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Distribution and Accumulation of Copper, Lead, Zinc, and Cadmium Contaminants in *Elsholtzia splendens* Grown in the Metal Contaminated Soil: A Field Trial Study

H.-Y. Peng, X.-E. Yang

MOE Key Laboratory of Environmental Remediation and Ecosystem Health, College of Natural Resources and Environmental Science, Zhejiang University, Hangzhou, 310029, People's Republic of China

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Much research work has been conducted in China on phytoremediation of heavy metal-polluted soil by *Elsholtzia splendens* (Yang *et al*., 2002;Yang *et al*., 1998; Jiang *et al*., 2002b, 2004ab). *E. splendens*, the dominant plant in the old copper mining deposit, has evolved high tolerance to Cu over many years (Jiang *et al*., 2002b; Tang *et al*., 1999, 2001; Yang *et al*., 1998; Yang *et al*., 2002). In the field of the mined area, root and shoot Cu were 2,288 mg kg^{-1} and 304 mg kg^{-1}, respectively. In solution culture, the stimulated plant growth was found at 50 μmol L^{-1} Cu supplies, and shoot Cu of above 1,000 mg kg^{-1} on a dry weight basis can be achieved at 500 μmol L^{-1} Cu supplies (Yang *et al*., 2002). Phytoextraction by *E. splendens* can effectively reduce the plant-available Cu levels in the polluted soils (Jiang *et al*., 2004b), and its shoot biomass yield as high as 11 t ha^{-1} can be achieved when grown in the field with favorable water and nutrients supplies (Jiang *et al*., 2004b).

To date, minimal information is available about the distribution and accumulation of Cu, Pb, Zn and Cd contaminants in *E. splendens* grown in the metal-polluted site in a field trial as affected by soil amendments. The multi-metal contaminated site was located at Huanshan Village, Fuyang County, Zhejiang Province of China, formerly as agricultural soil (an Alluvial loam, paddy soil). Soil amendments like organic manure (M), furnace slag (F), and soil preparations like soil capping (S) and discing (D) have been applied to the site (Jiang *et al*., 2004b). In this study, a field trial of *in situ* phytoremediation of the site by *E. splendens* was conducted in combination of soil amendments like M and F, to investigate plant growth response, the accumulation and translocation of Cu, Pb, Zn and Cd contaminants in different portions (roots, stems, leaves and flowers) of the plants before flowering and at harvesting, for evaluating the phytoremediation potential by *E. splendens* from the metal contaminated site as affected by soil amendments.

MATERIALS AND METHODS

The soil of metal contaminated site, located at Huanshan Village, Fuyang County, Zhejiang Province of China, contained high levels of Cu, Pb, Zn and Cd in the topsoil (0-15cm) due to metal smelting activities. The main agrochemical properties of the tested 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},

Correspondence to: H.-Y. Peng, College of Environmental and Resource Sciences, Zhejiang University, Huajiachi Campus, Hangzhou, 310029, People's Republic of China

available N, P, and K of 137.1, 37.2, and 24.6 mg kg^{-1}, respectively. Total Cu, Pb, Zn and Cd were 1,580, 1,500, 2,001 and 21.1 mg kg^{-1} respectively, and NH_4OAc extractable Cu, Pb, Zn and Cd were 79.4, 49.0, 18.1 and 3.2 mg kg^{-1}, respectively, at the soil layer of 0-15 cm. Five soil treatments were adopted and some soil properties after one-year treatments are shown in Table 1. In the field experiment, the area of each plot was 15 m^2, with one-meter distance between the two neighboring plots and the edges of each plot were separated by a plastic sheet to prevent heavy metals movement between the plots. All the plots were randomly arranged with four replications for each treatment. The five soil treatments were: A. control; B. M (1,500 kg ha^{-1} organic manure); C. MF (1,500 kg ha^{-1} organic manure plus 3,750 kg ha^{-1} furnace slag); D. S+MF (soil capping with 750,000 kg ha^{-1} red clay soil + C); and E. D+MF (soil discing by digging the topsoil (0-15cm) and the plow soil (0-5cm), putting the topsoil at the bottom and plow soil at the surface, respectively, + C).

