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Characteristics of Copper and Lead Uptake and Accumulation by Two Species of *Elsholtzia*

H. -Y. Peng · X. -E. Yang

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Copper (Cu) is an essential micronutrient for healthy organisms. A copper level of 3 to 20 mg kg^{-1} is needed for the normal growth of plants, and a level of 30 µg kg^{-1} is needed for adult uptake in daily diet based on his or her weight. However, lead (Pb) is a toxic metal with neuro-virulent properties, and animals and humans are very sensitive to it. In the People's Republic of China, millions of hectares of land have been contaminated with metals such as Cu and Pb, including mixed, transboundary, and regional properties, mainly because of the use of sludge or urban compost, pesticides, fertilizers, and the residues from metalliferous mining and metal smelting industries. Most physical and chemical resolutions of remediating the polluted soil are expensive and are ultimately unable to resolve this problem. However, phytoremediation, a plant-based technology, has become an attractive approach with advantages of location in situ, lower cost, minimal environmental disturbance, elimination of secondary pollution, and public acceptance (Salt et al., 1998). *Elsholtzia splendens* (Haizhou *Elsholtzia*) has a local nickname of ''copper flower'' because its growth is confined to highly Cu contaminated soils. It has been identified as a Chinese native Cu-tolerant plant species, and it has been widely investigated for phytoremediation of Cu contaminated soil (Yang et al., 2002; Song et al., 2004; Jiang et al., 2004a; Yang et al., 2005; Weng et al., 2005; Peng et al., 2005; 2006). *E. splendens* can cotolerate total levels of 1500 mg kg^{-1} Cu, 2001 mg kg^{-1} Pb, 1500 mg kg^{-1} Zn and 21.1 mg kg^{-1} Cd, respectively, in the field trial (Peng and Yang, 2005). *Elsholtzia argyi* (Purple Flower *Elsholtzia*), plants endemic to Pb/Zn mine waste deposits, have been reported as another Chinese native Cu-tolerant plant species (Jiang et al., 2004b). Both *Elsholtzia* plants belong to the family Labiatae. In this study, growth response and accumulation of Cu and Pb in both *E. splendens* and *E. aryi* were compared using solution and pot experiments for assessing their tolerance to Cu and Pb toxicity.

H. -Y. Peng (✉) · X. -E. Yang
Ministry of Education Key Laboratory of Environmental Remediation and Ecological Health, College of Natural Resources and Environmental Science, Zhejiang University, 310029 Hangzhou, People's Republic of China
e-mail: penghongyun@zju.edu.cn

Materials and Methods

Seeds of *E. splendens* and *E. argyi*, collected from mature plants growing on Cu mine and Pb/Zn mine waste deposits, respectively, of Zhejiang province of the People's Republic of China, were surface sterilized, rinsed, and sown in substrate having a combination of perlite + vermiculite in 3:1 ratio moistened with distilled water. After emergence of seedlings, ¼-strength basal nutrient solution was supplied until 4-leaf seedlings were established, and then the uniform plants were selected and transferred to hydroponics culture in 3.6 L plastic containers (5 plants per container) with a full-strength aerated nutrient solution for another 2 weeks preculture. The plants were treated with different Cu (0.016, 6.4, 32 mg L^{-1}) and different Pb (0, 10, 25, 50, 100, 200, 400 mg L^{-1}). Copper sulfate ($CuSO_4 \cdot 5H_2O$) and $Pb(NO_3)_2$ were analytical grade with purities of 99.0%. The levels of Cu and Pb in nutrient solutions were carefully calculated and measured with AAS, which was close to the real values, respectively, with 1% error for each named item. The composition of nutrient solution used for Cu treatments was (in µmol L^{-1}): 700 K_2SO_4, 100 KCl, 2000 $Ca(NO_3)_2 \cdot 4H_2O$, 500

