Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Chelators effect on soil Cu extractability and uptake by *Elsholtzia splendens*[*]

JIANG Li-ying (姜理英)[†1,2], YANG Xiao-e (杨肖娥)[1]
([1]*College of Natural Resources and Environmental Science, Zhejiang University, Hangzhou 310029, China*)
([2]*Department of Environmental Engineering, College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, China*)
[†]E-mail: jiangly111311@sina.com

Received July 16, 2003; revision accepted Nov. 19, 2003

Abstract: Phytoremediation is emerging as a potential cost-effective solution for remediation of contaminated soils, and bioavailability of metal in the soil for plant uptake is an important factor for successful phytoremediation. This study aimed at investigating the ability of EDTA and citric acid for enhancing soil bioavailability of Cu and phytoremediation by Elsholtzia splendens in two types of soils contaminated with heavy metals [i.e. mined soil from copper mining area (MS), and paddy soil (PS) polluted by copper refining]. The results showed that addition of 2.5 mmol/kg EDTA significantly increased the H_2O extractable Cu concentration from 1.20 to 15.78 mg/kg in MS and from 0.26 to 15.72 mg/kg in PS, and that shoot Cu concentration increased 4-fold and 8-fold as compared to the control. There was no significant difference between the treatment with 5.0 mmol/kg EDTA and that with 2.5 mmol/kg EDTA, probably because that 2.5 mmol/kg EDTA was enough for elevating Cu bioavailability to the maximum level. As compared with the control, citric acid had no marked effect on both soil extractable Cu and shoot Cu concentration or accumulation. The results indicated that EDTA addition can increase the potential and efficiency of Cu phytoextraction by *E. splendens* in polluted soils.

Key words: EDTA, Citric acid, *Elsholtzia splendens*, Phytoremediation, Cu
Document code: A **CLC number:** X53

INTRODUCTION

Phytoremediation, that use of green plants to decontaminate Cu and other heavy metals in soils, is an emerging technique with advantages of being in situ, cost-effective and environmentally sustainable (Chany *et al.*, 1997; Cunningham *et al.*, 1997; Salt *et al.*, 1998). The availability of metal in the soil for plant uptake is one important limitation for successful phytoremediation (Blaylock *et al.*, 1997). For example, lead (Pb) is one of the most important environmental pollutants, has limited solubility in soils, and is available for plant uptake due to complexation with organic matter. To solve this problem, chelators such as EDTA or HEDTA had been added to Pb contaminated substrates to promote metal translocation from roots to shoots in many species. For instance, Huang and Cunningham (1996) tested N-(2-hydroxyethyl)-ethylenediaminetriacetic acid (HEDTA) on Pb accumulation enhancement and found that 1 week after transplantation, the shoot Pb concentration increased from 40 to 10600 mg/kg. In addition to shoot Pb concentration, the shoot to root Pb content increased

---

[*]Project supported by the National Natural Science Foundation of China (No. 29977017) and the Science and Technology Ministry of China (No. 2002CB410804)

from 0.2 to 1.2. Beside Pb, EDTA adding could also increase other metals, such as Cu, Cd, Ni, bioavailability and uptake by plants. Blaylock *et al.*(1997) showed that a 2.5 mmol/kg EDTA treatment to soil containing Cd, Cu, Ni, Pb and Zn substantially increased the uptake of those metals to the shoots of *B.juncea*. Deram *et al.*(2000) found that accumulation of Cu, Co and Ni in *Arrhenatherum elatius* increased significantly because acid-extractable Cu concentration (1 mol/L hydrochloric acid) increased from 200 to 7500 mg/kg, Co from 40 to 175 mg/kg and Ni from 8 to 1276 mg/kg in the supporting soils after addition of 4 g/kg EDTA. In addition, some low molecular weight organic acids (LMWOA) are of particular importance due to their metal chelating complexing properties for mobilization of heavy metals (Mench and Martin, 1991). Krishnamurti *et al.*(1997) demonstrated that various LMWOA could influence the rate of Cd release from different soils and increase the solubility of Cd in bulk soil through the formation of soluble Cd-LMWOA complexes.

