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Growth response and phytoextraction of copper at different levels in soils by *Elsholtzia splendens*

L.Y. Jiang [a,b], X.E. Yang [a,*], Z.L. He [a,c]

[a] *Ministry of Education Key Lab of Environmental Remediation and Ecosystem Health, Department of Natural Resource Science, College of Natural Resource and Environmental Science, Zhejiang University, Huajiachi Campus, Hangzhou 310029, China*

[b] *Department of Environmental Engineering, College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310014, China*

[c] *Institute of Food and Agricultural Science, University of Florida, Indian River Research and Education Center, 2199 South Rock Road, Fort Pierce, FL 34945-3138, USA*

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Abstract

Phytoremediation is a promising approach for cleaning up soils contaminated with heavy metals. Information is needed to understand growth response and uptake mechanisms of heavy metals by some plant species with exceptional capability in absorbing and superaccumulating metals from soils. Greenhouse study, field trial, and old mined area survey were conducted to evaluate growth response and Cu phytoextraction of *Elsholtzia splendens* in contaminated soils, which has been recently identified to be tolerant to high Cu concentration and have great potential in remediating contaminated soils. The results from this study indicate that the plant exhibited high tolerance to Cu toxicity in the soils, and normal growth was attained up to 80 mg kg^{-1} available soil Cu (the NH_4OAc extractable Cu) or 1000 mg kg^{-1} total Cu. Under the field conditions, a biomass yield of 9 ton ha^{-1} was recorded at the soil available Cu level of 77 mg kg^{-1}, as estimated by the NH_4OAc extraction method. Concentration-dependent uptake of Cu by the plant occurred mainly at the early growth stage, and at the late stage, there is no difference in shoot Cu concentrations grown at different extractable soil Cu levels. The extractability of Cu from the highly polluted soil is much greater by the roots than that by the shoots. The NH_4OAc extractable Cu level in the polluted soil was reduced from 78 to 55 mg kg^{-1} in the soil after phytoextraction and removal of Cu by the plant species for one growth season. The depletion of extractable Cu level in the rhizosphere was noted grown in the mined area, even at high Cu levels, the NH_4OAc extractable Cu in the rhizosphere was 30% lower than that in the bulk soil. These results indicate that phytoextraction of *E. splendens* can effectively reduce the plant-available Cu level in the polluted soils.

Keywords: Copper; *E. splendens*; Foreign soil; Furnace slag; Phytoextraction; Polluted soils

1. Introduction

A large area of land is contaminated with heavy metals due to use of sludge or municipal compost, pesticides, fertilizers and emissions from municipal wastes incinerators, car exhausts, residues from metalliferous mined, and smelting industries. Excessive metal concentrations in the contaminated soils can result in soil quality degradation, crop yield reduction, and poor quality of agricultural products (McGrath, 1998; Yang et al., 2002b). Heavy metal contamination of soils can be of concern for human and animal health, as the metals may be transferred and accumulated in the bodies of

* Corresponding author. Tel./fax: +86-571-86971907.
E-mail addresses: xyang@zju.edu.cn, xyang@mail.ifas.ufl.edu (X.E. Yang).

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animals or human beings through food chain. Copper is one of the highly toxic heavy metals and its contamination to soils occurs frequently due to its widespread industrial and agricultural use. The clean-up of soils contaminated with Cu and other heavy metals is one of the most difficult tasks for environmental engineering. Use of green plants to remove Cu and other heavy metals from the contaminated soils, known as phytoremediation, is an emerging technique that offers the benefits being in situ, low cost and environmentally sustainable (Salt et al., 1998). The phytoremediation is composed of phytoextraction, phytostabilization, phytovolatilization, and rhizofiltration (Salt et al., 1995; Long et al., 2002). The phytoextraction approach is the use of metal hyperaccumulating or high biomass plants to extract Cu and other heavy metals from the soils by concentrating them in the harvestable parts. This approach is based on the abnormal capacity of some plant species in accumulating or hyperaccumulating heavy metals (Chaney et al., 1997; Cunningham et al., 1997). Metal tolerance is a pre-requisite for plants in phytoremediation technique, and it is important to identify plant genotypes that have a large biomass and a great ability to take up Cu from the soil.

To data, at least 25 plant species of copper hyperaccumulators and a great number of copper accumulators, excluders, and indicators have been identified (Brooks et al., 1978, 1980; Malaisse et al., 1979; Baker, 1981, 1983; Malaisse et al., 1994). In China, *Elsholtzia splendens* (*Elsholtzia haichowensis*) has been identified as Cu tolerant and accumulating plant species in the mined area and nutrient solution at high Cu levels (Yang et al., 1998, 2002a). *E. splendens* grows vastly over copper mined areas and was first recognized for its value in the exploration of copper ores in the 1950s (Hsieh and Hsu, 1954). It has a local nickname "copper flower" because of its growth confined in highly Cu contaminated soils. It has a large biomass with shoot dry matter yield reached as high as 10 ton ha^{-1} under field conditions. In nutrient solution, the growth of *E. splendens* was found to be optimal at Cu supply levels up to 100 μM, and was not dramatically reduced at Cu supply levels up to 500 μM (Yang et al., 2002a). However, copper accumulation in the shoots of the plant exceeded 1000 mg kg^{-1}, which was regarded as the threshold for hyperaccumulator (Brooks et al., 1980), only when grown at its toxic Cu levels in both nutrient solution and mined area (Yang et al., 1998, 2002a). At Cu supply levels lower than 500 μM, copper concentration in shoots of *E. splendens* did not reach the threshold of hyperaccumulator (Tang et al., 1999, 2001; Ke et al., 2001). However, little information is available about the extent of tolerance and phytoextractability of the plant species for Cu at different levels in soils. The characteristics of large biomass and high tolerance to Cu of the plant species are useful traits for phytoremediation, but effectiveness of the plant for decontaminating Cu in soils is unknown. The objectives of this study were to examine the growth response and Cu uptake by *E. splendens* at different levels in contaminated soils.