Seeds of *E. splendens* were collected from mature plants grown on the copper mined deposit (Zhuji County of Zhejiang Province, China), and germinated in the substrate. The germinated seeds were supplied with nutrient solution until the 40-day-old plant seedlings were established, then the uniform plant seedlings were selected to grow in each plot with a density of 20 cm × 20 cm in the field experiment at the beginning of May, 2002. No fertilizers and pesticides were applied, but weeding was made twice during the field experiment. The plant samples were collected at the two physiological stages, before flowering and at harvesting, thoroughly washed first with tap water and then distilled water, blotted dried, and oven dried at 65°C. The dry weight (DW) were recorded and the dried plant materials were ground to < 1 mm with a stainless steel mill. A sub-sample was ashed at 550°C for 6h, and dissolved in 1:1(V:V) HNO_3. The concentrations of Cu, Pb, Zn and Cd in the plant digestions were measured using an Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, Model IRAS-AP, TJA).

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

RESULTS AND DISCUSSION

High levels of Cu, Pb, Zn and Cd accumulated in the topsoil (0-15cm) of the metal contaminated site, located at Huanshan Village, Fuyang County, Zhejiang Province of China, due to metal smelting activities (Table 1), resulted in the harsh conditions that no crops can survive well. However, *E. splendens* can grow better in this site with large vegetation area and high shoot biomass yield (Jiang *et al.*, 2004b).

As the soil treatment with control, M, MF, S+MF and D+MF, the levels of Cu, Pb, Zn and Cd in the contaminated site decreased (Table 1). For both sampling times, plant growth of *E. splendens* was slightly depressed with the increasing of metal contaminants in soil (Fig.1). Before flowering, DW of *E. splendens* grown in each

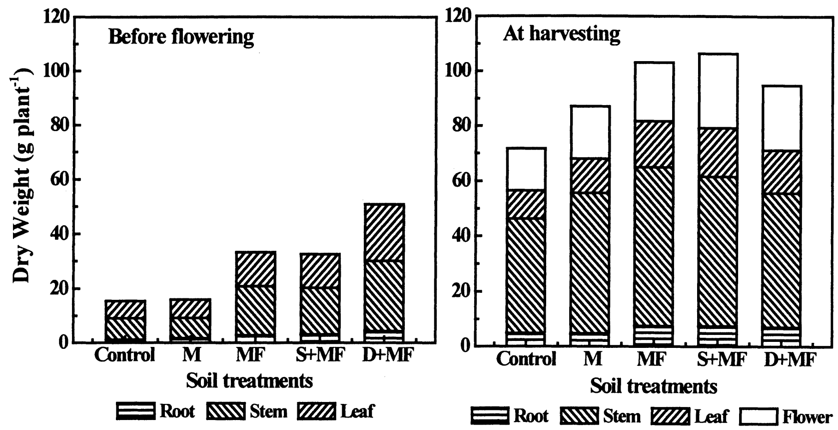

Figure 1. Dry weight of roots, stems, leaves and flowers of *E. splendens* grown in the multi-metal contaminated site at two sampling times (before flowering and at harvesting). All the data are means of 6 replications.

treated soil was: stem > leaf > root; at harvesting, it was stem > flower > leaf > root. Stem was the plant part with the greatest biomass (>50% of total weight of plant). Soil treatments can affect the growth of *E. splendens*. Before flowering, D+MF treatment caused the greatest biomass of *E. splendens*, while at harvesting, it was noted at S+MF treatment, followed by MF treatment (Fig. 1). The reasons may come from the significantly decreased levels of total and available Cu, Pb, Zn and Cd in soil at S+MF and D+MF treatment, although the two soil treatments significantly decreased the contents of NPK and organic matter in soil, so as to the lower soil fertility, as compared to control (Table 1). Before flowering, DW of *E. splendens* was 18-50 g plant^{-1}, while at harvesting, it was increased to 70-110 g plant^{-1}, which was mainly attributed to the fast increased stem DW and flower DW.

