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The role of arbuscular mycorrhiza on change of heavy metal speciation in rhizosphere of maize in wastewater irrigated agriculture soil

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Abstract: To understand the roles of mycorrhiza in metal speciation in the rhizosphere and the impact on increasing host plant tolerance against excessive heavy metals in soil, maize (*Zea mays* L.) inoculated with arbuscular mycorrhizal fungus (*Glomus mosseae*) was cultivated in heavy metal contaminated soil. Speciations of copper, zinc and lead in the soil were analyzed with the technique of sequential extraction. The results showed that, in comparison to the bolked soil, the exchangeable copper increased from 26% to 43% in non-infected and AM-infected rhizoshpere respectively; while other speciation (organic, carbonate and Fe-Mn oxide copper) remained constant and the organic bound zinc and lead also increased but the exchangeable zinc and lead were undetectable. The organic bound copper, zinc and lead were higher by 15%, 40% and 20%, respectively, in the rhizosphere of arbuscular mycorrhiza infected maize in comparison to the non-infected maize. The results might indicate that mycorrhiza could protect its host plants from the phytotoxicity of excessive copper, zinc and lead by changing the speciation from bio-available to the non-bio-available form. The fact that copper and zinc accumulation in the roots and shoots of mycorrhia infected plants were significantly lower than those in the non-infected plants might also suggest that mycorrhiza efficiently restricted excessive copper and zinc absorptions into the host plants. Compared to the non-infected seedlings, the lead content of infected seedlings was 60% higher in shoots. This might illustrate that mycorrhiza have a different mechanism for protecting its host from excessive lead phytotoxicity by chelating lead in the shoots.

Keywords: heavy metal speciation; arbuscular mycorrhiza; *Glomus mosseae*; rhizosphere

Introduction

Heavy metal contamination of agricultural land is becoming one of increasingly crucial environmental concerns in China. It is particularly severe in northern China where water resources are short and wastewater irrigation provides one of the more significant input sources of metals to farmland (Zhang, 1996). This may lead to a risk to environmental quality and sustainable food production. Exploration to solutions of this environmental problem has been attracting continuous interests of scientific communities of environmental conservation and protection.

Some studies have shown a positive impact of arbuscular mycorrhizal (AM) fungi on their associated plants in resisting heavy metal contamination from soil (Heggo, 1990; Hetrick, 1994; Davies, 2001). Studies on the mechanism of this impact indicated that cell well of mycorrhizal root or fungal slim may chelate the metal ions, and mycorrhizal fungi mycelia may filter the excessive metals (Bradley, 1981; 1982; Duck, 1986; Hartley, 1997). Some studies supported that mycorrhiza enhanced tolerance of host plants by improving the P absorption (Liu, 2000; Davies, 2001). It is obvious that early studies focused on fungus-plant system analysis to understand ways that mycorrhizal fungi improve their host plants' resistance to heavy metals. However, heavy metal uptake by plant might be also influenced by soil conditions, especially environment of rhizosphere, and uptake of heavy metals by plants can be closely correlated with the environment of rhizosphere (Chen, 1992; Atkinson, 2000). Li's findings (Li, 2001) on soil solution indicated that AM changed pH and limited Zn mobilization in soil solution to protect its host plant from stress of excessive Zn. It is speculated that mycorrhizal fungi might directly influence environment of rhizosphere to induce change and redistribution of heavy metal speciations that have different bioavailability. Thus, mycorrhizal fungi's protective effect may also associated with their role in influencing the chemical behavior of heavy metals, i.e. leading to changes of heavy metal speciation in rhizosphere and hence the absorption and toxicity of heavy metals to plants.

This study will identify heavy metal speciation changes that might have occurred in the rhizosphere of AM maize (*Zea mays* L.), determine whether such changes could have altered the accumulation and distribution of heavy metals Cu, Zn and Pb, and ultimately investigate the role of AM in protecting the host plants from toxicity of heavy metals in contaminated soil.

1 Materials and methods

1.1 Soil

Hapalquoll soil samples were collected from a vegetable cultivation land located at Eastern Xuzhuangzi in Tianjin. Wastewater and sludge have been applied since 1958 and the soil is rich in organic matter and nutrients so that there is no need of fertilizer. A surface soil sample (0—20 cm) was collected and its properties were pH 8.1, CEC 32.2 meq/100 g, and organic carbon 37.8 g/kg. Before used for plant cultivation, the soil samples were air-dried, sifted through 1 mm nylon nets and autoclaved at 120℃ for 2 h, and then cooled down to room temperature.