Elsholtzia splendens (*Elsholtzia haichowensis*) had been identified as a Cu tolerant and accumulating plant species in mining area and high Cu level nutrient solution (Yang *et al.*, 1998; 2002). *E. splendens* grows abundantly over copper mining areas and was first recognized for its value in exploration of copper ores in the 1950s (Xie and Xu, 1953). It has large biomass with shoot dry matter yield reaching as high as 10 tons/ha under field conditions. In nutrient solution, the growth of *E. splendens* was found to be optimal at Cu supply levels up to 100 μmol/L, and was not dramatically reduced at Cu supply levels of up to 500 μmol/L (Yang *et al.*, 2002). Copper accumulation of over 1000 mg/kg in the shoots of *E. splendens*, 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; 2002). At Cu supply levels lower than 500 μmol/L, Cu concentration in shoots of *E. splendens* did not reach the threshold of hyperaccumulator (Ke *et al.*, 2001; Tang *et al.*, 1999; 2001). Furthermore, Cu concentration in *E. splendens* root was much more than that in the shoots grown both in the mining area and in nutrient solution (Yang *et al.*, 2002; Tang *et al.*, 1999). If Cu uptake by root and translocation from root to shoot increased by adding some chelators, the phytoextraction will be more efficient. Song (2002) found Cu concentration was decreased sharply when 100 μmol/L EDTA was added to nutrient solution 1 week before *E. splendens* was harvested, but effectiveness of *E. splendens* to decontaminate Cu by chelators addition in the soils is unknown. This study aimed at investigating the effects of chelators addition on Cu availability in the soil and Cu phytoextraction by *E. splendens*.

MATERIALS AND METHODS

Soil samples and characteristics

Two types of soils tested in this study included mined soil (MS) collected from one copper mining area in Zhuji County of Zhejiang Province, and paddy soil (PS) which was an agricultural soil in Fuyang County of Zhejiang Province that was severely contaminated by heavy metal emission from many copper refining plants (Jiang *et al.*, 2002). Soil was collected from the surface (0−20 cm), air dried and sieved to pass through 2 mm for analysis. Some agrochemical properties of these two contaminated soils are shown in Table 1.

Table 1 Agrochemical properties of the contaminated soils tested in this study

Soil properties	Mined soil (MS)	Paddy soil (PS)
pH (H_2O)	6.06±0.25	7.40±0.09
CEC (cmol/kg)	5.65±0.21	7.09±0.32
OM (g/kg)	22.80±1.21	42.02±4.62
Total-N (g/kg)	0.42±0.06	1.28±0.12
Total-P (g/kg)	0.78±0.11	1.12±0.04
Total Cu (mg/kg)	1631±22.50	1022 ±15.38
NH_4OAc extractable Cu (mg/kg)	88.64±2.37	86.91±5.65
DTPA extractable Cu (mg/kg)	93.82±2.33	93.26±1.38

* CEC refers to cation exchange capacity; OM refers to organic matter content

Pot experiment

Pot experiment was conducted using air-dried soil samples passed through 2 mm and treated as follows: 1) Control: without adddition of chelating agents; 2) EDTA1: 2.5 mmol/kg EDTA; 3) EDTA2: 5.0 mmol/kg EDTA; 4) CA: 5.0 mmol/kg citric acid; and 5) EDTA2+CA: 5.0 mmol/kg EDTA plus 5.0 mmol/kg citric acid.

Soil (1.5 kg oven dried) was placed in each pot. Basal fertilizers (0.1 g/kg urea and 0.2 g/kg KH_2PO_4) were applied. The seeds of *E. splendens* 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 a nutrient solution prepared for growing seedlings (Yang *et al.*, 2002). After *E. splendens* grew for 40 days, two plants were transplanted to each pot. All pots were adjusted daily to 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 ºC and day/night humidity of 40/60%. After *E. splendens* had been planted for 90 days, EDTA and citric acid treatment was added as 5% and 20% solution respectively. The plants were harvested 10 days after treatment with EDTA or CA. Shoots were cut at the soil surface, rinsed with distilled water, blotted dry. Plant tissues were oven-dried at 70 ºC, 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 pot, air-dried, and passed through a 1.0 mm plastic sieve for chemical analysis.

