Bull Environ Contam Toxicol (2007) 78:384–389
DOI 10.1007/s00128-007-9197-4

Effect of *Elsholtzia Splendens*, Soil Amendments, and Soil Managements on Cu, Pb, Zn and Cd Fractionation and Solubilization in Soil under Field Conditions

H.-Y. Peng · X.-E. Yang

Received: 5 January 2007 / Accepted: 26 May 2007 / Published online: 7 July 2007

Heavy metal contamination into soil poses potential impacts on ecosystem quality and human health (He et al. 2005). Phytoremediation, a plant-based technology, has become an attractive approach with advantages of being in situ, lower cost, minimal environmental disturbance, elimination of secondary pollution and public acceptance (Salt et al. 1998). *Elsholtzia splendens*, dominantly growing on old copper mine waste deposits of China, has been identified as Chinese native Cu tolerant plant species, and widely investigated through greenhouse and field experiments for phytoremediation of Cu contaminated soils (Yang et al. 2005; Jiang et al. 2004; Song et al. 2004; Peng et al. 2005; Peng and Yang 2005, 2007). Furthermore, *E. splendens* can cotolerate Cu, Pb, and Zn in soil (Weng et al. 2004) as it has been shown for total levels of Cu, Pb, Zn of 223, 1,068, and 232 mg kg^{-1}, respectively in the pot experiments, and Cu, Pb, Zn, and Cd levels of 1500, 2,001, 1,500 and 21.1 mg kg^{-1}, respectively in the field trial (Peng and Yang 2005). The main factors for enhancing phytoremediation effectiveness include metal bioavailability in soil, uptake of available metals by roots and translocation to shoots. It has been reported that soil amendments like organic manure (M) and slag furnace (F) can enhance Cu bioavailability and increase phytoremediation effectiveness of Cu by *E. splendens* (Yang et al. 2005; Peng and Yang 2005). In the multi-metal contaminated site with Cu, Pb, Zn and Cd, located at Huanshan Village, Fuyang County, Zhejiang Province of China, soil amendments like organic manure (M) and slag furnace (F), and soil managements like soil capping (S) and soil discing (D) (Peng and Yang 2005) have been employed. But, little is reported about the solubility and fractionation of Cu, Pb, Zn, and Cd in this multi-metal contaminated soil, particularly in the rhizospheric soil of *E. splendens*, when soil amendments and soil managements are used for improving plant growth and metal uptake. The purpose of this study was to clarify changes in Cu, Pb, Zn, and Cd speciation in this contaminated soil and rhizospheric soil of *E. splendens* under field conditions.

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, Hangzhou 310029, China

H.-Y. Peng (✉)
College of Environmental and Resource Sciences, Zhejiang University, Huajiachi Campus, Hangzhou 310029, People's Republic of China
e-mail: penghongyun@zju.edu.cn

Materials and Methods

The field trial site was located at the multi-metal contaminated agricultural soil in Huanshan Village, Fuyang County, Zhejiang Province, China, which was 30 m away from the Cu refining factory. The main agrochemical properties of soil in this site have been determined. Copper, Pb, Zn, and Cd are the main contaminants at the top 0–15 cm soil. Five soil treatments: control, M, MF, S+MF, D+MF, have been applied to the site (Peng and Yang 2005), and the soil properties after 1 year of treatment application were evaluated. In the field experiment, the area of each plot was 15 m^2, with 1-m 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. Seeds of *E. splendens*, collected from mature plants grown in copper mine waste deposits (Zhuji County of Zhejiang Province, China), were germinated in the substrate, and supplied with nutrition solution to improve establish of the 40-day-old seedlings. Seedlings were transplanted to experimental plots of the field experiment at the beginning of May 2002, except for the not-planted plots, with a planting distance of 20 × 20 cm. Each soil treatment has one not-planted plot used as blank. All the plots were randomly arranged with four replications for each soil treatment. Plants grew for 170 days, and 1.0 kg topsoil (0–15 cm) samples of bulk soil, rhizospheric soil (soil attached to the roots after shaking and separated from the roots by hand), and the not-planted soil were taken, respectively, air-dried and passed through a 1.0 mm plastic sieve for chemical analysis.