$NgSO_4{\cdot}7H_2O$, 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); and for Pb treatments was (in μmol L^{-1}): 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 (Jarvis and Leung, 2001). Each treatment was replicated thrice. All solutions were aerated continuously, adjusted pH 5.5 ± 0.3 daily with 0.1 mol L^{-1} HCl or 0.1 mol L^{-1} NaOH, and renewed after every 4 d during the experiment, which was conducted in a greenhouse in September, 2003, with the temperature ranging from 28 ± 2°C (day) to 15 ± 2°C (night), without supplementary light. The Pb-treated plants were harvested at 0, 8, and 16 d, and the Cu-treated plants were harvested at 0, 7, 14, 21, and 28 d, respectively. At each harvest, roots of intact plants were rinsed with distilled water. The Pb-treated plants were immersed in 0.5 mmol L^{-1} Na_2EDTA for 30 min to remove Pb (Jarvis and Leung, 2001), and the Cu-treated plants were in 5 mmol L^{-1} $Pb(NO_3)_2$ for 20 min to remove Cu (Harrison et al., 1979) adhering to the root surfaces, respectively, and then washed with distilled water and blotted dry. Roots, stems, and leaves were separated and oven-dried at 105°C for 30 min, and then they were dried at 65°C to constant weight, and their dry weights (DW) were recorded.

In the pot experiment, two different soils were used: one was an Alluvial loam paddy soil of Fuyang county in Zhejiang Province of the People's Republic of China, where the soil is severely polluted with metals because of the Cu refining activities; this was polluted soil (PS). The other soil was an Alluvial soil (Flurio-marine yellow loamy soil) from a farm in Huajiachi campus of Zhejiang University of the People's Republic of China, and this soil was considered as nonpolluted soil (NPS). The top 10 cm of PS (10 m nearest to the Cu refining plants) and NPS were collected. The chemical properties of PS were as follows: pH (H_2O) 7.03, 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. For NPS, the chemical properties were: pH (H_2O) 6.03, organic matter 16.0 g kg^{-1}, cation exchange capacity (CEC) 14.6 cmol kg^{-1}, total N and P of 2.40 and 1.37 g kg^{-1}, available N, P, and K of 268.3, 41.1, and 30.6 mg kg^{-1}, respectively. Total Cu and Pb of PS were 2800 and 1150 mg kg^{-1} with NH_4OAc extractable Cu and Pb of 201 and 151 mg kg^{-1}, respectively. Total Cu and Pb of NPS were 100 and 40 mg kg^{-1} with NH_4OAc extractable Cu and Pb of 1.29 and 0.96 mg kg^{-1}, respectively. These soils were air-dried, passed through a 2.0-mm sieve, and placed in plastic pots. Each pot contained 1 kg of soil. Seeds of *E.splendens* and *E. argyi* were germinated directly in PS and NPS, respectively. After forty-five days of growth, five uniform seedlings were left in each pot. A randomized, complete block experimental design was used with each treatment having 6 replications. Soil moisture was maintained at 60% to 70% of the maximum field water-holding capacity by adding distilled water during the experimental period. Plants were grown under greenhouse conditions in April, 2004, with the natural light, day/night temperature of 30°C /25°C and day/night humidity of 65%/80%. After another 45 d growth, all plants were harvested, rinsed with distilled water, and blotted dry. Roots, stems, and leaves were separated, oven-dried at 105°C for 30 min, and then dried at 65°C to constant weight, and their DW were recorded.

The dried plant materials were ground with a stainless steel mill and were passed through a 60-mesh sieve, ashed at 550°C, and dissolved in 10 mL 1:1(V:V) HNO_3. Copper and Pb concentrations in the plant digestion were measured by an Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, Model IRAS-AP, TJA). The detection limitations of Cu and Pb were 0.01 mg L^{-1} and 0.02 mg L^{-1}, respectively. Tea leaf samples GBW–07605 (GSV-4) and soil samples GBW-07405 were used as standard reference substance with 0.5 mg L^{-1} Cu and 0.1 mg L^{-1} Pb used to control the accuracy of the instrument. All data were presented as mean values of at least 3 (solution experiment) or 6 replicates (pot experiment). SPSS statistical software package (Version 11.5) was used. One-way ANOVA with Tukey test post hoc procedure was employed to evaluate whether the means were significantly different at $p < 0.01$ or $p < 0.05$.