2. Materials and methods

2.1. Pot experiment

The soil used for the pot experiment is an Alluvial soil (Fluvio-marine yellow loamy soil), which was collected from the 0–20 cm depth in the farm of Zhejiang University. The main agrochemical properties of the tested soil are: pH 6.30, organic matter 15.8 g kg^{-1}, total N 2.4 g kg^{-1}, Olsen-P 41.1 mg kg^{-1}, and NH_4OAc-extractable K 30.6 mg kg^{-1}, total, DTPA- and NH_4-OAc-extractable Cu of 32.5, 4.26 and 0.43 mg kg^{-1}, respectively. Copper treatment levels were: 0, 100, 200, 400, 600, 800, 1000, and 1200 mg kg^{-1}, added as $CuSO_4 \cdot 5H_2O$. The copper salts were dissolved in stilled water, sprayed on the soil samples, and mixed thoroughly. Then, the treated soil samples were incubated in a large plastic container (60×40×15 cm) at 70% of maximum field water-holding capacity for 12 weeks. Soil extractable Cu (by deionized water, 1.0 M NH_4-acetate (pH 7.0), 1.0 M NH_4NO_3 (pH 7.0) and 50 mM EDTA was measured at the end of the incubation.

Pot experiment was conducted with the incubated soil samples to evaluate Cu tolerance and uptake of *E. splendens* at different soil Cu levels. Each pot contained 1 kg of soil thoroughly mixed with 0.1 g urea, 0.2 g KH_2PO_4 as basal fertilizers. Each treatment consisted of four replicates. The seeds of the plant were collected from the old mined area in Zhejiang Province, China and germinated on wetted filter paper in the dark. The germinated seeds were sown on quartz sand with nutrient solution prepared for establishing seedlings (Yang et al., 2002a). Two plants of 40-day-old seedlings were transplanted to each pot. A randomized complete block experimental design was used with each treatment replicated four times. Soil moisture was maintained at 60–70% of the maximum field water-holding capacity by adding distilled water during the experimental period. Plants were grown under glasshouse conditions with natural light, day/night temperature of 30/25 °C and day/night humidity of 40/60%. After growth for 70 days, the plants were harvested. Shoot length was recorded and shoots were cut at the soil surface, rinsed with distilled water, blotted dried. Roots were washed out from the soil with tap water, rinsed with distilled water, and blotted dried. Plant tissues were oven-dried at 70 °C, and dry weights were recorded. The dried plant materials were ground to less than 1-mm with a stainless steel mill for chemical analysis. Soil samples were collected from each pot, air-dried, and passed through a 1.0 mm plastic sieve prior to chemical analysis.

2.2. Field experiment

The tolerance and phytoextraction of Cu by *E. splendens* were examined under field conditions in 2001. The field experiment site was located in an agricultural field (an Alluvial loam, paddy soil) in Fuyang county of Zhejiang Province, where the soil was severely contaminated by heavy metal emission from many Cu refining plants (Jiang et al., 2002). Some physical and chemical properties of the soil were pH (H_2O) 7.5; organic matter 42.0 g kg^{-1}, cation exchange capacity (CEC) 7.1 cmol kg^{-1}, total N and P of 1.28 and 1.12 g kg^{-1}, available N, P, and K of 137.1, 37.2, and 24.6 mg kg^{-1}, respectively. Total and NH_4OAc extractable Cu were 1580.2 and 79.4 mg kg^{-1}, respectively, at the soil layer of 0–15 cm. In order to make different levels of Cu availability, five treatments adopted and some soil properties after the amendments for one year were shown in Table 1. The area of each field plot was 15 m^2, distance between the two neighboring plots was one meter and the edges of each plot were covered with a plastic sheet in order to prevent Cu movement between the plots. All the plots of the field experiment were randomly arranged with each treatment replicated four times. The seeds of the plant were collected from an old mined area in Zhejiang Province, and germinated in the wetted filter papers. The germinated seeds were sown on quartz sand with nutrition solution to establish the seedlings. The 40-day-old seedlings of the plant were transplanted to each plot of the field experiment with planting density of 20×20 cm^2. No fertilizers and pesticides were applied but interval weeding was made twice during the growth of the plants in the field. The plant and soil samples were collected at different growth stages, i.e. 70 (vegetative stage) and 170 days (flowering or reproductive stage) after transplanting. Shoots were cut at the soil surface, washed with tap water, rinsed with distilled water, blotted dried, and oven dried at 70 °C. Roots were collected from the soil and washed with tap water, rinsed with distilled water, and oven dried. The dry matter yields were then recorded and the dried plant materials were ground to <1 mm with a stainless steel mill for Cu analysis. The collected soil samples were air-dried and passed through a 1.0 mm plastic sieve for chemical analysis.