Soil metal fractionation was assayed according to the method of Tessier et al. (1979). Soil soluble metal concentrations were extracted with 1.0 mol L^{-1} NH_4OAc (pH 7.0) at a ratio of 1:5 soil/solution (W:V) and 1 mol L^{-1} NH_4NO_3 (pH 7.0) of 1:5 soil/solution (W:V) respectively (Ernst 1996). Heavy-metal concentrations in the soil extracts were measured by an Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, Model IRAS-AP, TJA). The detection limitations of Cu, Pb, Zn and Cd were 0.005, 0.01, 0.005, and 0.001 mg L^{-1}, respectively, with soil samples GBW-07405 used as standard reference substance for controlling accuracy of the instrument. All the data were presented as mean values of at least four replicates. SPSS statistical software package (Version 11.5) was used. One-way ANOVA with Tukey test was employed to evaluate whether the means were significantly different at $p < 0.05$.

Results and Discussion

Copper smelting activities in Huanshan Village of Fuyang County, Zhejiang Province of China, resulted in heavily contaminated soil with Cu, Pb, Zn, and Cd, especially in the top 15 cm soil, which was formerly an agricultural soil (Alluvial loam, paddy soil) (Peng and Yang 2005). The changes of these metals in soil were estimated by analyzing differences in Cu, Pb, Zn, and Cd fractionation using a sequential extraction method with and without soil amendments and soil physical managements before and after phytoremediation by *E. splendens*. In the not-planted soil, Cu, Pb, and Zn were mainly bound to Fe/Mn oxides and organic substance while Cd was bound to carbonates (Fig. 1). And in this not-planted soil, as compared to control, soil amendments with M and MF (without soil managements like S and D) increased H_2O soluble, exchangeable and Fe/Mn oxide bound Cu soil fractions, but decreased carbonate-bound Cu soil fraction and slightly changed residual Cu soil fraction (Fig. 2). This was in accordance with Yang et al. (2005) that application of M to this contaminated soil in pot experiment could decrease carbonate-bond soil fractions with an increase in the exchangeable and organic bond Cu soil fractions. Soluble organic substances from M may activate soil Cu, which is partly attributed from the elevated soil H_2O soluble Cu at the application of M to the contaminated soil (Yang et al. 2002). So, M amendment to this contaminated soil could enhance Cu solubility, resulting in elevated Cu extractability in soil (Yang et al. 2005). While for Pb, Zn, and Cd, M application to this contaminated soil increased exchangeable and Fe/Mn oxide bound Pb soil fractions but

