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Phytofiltration of Copper from Contaminated Water: Growth Response, Copper Uptake and Lignin Content in *Elsholtzia splendens* and *Elsholtzia argyi*

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Abstract Phytofiltration of Cu from water by *Elsholtzia* plants were investigated hydroponically. Both *E. splendens* and *E. argyi* could effectively clean up lower Cu contaminated water, probably attributed to their extremely high surface area of roots. The removal rate of Cu was concentration-dependent and showed a monotonic decline with time. At higher external Cu concentration, the growth of *E. argyi* has been considerably inhibited while *E. splendens* still grew normally. *E. splendens* has the greater capacity to absorb Cu to roots from water and translocate Cu from roots to shoots. Lignin in roots probably has no significant effect on immobilizing Cu.

Keywords Phytofiltration · Cu · *E. splendens* · *E. argyi*

As essential micronutrient, copper (Cu) concentration ranging from 3 to 20 mg kg^{-1} is required for the normal growth of plants (Robson and Reuter 1981), and of 30 μg kg^{-1} for adult uptake in daily diet based on his/her body weight. In China, the highest Cu concentration in drinking water reported is 1 mg L^{-1}, less than U.S. EPAs Drinking Water Equivalent level (1.3 mg L^{-1}) (USEPA 1991) and WHO guideline value (2 mg L^{-1}) (WHO 1993). However, most of reservoirs, for example, 13 reservoirs monitored in Gansu Province, have been polluted with metals such as Cu ion level above the threshold of drinking water being over 33.3%. Copper pollution to water system is mainly due to the effluents from Cu smelting activities and wastewater disposal activities and so on. The toxicity of Cu ions in water is largely dependent on such properties as pH, natural organic matter (NOM), oxygen concentration, and etc. Copper toxicity can be considerably decreased by forming complexes with NOM, and toxicity can be negligible when Cu exists in this form. However, some low-cost technologies are still needed to be developed for effective cleanup of excess Cu in effluents from Cu smelting activities and wastewater disposal activities.

Phytofiltration, the use of plant roots to remove contaminants from water, is an emerging technology (Dushenkov et al. 1995; Dushenkov and Vasudev 1997). The initial research on phytofiltration of contaminants from water began with the use of wetlands for water purification (Kadlee and Knight 1996). Chandra et al. (1997) demonstrated that vascular aquatic plants *Scirpus lacustris* and *Phragmites karka* can effectively remove Cr from contaminated water. Huang et al. (2004) designed a hydroponic system using arsenic-hyperaccumulating ferns to remove arsenic from contaminated water. Chinese native Cu-tolerant and accumulating plants, *Elsholtzia splendens* (Haizhou Elshotlzia) and *Elsholtzia argyi* (Purple Flower Elshotlzia), are endemic to Cu and Pb/Zn mine waste deposits respectively (Jiang et al. 2004a, b). When hydroponically cultivated, fibrous root system covered with root hairs of both *Elsholtzia* plants can create an extremely high surface area and an advanced uptake mechanism for Cu absorption to the surface of plant roots. Moreover, both *Elsholtzia* plants have different phytochemical properties such as lignin content despite of their belonging to the same family *Labiatae*. Whether root lignification can affect phytofiltration efficacy still need further investigation. Therefore, the objectives of this study were to examine the growth, Cu removal and lignin content in both *Elsholtzia*

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plants when they were used to phytofiltrate Cu from the artificial contaminated water.

Materials and Methods

Seeds of terrestrial plants *E. splendens* and *E. argyi*, collected from mature plants growing on Cu mine and Pb/Zn mine waste deposits of Zhejiang province of China respectively, were surface sterilized, rinsed, and then sowed in quartz sand moistened with distilled water. After 7-day emergence of seedlings, 0.2-strength nutrientsolution was supplied for another 2-week growth. Then the uniform plant seedlings were transferred to hydroponic solution in 2 L plastic containers (seven plants per container) with a full-strength aerated nutrient solution for another 4-week preculture in a glasshouse in Oct 2004, with temperature ranging from 28 ± 2°C (day) to 15 ± 2°C (night) and natural light intensity. The composition of nutrient solution was (in μmol L^{-1}): 700 K_2SO_4, 100 KCl, 2,000 $Ca(NO_3)_2 \cdot 4H_2O$, 500 $MgSO_4 \cdot 7H_2O$, 100 KH_2PO_4, 10 H_3BO_3, 0.5 $MnSO_4 \cdot H_2O$, 0.5 $ZnSO_4 \cdot 7H_2O$, 0.2 $CuSO_4 \cdot 5H_2O$, 0.01 $(NH_4)_6Mo_7O_{24}$, 100 Fe-EDTA (Yang et al. 2002). All solutions were aerated continuously, adjusted pH 5.8 ± 0.3 daily with 0.1 mol L^{-1} HCl or 0.1 mol L^{-1} NaOH, and the nutrient solution was replaced every 2 days.

