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Copper Phytoavailability and Uptake by *Elsholtzia Splendens* from Contaminated Soil as Affected by Soil Amendments

Hong-Yun Peng,[1] Xiao-E Yang,[1] Li-Ying Jiang,[1]
and Zhen-Li He[1,2]

[1]MOE Key Lab of Environmental Remediation and Ecosystem Health, College of Natural Resources and Environmental Science, Zhejiang University, Hangzhou, China
[2]University of Florida, Institute of Food and Agricultural Sciences, Indian River tj1380 Research and Education Center, Fort Pierce, Florida, USA

Pot and field experiments were conducted to evaluate bioavailability of Cu in contaminated paddy soil (PS) and phytoremediation potential by *Elsholtzia splendens* as affected by soil amendments. The results from pot experiment showed that organic manure (M) applied to the PS not only remarkably raised the H_2O exchangeable Cu, which were mainly due to the increased exchangeable and organic fractions of Cu in the PS by M, but also stimulated plant growth and Cu accumulation in *E. splendens*. At M application rate of 5.0%, shoot Cu concentration in the plant increased by four times grown on the PS, so as to the elevated shoot Cu accumulation by three times as compared to the control. In the field trial, soil amendments by M and furnace slag (F), and soil preparations like soil capping (S) and soil discing (D) were performed in the PS. Soil capping and discing considerably declined total Cu in the PS. Application of M solely or together with F enhanced plant growth and increased H_2O exchangeable Cu levels in the soil. The increased extractability of Cu in the rhizosphere of *E. splendens* was noted, which may have mainly attributed to the rhizospheric acidification and chelation by dissolved organic matter (DOM), thus resulting in elevating Cu uptake and accumulation by *E. splendens*. Amendments with organic manure plus furnace slag (MF) to the PS caused the highest exactable Cu with saturated H_2O in the rhizospheric soil of *E. splendens* after they were grown for 170 days in the PS, thus achieving 1.74 kg Cu ha^{-1} removal from the contaminated soil by the whole plant of *E. splendens* at one season, which is higher than those of the other soil treatments. The results indicated that application of organic manure at a proper rate could enhance Cu bioavailability and increase

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Address correspondence to Xiao-E Yang, MOE Key Lab of Environmental Remediation and Ecosystem Health, Zhejiang University, Hanghzou, China; E-mail: zhe@mail.ifas.ufl.edu or xyang@zju.edu.cn

effectiveness of Cu phytoextraction from the contaminated soil by the metal-tolerant and accumulating plant species (*E. splendens*).

Key Words: Elsholtzia splendens; Copper uptake; Organic manure; Phytoavailability; Phytoremediation.

INTRODUCTION

A number of plant species endemic to metalliferous soil have been evolved to accumulate large amounts of heavy metals that greatly exceed their phytotoxicity in their aboveground biomass.[1] These plant species have the potential to clean up metal-contaminated soil.[2–4] Two types of plants are currently investigated widely in laboratories and greenhouses. The first type is naturally occurring metal hyperaccumulator such as *Thlaspi caerulescens*, a Zn hyperaccumulator with the capability of accumulating and tolerating above 10,000 mg kg^{-1} Zn in its aboveground biomass, but its application in the field is limited due to its small size and slow growth.[5–7] The second type is a metal-tolerant plant such as *Brassica juncea* (Indian mustard), which can factually take up large quantities of metal contaminants from soil due to its high biomass, despite lower metal concentration in plant.[6,7] To extract significant quantity of metals from soil by plant accumulation, the ideal plant species should have a greater biomass potential while accumulating higher concentrations of metal contaminants. Chinese native herbs *E. splendens*, dominant plants in the old copper mining deposit, have evolved metal tolerance over the years.[8–10] *E. splendens* can tolerate high levels of Cu, which has been confirmed by field surveys in the old mined areas[8,9,11,12] and solution culture studies.[13] In the field of the mined area, this plant accumulated 2288 mg kg^{-1} Cu in the root and 304 mg kg^{-1} Cu in the shoots. In solution culture, the growth of *E. splendens* was found to be optimal at Cu supply levels of up to 100 μmol L^{-1}, and at 500 and 1000 μmol L^{-1} Cu supplies levels, shoot Cu concentrations achieved 1133 and 3417 mg kg^{-1} on a dry-weight basis, respectively.[13] Moreover, *E. splendens* has an average shoot biomass of 11,000 kg ha^{-1} and an average root biomass of 2420 kg ha^{-1} in the field experiment.[14] The characteristics of large biomass and the great tolerance and accumulation of Cu in *E. splendens* make it a great candidate for the phytoremediation of Cu-contaminated soil.

Physiological analysis of metal accumulation and tolerance,[15] and ecological aspects of metal accumulation, uptake, and distribution in *E. splendens* have been investigated[8,14,16–18] in laboratory and greenhouse. Unlike other heavy metals such as cadmium (Cd), nickel (Ni), and zinc (Zn), copper bioavailability and mobility in soil are relatively low, which limits the efficiency of Cu removal by phytoextraction from the soils. Retention of Cu by soils highly depends on soil physicochemical characteristics, particularly soil pH and soil soluble ligands, which are particularly important factors in influencing the degree of complexation with Cu for plant uptake.[19] If soil Cu availability and mobility for root uptake and translocation from roots to shoots were increased by some additional amendments, the phytoextraction efficiency could be enhanced.

Incorporation of carbon-rich organic manures into soils has been shown to increase metal mobility through the formation of soluble metal-organic complexes.[13,20] Crude organic matter in soil could effectively increase the activity of metals[21] and improve metal mobility and distribution in soil. He and Singh[22] discovered that 320 g kg^{-1} turves added to sandy soils elevated Cd concentration in exchangeable fraction from 27% to 54%, while Fe-Mn oxide fraction decreased from 19% to 13%. Soil amendment substances organic manure (M) and furnace slag (F) could be used to amend metal-contaminated soil.[23] But the field performance of phytoremediation in metal-contaminated soil by *E. splendens* as affected by soil amendments is limited. In this study, pot experiment and a field trial of phytotremediation were conducted to examine bioavailability of Cu in soil, copper uptake and accumulation in plant, and copper removal from soil for assessing phytoremediation effectiveness of Cu from the contaminated site by *E. splendens* as affected by soil amendments like M and F.