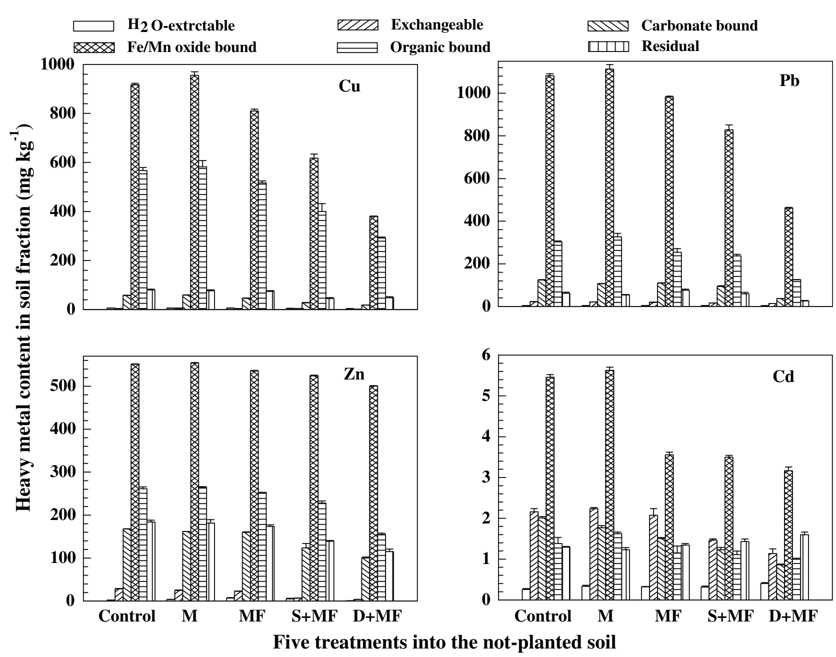

Fig. 1 Effects of soil amendments and managements on the distribution of Cu, Pb, Zn and Cd in the fraction of the not-planted soil in the field experiment. Data are means of 4 replications, and bars depict SE

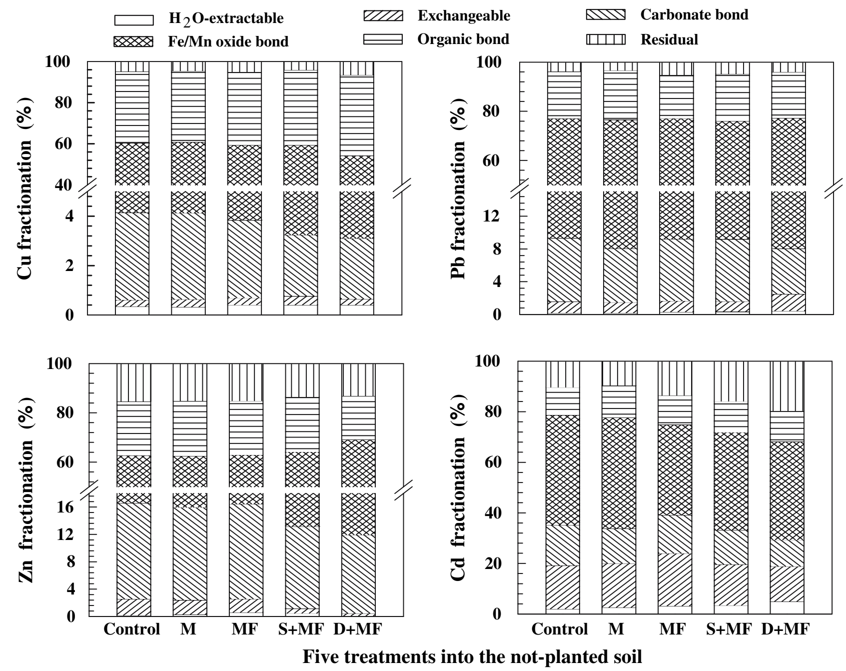

Fig. 2 Effects of soil amendments and managements on the fractionation of Cu, Pb, Zn and Cd in the not-planted soil in the field experiment. Data are means of 4 replications

decreased carbonate-bound Pb soil fraction, MF amendment increased H_2O soluble Zn, Cd soil fractions and exchangeable Cd soil fraction but decreased Fe/Mn oxide bound Cd soil fraction (Fig. 2). In this soil, as compared to control, soil managements like S and D, especially D, greatly reduced carbonate-bound Cu, Pb, Zn, and Cd soil fractions, but increased H_2O soluble Cu, Pb, Cd soil fractions, and exchangeable Cu soil fraction.

As compared to the not-planted soil, no significant changes in Cu fractionation were found in the bulk soil for the five soil treatments. While for rhizospheric soil of *E. splendens*, for the five soil treatments, the increased H_2O soluble and exchangeable Cu fractions, and the greatly enhanced organic bound and Fe/Mn oxide bound Cu fractions were observed, but carbonate-bound Cu fraction decreased significantly with slight change in residual Cu fraction, when compared to both the not-planted soil and bulk soil (Fig. 3). These indicate that, for the five soil treatments, *E. splendens* can activate Cu in the rhizospheric soil, so as to the decreased carbonate-bound Cu fraction with an elevation in organic bound and Fe/Mn oxide bound Cu fractions, especially H_2O extractable and exchangeable bond Cu fractions in the rhizospheric zone for plant uptake. For the five-soil treatment, soil extractability of Cu changed slightly for the bulk soil as compared to the corresponding not-planted soil. While after *E. splendens* grown in these treated soils for 170 days, the NH_4NO_3 extractable-Cu in the rhizospheric zone of *E. splendens* significantly increased as compared to the corresponding bulk soil (Fig. 4), suggesting that rhizospheric effects of *E. splendens* elevated extractability of Cu in rhizospheric soil. M and MF soil amendments enhanced mostly the levels of NH_4NO_3 extractable-Cu in the rhizospheric zone for Cu uptake to the root of *E. splendens* (Fig.5).