After preculture, some plants were selected for the determination of root morphological parameters before Cu treatment, using a automatic root canning apparatus (MIN M AC, STD1600+) with the analysis software of WinRHIZO offered by Regent Instruments Co. And the roots of intact plants were rinsed with tap water, immersed in distilled water for 20 min to remove metal ions adhering to the root surfaces. Then the roots of *E. splendens* and *E. argyi* were respectively immersed in the artificial Cu contaminated water with one plant in a PVC plastic container containing 500 mL solution for Cu phytofitration experiments (Huang et al. 2004). Control contained aerated Cu contaminated water without plants. Copper sulfate ($CuSO_4 \cdot 5H_2O$), analytical grade with the purities of 99.0%, were added to the distilled water to achieve the artificial Cu contaminated water with different concentrations of 10, 25, 100, 200 mg L^{-1}. A randomized complete block experimental design was used with each container having three repetitions. After 1, 2, 4, 8, 12, 24, 48, and 72 h, 1-mL aliquots were removed from each container for Cu concentration analyses. The total volume of the solution was kept constant by adding deionized water to compensate for water lost through plant transpiration, sampling, and evaporation. After 3-day experiment, plants were harvested. Roots of intact plants were rinsed with distilled water, and then immersed in 5 mmol L^{-1} $Pb(NO_3)_2$ for 20 min to remove Cu adhering to the root surfaces (Harrison et al. 1979) and washed with distilled water, blotted dried. Roots and shoots were separated, oven-dried at 105°C for 30 min, then dried at 65°C to constant weight, and their dry weight (DW) were recorded. The dried plant materials were ground with a stainless steel mill and passed through a 0.25-mm sieve, ashed at 550°C, and dissolved in 10 mL 1:1 (V:V) HCl. Copper concentrations in the plant digestion and one-milliliter aliquots taken from each container were determined by AAS (Shimaduz, AA-6800). The detection limitation of Cu was 0.4 mg L^{-1}. Lignin content in root, stem and leaf of plants was estimated according to the method described by Шочинок (1981). All data were presented as mean values of at least 3 replicates. SPSS statistical software package (Version 11.5) was used. One-way ANOVA was employed to evaluate whether the means were significantly different at $p < 0.05$.

Results and Discussion

The hydroponically cultivated roots of 8-week *E. splendens* and *E. argyi* have developed much longer, finery root system. Their root morphological parameters showed that root length before exposure to Cu were 8841.6 ± 356.8 and 8954.5 ± 556.8 cm, with root surface area of 3059.1 ± 248.6 and 3222.9 ± 189.7 cm^2, root volume of 88.2 ± 10.3 and 92.3 ± 11.4 cm^3, and root average diameter of 1.4 ± 0.1 and 1.5 ± 0.1 mm, respectively. And average root DW was 0.50 ± 0.05 g $plant^{-1}$ for both *Elshotzia* plants. For the lower Cu (10 mg L^{-1}) contaminated water, roots of *E. splendens* reduced Cu concentration to 3.08 mg L^{-1} in 8 h, while for *E. argyi*, it was reduced to 4.81 mg L^{-1} in 8 h. The initial Cu concentration of 10 mg L^{-1} was reduced to zero after treating with *E. splendens* for 48 h and with *E. argyi* for over 72 h (Fig. 1). Both *E. splendens* and *E. argyi* can effectively clean up this lower Cu contaminated water with the former better than the latter. The results indicated that the roots of both *Elshotzia* plants can absorb and immobilize Cu ions in the artificial contaminated water with *E. splendens* better than *E. argyi*. Hydroponically cultivated roots of several terrestrial plants were recently discovered to be effective in absorbing, concentrating, or precipitating metals from polluted effluents (Salt et al. 1995). This process termed “rhizofiltration”. Using terrestrial plant roots in the remediation of contaminated water (Dushenkov et al. 1995; Dushenkov and Vasudev 1997; Salt et al. 1995; Cunningham et al. 1996), which is mainly attributed to the extremely high surface area of plant roots and its capacity to absorb metals in contaminated water.