2.3. Mined area investigation

In order to characterize the relationship of soil Cu levels and plant growth and Cu extraction under naturally growing conditions of *E. splendens*. A survey in the mined area was conducted in 1999. The investigation area is located in Zhuji city, Zhejiang Province of China, which is 29° latitude north and is 120° longitude east. The sampling sites had different soil Cu levels, with the highest one in the center of the residual Cu ore piles, where had been almost naturally colonized by *E. splendens* completely, few or no other plant species survived. Plant samples of *E. splendens* with four replications were collected, separated into roots and shoots, washed with distilled water, blotted dried, and then oven dried at 70 °C. At each site, corresponding samples (0–15 cm) of both plant rhizosphere and bulk soils were collected, air-dried, and passed through 1-mm plastic sieve for analysis. The dried plant materials were ground to <1 mm with a stainless steel mill for Cu analysis.

Table 1
Total and available copper and some other element contents of the amended soils of the field experiment[a]

Plots	Treatments	pH (H_2O)	Organic matter (g kg^{-1})	Total (g kg^{-1})			Extractable (mg kg^{-1})			
				N	P	Cu	N	P	K	Cu
A	Control	7.54	55.7	2.43	1.11	1.43	298	25.2	29.5	77
B	Manure (1.5 ton ha^{-1})	7.54	55.9	2.55	0.94	1.43	327	25.4	29.8	78
C	Manure + furnace slag (1.5 ton ha^{-1} + 3.75 ton ha^{-1})	7.47	59.9	2.60	0.99	1.23	317	24.7	31.1	71
D	Manure + furnace slag + foreign soil (1.5 ton ha^{-1} + 3.75 ton ha^{-1} + 750 ton ha^{-1})	7.76	39.8	1.43	0.69	0.39	167	10.7	36.9	39
E	Inverting soil surface layer with the lower Cu level up to the plow layer (15 cm)	7.75	46.9	1.93	0.85	0.32	208	15.6	26.6	25

[a] All the analyses were conducted after the amendments had been applied to the polluted soil for one year.

2.4. Chemical and data analyses

Soil pH was measured in deionized water with a soil/solution ratio of 1:2.5 (W:V). Organic matter content, CEC and total N, P, and K in the soil were determined following the methods described in the Physical Chemical Analysis of Soils (SSICA, 1980). Soil available N was hydrolyzed by 1.0 M NaOH, available P extracted by 50 mM $NaHCO_3$, and available K extracted by 1.0 M NH_4OAc (SSICA, 1980). Soil total Cu was extracted with HF-$HClO_4$ (SSICA, 1980) and available Cu was extracted by 1.0 M NH_4-acetate (pH 7.0, 1:20 of soil: solution (W:V)), deionized water (1:1 of soil: water), 50 mM EDTA (1:2.5), and 1.0 M NH_4NO_3 (1:2.5), respectively (MAFF, 1986; Ernst, 1996). Sub-samples of the ground plant samples were ashed at 550 °C, dissolved in 10 ml 1:1 (v:v) HCl. The Cu concentrations in the soil extracts and the plant digests were measured using an Inductively Coupled Plasma-optical Emission Spectroscopy (Model IRAS-AP, TJA).

All the data sets were analyzed using the SPSS computer program, using one-way ANOVA. Means of treatments were compared using the Duncan test at the significance level $p < 0.05$.

3. Results

3.1. Dry matter production of E. splendens at different soil Cu levels

Shoot growth of *E. splendens* increased with increasing Cu levels in the soil, and peaked at the NH_4OAc extractable Cu of 62.6 mg kg^{-1}, and then slightly decreased in the pot experiment (Fig. 1). The shoot dry weight at the highest soil Cu level was 6.68

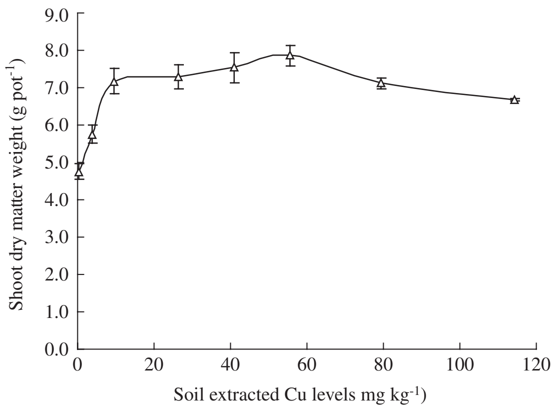

Fig. 1. Shoot dry mater yields of *E. splendens* as a function of the NH_4OAc extractable Cu levels in the soil from the pot experiment. The plants were grown for 70 days in the pot before harvested.