Both soil amendments and rhizospheric effects by *E. splendens* can affect Pb, Zn and Cd solubility and extractability in this soil. Compared to the bulk soil, no significant changes were found in H_2O soluble and exchangeable Pb soil fractions in the rhizospheric zone except D+MF soil treatment, while organic bound Pb soil fraction increased considerably in the rhizospheric zone for the five soil treatments (Fig. 3). MF treatment markedly reduced carbonates bound Pb fraction, but enhanced Fe/Mn oxide bound Pb and organic bound Pb fractions in the rhizospheric soil (Fig. 3). While for Zn, as compared to the bulk soil, both M and MF amendments increased exchangeable Zn fraction, M amendment reduced Fe/Mn oxide bound Zn fraction and MF amendment reduced carbonates bound Zn fractions in the rhizospheric zone of *E. splendens*. The increased carbonates bound Cd fraction in the rhizospheric soil were noted for the control and M-amended soil, while soil treatments with MF, S+MF and D+MF decreased carbonates bound Cd fraction in the rhizospheric zone of *E. splendens*, as compared to the bulk soil. MF amendments increased exchangeable and residual Cd fractions in the rhizospheric zone. Both S+MF and D+MF soil treatments increased exchangeable Cd fractions in the rhizospheric zone of *E. splendens* (Fig. 4). As compared to the not-planted soil, the extractability of Pb, Zn and Cd in the bulk soil changed slightly, but it was reduced as the soil treatment with control, M, MF, S+MF and D+MF (Fig. 5). As compared to the bulk soil, for the