Results and Discussion

Copper level of 0.25 μmol L^{-1} (i.e., 0.016 mg L^{-1}) in nutrient solution can be regarded as a control in this study, for it is generally adequate for optimum growth of the majority of higher plants (Marschner, 1995). For the control, DW of root, stem and leaf of both *Elsholtzia* plants increased pronouncedly, and total DW of *E.argyi* elevated significantly than that of *E. splendens*, as Cu exposure time increased, indicating that *E. argyi* grows better than *E. splendens* under uncontaminated condition. Cu at 6.4 mg L^{-1} for 28 d duration, DW of *E. argyi* decreased by 90%, whereas that of *E. splendens* significantly reduced by 26%, as compared to controls. At 32 mg L^{-1} Cu for 28 d duration, DW of both *Elsholtzia* plants significantly decreased as compared to controls (Fig. 1). With increasing exposure time for 6.4 mg L^{-1} Cu, both root length and shoot height of *E.splendens* increased nonsignificantly as compared to control, whereas for *E. argyi*, shoot height increased but root length decreased, and both were significantly less than the control (Fig. 2). *E. splendens* can tolerate 6.4 mg L^{-1}

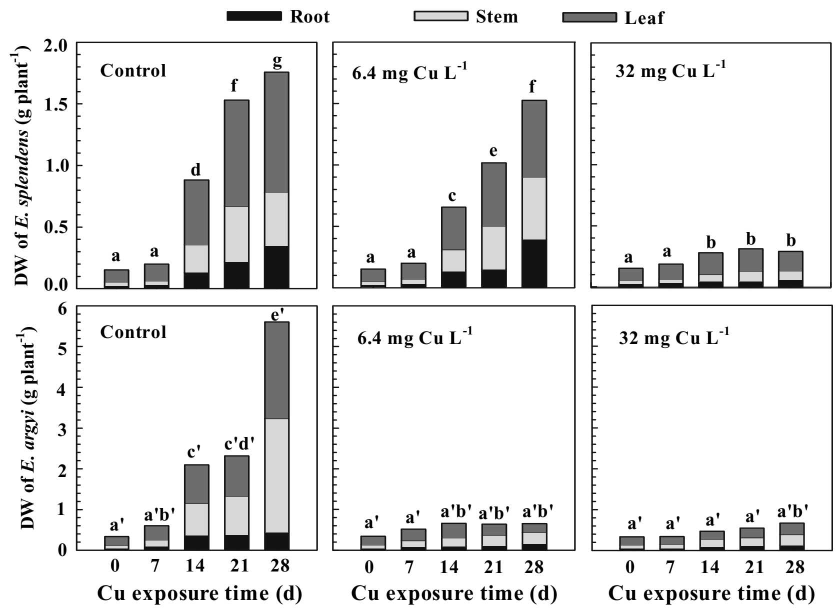

Fig. 1 Dry weight (DW) of *E. splendens* and *E. argyi* exposed to different Cu for 0, 7, 14, 22, and 28 d. Data are means of 3 replications. Lower-case letters a, b, c, d, etc., represent significant differences at $p < 0.05$

Cu better than *E. argyi* for 28-d Cu exposure time in solution culture. At 6.4 mg L^{-1} Cu, tissue accumulations in both plants decreased as follows: root > leaf > stem, and they were enhanced as Cu exposure time was extended. After treatment with 6.4 mg L^{-1} Cu for 14 d, more Cu was discovered in leaves of *E. splendens* than *E. argyi* (Fig. 3). When exposured to 6.4 mg L^{-1} Cu for 28 d, shoots Cu accumulation in *E. splendens* and *E. argyi* were 0.30 and 0.13 g $plant^{-1}$ DW, respectively, with shoot biomass of 1.13 and 0.48 g $plant^{-1}$, respectively, indicating that *E. splendens* can remove more Cu than *E. argyi* at this growing condition. Tian et al. (2006) discovered that the significantly higher efficiency of Cu phytofiltration by *E. splendens* is associated with its better ability to tolerate higher Cu concentrations and to absorb more Cu ions to plant roots as well as to translocate absorbed Cu from roots to shoots as compared to *E. argyi*. In this study, *E. splendens* showed better potential for the phytoremdiation of Cu pollution than *E. argyi* because of its greater capacity for tolerating high Cu toxicity and accumulating higher levels of Cu in its shoot.