Chemical and data analyses

Soil pH was measured in distilled water with a soil/solution ratio of 1:2.5 (W:V). Organic matter content, cation exchange capacity (CEC), and total N and P in the soil were determined according to the methods described by SSICA (1980). Soil total Cu was extracted with Aqua regia and plant available Cu was extracted with 1.0 mol/L NH_4OAc (pH 7.0), distilled water and 1.0 mol/L NH_4NO_3 with the soil: extractant ratios of 1:20, 1:1 and 1:2.5, respectively (Ernst, 1996; MAFF, 1986). Subsamples 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 digestion were measured by an Inductively Coupled Plasma-optical Emission Spectroscopy (ICP-OES, Model IRAS-AP, TJA).

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

RESULT

Chelators effect on soil H_2O extractable Cu

H_2O extractable Cu is regarded as Cu ion in soil solution, which can be taken up directly by plants (Schramel *et al.*, 2000). Addition of EDTA significantly increased H_2O extractable Cu in the two contaminated soils as compared with the control (Fig.1). Addition of EDTA with 2.5 mmol/kg increased H_2O extractable Cu from 1.20 to 15.78 mg/kg in MS and from 0.26 to 15.72 mg/kg in PS. But when EDTA levels increased to 5 mmol/kg, H_2O extractable Cu was not increased significantly when compared to 2.5 mmol/kg EDTA treatment, which may indicate that EDTA level of 2.5 mmol/kg was high enough to elevate Cu bioavailability to the maximum level.

As compared with the control, adding 5 mmol/kg citric acid (CA) did not affect H_2O extractability of Cu markedly (Fig.1). Moreover, there was no significant difference between the treatment with 5.0 mmol/kg EDTA (EDTA2) and that with 5.0 mmol/kg EDTA plus 5.0 mmol/kg CA (EDTA2+CA). Little differences in the concentration of H_2O extractable Cu among EDTA1, EDTA2 and EDTA2+CA treatments were noted, which suggested that EDTA had higher capacity than citric acid to chelate and mobilize Cu in the soils.

Chelators effect on NH_4NO_3 and NH_4OAc extractable Cu in the soils

Soil Cu extracted by NH_4NO_3 and NH_4OAc is absorbed weakly by soil particles, which can dissol-

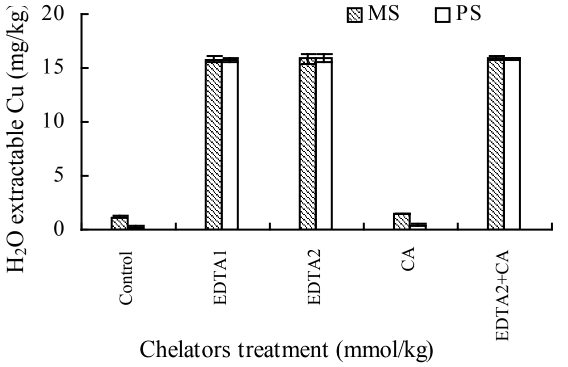

Fig.1 Effects of chelators application on H_2O extractable Cu in the two contaminated soils

* MS: Soil from copper mined area; PS: paddy soil polluted by copper refining;
Control: with no chelating agents; EDTA1: 2.5 mmol/kg EDTA ; EDTA2: 5.0 mmol/kg EDTA ;
CA: 5.0 mmol/kg citric acid; EDTA2+CA: 5.0 mmol/kg EDTA together with 5.0 mmol/kg citric acid

ve or be desorbed and then be uptaken by plant. Hence, this extractable Cu has great correlation with plant Cu (Ernst, 1996; Schramel *et al.*, 2000). Addition of EDTA had no marked effect on NH_4NO_3 extractable Cu in MS, but increased significantly NH_4NO_3 extractable Cu in PS (Fig.2), which suggested that increasing of labile mobile Cu by EDTA was dependent on soil type. Furthermore, it was strange that citric acid added decreased the NH_4NO_3 extractable Cu in both MS (10.6%) and PS (21.2%), and similarly, NH_4OAc extractable Cu was decreased by citric acid in the PS. Besides, all other treatments had no significant influence on NH_4OAc extractable Cu as compared with the control (Fig.3).