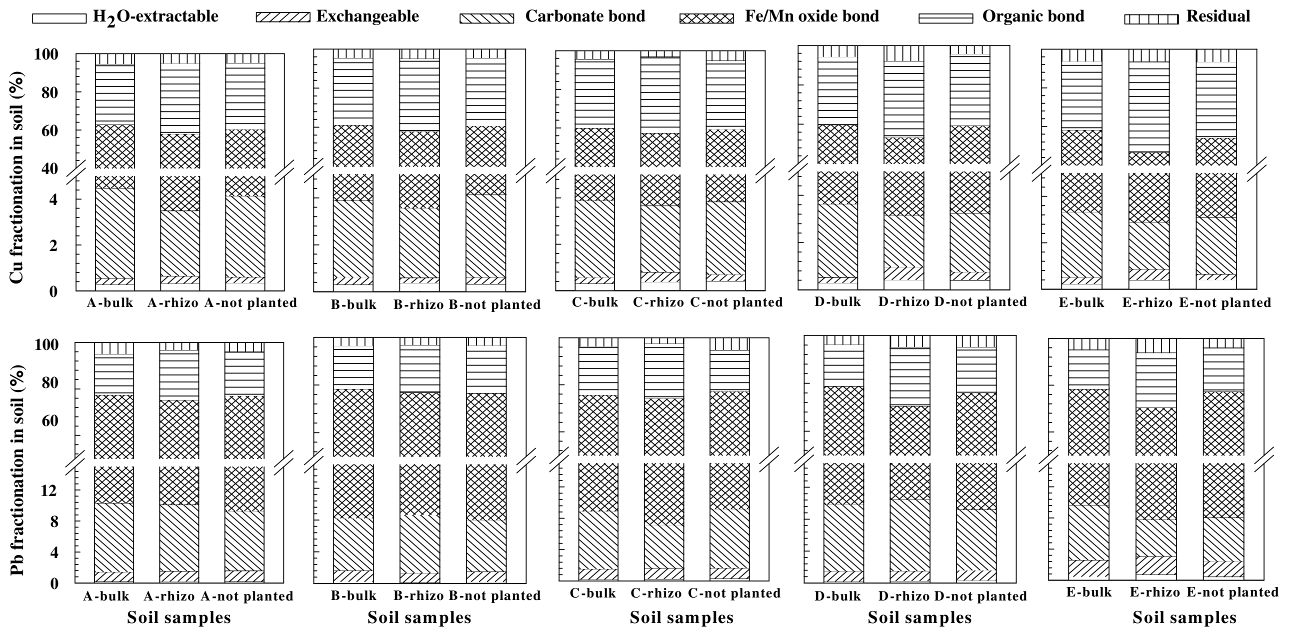

Fig. 3 Effects of soil amendments and managements on Cu, Pb fractionation in the not-planted soil, bulk soil and rhizospheric soil after *E. splendens* grown for 170 days in the filed experiment (A, B, C, D, E refer to the five soil treatments: Control, M, MF, S+MF and D+MF, respectively; bulk, rhizo and not planted refer to the bulk soil, rhizospheric soil and not planted soil, respectively)

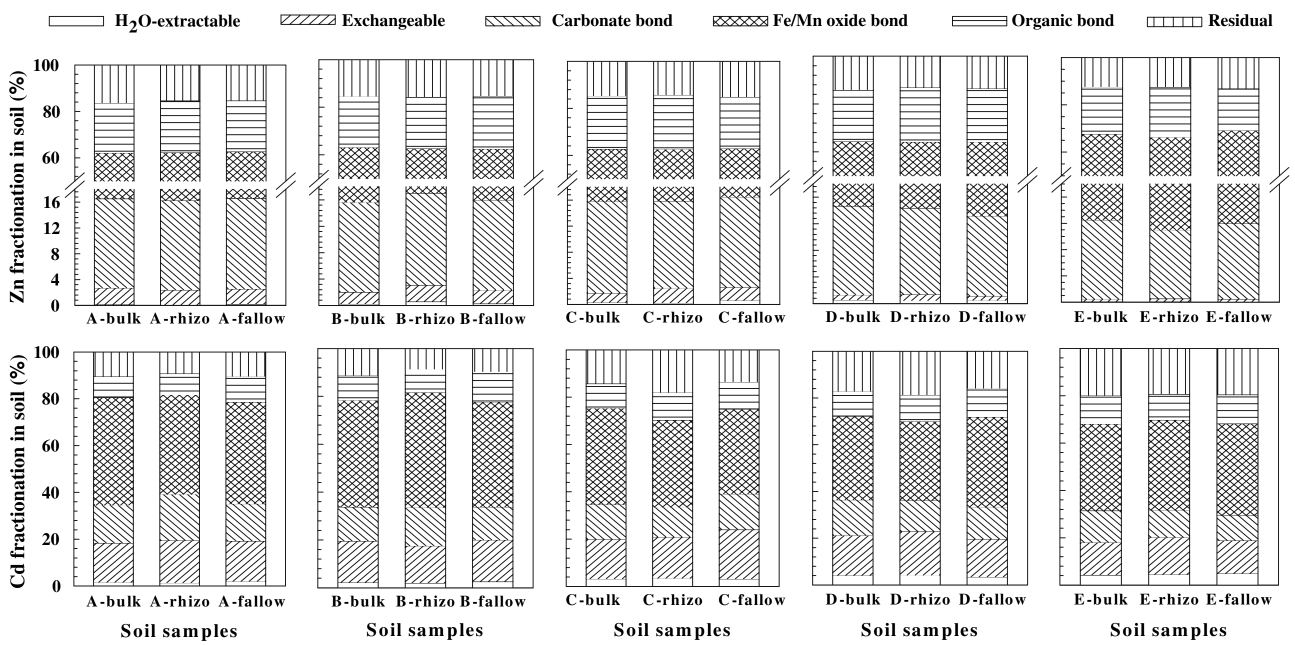

Fig. 4 Effects of soil amendments and managements on Zn, Cd fractionation in the not planted soil, bulk soil and rhizospheric soil after *E.splendens* grown for 170 days in the filed experiment (A, B, C, D, E refer to the five soil treatments: Control, M, MF, S+MF and D+MF, respectively; bulk, rhizo and not planted refer to the bulk soil, rhizospheric soil and not planted soil, respectively)

