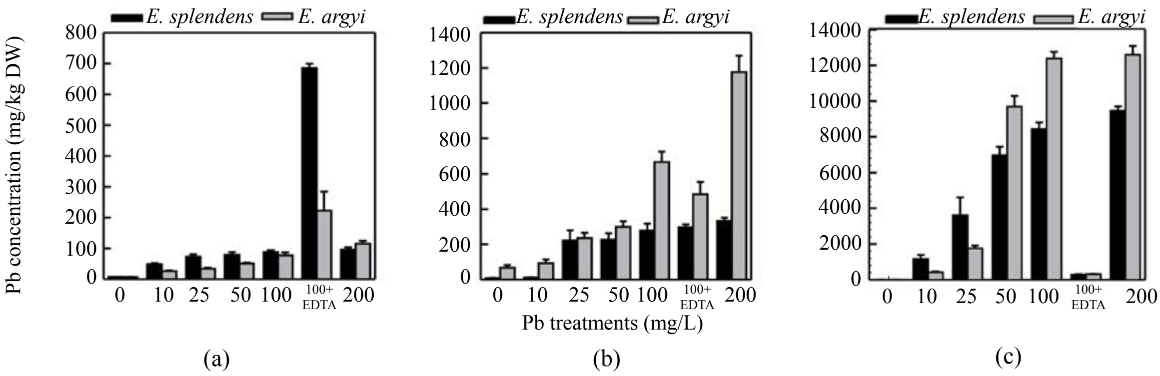


**Fig.3 Lead concentrations in root, stem and leaf of both *Elsholtzia* species exposed to Pb with and without 50 mmol/L EDTA addition for 8 d's growth. Data are means of three replications, and bars depict SE (a) Leaf; (b) Stem; (c) Root**

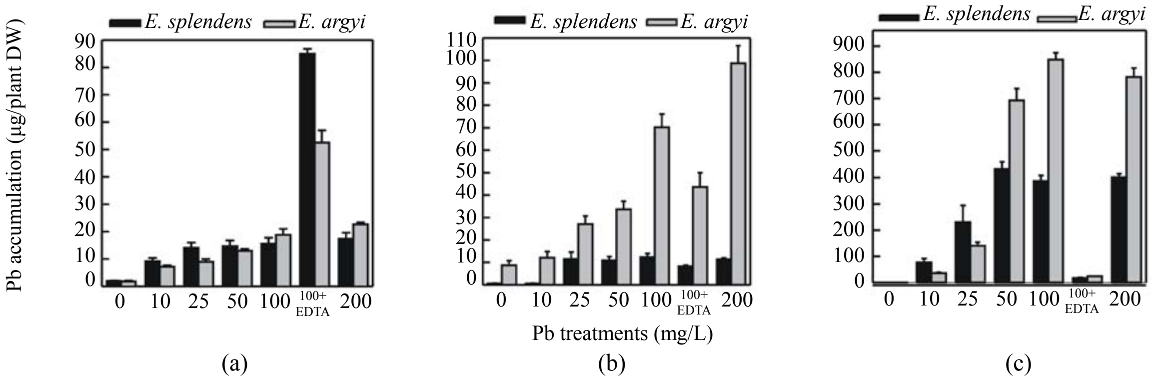


**Fig.4 Lead accumulations in root, stem and leaf of both *Elsholtzia* species exposed to Pb with and without 50 mmol/L EDTA addition for 8 d's growth. Data are means of three replications, and bars depict SE (a) Leaf; (b) Stem; (c) Root**

normally exceed 5 mg/kg, Pb≥0.1 mg/L in nutrient solution inhibited the normal growth of plant. In this study, 8 d's treatment with Pb≥10 mg/L significantly reduced the DW of *E. splendens*, while for *E. argyi*, 10 mg/L Pb didn't affect its DW, but Pb≥25 mg/L pronouncedly decreased its DW. As increasing Pb, DW of *E. splendens* reduced faster than that of *E. argyi* (Fig.1), indicating that the growth of *E. splendens* is restrained heavily than *E. argyi* in response to Pb toxicity.