Copper removal rate was concentration-dependent and showed a monotonic decline with time (Fig. 1). The roots

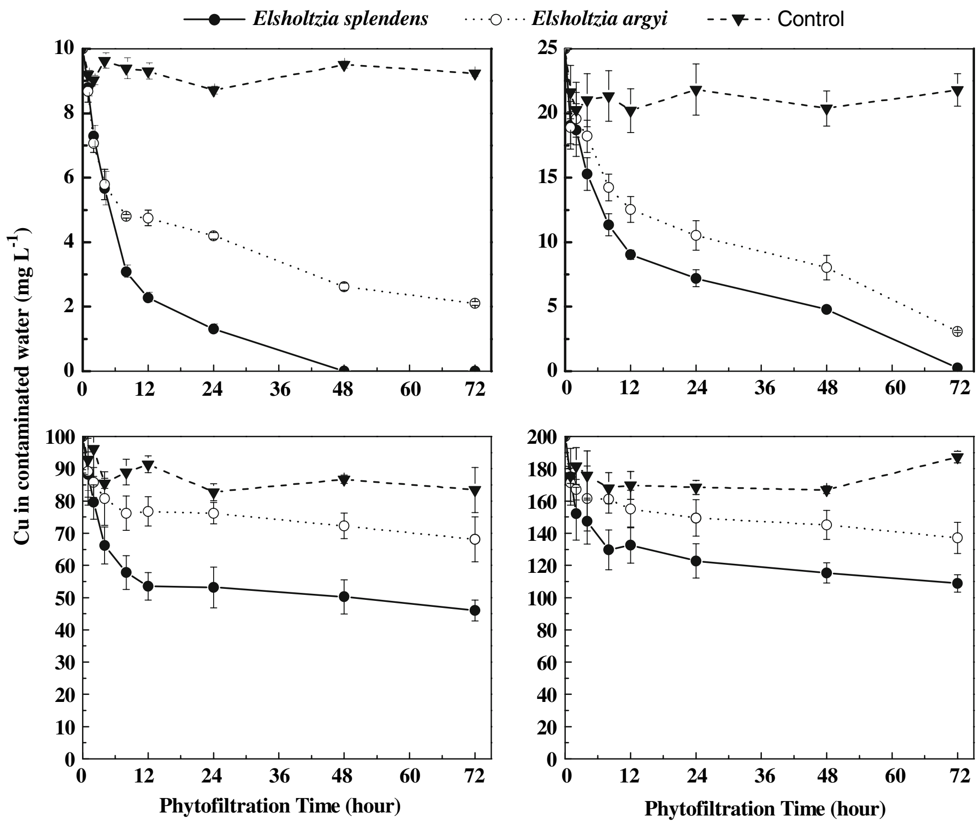

Fig. 1 Phytofiltration of Cu by *E. splendens* and *E. argyi* exposed to Control, 10, 25, 100, 200 mg L^{-1} Cu for 3 days. Control contained aerated Cu contaminated water without plants. All the data are means of three replications, and bars depict SE

of *E. splendens* immersed in 25 mg L^{-1} Cu reduced this concentration by 54.6%, 63.9%, 71.2%, 80.8%, 98.9%, respectively, in 8, 12, 24, 48 and 72 h. While for *E. argyi*, it was reduced by 43.0%, 49.9%, 57.9%, 67.9%, 87.6%, respectively, in 8, 12, 24, 48 and 72 h. The decrease in Cu concentration during the experiment can be calculated as $Y = be^{kx}$, e.g., it was $Y = 91.46e^{-0.0529x}$ for *E. splendens*, and $Y = 79.928e^{-0.0244}$ for *E. argyi*. Therefore, the constant k can be deduced to: $k_{E.\ splendens}$ (h) = −0.0529, and $k_{E.\ argyi}$ (h) = −0.0244. The Cu concentration in water declined faster when phytofiltrated by *E. splendens* than by *E. argyi*. In the artificial contaminated water with 200 mg L^{-1} Cu, about 45.6% and 31.5% Cu in the water can be phytofiltrated by *E. splendens* and *E. argyi* after 72 h, respectively (Fig. 1). Copper concentration in the contaminated water phytofitrated by both *Elsholtzia* plants decreased faster in the first 12 h and then exhibited a lower decrease process after 12 h, indicating that Cu ions absorption to the surface of plant roots is a rapid process, which is completed in the first 12 h. The lower decrease of Cu in medium after 12 h may be due to Cu uptaken by plant roots through intracellular Cu uptake and translocation from roots to shoots (Harrison et al. 1984). Phytofitration of Cu ions from contaminated waters by both *Elsholtzia* plants include these processes, so as to a rapid process in the first 12 h and a lower process after 12 h.