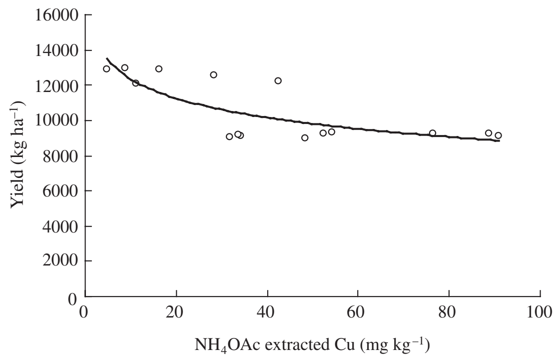

Fig. 2. Shoot dry matter yields of *E. splendens* as a function of the NH_4OAc extractable Cu levels in the soil from the field experiment. The plants were grown for 170 days in the field before harvested.

g pot^{-1}, approximately 40% greater than the control (Fig. 1), implying that the plant species might grow better in the Cu contaminated soil. The results show that the plant of *E. splendens* exhibited high tolerance to high Cu in the soils, up to 80 mg kg^{-1} available soil Cu (the NH_4OAc extractable Cu) and 1000 mg kg^{-1} total Cu in the pot experiment under glasshouse conditions.

The field experimental site was originally a bare area on the agricultural paddy rice farm resulted from high toxicity of the polluted heavy metals. Under field condition, shoot growth of *E. splendens* was normal with NH_4OAc extractable Cu over 80 mg kg^{-1} (Fig. 2). Whereas, the shoot dry matter yields reached 12–13 ton ha^{-1} at soil NH_4OAc extractable Cu levels of 25–40 mg kg^{-1} (Fig. 2). These results indicate that *E. splendens* has an extraordinary ability to tolerate high Cu levels in the soils under field conditions, which is of great importance to use it for the re-plantation of Cu contaminated sites and decontamination of the polluted agricultural soils.

3.2. Copper concentration and accumulation

In the pot experiment, shoot and root Cu concentrations in *E. splendens* increased with increasing soil Cu levels (Fig. 3). Copper concentrations in the roots reached as high as 1751 mg kg^{-1} at the NH_4OAc extractable soil Cu level of 114 mg kg^{-1}, which was 180 times greater than that in the shoots (Fig. 3). These results indicate that the extraction of Cu from the soil by the plant is mainly through root acquisition.

In the field trial, shoot Cu concentrations differed with different growth stages, over 250 mg kg^{-1} at the early stage (70 days), but only about 50 mg kg^{-1} at the late growth stage (170 days) (Fig. 4), which might be due to the dilution of plant growth. Root Cu concentrations were 720–1260 mg kg^{-1} under field conditions, which was

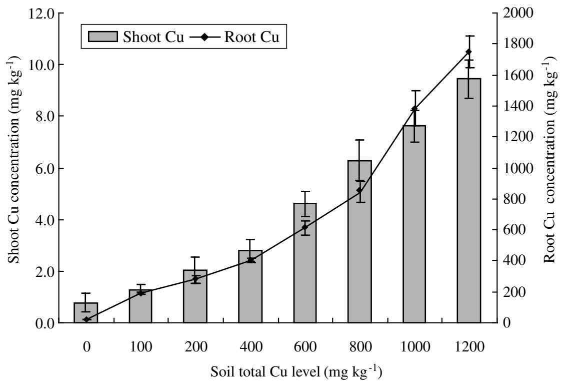

Fig. 3. Root and shoot Cu concentrations of *E. splendens* grown at different Cu levels for 10 weeks under glasshouse conditions.

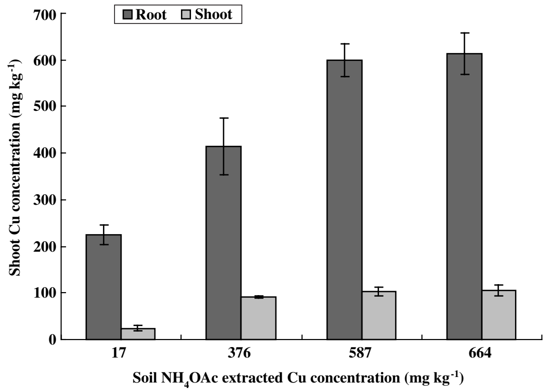

Fig. 5. Shoot and root Cu concentrations of *E. splendens* grown at different Cu levels in the old mined area. Plants were harvested at the flowering stage, which had a similar age as those of 170-day old plants in the field experiment.

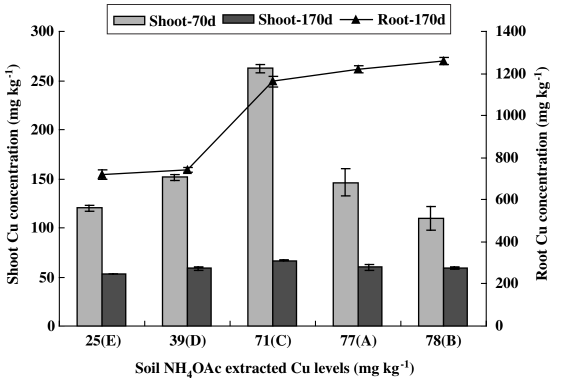

Fig. 4. Copper concentrations in the shoots (a) and the roots (b) of *E. splendens* grown at different extractable Cu levels for 70 and 170 days under field conditions.