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1.2 Host plant

After 30 min shaking in 10% H_2O_2, the sterilized seeds of maize(*Zea mays* L.) were pre-geminated in Petri dishes for 3 d and then ready for sowing.

1.3 Mycorrhizal inoculum

The inoculum was consisted of AM mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) spores with sandy soil. The original inoculum was kindly provided by Prof. Li Xiaolin of China Agriculture University(Li, 2001).

1.4 Cultivation system

The cultivation was conducted using a rhizobox similar to that used by Zoysa *et al*.(Zoysa, 1997) and modified by Tao *et al*.(Tao, 2003). It consists of two square plastic boxes. The upper part is 70 mm in width and 50 mm in depth and contains 50 g of autoclaved soil. The top is opened and the bottom was sealed with a 24 μm(pore diameter) polyester mesh, which design to restrict the roots growing to the lower box. The lower compartment is slightly bigger with a width of 74 mm and a depth of 50 mm. After packed with 100 g of autoclaved soil, a polyester mesh is placed on top of the soil in lower box and 8 g of soil is evenly spread onto the mesh to form a 2 mm thick soil layer. Then, the upper box is put on the top of the lower box to tightly contact with the layer of the soil.

1.5 Experimental design and cultivation procedures

Simple design for comparison analysis was used for this experiment. The experiment included 2 treatments(inoculated maize and not inoculated maize) and a control(no plants) with 4 replications, which made a total of 12 experimental units in this experiment.

Mycorrhizal treatment received 10 g of fresh inoculum while non-mycorrhizal plant received the same amount of sand soil in upper box. 20 seedlings were sown into each upper box and 20 ml deionized water was added to each rhizobox at the beginning of the cultivation stage to reach moisture of 30%(saturation poit). All the samples were placed in a growth chamber(13 h light with 180 μmol/(m^2·s) at 30℃ and 11 h darkness at 25℃) for 7 weeks. During the period, the plants were watered with deionized water daily to maintain 15% moisture in the soil.

After one week growing in the cultivation device, a layer of root pad was formed on the polyester mesh on the bottom of upper boxes. According to Kuchenbuch and Jungk (Kuchenbuch, 1982), root action can influence soil for a distance of several millimeter within several days. Thus, the 2 mm stick soil layer tightly stacked with the polyester mesh on bottom of upper box could be considered to represent the rhizosphere soil. Control boxes were setup in identical mode without maize seedlings.

After 4 weeks growing, roots of inoculated maize were randomly sampled for straining to identify the infected roots according to Vierheilig(Vierheilig, 1998). The infection rate was greater than 90%. After 7 weeks of cultivation, the upper box and the lower box of each growth container were separated. Plants were washed carefully in deionized water and separated into shoot and root, then dried at 105℃ for 24 h. The dry mass was recorded before element analysis. The 2 mm layer of soil between the polyester mesh of upper and lower boxes was placed into a 25 ml beaker and finely mixed. 2 soil samples were collected from each rhizobox for separate analyses. Both plant and soil samples were ground with an agate mortar of pestle before digestion.

1.6 Extraction and analysis

Two-tenth of a gram of dried plant sample was digested with 10 ml 70% HNO_3 in a microwave oven (CEM-MDS 2000) at 50% energy(635 W) and 120 psi for 60 min. The digestion solution was fixed to 50 ml and ready for element analysis.

Soil samples were proposed to analyze total heavy metal concentration and speciation. For determination of total concentration, 0.2000 g air-dried soil samples were digested in the microwave oven (CEM-MDS 2000) with 5 ml 70% HNO_3, 2 ml $HClO_4$ and 3 ml HF for 60 min at 50% energy (635 W) and 120 psi. For metal speciation, the soil samples were sequentially extracted with an operationally defined sequential fractionation procedure according to Tessier *et al*. (Tessier, 1979) in which increasingly strong extractants were used to release different metal associated soil fractions. 0.2000 g air-dried soil samples were used for the extraction and four fractions were extracted in the following sequence: Step 1. Exchangeable(a 5 ml of 1.0 mol/L $MgCl_2$ for 120 min at room temperature); Step 2. Carbonate-associated(a 5 ml aliquot of NaAc, for 6 h at room temperature); Step 3. Amorphous iron-manganese oxides (5 ml of 0.04 mol/L NH_2OHHCl in 25% HAc for 6 h at 96℃); and Step 4. Organic bound(3 ml and 2 ml of a mixture of H_2O_2 and 0.02 mol/L HNO_3 adjusted to pH 2 at 85℃ for 1 h and 2 h in sequence, followed by 5 ml of a mixture of 0.8 mol/L NH_4Ac and 20% HNO_3 for 30 min). Subtracting these four fractions from the total derived the residual fraction. All analyses of elements were performed with a flame atomic absorption spectrophotometer (AAS Hitachi 180—80 flame) with a detection limit of Cu, 0.01 mg/ml, Zn 0.02 mg/ml, Pb 0.01 mg/ml.