Copper removal rate was also dependent on plant species. At the initial Cu concentration of 10, 25, 100, 200 mg L^{-1} in the artificial contaminated water, the concentrations decreased by 86.9%, 71.2%, 46.9%, 38.6% when phytofiltrated by *E. splendens* in 24 h, and by 58.0%, 57.9%, 23.8%, 25.3% when phytofiltrated by *E. argyi* in 24 h, respectively (Fig. 1). The removal efficacy for Cu was 1.5, 1.2, 2.0, and 1.5-fold more effective for *E. splendens* than for *E. argyi*. Higher efficiency of Cu phytofiltration by *E. splendens* are mainly attributed to the roots of *E. splendens* which could absorb and immobilize more Cu ions in the artificial contaminated water, but the mechanism need to be further investigated.

When phytofiltration of Cu from contaminated water, at day 1 after 100 mg L^{-1} Cu was added, the dark-brown roots of *E. argyi* were noted, but the plant remained alive except for a few leaves wilted for water deficit. The growth of *E. argyi* has been considerably inhibited when grown at 100 mg L^{-1} Cu for 3 days. In contrast, *E. splendens* can still grow normally at 100 mg L^{-1} Cu contaminated water despite of the appearance of black roots at day 1 after Cu was added. As Cu concentration increased up to 100 mg L^{-1} in water, root DW were reduced by 17.2% and 24.7%, and shoot DW by 49.0% and 50.0% for *E. splendens* and *E. argyi* respectively, as compared to those when plants were exposed to 10 mg L^{-1} Cu contaminated water.

The root biomass reduced more dramatically in *E. argyi* than in *E. splendens*, and *E. splendens* was more tolerant to high Cu than *E. argyi* (Fig. 2).

When Cu level in water increased to 200 mg L^{-1}, Cu concentration in roots and shoots of both plants, especially of *E. splendens*, increased sharply (Fig. 3). In the contaminated water with 10 mg L^{-1} Cu, no marked difference were noted for both shoot and root Cu concentrations between *E. splendens* and *E. argyi*, while considerably increase in root and shoot Cu concentration of *E. splendens* occurred at $\geq$100 mg L^{-1} Cu, which were higher as compared to *E. argyi*. For example, at 10 mg Cu L^{-1} in water, shoot Cu concentration in *E. splendens* and *E. argyi* was 220.7 and 210.2 mg kg^{-1} respectively, root Cu concentration was 7,329.7 and 8,689.3 mg kg^{-1}, respectively. At Cu level up to 100 mg L^{-1} in water, shoot Cu concentration of *E. splendens* and *E. argyi* increased to 851.8 and 486.4 mg kg^{-1} respectively, and root Cu concentrations reached to 44,693.3 and 38,778.0 mg kg^{-1}, respectively. Shoot and root Cu concentrations in *E. splendens* were 2.6 and 1.6-fold higher than those in *E. argyi* when plants were grown at Cu contaminated level of 200 mg Cu L^{-1}.

When phytofitration of Cu $\geq$25 mg L^{-1} in the contaminated water, greater Cu can be uptaken (calculated by Cu concentration × biomass) by both roots and shoots of *E. splendens* as compared to *E. argyi*.This may be due to the fact that *E. splendens* could transport much more Cu from roots to shoots by its more effective Cu transportation system, as compared to *E. argyi*. At Cu level of 200 mg L^{-1} in water, the plants of *E. splendens* and *E. argyi* can respectively remove 2.4% and 1.6% Cu (based on dry weight biomass) from the water after 72-h treatment. Therefore, *E. splendens* has the greater capacity to absorb Cu to roots from water and translocate the absorbed Cu from roots to shoots at Cu concentration $\geq$25 mg L^{-1} Cu in water.

Lignin is found principally in sclerenchyma and in the tracheids and vessels of the xylem. It is also found in other cells in response to infection or certain other external stimulators. Whether the lignifications of plant root and shoot can affect Cu ions diffusing within plants is still needed to be investigated. Lignin concentration in both *E. splendens* and *E. argyi* decreased in the order: root > stem > leaf, and greater lignin percentages in root, stem and leaf of *E. splendens* were noted as compared to *E. argyi*