12–21 times greater than that in the shoots (Fig. 4). At early growth stage (70 days), shoot Cu concentration of *E. splendens* increased by 80% with addition of manure and furnace slag together, but at the late stage there is no difference in shoot Cu concentrations grown at different amendment treatments. These results indicate that concentration-dependent uptake of Cu by *E. splendens* occurs mainly at the early growth stage.

In the mined area, shoot Cu concentration increased gradually with soil available and total Cu levels, but leveled off at NH_4OAc extractable soil Cu levels around 300 mg kg^{-1}, whereas the root Cu concentration increased sharply with increasing soil available Cu (Fig. 5). At the total and NH_4OAc extractable soil Cu levels of 3454 and 660 mg kg^{-1}, copper concentration in the roots was as high as 600 mg kg^{-1}, about 4 times greater than that in the shoots. These results again indicate that the extractability of Cu from the highly polluted soil is much greater by the roots than by the shoots of *E. splendens*.

3.3. Copper extraction and depletion from the soils by *E. splendens*

The total Cu removal by the whole plant of *E. splendns* was 57–500 μg plant^{-1} when the plants were grown at different soil Cu levels in the pot for 10 weeks (70 days) under glasshouse conditions (Fig. 6a). Under field conditions, however, the amount of Cu removal by the shoots was 550–720 g ha^{-1}, and was 1.2–1.7 kg ha^{-1} by the whole plants when grown at different soil Cu levels in the polluted agricultural field for 170 days (Fig. 6b). Soil available Cu levels significantly affected the phytoextraction of Cu by *E. splendens*. In the pot experiment, significant differences in plant available Cu (extracted either by NH_4NO_3 or NH_4OAc) were noted between the treatments with plant and that without plant (Fig. 7a). The extent of the differences was enlarged with increasing Cu supply levels, which is in agreement with the total amount of Cu removal by the whole plant. Under field conditions, the NH_4OAc extractable Cu in the soil decreased after the plants were grown for 170 days at each Cu level (Fig. 7b). For instances, the NH_4OAc extractable Cu level in the polluted soil without any amendment was reduced from 78 to 55 mg kg^{-1} in the soil after phytoextraction of Cu by the plant for one season. The depletion of extractable Cu level in the rhizosphere was noted for the plant species grown in the mined area (Fig. 8). Even at high Cu levels, the NH_4OAc extractable Cu in the rhizosphere was 30% lower than that in the bulk soil. These results indicate that phytoextraction of *E. splendens* can effectively reduce the plant-available Cu level in the polluted soils.

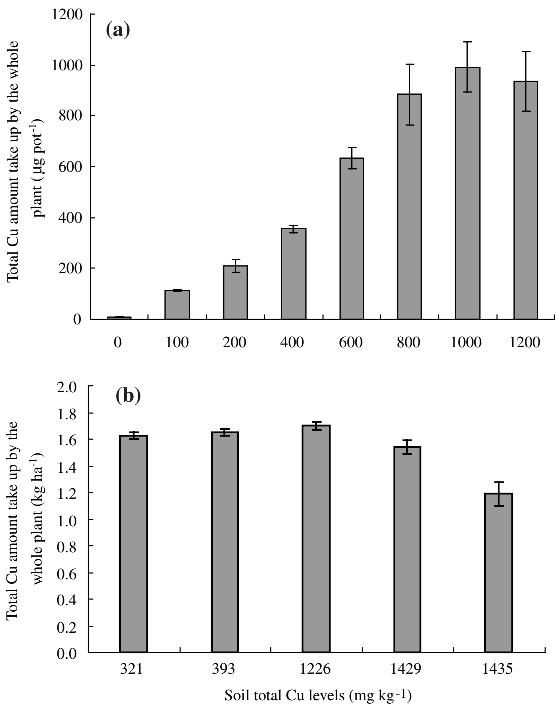

Fig. 6. Copper removal by the whole plant of *E. splendens* grown at different Cu levels from the polluted soils: (a) pot experiment, (b) field experiment.

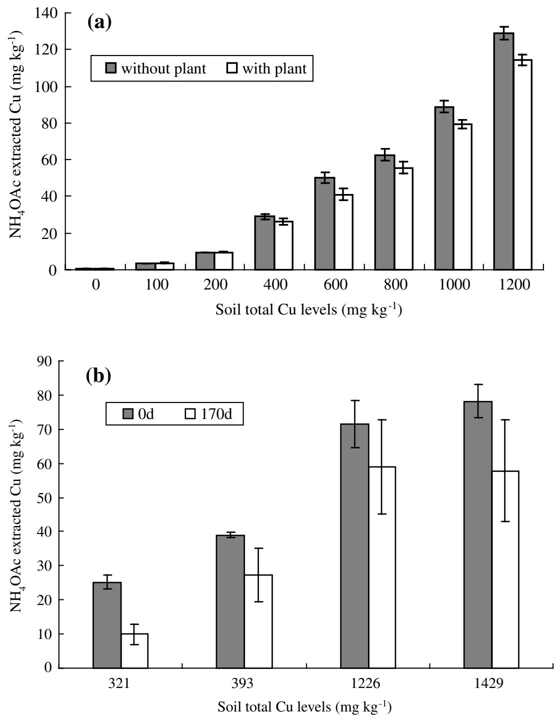

Fig. 7. Effects of Cu phytoextraction by *E. splendens* on the depletion of NH_4OAc extractable Cu in the polluted soils: (a) pot experiment, (b) field experiment.