MATERIALS AND METHODS

Pot Experiment

The soil used for the pot experiment is a paddy soil (PS), collected from Fuyang County of Zhejiang Province of China, where the soil was heavily contaminated by heavy metal emission from many Cu refining plants.[24] Soil was collected from the surface layer (0–20 cm). The main agrochemical properties of the tested soil are shown in Table 1. Commercial organic manure was chosen, oven-dried at 70°C, and ground to pass through 1.0 mm sieve for analysis. The chemical properties and nutrient contents of organic manure were as follows: pH (H_2O) of 8.80, OM (g kg^{-1}) of 882.30, total-N and P (g kg^{-1}) of 16.57 and 6.57, respectively, and total Cu of 199.76 mg kg^{-1}.

Pot experiment was conducted using air-dried soil samples passed through 2.0 mm. Each pot contained 1 kg of soil thoroughly mixed with 0.1 g urea, 0.2 g KH_2PO_4 as basal fertilizers. For assessing the phytoextraction potential of *E. splendens* as affected by organic manure, five rates of organic manure were 0, 0.5, 1.0, 2.5, and 5.0% based on the air-dried soil of the PS. The seeds of the plant were collected from the old mined area in Zhejiang Province, China, and germinated on wet filter paper in the dark. The germinated seeds were sown on quartz sand with nutrient solution prepared for establishing seedlings.[15] Two plants of 40-day-old seedlings were transplanted to the pots except for the blank. A randomized complete block experimental design was used and each treatment had six replications, with four replicates being transplanted with *E. splendens*

Table 1: Physicochemical characteristics of the metal-polluted soil.

Characteristics	pH (H_2O)	Organic matter (g kg^{-1})	CEC (cmol kg^{-1})	Total contents (g kg^{-1})					Available contents* (mg kg^{-1})			
						Cu						
				N	P	0–5 cm	5–15 cm	15–35 cm	N	P	K	Cu
Data	7.5	42	7.1	1.28	1.12	1.95	0.56	0.044	137.1	37.2	24.6	79.4

Note: Soil sampling and analyses were conducted prior to the application of five soil treatments to the metal-polluted site.

*Background value of Zhejiang Province: Cu is 0.198 g kg^{-1}(24).

seedlings after a four-week incubation. Another two replicates incubated for eight weeks were used as the blank without any plants for determining the extractability of Cu and Cu distribution in soil. 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 greenhouse conditions with natural light, day/night temperature of 30/25°C, and day/night humidity of 65/80%. Eight weeks later, the plants in the pots were harvested for analysis. Shoots were cut at the soil surface, rinsed with distilled water, and blot-dried. Plant tissues were oven-dried at 65°C, and dry weights were recorded. The dried plant materials were ground with a stainless steel mill for chemical analysis. Soil samples were collected from each pots, air-dried, and passed through a 1.0 mm plastic sieve for chemical analysis.

Field Experiment

The field experiment was conducted in an agricultural field (an alluvial loam, paddy soil) in Fuyang County of Zhejiang Province, where the soil was heavily contaminated by heavy metal emission from many Cu refining plants.[24] The main agrochemical properties of the tested soil are listed in Table 1. Five treatments were adopted and some soil properties after one-year treatments are shown in Table 2. 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. Five soil treatments were used: A. Control; B. M (application of 1500 kg ha^{-1} organic manure); C. MF (co-application of 1500 kg ha^{-1} organic manure and 3750 kg ha^{-1} furnace slag); D. S and MF (soil capping with 750,000 kg ha^{-1} red clay soil + C); E. D and MF (soil discing by digging the topsoil (0–15 cm) and the plow soil (0–5 cm), putting the topsoil at the bottom and plow soil at the surface, respectively, + C). Organic manure was used as described in the pot experiment, and the properties of furnace slag

Table 2: Physicochemical properties of the amended soil in the field trial.

Soil treatments	pH (H_2O)	Organic matter (g kg^{-1})	Total contents (g kg^{-1})			Available contents* (mg kg^{-1})			
			N	P	Cu	N	P	K	Cu
Control	7.54	55.7	2.43	1.11	1.43	297.73	25.17	29.50	76.7
M	7.54	55.9	2.55	0.94	1.43	326.67	25.36	29.77	78.2
MF	7.47	59.9	2.60	0.99	1.23	317.33	24.64	31.09	71.4
S+MF	7.76	39.8	1.43	0.69	0.39	167.07	10.74	36.94	39.0
D+MF	7.75	46.9	1.93	0.85	0.32	208.13	15.60	26.58	25.2

Note: 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 was performed.
*Available contents of heavy metals were extracted with 1mol L^{-1} NH_4OAc.

were: pH (H_2O) of 8.7; OM (g kg^{-1}) of 1.5; total N, P, and K (g kg^{-1}) of 0.47, 3.30, and 1.40, respectively; total Cd, Cu, Zn, and Pb (mg kg^{-1}) of 3.51, 37.9, 43.2, and 46.6, respectively.

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 wet 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 the plots of the field experiment except for the fallow plots with planting density of 20 cm × 20 cm (each soil treatment had one fallow plot used as blank). No fertilizers and pesticides were applied, but weeding was done twice during the field experiment. The plant and soil samples were collected after plants were grown for 170 days. Shoots were cut at the soil surface, washed with tap water, rinsed with distilled water, blot-dried, and oven-dried at 65°C. 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 samples of bulk soil, rhizospheric soil (soil attached to the roots after shaking and separated from the roots by hand), and the fallow soil taken from the field were air-dried and passed through a 1.0 mm plastic sieve for chemical analysis.