For both sampling times, Cu, Pb, Zn and Cd concentrations in the plant organs (roots, stems, leaves and flowers) were increased with the rise of these heavy metal levels in soil, this effect has been observed for several plants (Denny and Wilkins, 1987; Poschenrieder *et al.*, 2001). Zn is necessary as a micronutrient and it is known that plant have special zinc transporters to absorb this metal (Guerinot, 1997). However, an excessive accumulation of Zn in living tissues leads to toxicity symptoms (Marschner, 1985). In our experiment, leaf is the major organ of Zn accumulation. Brune *et al.* (1994) showed that Zn accumulation in leaf could be a mechanism to avoid toxic effects of Zn in stem. At both sampling times, and with soil contamination levels, the maximum concentration of Pb and Cu in *E. splendens* was found in root (Table 2), which was also observed by Poschenrieder *et al.* (2001) for several plants. Copper is an essential micronutrient for plant life but it is toxic at high concentration (Burzynski and Buczek, 1997). *E. splendens* accumulated much more Cu in the root, escaping the high Cu toxicity on shoot parts. Soil Pb especially soluble Pb is very toxic to plant growth. Pb concentration in the ordinary plant is 0.02-3.0 mg kg^{-1}, and most of plant can mobilize Pb to the

Table 1. Physicochemical properties of the amended soil in the field trial .*

Soil treatments	pH (H_2O)	OM** ($g\ kg^{-1}$)	Total contents ($g\ kg^{-1}$)						Available contents ** ($mg\ kg^{-1}$)						
			N	P	Cu	Pb	Zn	Cd	N	P	K	Cu	Pb	Zn	Cd
Control	7.54	55.7	2.43	1.11	1.43	1.50	2.00	0.021	297.7	25.2	29.5	76.7	49.0	18.1	3.2
M	7.54	55.9	2.55	0.94	1.43	1.47	1.99	0.021	326.7	25.4	29.8	78.2	50.0	18.3	3.4
MF	7.47	59.9	2.60	0.99	1.23	1.44	1.98	0.020	317.3	24.6	31.1	71.4	44.5	18.0	3.0
S+MF	7.76	39.8	1.43	0.69	0.39	0.84	1.56	0.016	167.1	10.7	36.9	39.0	16.9	14.8	1.5
D+MF	7.75	46.9	1.93	0.85	0.32	0.50	1.27	0.013	208.1	15.6	26.6	25.2	2.9	13.3	1.0

* Soil sampling and analyses were conducted one year after five soil treatments were applied to the metal polluted site before the field trial of phytoremediation were performed.

** Available contents of heavy metals were extracted with 1mol L^{-1} NH_4OAc. OM refers to organic matter.

Table 2. Heavy metal concentrations in *E. splendens* before flowering and at harvesting.

Heavy metal	Soil treatments	Heavy metal concentration in plant (mg kg^{-1})						
		Before flowering			At harvesting			
		Root	Stem	Leaf	Root	Stem	Leaf	Flower
Cu	Control	1312 b	71 a	169 ab	1302 a	54 a	135 b	77 b
	M	1305 b	70 a	171 ab	1297 a	54 a	161 a	79 b
	MF	1384 a	75 a	207 a	1314 a	53 a	159 a	88 a
	S+MF	896 c	60 b	136 b	886 b	46 b	132 b	61 c
	D+MF	872 c	60 b	102 b	855 b	51 a	121 c	64 c
Pb	Control	571 b	33 a	50 a	492 a	26 a	50 a	28 ab
	M	682 a	32 a	53 a	543 b	28 a	51 a	27 ab
	MF	572 b	30 a	56 a	467 c	28 a	50 a	32 a
	S+MF	297 c	26 b	36 b	288 d	18 b	36 b	20 b
	D+MF	210 d	16 c	20 c	208 e	11 c	18 c	13 c
Zn	Control	483 a	452 ab	491 bc	464 ab	236 a	340 b	228 b
	M	490 ab	472 a	516 ab	460 b	212 a	402 a	202 c
	MF	498 a	426 b	535 a	486 a	214 a	343 b	265 a
	S+MF	397 c	399 c	540 a	386 c	165 b	399 a	183 d
	D+MF	381 d	345 d	458 c	362 d	185 b	335 b	159 e
Cd	Control	8.6 a	2.4 b	3.2 b	8.4 a	2.1 c	2.6 bc	2.9 b
	M	8.4 a	2.7 a	3.2 b	8.7 a	2.7 b	2.8 ab	3.0 a
	MF	8.1 a	2.9 a	4.7 a	7.9 a	3.2 a	2.9 a	2.7 c
	S+MF	5.1 b	2.0 c	2.4 c	4.9 b	2.0 c	2.4 c	1.6 d
	D+MF	4.3 b	1.0 d	1.1 d	4.0 c	1.5 d	1.4 d	1.2 e

Letters a, b, c, d and e show the significant differences between the five soil treatments. Different letters indicate statistical significance at $p < 0.05$.