five soil treatments, M and MF soil amendments elevated NH_4NO_3 and NH_4OAc extractability of Pb and Cd in the rhizospheric soil of *E. splendens*, while NH_4NO_3 and NH_4OAc extractability of Zn in the rhizospheric zone changed slightly (Fig. 5). Both soil amendments with MF and the rhizospheric effects of *E. splendens* can activate Pb and Cd in the rhizospheric soil of *E. splendens*, so as to the elevated phytoavailability in the rhizospheric soil.

In this contaminated site, there might be existence of competition mechanisms between Cu, Pb, Zn and Cd in the rhizospheric soil of *E. splendens* for plant uptake. Mench and Harin (1991) has confirmed that the soluble humus

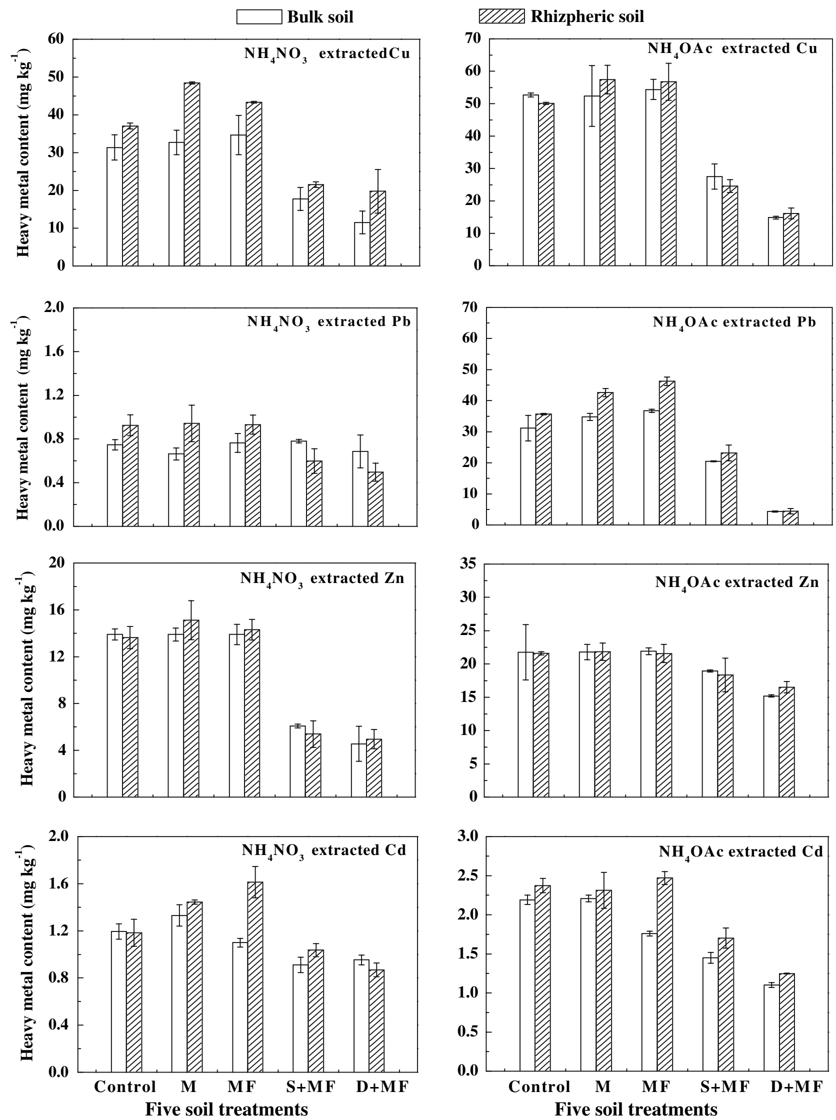

Fig. 5 Extractable Cu, Pb, Zn and Cd of the treated soil after *E.splendens* grown for 170 days in the filed experiment. All the data are means of 4 replications, and bars depict SE