Chemical and Data Analyses

Soil pH (H_2O) was measured in deionized water with a soil/solution ratio of 1:2.5 (W:V). Organic matter content, cation exchange capacity (CEC), and total and available N, P, and K in the soil were determined according to the methods of SSICA.[25] Dissolved organic matter (DOM) was determined as follows: 15.00 g of dried soil were extracted with 80 ml 0.5 mol L^{-1} K_2SO_4 at the rate of 200 rpm for 0.5 h (20°C), then centrifuged at 4200 r min^{-1} for 10 min at 20°C, filtered with 0.45 μm films, and assayed with TOC analyzer (Shimaduz, TOC-5000A, Japan). Soil total Cu concentrations were extracted with HF-$HClO_4$,[25] and available Cu concentrations were extracted by saturated water, deionized water (1:1 of soil:water), 1.0 mol L^{-1} NH_4-OAc (pH 7.0, 1:20 soil:solution (W:V), and 1 mol L^{-1} NO_3NH_4 (1:5 soil:solution), respectively.[26] Plant shoots were thoroughly washed with tap water and distilled water, oven-dried (65°C) to constant weight, ground, and sieved. A subsample was ashed at 550°C for 6 h, and dissolved in 1:1(V:V) HNO_3. The Cu concentrations in the soil extracts and plant digests were measured by an Inductively Coupled Plasma-optical Emission Spectroscopy (ICP-OES, Model IRAS-AP, TJA).

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

RESULTS

Effects on pH Changes and DOM Content in the Contaminated Soils

The increasing rates of M applied to PS in the pot experiment caused the elevated soil pH after either one-week or eight-week soil incubations (Table 3). For example, 0.3 units increase in soil pH was noted after one-week incubation at 5% M rate. However, decreased soil pH was found at the increasing of soil incubation time, indicating that the equilibrium of soil pH needs much longer time.

In the field experiment, M and M plus F treatments to PS slightly elevated soil pH, as compared to control (Table 2, Fig. 1). After *E. splendens* grown in soil for 170 days, only slight changes in soil pH were observed between the fallow soil and the corresponding bulk soil, while planting *E. splendens* caused a significant decrease in rhizospheric pH as compared with those of the fallow soil (Fig. 1A). Furthermore, no significant differences in DOM contents were noted between the fallow soil and the corresponding bulk soil, whereas growing *E. splendens* dramatically increased rhizospheric DOM contents, as compared with those of the fallow soil (Fig. 1B). However, there were no obvious changes in DOM contents of soil amended by application of M and F, when compared to the control.

Effects on Cu Extractability and Fractionation in the Contaminated Soils

The H_2O extractable Cu significantly increased in the PS with increasing M rates after incubated for one week and eight weeks in the pot experiment (Table 4). For example, the H_2O extractable Cu was increased by 4.81 mg kg^{-1} when 5% M applied to the PS after one-week incubation, as compared to the control. However, NH_4OAc extractable Cu was decreased significantly in the PS with increasing of M rates (Table 4).

Copper distribution in different fractions for the PS was in the order: Fe/Mn oxide bound > organic bound > carbonates bound > residual > exchangeable

Table 3: Effect of organic manure application on soil pH in the contaminated soil (pot experiment).

	Soil incubation time	
Rate of organic manure (%)	1 week	8 weeks
0	7.34c	6.94d
0.5	7.34c	7.02c
1	7.37c	7.00c
2.5	7.47b	7.23a
5	7.64a	7.12b

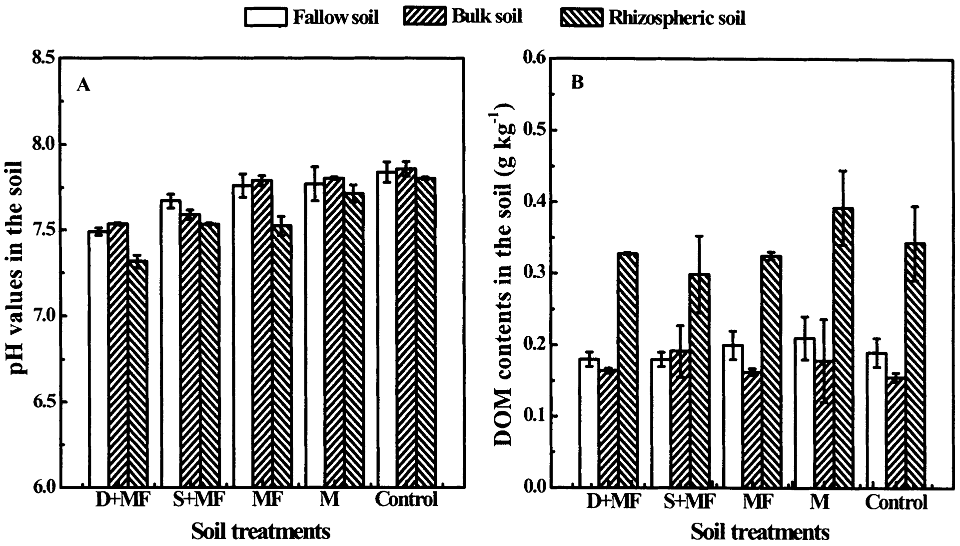

Figure 1: Changes in pH and DOM in the rhizosphere after *E. splendens* was grown in the contaminated site for 170 days in the field experiment. Data are means of six replications, and bars depict SE.

(Fig. 2). With increasing of M rates, copper distribution in Fe/Mn oxide bound and carbonate bound fractions decreased, and that in organic bound fraction increased in the PS (Fig. 2). For instance, application with M at 5% rate decreased oxide fraction from 44.2% to 34.5% and carbonate bound fraction by 5.5%, respectively, while it increased organic bound fraction from 23.2% to 30.4% in the PS.