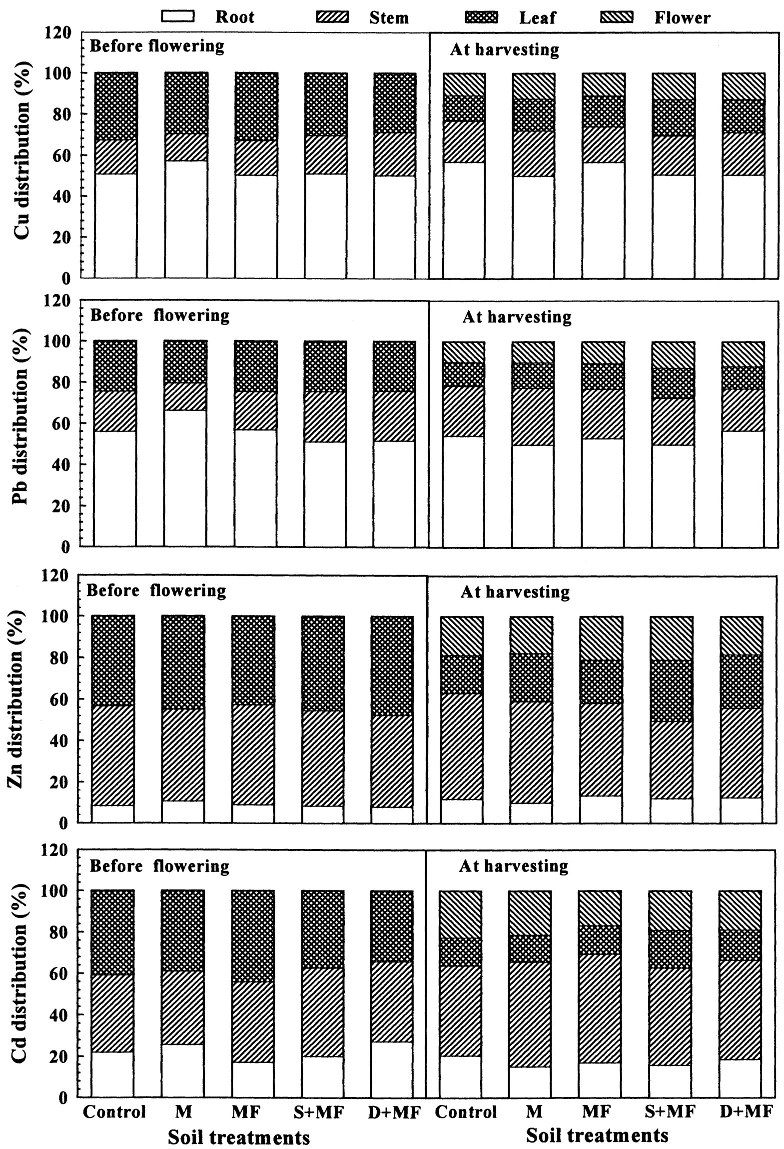

Figure 2. Distribution of Cu, Pb, Zn and Cd in the roots, stems, leaves and flowers of *E. splendens* grown in the multi-metal contaminated site at two sampling times (before flowering and at harvesting). All the data are means of 6 replications.

root, reducing its toxic to plant shoot. Shoot Pb in *E. splendens* was 11-33 mg kg^{-1}, but was much lower than root Pb. Cu and Pb in *E. splendens* were much more difficult to translocate from root to shoot. The major organ of Cd concentrated in *E. splendens* was the root. Studies of tobacco showed that the compartmentation of Cd in vacuoles of roots was a physiological mechanism of Cd tolerance (Vögeli and Wagner, 1990). For both sampling times, the concentration of Cd in stem, leaf