3.4. Relationship of plant extractability of soil Cu with soil test values

Chemical extraction is a common soil test for evaluating heavy metal availability. For example, extraction with deionized water or NH_4NO_3 gives readily available metal and extraction with organic solvents such as NH_4-acetate or EDTA may indicate the pool size of metals available to plants root over a period of years. The correlations between Cu uptake in the plant and soil available Cu measured with different chemical extraction methods were shown in Table 2. In the pot experiment, root and shoot Cu concentrations were significantly and positively correlated with total soil Cu, water, NH_4NO_3, NH_4-acetate, or EDTA extractable Cu, respectively. However, the correlation coefficients between the EDTA extractable Cu and root and shoot Cu were much smaller than those of other extractants. A strong linear relationship between root Cu and water or NH_4NO_3 extractable Cu was noted with the correlation coefficients being 0.978 and 0.975, respectively.

Under field conditions, significant and positive correlation was observed between shoot Cu uptake at the growth stage of 70 days, whereas the EDTA extractable Cu in the soil was not significantly correlated with the shoot Cu concentration nor with plant Cu uptake at 70-day stage (Table 2). From the survey in the mined area, NH_4OAc extractable Cu levels were closely correlated with shoot and root Cu concentrations in *E. splendens*. These results imply that soil Cu level extracted with NH_4OAc or NH_4NO_3 is the best indicators of plant available Cu for the Cu-tolerant and accumulating plant species.

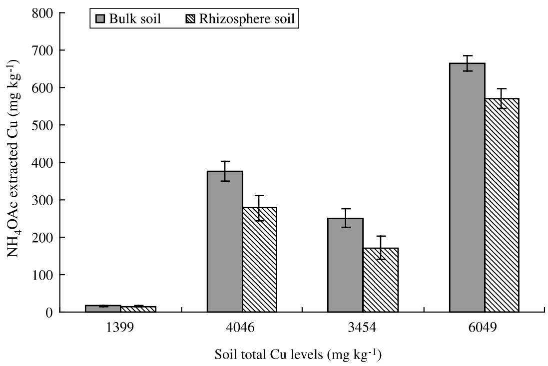

Fig. 8. Comparison of NH_4OAc extractable Cu between the rhizosphere and the bulk soils of *E. splendens* grown in the old mined soil.

Table 2
Correlations between Cu uptake by the plant of *E. splendens* and the extracted soil Cu in the soil samples from the pot and field experiments as well as the old mined area

Experiment	Items	Correlation coefficients				
		Cu_{Total}	Cu_{H_2O}	$Cu_{NH_4NO_3}$	Cu_{NH_4OAc}	Cu_{EDTA}
Pot Exp.	Root Cu	0.961**	0.978**	0.975**	0.965**	0.581**
($n = 24$)	Shoot Cu	0.890**	0.883**	0.856**	0.880**	0.582**
	Plant Cu	0.921	0.871**	0.882**	0.861**	0.626**
Field Exp.	Root Cu	0.662**	0.889**	0.723**	0.688**	0.701**
($n = 15$)	Shoot Cu	0.124	0.275	0.266	0.338	0.316
	Plant Cu	0.431	0.565*	0.578*	0.534*	0.543*
Mined area	Root Cu	0.862**	–	0.831**	–	–
($n = 12$)	Shoot Cu	0.869**	–	0.862**	–	–
	Plant Cu	0.914**	–	0.885**	–	–

$^{*}p < 0.05$; $^{**}p < 0.01$.

4. Discussions

Copper is highly toxic to normal plants, and critical tissue Cu concentrations at 10% reduction of dry matter (DM) yields were reported to be 5–30 mg kg^{-1}, depending on the crop plant species (Yang et al., 2002a). The critical soil total Cu levels were 140–180 mg kg^{-1} for different vegetable crops (Yang et al., 2002b). In the contaminated soil even after amendment with foreign soil, normal crop plants such as mustard could grow normally only when the NH_4OAc extractable Cu levels was reduced to about 25 mg kg^{-1}. At the NH_4OAc extractable Cu levels above 70 mg kg^{-1}, most of the crop plants died from Cu toxicity (unpublished data). *E. splendens* has been regarded as good geobotanical indicator of Cu since 1953 (Se and Sjuj, 1953). The plant can complete its life cycle (survive) at a total soil Cu level over 3000 mg kg^{-1} (Yang et al., 1998), and extractable soil Cu level of 600 mg kg^{-1} in the old mined soil. In nutrient solution, the shoot growth of the plant species was stimulated at Cu levels from 50 to 100 μM, and was not dramatically reduced at Cu levels up to 500 μM (Yang et al., 2002a). The exposure time in the nutrient solution is relatively short, usually less than 20 days. The results from the pot experiment of this study showed that shoot height and shoot dry matter yield slightly increased with increasing Cu supply levels, and peaked at the NH_4OAc extractable Cu of 60 mg kg^{-1}, and then reduced with further increasing Cu levels (Fig. 1). No significant reduction in shoot dry matter yields was noted at the NH_4OAc extractable Cu level as high as 120 mg kg^{-1}, as compared to the control. Under the field condition, the plant growth was normal at the heavily polluted paddy soil with total soil Cu and NH_4OAc extractable Cu levels over 1600 and 75 mg kg^{-1}, respectively, at which the common crop plants such as rice and mustard were unable to grow. Relatively lower shoot biomass yields (170 days) was obtained at soil available Cu higher than 60 mg kg^{-1}, as compared with those at the NH_4OAc-Cu lower than 40 mg kg^{-1} under field conditions (Fig. 2). Higher shoot dry matter yields obtained at the relatively lower Cu levels in the polluted soil may be due to the stimulation of Cu at sub-optimal levels on plant growth of *E. splendens*. The critical soil available Cu level for yield reduction compared with the optimal growth of the plant is approximately 60 mg kg^{-1} as estimated by the NH_4OAc extraction method. A plant biomass yield more than 9 ton ha^{-1} was obtained for the plant species at the NH_4OAc-extractable Cu up to 80 mg kg^{-1} in the soil polluted with Cu and other heavy metals. These results indicate that *E. splendens* is a highly Cu tolerant plant specie and can achieve a large biomass in the soils polluted with different levels of Cu as well as other heavy metals.