In the field experiment, extractable Cu levels in the soil differed greatly for the five soil treatments (Fig. 3, Table 5). Slight reduction in extractable Cu

Table 4: Effect of organic manure application on the extractability of Cu in the contaminated soil by single extraction (pot experiment).

Extractants	Rate of organic manure (%)	Soil incubation time 1 week	 8 weeks
H_2O	0	1.66 d	0.54 e
	0.5	1.81 d	0.67 d
	1.0	2.87 c	1.10 c
	2.5	3.23 b	1.21 b
	5	6.47 a	4.42 a
NH_4OAC	0	86.91 a	72.03 a
	0.5	80.44 b	70.94 c
	1.0	79.97 b	71.54 b
	2.5	77.20 c	69.96 d
	5	69.00 d	68.24 e

Note. Letters a, b, c, d, and e show the significant differences between the two treatments. Different letters indicate significant statistical differences ($p < 0.05$).

Table 5: Extractable Cu levels in the fallow and bulk soil sampled from the field Experiment.

		Extractable Cu levels (mg kg^{-1})	
Extractants	**Soil treatments**	**Fallow soil**	**Bulk soil**
Saturated water	Control	ab 0.291 ± 0.092 x	ab 0.326 ± 0.030 x
	M	ab 0.301 ± 0.047 x	a 0.263 ± 0.014 x
	MF	a 0.323 ± 0.048 x	b 0.383 ± 0.044 x
	S+MF	bc 0.184 ± 0.038 x	c 0.077 ± 0.031 y
	D+MF	c 0.148 ± 0.032 x	cd 0.053 ± 0.031 y
NH_4NO_3	Control	a 37.13 ± 0.01 x	a 31.38 ± 3.34 y
	M	a 36.72 ± 1.12 x	a 32.72 ± 3.26 x
	MF	a 33.78 ± 0.80 x	a 34.66 ± 5.22 x
	S+MF	b 26.79 ± 1.85 x	bc 17.76 ± 3.03 y
	D+MF	c 21.02 ± 1.91 x	c 11.51 ± 3.02 y
NH_4OAc	Control	a 59.55 ± 0.31 x	a 52.67 ± 0.61 y
	M	a 59.62 ± 6.47 x	a 52.37 ± 9.35 x
	MF	a 54.80 ± 4.62 x	a 54.36 ± 3.12 x
	S+MF	b 33.89 ± 1.02 x	b 27.50 ± 3.90 y
	D+MF	b 25.39 ± 2.08 x	c 14.85 ± 0.44 y

Note: Letters a, b, c, and d show the significant differences between the five soil treatments. Different letters indicate significant statistical differences ($p < 0.05$). Letters x and y show the significant differences between the fallow soil and bulk soil. Different letters indicate significant statistical differences ($p < 0.05$).

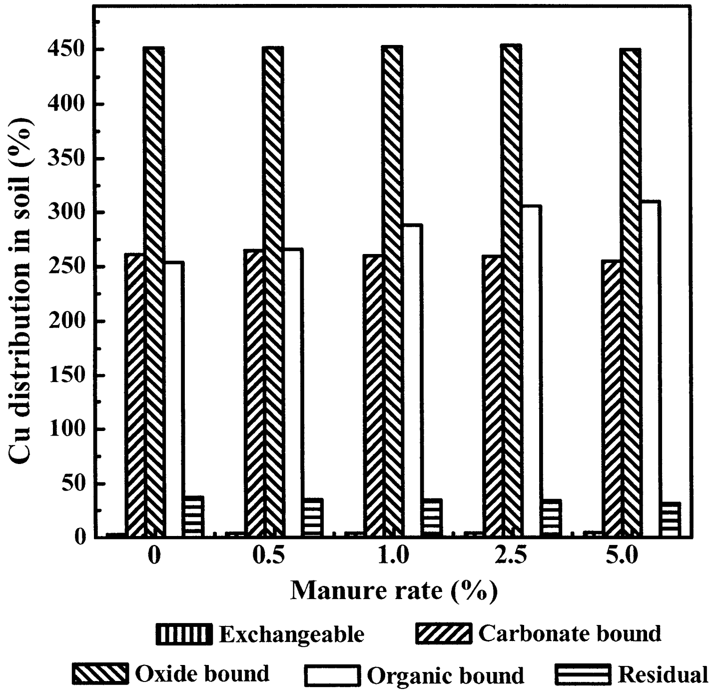

Figure 2: Effects of M application at different rates on Cu distribution in the contaminated soils after eight-week incubation (sequence extraction) in the pot experiment. Data are means of six replications, and bars depict SE.