With increasing Pb level in solution, DW of root, stem, and leaf of both *E. splendens* and *E. argyi* considerably decreased at day 16 after Pb treatment (Fig. 4), and the former decreased faster than that of the latter. Up to 100 mg L^{-1} Pb in solution, root DW of *E. splendens*, was depressed by 70% whereas that of *E. argyi* by 5%, stem DW of *E. splendens*, and *E. argyi* by 70% and 10%, respectively, and leaf DW by 60%, respectively, as compared to controls, so as to much more biomass reduction of *E. splendens* than

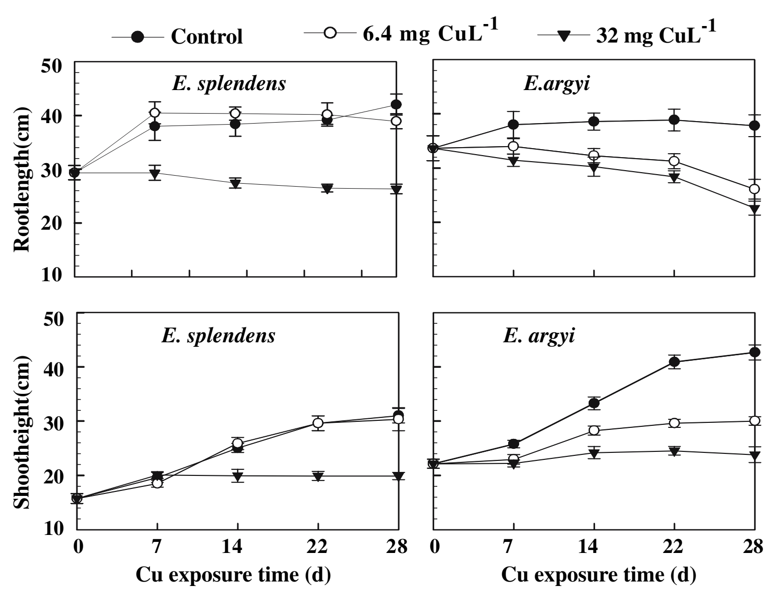

Fig. 2 Root length and shoot height of *E. splendens* and *E. argyi* exposed to different Cu for 0, 7, 14, 22, and 28 d. Data are means of 3 replications, and bars depict SE

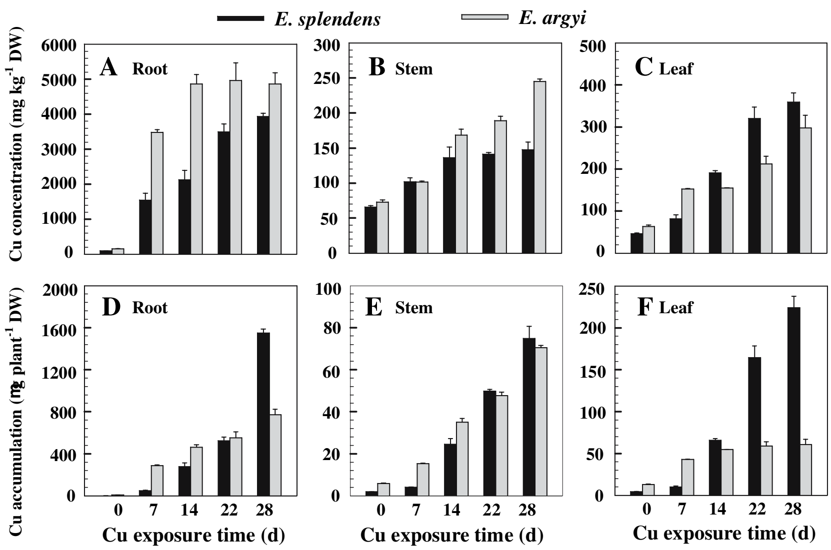

Fig. 3 Copper concentrations and accumulations in roots, stems, and leaves of *E. splendens* and *E. argyi* exposed to 6.4 mg L^{-1} Cu for 0, 7, 14, 22, and 28 d. Data are means of 3 replications, and bars depict SE