from root exudates can dissolve soil heavy metals such as Cu, Pb, Zn and Cd, and transport them to the rhizospheric zone of plants. Both Cu and Pb have high affinity to the soluble humus and mucilage from root, so as to their difficulty to flux to the root surface than Zn and Cd (Morel et al. 1986). Soil pH and DOM contents are key factors highly affecting the degree of complexation with metals (Harter 1983), subsequently Cu, Pb, Zn and Cd mobility and extractability in the soil, particularly in the rhizospheric soil. Both acidification and elevated DOM content in the rhizospheric zone of *E. splendens* suggested that secretion of acid substances and DOM from plant roots improved the physicochemical properties of rhizospheric soil (Peng et al. 2005), thus enhancing the solubility and extractability of Cu, Pb, Zn and Cd, especially Cu, in the rhizospheric zone, for example, M or MF affected mostly the NH_4NO_3 extractable Cu in soil as compared to Pb, Zn, and Cd, which maybe helpful for metal uptake by plant root. But the activation mechanism in the rhizospheric zone of *E. splendens* in this contaminated soil to absorb more Cu, Pb, Zn, and Cd need to be further investigated.

Acknowledgments This project was supported by the National Natural Science Foundation of China (NSFC, 20307008) and the "973" Program from Science and Technology Ministry of China (2002CB410804).

References

Ernst WHO (1996) Bioavailability of heavy metals and decontamination of soils by plants. Appl Geochem 11:163–167

Harter RD (1983) Effect of soil pH on absorption of lead, copper, zinc and nickel. Soil Sci Soc Am J 47:47–51

He ZL, Yang XE, Stoffella PJ (2005) Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol 19:125–140

Jiang LY, Yang XE, He ZL (2004) Growth response and phytoextraction of copper at different levels in soils by *Elsholtzia splendens*. Chemosphere 55:1179–1187

Mench M, Martin E (1991) 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 132:187–196

Morel JL, Guckert A, Mench M (1986) Measurement of Pb^{2+}, Cu^{2+} and Cd^{2+} binding with mucilage exudates from maize (*Zea mays* L.) roots. Biol Fertil Soil 2:29–34

Peng HY, Yang XE (2005) Distribution and accumulation of copper, lead, zinc and cadmium contaminants in *Elsholtzia splendens* grown in the metal contaminated soil—a field trial study. Bull Environ Contam Toxicol 75: 1115–1122

Peng HY, Yang XE (2007) Characteristics of Cu and Pb uptake and accumulation in the two ecotypes of Elsholtzia. Bull Environ Contam Toxicol 78:142–147

Peng HY, Yang XE, Jiang LY, He ZL (2005) Copper phytoavailability and uptake by *Elsholtzia splendens* from contaminated soil as affected by soil amendments. J Environ Sci Health A 40:839–856

Salt DE, Smith RD, Raskin I (1998) Phytormediation. Ann Rev Plant Physiol Plant Mol Biol 49:643–668

Song J, Zhao FJ, Luo YM, McGrath SP, Zhang H (2004) Copper uptake by *Elsholtzia splendens* and *Silene vulgaris* and assessment of copper phytoavailability in contaminated soils. Environ Pollut 128:307–315

Tessier A, Cambell PC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 284:66–69

Weng GY (2004) Metal tolerance and phytoextraction of *Elsholtzia* plants. Master thesis of Zhejiang Univeristy

Yang XE, Peng HY, Jiang LY, He Z (2005) Phytoextraction of copper from contaminated soil by *Elsholtzia splendens* as affected by EDTA, citric acid and compost. Inter J Phytoremed 7:69–83

Yang XE, Calvert DV, He ZL, Stoffella PJ, Li YC, Zhang M (2002) Effect of compost amendment on solubility and transformation of copper in soils. Poster presentation in the Int. Sym of the 17th WCSS, Thailand, pp 14–21