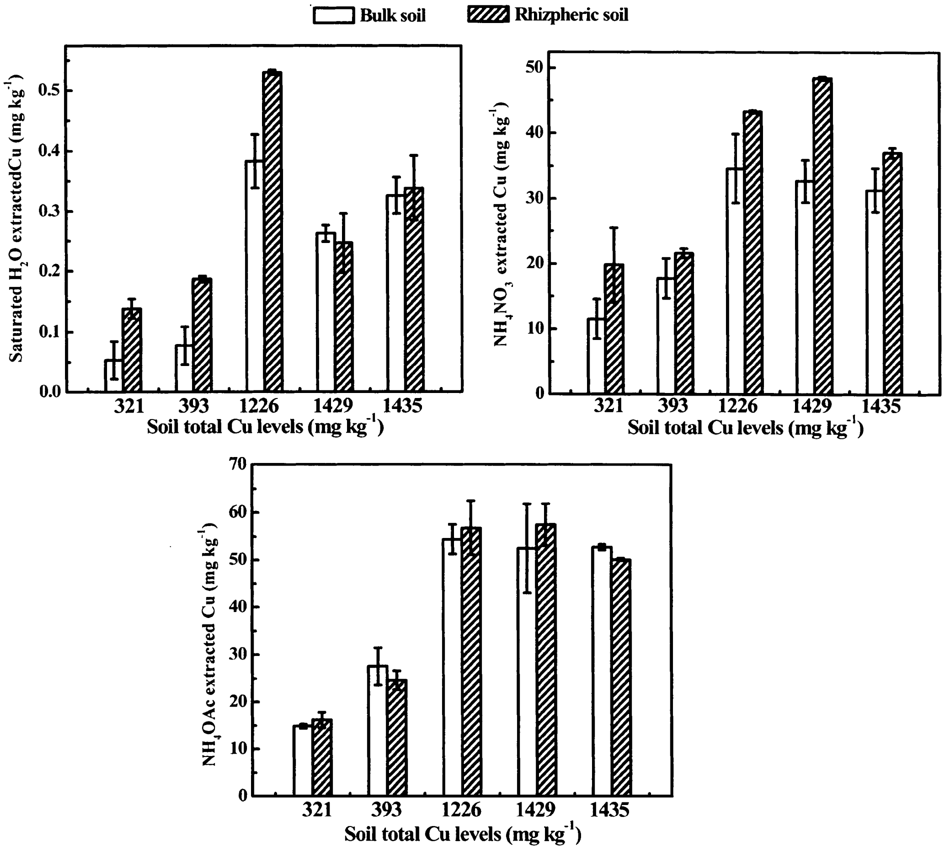

Figure 3: Extractable Cu of the amended soil by single extractant after *E. splendens* was grown in the contaminated site for 170 days in the field experiment. Data are means of six replications, and bars depict SE.

was found in the bulk soil as compared with those of the fallow soil. Moreover, extractable Cu levels were reduced, to a different extent, with decreasing total Cu in the soil. As compared to the control, application of M solely or together with F without any soil preparations caused no differences in the NH_4AC-extractable Cu in the soil. Soil preparations by either soil capping (S) or soil discing (D) pronouncedly declined extractable Cu in the fallow soil and the bulk soil due to the considerably decreased total Cu levels in the soil (Table 2). On the other hand, the pronounced increase in H2O-extractable or NH_4NO_3-extractable Cu levels in the rhizospheric soil of *E. splendens* was observed after plants were grown for 170 days in the treated soils (Fig. 3).

Effects on Plant Growth and Dry Matter Production

Shoot dry matter yield of *E. splendens* was pronouncedly increased by M application to the PS in the pot experiment (Fig. 4A). The maximum shoot biomass

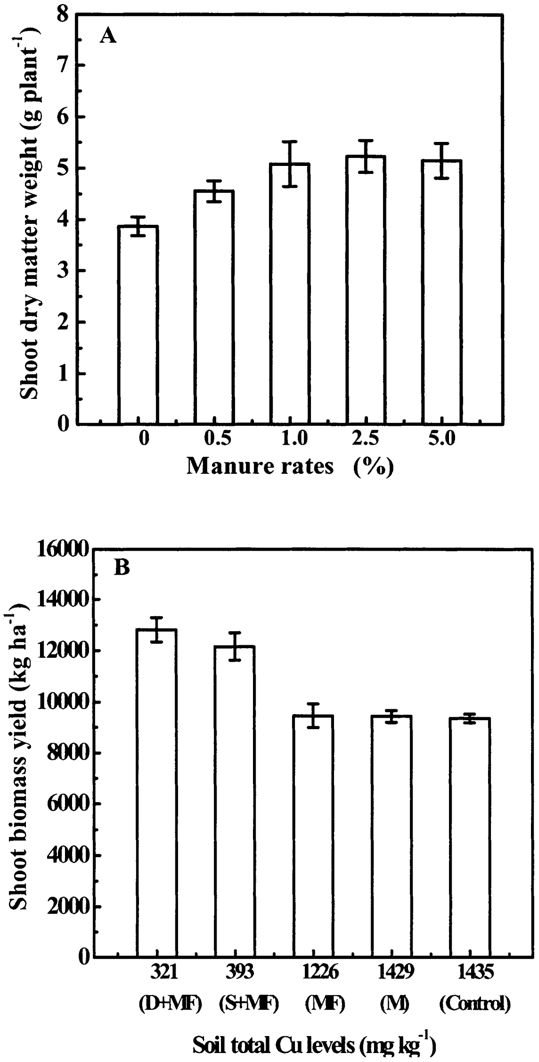

Figure 4: Shoot biomass of *E. splendens* grown in the contaminated soils. Data are means of six replications, and bars depict SE (*A*, pot experiment; *B*, field experiment).

was obtained at 2.5% M rate. For instance, shoot biomass yields increased by 35.5%, as compared with the control (Fig. 4). The increased root biomass was also noted as the increasing of M rate (data not shown). In the field experiment, shoot growth of *E. splendens* dramatically decreased with increasing total Cu levels in the soil after 170 days of growth in the contaminated site (Fig. 4B). In the control, total Cu concentrations in the soil were 1435 mg kg^{-1}, and shoot biomass of *E. splendens* was only 9200 kg ha^{-1}. In comparison, soil amended by MF significantly increased the shoot biomass production, despite the high levels of Cu (1226 mg kg^{-1}) in the soil. However, the increased levels in shoot biomass production were far lower than those with S+MF and D+MF.