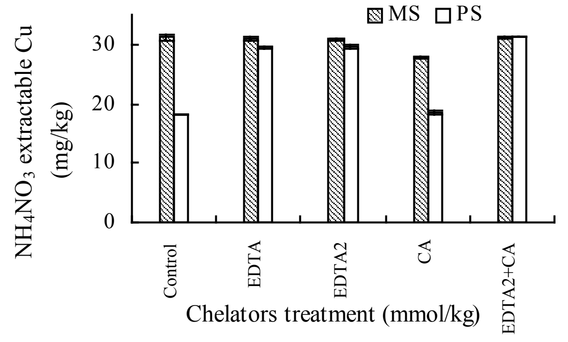

Fig.2 Effects of chelators application on NH_4NO_3 extractable Cu in the two contaminated soils

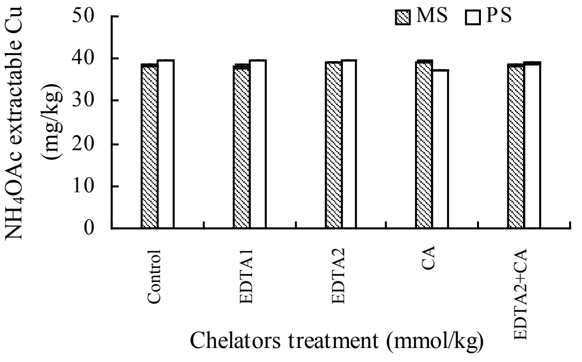

Fig.3 Effects of chelators application on NH_4OAc extractable Cu in the two contaminated soils

Chelators effect on plant growth

Addition of EDTA and citric acid led to yield reduction in shoot biomass. At EDTA treatment levels of 2.5 and 5 mmol/kg, the plants appeared to be wilting only at day 1 after the chelator was added, but remained alive except for a few fallen leaves at day 10. Shoot biomass at 2.5 mmol/kg EDTA treatment was decreased by 13% for MS and 24% for PS as compared with the control (Fig.4). No significant difference in shoot biomass existed between 2.5 mmol/kg EDTA and 5.0 mmol/kg EDTA treatments. Biomass reduction by addition of citric acid was probably due to pH change .

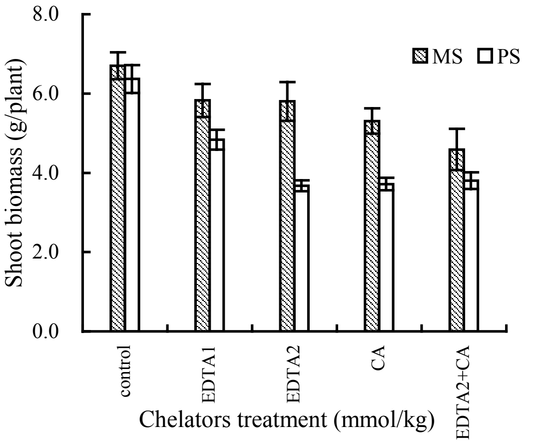

Fig.4 Effects of chelators application on the shoot biomass of *E. splendens* grown in the two contaminated soils

Chelators effect on Cu accumulation in plant

Addition of EDTA significantly enhanced shoot Cu concentration (Table 2), which was increased by more than 4-fold and 8-fold at 2.5 mmol/kg EDTA as a result of the increased H_2O extractable Cu in the soil, as compared with the control. No significant difference in shoot Cu concentration was observed between 2.5 and 5 mmol/kg EDTA treatments. And 5.0 mmol/kg citric acid also had no marked effect on shoot Cu as compared with the control.