The Cu uptake and translocation in the plant of *E. splendens* depend largely on Cu supply levels, growth stage, and growth conditions. Under nutrient solution culture conditions, shoot Cu concentration reached 1133 mg kg^{-1} at the Cu supply level of 500 μM (Yang et al., 2002a). However, shoot Cu concentrations of the plant species were lower than 400 mg kg^{-1} in most cases (Tang et al., 1999; Yang et al., 1998, 2002a). The results from this study show that shoot Cu concentrations of *E. splendens* were only up to 10 mg kg^{-1} grown under glasshouse conditions in the pot and as high as 250 mg kg^{-1} grown under the field conditions. However, root Cu concentrations were up to 1700 mg kg^{-1} in the pot experiment and 1260 mg kg^{-1} in the field experiment. It appears that the ability of the whole plant for extracting Cu from the polluted soil under field conditions was greater than under glasshouse conditions, and mainly relied on root acquisition from the soil. Copper uptake by the plant at the early growth stage was dependent on soil available Cu levels, but this was not the case at the late growth stage, implying the roots of *E. splendens* might has a great ability to explore soil Cu and take up Cu from the non-available pool with the advance of growth stages. Many researchers had reported that accumulating and hyperaccumulating plants excrete proton or special organic substance to acidify the rhizosphere and enhance metal availability, thereby metal uptake by root increases (Römheld, 1991; Kuiters and Mulder, 1993; McGrath et al., 2001). In the present pot experiment, the maximum value of root Cu concentration can reach 1751 $mg\,kg^{-1}$ at the corresponding soil Cu levels extracted by water, NH_4-acetate and NH_4NO_3 of 8.71, 129, and 30.6 $mg\,kg^{-1}$, respectively, probably because of the roots of the plant having activated the fixed Cu fractions in the soil by root exudation of specific organic or inorganic substances. These results indicate that the plants of *E. splendens*, especially the roots, have an extraordinary ability to take up Cu from the polluted soils.

Due to the high dry matter yields, the removal of Cu by the whole plant of *E. splendens* can be remarkable. The total amounts of Cu extracted by the whole plant were 57–495 $\mu g\,plant^{-1}$ in the pot experiment, and as high as 1.7 $kg\,Cu\,ha^{-1}$ in the field experiment. The amount of Cu extracted by the 1.0 M NH_4OAc or NH_4NO_3 was best correlated with plant Cu uptake for both the accumulating and conventional crop plants (Yang et al., 2001; Song, 2002). The water or NH_4OAc extractable soil Cu significantly decreased after the phytoextraction of Cu by the plant species in both pot and field experiments (Fig. 7). Unfortunately, the removal of Cu by the roots was greater than that by the shoots of *E. splendens*, which may limit its commercial use for phytoextraction. However, it may be useful for phytostabilization of metals in the mined area and rhizofiltration of metal in the polluted water.

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References

Baker, A.J.M., 1981. Accumulators and excluder-strategies in the response of plants to heavy metals. J. Plant Nutr. 3, 643–646.

Baker, A.J.M., 1983. Studies on copper and cobalt tolerance in three closely related taxa within the genus *Silene L.* (Caryophyllaceae) from Zaire. Plant Soil 73, 377–385.

Brooks, R.R., Morrison, R.S., Reeves, R.D., Malaisse, F., 1978. Copper and cobalt in Africa species of Aeolanthus Mart (Plectranthinae, Labitae). Plant Soil 50, 503–507.

Brooks, R.R., Reeves, R.D., Morrison, R.S., Malaisse, F., 1980. Hyperaccumulation of copper and cobalt—a review. Bull. Soc. Roy. Belgique. 113, 166–172.

Chaney, R.L., Malik, M., Li, Y.M., Brown, S.L., Brewer, E.P., Angle, J.S., Baker, A.J., 1997. Phytoremediation of soil metals. Curr. Opin. Biotechnol. 8, 279–284.