Effects on Cu Concentration and Accumulation in *E. Splendens*

With increasing of M rates from 2.5% to 5.0% in the PS, shoot Cu concentration in *E. splendens* increased by 150–400%, while no apparent changes in root Cu in plant were observed in the pot experiment (Fig. 5A). Copper accumulation

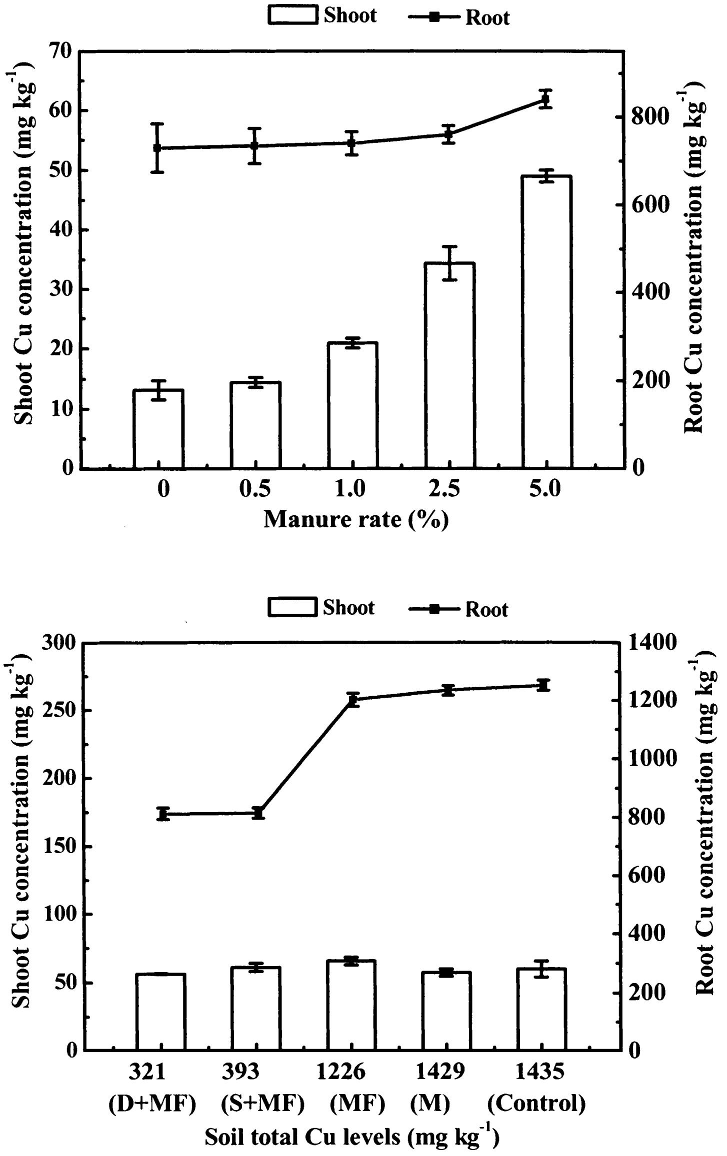

Figure 5: Copper concentration in *E. splendens* grown in the contaminated soils. Data are means of six replications, and bars depict SE (*A*, pot experiment; *B*, field experiment).

in both shoots and roots increased dramatically with increasing of M application rates, which may attribute mainly to the stimulated plant growth by M (Fig. 6A). However, root Cu accumulation was far above shoot Cu accumulation by *E. splendens*. The maximum Cu accumulation (4.6 mg plant^{-1}) by the whole plant of *E. splendens* was at 5% M rate after an eight-week growth of plant.

In the field experiment, when total Cu levels in the soil were higher than 1226 mg kg^{-1}, co-application of M and F significantly increased Cu

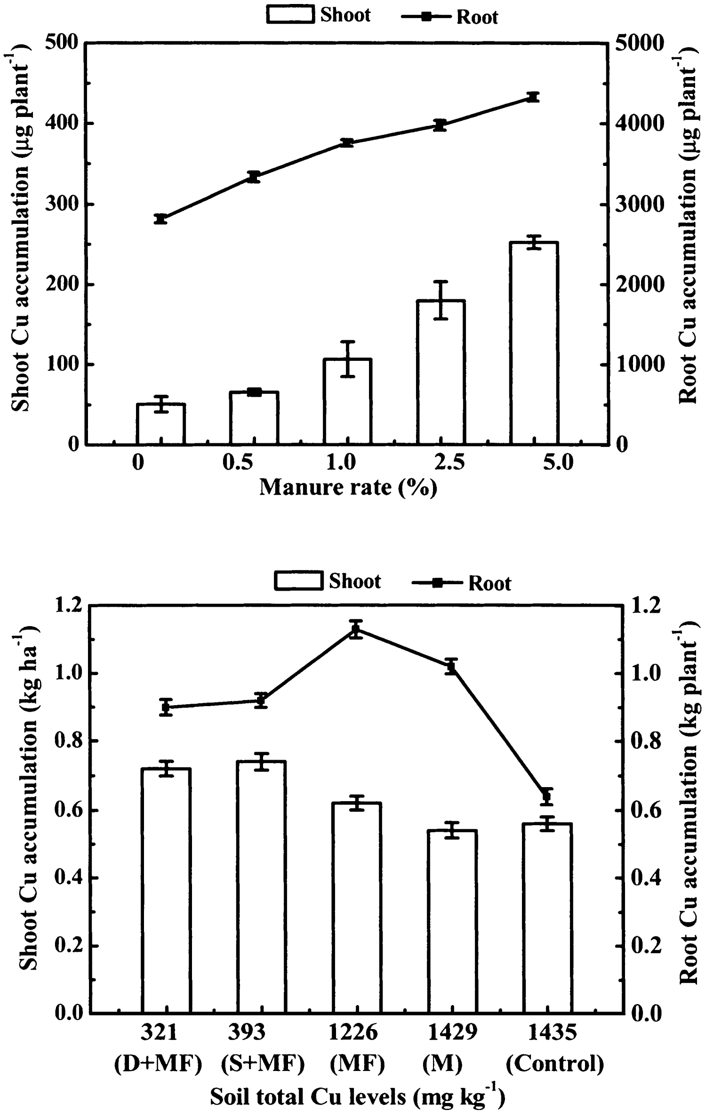

Figure 6: Copper accumulation in *E. splendens* grown in the contaminated soils. Data are means of six replications, and bars depict SE (*Upper*, pot experiment; *Lower*, field experiment).

concentrations in plants despite the lower levels of Cu (1226 mg kg^{-1}) in the soil as compared to the control (Fig. 5B). When total Cu levels in the soil were lower than 1226 mg kg^{-1}, shoot Cu concentrations considerably increased with the elevated total Cu levels in the soil, whereas the decreased shoot Cu concentrations in plants were noted when total Cu levels were higher than 1226. The root Cu concentration in plant increased with the increasing of total Cu in the soil (Fig. 5B).