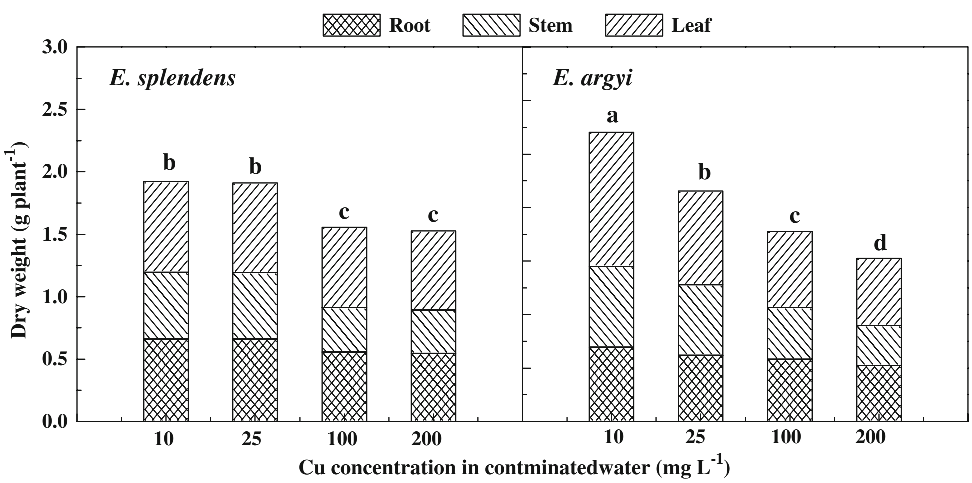

Fig. 2 Dry weight of roots, stems and leaves of *E. splendens* and *E. argyi* exposed to Cu for 3 days, data are means of three replications. Letters a, b, c and d represent significant difference at $p < 0.05$

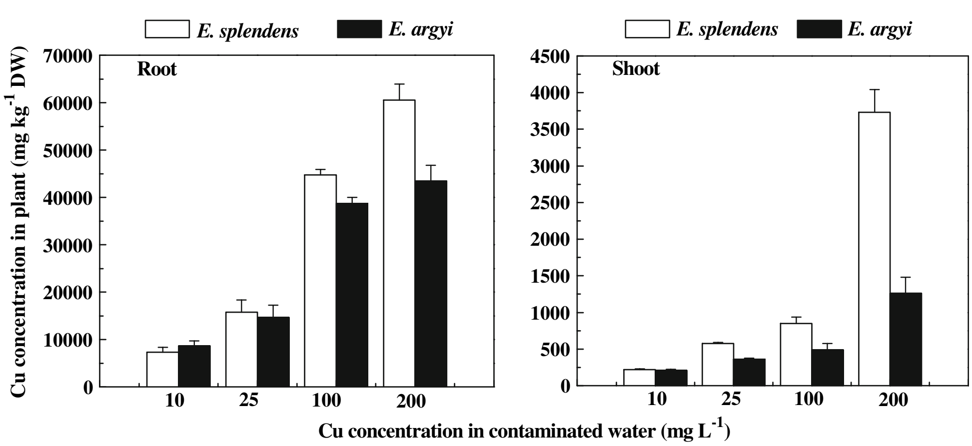

Fig. 3 Copper concentrations in the roots and shoots of *E. splendens* and *E.* argyi exposed to Cu for 3 days, data are means of three replications, and bars depict SE

Table 1 Lignin percentage (%) in tissues of *E. splendens* and *E. argyi* exposed to different Cu for 3 days

Copper concentration (mg L^{-1})	Lignin percentage (%)		
	Root	Stem	Leaf
E. splendens			
10	29.86 ab	25.36 ab	22.31 a
25	30.11 a	26.85 a	23.56 a
100	29.96 ab	25.52 ab	20.56 ab
200	29.27 ab	25.11 ab	22.98 a
E. argyi			
10	19.05 ab	16.23 a	12.50 a
25	21.82 a	16.36 a	13.28 a
100	20.49 a	14.02 ab	13.16 a
200	18.92 ab	15.36 a	12.93 a

Letters a and b in the column indicate the significant statistical at $p < 0.05$ between treatments

(Table 1). When phytofitration of Cu from contaminated water, lignin content (i.e., calculated by lignin percentage × biomass) in the roots and shoots of *E. splendens* could be attained to 16–20 and 23–31 g $plant^{-1}$, while those of *E. argyi* were 10–14 and 13–28 g $plant^{-1}$, respectively. However, the root-to-shoot transportation rate of Cu (calculated by the data in Fig. 3) was higher in *E. splendens* rather than in *E. argyi*. *E. argyi* and *E. splendens* are endemic plants to Cu and Pb/Zn mine waste deposits in China. The removal rate of Cu by both plants depended on the initial Cu level in the artificial contaminated water and the lignin content of plant species. The significantly higher efficiency of copper phytofiltration by *E. splendens* appeared to be associated with its better ability to tolerate higher Cu concentrations and absorb more Cu ions to plant roots as well as translocate absorbed Cu from roots to shoots as compared to *E. argyi*. Our results demonstrate that these two plants especially *E. splendens* may provide the basis for a phytoremedation technique that enables small-scale cleanup of Cu contaminated water.

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