The successes of phytoremediation depended on both shoot biomass and shoot Cu concentration, so the potential effectiveness of each plant for phytoremediation was evaluated by copper accumulation (metal concentration in plant×dry weight). As compared with the control, the addition of EDTA increased heavy metal accumulation by plants. This was due to the sharp increase in shoot Cu concentration caused by EDTA application. Shoot Cu accumulation was increased to 0.78 mg/plant in MS and 0.39 mg/plant in PS when 2.5 mmol/kg EDTA was added (Table 2). Citric acid addition did not increase Cu accumulation in *E. splendens* due to its minimal effect on shoot biomass and Cu concentration.

Table 2 Copper concentration and accumulation in shoots of *E. splendens* grown in two contaminated soils treated with chelators

Soil type	Treatment	Cu concentration (mg/kg)	Cu accumulation (μg/plant)
MS	Control	31.40±0.83	211.2±1.53
	EDTA1	135.0±4.42	784.3±10.6
	EDTA2	161.0±16.9	944.1±130
	CA	31.44±1.10	166.5±1.49
	EDTA2+CA	182.1±14.3	835.6±51.1
PS	Control	9.610±0.63	61.47±5.68
	EDTA1	82.77±10.7	397.9±47.6
	EDTA2	102.8±13.0	376.5±44.1
	CA	13.64±0.54	50.75±2.71
	EDTA2+CA	160.9±30.7	616.1±74.1

DISCUSSION

In soil, the applied chelator acts first to complex the soluble metals in the soil solution. As the free metal activity decreases, dissolution of bound metal ion begins to compensate for the shift in equilibrium (Blaylock *et al.*, 1997). This process continues until the chelator is saturated, the supply of metal from solid phase is exhausted, and or equilibrium is achieved and the insolubility of the solid phase restricts the activity of the free-metal (Blaylock *et al.*, 1997). In the case of EDTA, the formation of Cu-EDTA is expected to be the dominant metal-EDTA complex in most soils with metal binding constants of as high as 18.8 for EDTA (Smith *et al.*, 1995). As a result, if EDTA is added in sufficient amounts, nearly all of the soluble Cu will be complexed as Cu-EDTA with only very low activities of Cu^{2+}. For example, addition of 2.5 mmol/kg EDTA resulted in increasing of H_2O extractable Cu from 1.20 to 15.78 mg/kg in MS and from 0.26 to 15.72 mg/kg in PS. The amount of Cu in shoots was always directly proportional to the amount of soluble Cu in the soil, which suggested that the amount of soluble Cu in the soil appears to be a key factor in the enhancement of Cu uptake. But total soil Cu is limiting, the soluble Cu pool and corresponding Cu translocation from root to shoot may be limiting, even if more amount of chelator is added. For example, compared with the treatment with 2.5 mmol/kg EDTA, addition of 5.0 mmol/kg EDTA had no significant effect on soil extractable Cu and shoot Cu concentration.

Exudation of organic compounds by roots may influence ion solubility and uptake through their indirect effects on microbial activity, rhizosphere physical properties and root growth dynamics, and directly through acidification, chelation, precipitation and oxidation-reduction reactions in the rhizosphere (Marschner, 1995). Of these compounds, low molecular weight organic acids (LMWOA) are of particular importance due to their metal chelating/complexing properties for mobilization of heavy metals (Mench and Martin, 1991). In this study, citric acid addition had no effect on soil extractable Cu and shoot Cu concentration as compared to the control. This is in agreement with the reported results by Wu *et al.*(2003) that LMWOA had very small effect on the concentrations of Cu, Zn, Pb and Cd in soil solution as compared to EDTA. The main reason was that citric acid was weak acid and had no significant effect on soil pH (Wu *et al.*, 2003).