For the control, copper accumulation by *E. splendens* was 0.56 kg ha^{-1} in the shoots and 0.64 kg ha^{-1} in the roots of plant. Although soil treatments with S and D decreased total and available Cu concentrations in the soil, total Cu accumulation in plants increased significantly with elevated biomass production of the plant. In comparison with control, treatments with S+MF and D+MF caused the elevated Cu accumulation by 1.66 kg ha^{-1} and 1.62 kg ha^{-1} by the whole plant of *E. splendens*. In comparison, co-application of M and F to the PS resulted in the maximum Cu accumulation of 1.75 kg ha^{-1} by the whole plant of *E. splendens*, and the maximum Cu accumulation in the roots of the plant was 1.13 kg ha^{-1}.

DISCUSSION

The effectiveness of phytoremediation of the heavy metal–polluted soil is strongly dependent on metal bioavailability and mobility in the soil, and metal uptake and translocation capacity of the plant.[27,28] Copper bioavailability in soil highly depends on soil pH, organic matter content, soil solution ionic strength, Mn and Fe oxides, redox potential, and the nature of soil surface. The Cu-contaminated paddy soil, formerly an agricultural soil (an alluvial loam, paddy soil), contained 1946 mg kg^{-1} Cu in the surface soil (0–5 cm) due to metal smelting activities.[24] At the profile of 5 to 15 cm, total Cu concentration was reduced to 560 mg kg^{-1}, but still far above soil Cu background levels in Zhejiang Province, China.[24] Soil amendments like organic manure (M), furnace slag (F), and soil preparations such as soil capping (S) and soil discing (D) have been performed at the site.[10] Treatments with M solely or together with F slightly elevated soil pH (Table 1), which is perhaps due to the alkalinity of M;[16] however, water extractable Cu levels increased with increased M application. Available Cu levels with M or together with F were still too high to be beneficial for crop production.[17] Treatments with S and D significantly reduced total Cu, but had minimal influences on Cu bioavailability. In the PS, the binding capacity of Cu to different soil fractions followed the order of Fe/Mn oxide > organic matter > carbonates > residual state > exchangeable state (Fig. 2), implying that the Cu are mainly combined with Fe/Mn oxides and organic matter.[16] Increased application of M decreasing Cu concentration in carbonates and residual fractions while slightly increasing Cu concentration in the exchangeable and organic fraction (Fig. 2), and thus enhanced Cu extractability and movement in the PS. Perhaps application of M to the soil enhanced the binding capacity between humus and Fe, Al, Mn, and Cu, decreased the binding capacity of Fe/Mn oxides and Cu, resulting in enhanced Cu transfer from Fe/Mn oxides to organic combinative fraction in the soil. Soluble organic substances from M might have activated soil Cu,[16,29] which may be partially attributed to elevated H_2O extractable Cu by M application in the PS. Yang et al.[16] confirmed that application of M enhanced Cu solubility, resulting in the elevated extractable Cu concentration in the soil. It appears that application of organic manure at proper rate is an effective measure to increase Cu bioavailability in the polluted soil.

The phytoavailability in the rhizosphere of the metal-tolerant and accumulating plant species is particularly important for improving phytoremediation efficiency. Soil pH and DOM contents are important factors strongly affecting the degree of complexation with metals,[19] and subsequently Cu bioavailability and mobility in the soil, particularly in the rhizospheric soil. Significant reduction in rhizospheric pH of *E. splendens* was observed as compared to the bulk soil, and as the increasing of the available Cu in the soil (Fig. 5). Furthermore, significantly greater rhizospheric DOM contents of *E. splendens* were found and the rhizospheric DOM contents changed slightly with increasing total and available Cu in the soil, as compared to the bulk soil, despite the application of M to the PS. Both rhizospheric acidification and elevated DOM content in the rhizosphere suggested that secretion of acid substances and DOM from roots of *E. splendens* improved the physicochemical properties of rhizospheric soil,[29,30,31] thus enhancing Cu solubility and resulting in elevated phytoavailable Cu levels in the rhizospheric zone. A pronounced increase in extractable Cu levels in the rhizosphere was observed after *E. splendens* had been grown for 170 days in the treated soils (Fig. 3). When the total Cu levels in the soil were above 1226 mg kg^{-1}, co-application of M and F significantly enhanced the Cu levels in the rhizospheric zone, especially with the saturated H_2O extraction. The increased H_2O extractable Cu level in the rhizosphere of *E. splendens* due to combined application of M and F may be helpful for Cu uptake and accumulation by the plant, and result in the enhanced phytoremediation efficiency from the contaminated soil. The mechanisms of Cu activation in the rhizosphere of *E. splendens* need to be further clarified.

Total metal accumulation in the plants, calculated via the multiplication of biomass production by metal concentration in plant, can be used for characterization of metal removal from the contaminated soil.[4] *E. splendens* is a highly Cu-tolerant and accumulating plant species native to China with better adaptation to adverse growth conditions,[8,9,13] and has a great potential for the phytoremediation of Cu from the PS. Its shoot biomass production reached 9200–12,100 kg ha^{-1}, shoot Cu concentration was 50–70 mg kg^{-1}, and root Cu was 800–1200 mg kg^{-1} grown under field conditions (Figs. 4 and 5). Application of M significantly increased shoot biomass production of *E. splendens*, and the highest shoot Cu concentration and maximum Cu accumulation were noted at 5% M rate. The Cu removal was 0.56–0.74 kg Cu ha^{-1} by shoots and 0.64–1.13 kg Cu ha^{-1} by roots of *E. splendens* from the PS per season in the field experiment. The greater Cu removal by *E. splendens* with M at 5% rate may result from both the increased H_2O extractable Cu and enhanced Cu uptake and accumulation in the plant by M. Combined application of M and F to the PS enhanced the plant to remove much more Cu than other soil amendments, with 1.74 kg Cu ha^{-1} extracted from the PS by the whole plant of *E. splendens* at one season. Therefore, *E. splendens* can be used as good plant material for phytoremediation of Cu-contaminated soil.