Cunningham, S.D., Shann, J.R., Crowley, D.E., Anderson, T.A., 1997. Phytoremediation of contaminated water and soil. J. Environ. Qual. 28, 760–766.

Ernst, W.H.O., 1996. Bioavailability of heavy metals and decontamination of soils by plants. Appl. Geochem. 11 (1–2), 163–167.

Hsieh, Hsu, 1954. On *Elsholtzia haichowensis*: an indicator plant for copper. Bull. Geol. Soc. China 32 (4), 360–367 (in Chinese).

Jiang, L.Y., Ye, H.B., Yang, X.E., Shi, W.Y., Jiang, Y.G., 2002. Effect of copper refining on spatial distribution of heavy metal in surrounding soils and crops. J. Zhejiang Univ. (Agric. Life Sci.) 28 (6), 689–693 (in Chinese).

Ke, W.S., Xi, H.A., Yang, Y., Wang, W.X., Chen, S.J., 2001. Analysis on characteristics of phytogeochemistry of *Elsholtzia haichowensis* in Daye Tonglushan copper mine. Acta Ecol. Sinica 6, 907–912 (in Chinese).

Kuiters, A.T., Mulder, W., 1993. Water-soluble organic matter in forest soils. Plant Soil 152, 215–235.

Long, X.X., Yang, X.E., Ni, W.Z., 2002. Current situation and prospect on the remediation of soils contaminated by heavy metals. Chinese J. Appl. Ecol. 13 (6), 757–762 (in Chinese).

Malaisse, F., Gregoire, J., Morrison, R.S., Brooks, R.R., Reeves, R.D., 1979. Copper and cobalt in vegetation of Fuangurume, Shaba Province, Zaire. Oikos 33, 472–478.

Malaisse, F., Brooks, R.R., Baker, A.J.M., 1994. Diversity of vegetation communities in relation to soil heavy metal content at the Shinkolobwe copper/cobalt/uranium mineralization, upper Shaba, Zaire. Belg. J. Bot. 127 (1), 3–16.

McGrath, S.P., 1998. Phytoextraction for soil reclamation. In: Brooks, R.R. (Ed.), Plants that Hyperaccumulate Heavy Metal. Their Role in Phytoremediation. Microbiology, Archaeology, Mineral Exploration and Phytomining. CAB International, Wallingford, pp. 261–287.

McGrath, S.P., Zhao, F.J., Lombi, E., 2001. Plant and rhizosphere process involved in phytoremediation of metal-contaminated soils. Plant Soil 232, 207–214.

MAFF (Ministry of Agriculture Fisheries and Food), 1986. The Analysis of Agricultural Materials. London, Reference Book 427. HMSO, London.

Römheld, V., 1991. The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: an ecological approach. Plant Soil 130, 127–134.

Salt, D.E., Blaylock, M., Nanda Kumar, P.B.A.N., Duschenkov, S., Ensley, B.D., Chet, I., Raskin, I., 1995. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 31, 468–474.

Salt, D.E., Smith, R.D., Raskin, I., 1998. Phytormediation. Annual Rev. Plant Physiol. Plant Mol. Biol. 49, 643–668.

Se, S.T., Sjuj, B.L., 1953. *Elsholtzia haichowensis* Sun—A plant that can reveal the presence of copper-bearing strata. Dichzi Sjuozheo. 32, 360–368.

Song, J., 2002. Assessment of phytoavailability of soil metals and phytoremediation of soils contaminated with copper.

Ph.D. thesis, Soil Sci Res. Institute, Chinese Academica Sinica, pp. 44–57 (in Chinese).

SSICA (Soil Sci. Ch. Acad.), 1980. Physical and Chemical Analyses of Soils. Shanghai Academic Press, China (in Chinese).

Tang, S.R., Wilke, B.M., Huang, C.Y., 1999. The uptake of copper by plants dominantly growing on copper mined spoils along the Yangtze River, the People's Republic of China. Plant Soil 209, 225–232.

Tang, S.R., Wilke, B.M., Brooks, R.R., 2001. Heavy-metal uptake by metal tolerant *Elsholtzia haichowensis* and *Commelina communis* from China. Commun. Soil Sci. Plant Anal. 32, 895–905.

Yang, X.E., Shi, W.Y., Fu, C.X., Yang, M.J., 1998. Copper-hyperaccumulators of Chinese native plants: characteristics and possible use for phyto-remediation. In: Bassam, N.E.L. (Ed.), Sustainable Agriculture for Food, Energy and Industry. James and James Publishers, London, pp. 484–489.

Yang, X.E., He, Z.L., Li, Y.C., Calvert, D.V., Stoffella, P.J., 2001. Effect of pH on copper availability and transformation in soils. Poster presentation. In: Proc. of the 95th Annual Meeting of ASA-CCSA-SSSA, p. 567.

Yang, M.J., Yang, X.E., Römheld, V., 2002a. Growth and nutrient composition of *Elsholtzia splendens* Nakai under copper toxicity. J. Plant Nutr. 25 (7), 1359–1375.

Yang, X.E., Long, X.X., Ni, W.Z., He, Z.L., Stoffella, P.J., Calvert, D.V., 2002b. Assessing copper thresholds for phytotoxicity and potential toxicity in selected crops. J. Environ. Sci. Health B 37, 625–635.