Under Pb toxicity, root morphology of both *Elsholtzia* species exhibited significant difference. In this study, as increasing Pb treatment for 8 d, Pb treatment affected mostly the length of $0.2 \leq D \leq 0.6$ mm root for *E. argyi*, and $D \leq 0.6$ mm root for *E. splendens*. Significant decrease in surface area of $D \leq 1.0$ mm root of *E. splendens* was found at Pb≥10 mg/L, while for *E. argyi*, it was at Pb≥25 mg/L. Compared to *E. argyi*, the length of $D \leq 0.2$ mm root and surface area of $D>1$ mm root of *E. splendens* decreased rapidly. All those above suggested that, compared to *E. splendens*, the root of *E. argyi* can tolerate more Pb, and its greater root length and root surface area as well as root volume (Fig.2) is helpful for Pb uptake by plant root.

It has been reported that root morphology can directly affect the uptake of water, minerals and heavy metals (Marschner, 1995). More Pb concentrated and accumulated in the root of *E. argyi* than in the root of *E. splendens*, which mainly attributed to its greater root parameters, maybe beneficial for Pb translocation from root to shoot of plant. In this study, much higher stem Pb concentration and accumulation were observed in *E. argyi* than in *E. splendens* at Pb>50 mg/L. However, at Pb<100 mg/L, more Pb in the leaf of *E. splendens* was noted than in the leaf of *E. argyi*. There must be different mechanism for Pb transport between both *Elsholtzia* species. Pb treatment at 50−100 mg/L, 25.6−27.9 and 46.6−89.0 μg/plant DW Pb accumulated in *E. splendens* and *E. argyi* shoot with biomass of about 0.23−0.22 and 0.37−0.35 g/plant, respectively. *E. splendens* is an endemic Cu-tolerant and accumulating plant species in the old mining areas (Yang *et al*., 1998; Song *et al*., 2004), and can tolerate and accumulate Pb in its shoot, but much less than that in the shoot of *E. argyi* which is endemic to the Sanmen Pb/Zn mined area of Zhejiang Province.

EDTA has proven to be very effective in facilitating the uptake of Cd, Cu, Ni, Pb and Zn by plant root to its shoot tissues when applied to the rhizospheric zone of the plant several days before harvest (Raskin *et al*., 1997). Treatment with 100 mg/L Pb plus 50 mmol/L EDTA increased leaf Pb concentration from 87.7 to 684 mg/kg for *E. splendens*, and from 76.8 to 222.5 mg/kg for *E. argyi*, as compared to 100 mg/L Pb treatment, alone. Leaf Pb accumulation increased from 16.8 to 84.9 g/plant for *E. splendens*, and that of *E. argyi* increased from 18.8 to 52.5 g/plant after treatment with 100 mg/L Pb plus 50 mmol/L EDTA. Application of chelators to high crop plants such as *Brassica juncea* (Indian mustard), corn and sunflower, led to significant accumulation of Pb in their shoot tissues (Huang and Cunningham, 1996; Blaylock *et al*., 1997). This study revealed that compared to *E. argyi*, Pb translocation from root to shoot of *E. splendens* can be much enhanced at 100 mg/L Pb plus 50 mmol/L EDTA. However, compared to 100 mg/L Pb, 100 mg/L Pb plus 50 mmol/L EDTA significantly decreased root length, root surface area and root volume for both *Elsholtzia* species, with root average diameter of both *Elsholtzia* species decreased slightly (Tables 1−2, Fig.2), thus resulting in the significantly decreased DW of both *Elsholtzia* species. Moreover, treatment with 100 mg/L Pb plus 50 mmol/L EDTA significantly reduced length and surface area of $D<0.2$ mm root and increased those of $0.2<D<0.8$ mm root for the case of *E. argyi*, while for *E. splendens*, length and surface area of $D<0.6$ mm root were reduced considerably, suggesting the root of *E. splendens* is more sensitive than *E. argyi* to 50 mmol/L EDTA. Treated only with 100 mg/L Pb, *E. argyi* showed great potential for Pb uptake to shoot tissues as compared to *E. splendens*, while at the treatment of 100 mg/L Pb plus 50 mmol/L EDTA, the much more Pb transported to leaf of *E. splendens* than to leaf of *E. argyi* was observed (Fig.4), which probably mainly resulted from the significant difference between their root morphologies and their transport system, but its mechanism should be further investigated.

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