EDTA has proven to be very effective in facilitating plant uptake of Cd, Cu, Ni, Pb and Zn when applied to established plants several days before harvest (Raskin *et al.*, 1997). In this study, 2.5 mmol/kg EDTA addition increased shoot Cu concentration by 100 mg/kg, but decreased shoot biomass by 13% for MS and 24% for PS as compared with the control. These results indicated that EDTA had less severe effect on plant growth when EDTA was applied to established plants. Hence, Cu accumulation in shoot of *E. splendens* was increased by 0.87 μg/plant for MS and 0.39 μg/plant for PS when 2.5 mmol/kg EDTA was applied. It was found that in other high crop plants such as Indian mustard, corn and sunflower, addition of metal chelators induced significant accumulation of Pb (Huang and Cunningham, 1996; Blaylock *et al.*, 1997). But the mechanisms involved in metachelator induced plant uptake and translocation of metals are not well understood.

The results from our experiments demonstrated that Cu accumulation by *E. splendens* can be enhanced with application of EDTA applied, but EDTA addition also caused metal leaching through the soil profile and had toxic effects on test plants and soil microorganisms (Grěman *et al.*, 2001). Hence, further study must focus on the manipulation of EDTA applied in field experiment and avoidance of groundwater pollution.

References

Blaylock, M., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I., 1997. Enhanced accumulation of Pb in Indian Mustard by soil-applied chelating agents. *Environ. Sci. Technol.*, **31**:860-865.

Brooks, R.R., Reeves, R.D., Morrison, R.S., Malaisse, F., 1980. Hyperaccumulation of copper and cobalt a review. *Bulletin de la Societie royale de 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.

Deram, A., Petit, D., Robinson, B., Brooks, R., Gregg, P., Van-Halluwyn, C., 2000. Natural and induced heavy-metal accumulation by *Arrhenatherum elatius*: implications for phytoremediation. *Commun. Soil Sci. Plant Anal.*, **31**:413-421.

Ernst, W.H.O., 1996. Bioavailability of heavy metal and decontamination of soils by plants. *Applied geochemistry*, **11**(1-2):163-167.

Grěman, H., Velikonja-Bolta, Š., Vodnik, D., Kos, B., Leštan, D., 2001. EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. *Plant soil*, **235**:105-114.

Huang, J.W., Cunningham, S.D., 1996. Lead phytoextraction: species variation in lead uptake and translocation. *New phytol.*, **134**:75-84.

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 Unv. (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 ecologica sinica*, **6**:907-912 (in Chinese).

Krishnamurti, G.S.R., Huang, P.M., Van Rees, K.C.J., 1997. Kinetics of cadmium release from soils as influenced by organic: implications in cadmium availability. *J. Environ. Qual.*, **26**(1):271-277.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. 2nd Ed, Academic Press, London.

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**(2):187-196.

MAFF (Ministry of Agriculture Fisheries and Food), 1986. The analysis of agricultural materials. HMSO, London.

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

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

Schramel, O., Michalke, B., Kettrup, A., 2000. Study of the copper distribution in contaminated soils of hop fields by single and sequential extraction procedures. *The Science of the Total Environment*, **263**:11-22.

Smith, R.M., Martell, A.E., Motekaitis, R.J., 1995. NIST Critical Selected Stability Constants of Metal Complexes Database. Department of Commerce, U.S.A.

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, p.75-83 (in Chinese).

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

Tang, S.R., Wilke, B.M., Huang, C.Y., 1999. The uptake of copper by plants dominantly growing on copper mining 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**(5-6):895-905.

Wu, L.H., Luo, Y.M., Christie, P., Wong, M.H., 2003. Effects of EDTA and low molecular weight organic acids on soil solution properties of a heavy metal polluted soil. *Chemosphere*, **50**:819-822.

Xie, X.J., Xu, B.L., 1953. On *Elsholtzia haichowensis*: an indicator plant for copper. *Bull. Geol. Soc. China*, **32**(4):360-367 (in Chinese).

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. (Eds.), Sustainable Agriculture for Food, Energy and Industry. James and James Publishers, London, p.484-489.

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