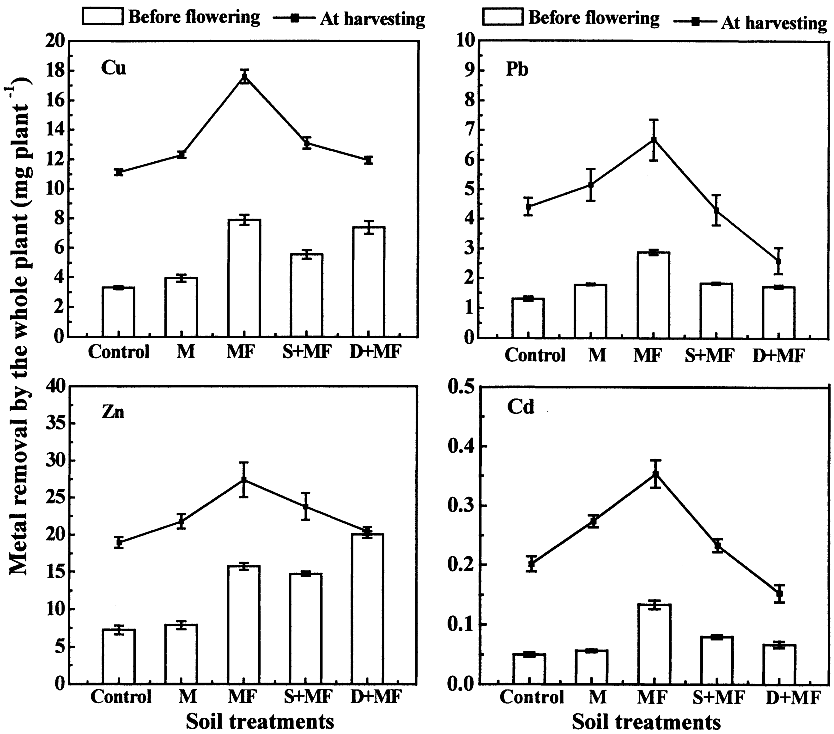

Figure 3. Metal removal by the whole of *E. splendens* grown in the multi-metal contaminated site at two sampling times (before flowering and at harvesting). All the data are means of 6 replications, and the bar depicts the least significant difference ($LSD_{0.05}$).

and flower of *E. splendens* were found at the same level (about 1.0-3.2 mg kg^{-1}). At harvesting, Zn, Cu and Pb concentrations in *E. splendens* were decreased, as compared to those collected before flowering, which was mainly attributed to the dilution of heavy metals in plant because of the fast growth of plant. The faster growth of stem resulted in the faster decreased concentrations of Zn, Cu and Pb in stem of *E. splendens*.

The maximum accumulation of heavy metals in the aerial parts of plants makes it the best candidate for the phytoremediation of metal contaminated soil (Saxena *et al.*, 1999). In our study, for both sampling times, accumulation much more Cu and Pb in root than in shoot parts of *E. splendens* were noted, while the accumulation of Zn and Cd was the contrary (Fig.2). The much greater biomass of *E. splendens* at harvesting than before flowering showed that sampling at harvesting is the optimum time that the maximum accumulation of heavy metals could be achieved, and MF treatment resulted in the greatest Cu, Pb, Zn and Cd removal by the whole plant of *E. splendens* when collected plant at harvesting (Fig.3). Before flowering, about 8 mg Cu $plant^{-1}$, 3 mg Pb $plant^{-1}$, 15 mg Zn $plant^{-1}$ and 0.14 mg Cd $plant^{-1}$ can be taken away from MF-treated soil by *E. splendens*. While at harvesting, *E. splendens* can remove about 17 mg Cu $plant^{-1}$, 6.8 mg Pb $plant^{-1}$, 27 mg Zn $plant^{-1}$ and 0.35 mg Cd $plant^{-1}$ from MF-treated soil. *E. splendens* survive well at this Cu, Pb, Zn and Cd contaminated site, but soil amendments with MF greatly enhanced its phytoremediation effectiveness.

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