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REFERENCES

1. Baker, A.J.M.; Brooks, R.R. Terrestrial higher plants which hyperaccumulate metallic elements: A review of their distribution, ecology and photochemistry. Biorecovery **1989**, *1*, 81–126.

2. Salt, D.E.; Smith, R.D.; Raskin, I. Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol. **1998**, *49*, 643–668.

3. Nedelkoska, T.V.; Doran, P.M. Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Miner. Eng. **2000**, *13*, 549–561.

4. Raskin, I.; Smith, R.D.; Salt, D.E. Phytoremediation of metals: Using plants to remove pollutants from the environment. Curr. Opin. Biotechnol. **1997**, *8*, 221–226.

5. Vázquez, M.D.; Barcelo, J.; Poschenrieder, Ch.; Ma Dico, J.; Hatton, P.; Baker, A.J.M.; Cope, G.H. Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae), a metallophyte that can hyperaccumulate both metals. J. Plant Physiol. **1992**, *140*, 350–355.

6. Ebbe, S.D.; Tasat, M.M.; Brady, D.J.; Cornish, J.; Gordon, R.; Kochian, L.V. Phytoextraction of cadmium and zinc from a contaminated site. J. Environ. Qual. **1997**, *26*, 1424–1430.

7. Ebbs, S.D.; Kochian, L.V. Toxicity of zinc and copper to *Brassica* species: Implications for phytoremediation. J. Environ. Qual. **1997**, *26*, 776–781.

8. Yang, X.E.; Shi, W.Y.; Fu, C.X.; Yang, M.J. Copper-hyperaccumulators of Chinese native plants characteristics and possible use for phytoremediation. *Sustainable Agriculture for Food, Energy and Industry*. James & James, London: 1998.

9. Jiang, L.Y.; Shi, W.Y.; Yang, X.E.; Fu, C. X.; Chen, W.G. Cu hyperaccumulators in mining area. Chinese J. Appl. Ecol. **2002**, *13* (7), 906–908.

10. Jiang, L.Y.; Yang, X.E. Chelator effects on soil Cu extractability and uptake by *Elsholtzia splendens*. J. Zhejiang Univ. Sci. **2004**, *5* (4), 450–456.

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

12. Tang, S.R.; Wilke, B.M.; Brooks, R.R. Heavy-metal uptake by metal tolerant *Elsholtzia haichowensis* and *Commelina communis* from China. Commun. Soil Sci. Plant Anal. **2001**, *32* (5&6), 895–905.

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

14. Jiang, L.Y.; Yang, X.E.; He, Z.L. Growth response and phytoextraction of copper at different levels in soils by *Elsholtzia splendens*. Chemosphere **2004**, *55*, 1179–1187.

15. Yang, X.E.; Peng, H.Y.; Tian, S.K. Response of antioxidant enzyme system to copper toxicity and copper detoxification in copper tolerant and accumulating plant species (*Elsholtzia splendens*). J. Plant Nutr. **2004** (in press).

16. Yang, X.E.; Calvert, D.V.; He, Z.L.; Stoffella, P.J.; Li, Y.C.; Zhang, M. Effect of M amendment on solubility and transformation of copper in soils. Poster presentation in the International Symposium of the Seventeenth WCSS, Thailand, 2002; 14–21.

17. Yang, X.E.; Long, X.X.; Ni, W.Z.; He, Z.L.; Stoffella, P.J.; Calvert, D.V. Assessing copper thresholds for phytotoxicity and potential dietary toxicity in selected crops. J. Environ. Sci. Heal. B. **2002**, *37* (6), 625–635.

18. Jiang, L.Y.; Yang, X.E.; Ye, Z.Q.; Shi, W.Y. Uptake, distribution and accumulation of copper in plants of two ecotypes of *Elsholtzia*. Pedosphere **2003**, *13* (4), 359–366.

19. Harter, R.D. Effect of soil pH on absorption of lead, copper, zinc and nickel. Soil Sci. Soc. Am. J. **1983**, *47*, 47–51.

20. Zhou, L.X.; Wong, J.W.C. Effect of dissolved organic matter from sludge and sludge compost on soil copper sorption. J. Environ. Qual. **2001**, *30*, 878–883.

21. Cezary, K.; Singh, B.R. Fractionation and mobility of copper, lead, and zinc in soil profiles in the vicinity of a copper smelter. J. Environ. Qual. **2001**, *30*, 485–492.

22. He, Q.B.; Singh, B.R. Effect of organic matter on the distribution, extractability and uptake of cadmium in soils. J. Soil Sci. **1993**, *44*, 641–650.

23. William, R.B.; Scott, D.C. Phytostabilization of metals. In *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*; Raskin, I; Ensley, B.D., Eds., John Wiley & Sons: New York, 2000; 71–89.

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

25. SSICA (Soil Aci. Ch. Acad.). *Physical and Chemical Analyses of Soils*. Shanghai Academic Press: Shanghai, China, 1980.

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

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

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

29. Huang, Z.C.; Chen, T.B.; Lei, M. Effect of DOM derived from sewage sludge on Cd adsorption in different soils in China (I). Difference in latitudinal zonal soils. Acta Sci. Circums. **2002**, *22* (3), 349–353.

30. Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd Ed. Academic Press: San Diego, CA, USA, 1995.

31. Mench, M.; Martin, E. Mobilization of cadmium and other metals from two soils by root exudates of *Zea mays* L., *Nicotiana tabacum* L., and *Nicotiana rustica* L. Plant Soil **1991**, *132* (2), 187–196.