that of *E. argyi* (Fig. 4). As Pb level was increased, shoot height of both plants decreased, especially for *E. splendens*, which decreased faster as compared to *E. argyi* and exhibited the considerable reduction at 25 mg L^{-1} Pb. Root length of both plants significantly decreased at 25 mg L^{-1} Pb as compared to controls. These results indicated that Pb at ≥ 25 mg L^{-1} caused much more toxicity on the growth of *E. splendens* than that of *E. argyi*. Lead concentrations and accumulations in both plants were observed with root > stem > leaf increasing considerably with the increasing Pb level in solution. With Pb at < 50 mg L^{-1}, higher leaf Pb concentration and accumulation were observed in *E. splendens* as compared to *E. argyi*, indicating that *E. splendens* has better capacity of transporting Pb from roots to shoots under this condition. At Pb > 50 mg L^{-1}, much more Pb was transported to shoots of *E. argyi* than that of *E. splendens* (Fig. 5). For example, at 50–100 mg L^{-1} Pb in solution, shoot Pb concentration in *E. splendens* and *E. argyi* was 384–565 and 629–1422 mg kg^{-1}, respectively. Shoot Pb concentration in Pb accumulating ecotype of *Sedum alfredii* Hance was 514 mg kg^{-1} under the same condition (He et al., 2002). *E. argyi* could be used as another Pb accumulating plant species. With Pb at 50–100 mg L^{-1} in solution for 16 d, shoot Pb accumulation in *E. splendens* and *E. argyi* were 31.8–25.9 and 76.1–154.5 mg plant^{-1}, respectively, with shoot biomass of 0.27–0.16 and 0.32–0.31 g plant^{-1}, respectively. *E. splendens* is an endemic plant in old Cu mine; it can also tolerate and accumulate Pb in shoots, but much less than that of *E. argyi*, which came from old Pb/Zn mine waste deposit.

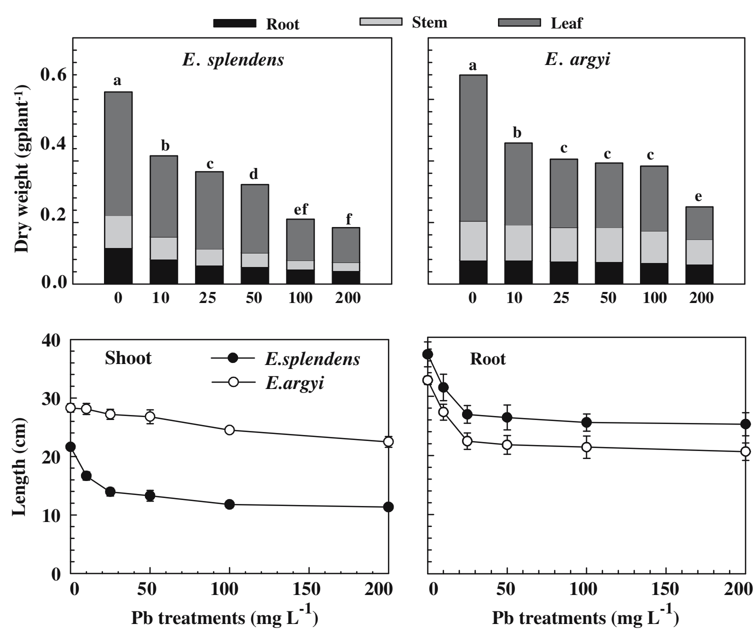

Fig. 4 Dry weight, shoot height, and root length of *E. splendens* and *E. argyi* exposed to different Pb for 16 d. Data are means of 3 replications. Letter a, b, c, d, and e represent significant differences at $p < 0.05$ (Supper), and the bars depict SE (Lower)

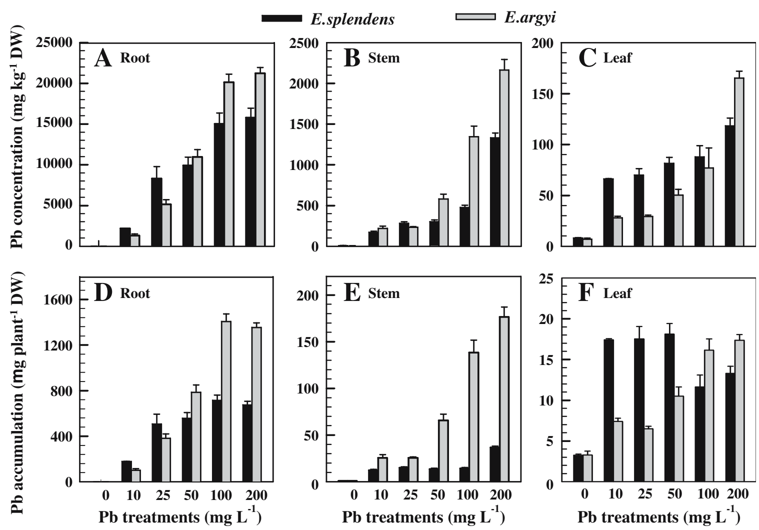

Fig. 5 Lead concentrations and accumulations in roots, stems, and leaves of *E. splendens* and *E. argyi* exposed to different Pb for 16 d. Data are means of 3 replications, and the bars depict SE

In the pot experiment, DW of root, stem, and leaf of *E. splendens* growing in PS were much higher that those in NPS, and shoot height increased, whereas there was a decrease in root length in PS as compared to those in NPS. Whereas for *E. argyi*, DW and root length, as well as shoot height, decreased more in PS than those in NPS (Table 1). For example, after growing in PS for 90 d, shoot height and root length of *E. splendens* were 29.0 and 25.5 cm, respectively, whereas shoot height and root length of *E. argyi* were only 6.5 and 2.5, cm respectively; DW of root, stem, and leaf of *E. splendens* were 29.7, 81.4, and 155.3 mg plant^{-1}, respectively, whereas those of *E. argyi* were 0.95, 1.43, 3.10 mg plant^{-1}, respectively (Table 1), indicating that *E. splendens* could survive better in PS than in NPS, but *E. argyi* grows better in NPS than in PS. In the pot experiment, Cu and Pb distribution in both plants were observed with root > shoot (Table 1), which results were in accordance with the report that most of Cu and Pb are located in plant roots, and only a small amount is translocated into plant shoots (Song et al., 2004; Begonia et al., 1998). Much higher Cu and Pb accumulated in root, stem, and leaf of *E. splendens* in PS than in NPS after 90 d plant growth, whereas for *E. argyi*, it was the opposite (Table 1). In this study,

Table 1 Growth response, metal concentrations, and accumulations in plants after 90-d growth in the pot experiment

Growth response

Soil type	Plant sample	Root length (cm)	Shoot height (cm)	DW (mg plant^{-1})		
				Root	Stem	Leaf
PS	E. splendens	25.50 C	29.00 A	29.70 B	81.40 A	155.3 A
	E. argyi	2.50 E	6.50 E	0.95 D	1.43 D	3.10 D
NPS	*E. splendens*	29.13 B	19.27 B	14.29 C	62.10 B	120.7 B
	E. argyi	21.23 D	17.77 C	40.67 A	39.30 C	98.00 C

Metal concentrations and accumulations

Soil type	Plant sample	Cu concentration (mg kg^{-1} DW)			Cu accumulation (µg plant^{-1} DW)			Pb concentration (mg kg^{-1} DW)			Pb accumulation (µg plant^{-1} DW)		
		Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
PS	*E. splendens*	827.81B	82.13B	66.62B	24.59A	6.68A	10.35A	367.3B	43.10B	116.9A	10.91A	3.51A	18.16A
	E. argyi	1698.0A	184.3A	114.8A	1.61B	0.26C	0.36C	754.3A	204.1A	115.3A	0.72C	0.29C	0.36B
NPS	*E. splendens*	59.92C	53.47C	53.51C	0.86B	3.32B	6.46B	58.66C	37.74B	15.10B	0.84C	2.34A	1.82B
	E. argyi	76.57C	45.85C	55.06C	3.11B	1.80B	5.40B	64.36C	47.21B	15.06B	2.62B	1.86B	1.48B

Values followed by capital letter A, B, C, and D represent significant differences at $p < 0.01$. PS and NPS refer to polluted paddy soil and nonpolluted soil, respectively, which has been defined in the Method Section

d, day

E. splendens survived well in PS with NH_4OAc extractable Cu and Pb of 201 and 151 mg kg^{-1}, shoot Cu and Pb concentrations were 71.95 and 91.52 mg kg^{-1} DW, and root Cu and Pb concentrations were 827.8 and 367.3 mg kg^{-1} DW, with root and shoot biomass of 29.7 and 236.7 mg $plant^{-1}$ DW, respectively. Plants harvested at 90 d resulted in shoot Cu and Pb accumulation in *E.splendens* of 17.03 and 21.66 μg $plant^{-1}$ DW and root Cu and Pb accumulation of 24.59 and 10.91 μg $plant^{-1}$, respectively. *E. splendens* have an extraordinary ability to tolerate Cu and Pb toxicity in this Cu/Pb mixed- polluted soil; the total amounts of Cu and Pb extracted by the whole plant were 41.62 and 32.57 μg $plant^{-1}$ DW in the pot experiment. As for *E. argyi*, its growth was not good in PS with much less shoot biomass and even the cessation of plant growth, which was mainly caused by its inability to tolerate high Cu toxicity in PS. Therefore, *E. splendens* has great potential for phytoremediation of this high Cu/Pb mixed contaminated paddy soils as compared to *E. argyi*.

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