Standard deviations were computed and ANOVAs tested the significance of treatments with Excel(Microsoft 2000). T-test assessed the significance between concentration of Cu, Zn and Pb in the shoots and roots of maize grown in AM inoculated and non-inoculated soils.

2 Results and discussion

2.1 Fractionation of Cu, Zn and Pb in the contaminated soil

The total content of Cu, Zn and Pb in the tested soil of this study was determined as 164.0 mg/kg, 334.5 mg/kg and

135.4 mg/kg respectively. The background concentration of Cu, Zn and Pb in uncontaminated soil in the region was 28.8 ± 9.2, 79.3 ± 22.0 and 21.0 ± 5.3 mg/g in average (Chinese Environ. Monitoring Center, 1990). Therefore the sample soil was considered to represent a typical contaminated soil in the wastewater irrigated area in North China.

Analyses of metal speciations (testing of soil samples from the control) showed that the distribution of metal fractions varies greatly among different metal elements (Table 1). Only very small amount of Cu presents in the exchangeable fraction, while exchangeable fraction of Zn and Pb was too low to be detected (lower than 0.02 mg/kg). Although the distribution of metal among the various fractions determined by the sequential extraction scheme is not chemically cut-clear, the results of the fractionation have provided an understanding of metal's relative mobility and bioavailablity. As the exchangeable metal fraction is considered to be the most important available fraction for plant uptake (Vulkan, 2000; Grezbisz, 1997; Onyatta, 1999; Sparks, 1983), comparing with Zn and Pb, the small and active part of Cu in Tianjin wastewater irrigated soil may provide a relative higher amount of Cu which is the most available for plant uptake and lead to heavy phytotoxicity.

Table 1 The distribution of Cu, Zn and Pb in wastewater implicated soil of Tianjin vegetable cultivation area (mg/kg; $n = 8$)

	Cu	Zn	Pb
Exch	0.67 ± 0.016	ud[a]	ud
Carb	5.08 ± 0.12	21.76 ± 0.44	19.16 ± 0.46
Ferr	22.11 ± 0.40	60.75 ± 0.73	41.73 ± 0.13
Org	49.87 ± 0.77	24 ± 0.12	34.31 ± 0.08
Residuals	46.19 ± 3.96	228.02 ± 11.86	58.19 ± 2.44

Notes: Exch. exchangeable; Carb. carbonated; Ferr. Fe-Mn oxide; Org. organic bound; a. ud: mean the content is lower than the limited detectable amount of the equipment. The detection limits for Cu was 0.3 mg/kg, Zn 0.2 mg/g and Pb 1.0 mg/kg

The organic bound Cu was the dominant fraction, accounting for 40% of the total amount, while oxide bound and residual Cu accounts for 17% and 37% of the total, respectively. The residual fraction of Cu was 30% and relative lower than that in uncontaminated soil. The result is consistent with other researcher's work (Schramele, 2000; Kabala, 2001). According to Nyamangara (Nyamangara, 1998), introduced metals in contaminated soil tending to go into the more reactive forms. Therefore, quite possibly, the Cu introduced to the soil through wastewater and sludge applications in Tianjin area may undergo an accumulation process from readily available to unavailable fraction but hardly reach the residual form.

The highest percentage of Zn and Pb associated with the residual fraction averages 68% and 38% respectively. An average 6.5%, 18%, 7% of Zn and 13%, 27%, 22% of Pb is associated with the carbonate, Fe-Mn oxide and organic forms respectively. The percentage of Zn and Pb fractions follows the order: residual > Fe-Mn oxides > carbonate > organic > exchangeable. The results are in line with other investigation in south China (Lu, 2003). The induced metal often bound with organic component in soil (Pichtel, 2000). It might be true that the high amount of organic matter induced by wastewater in sample combined with excessive Zn and Pb and lead to decrease of exchangeable fraction of them.

2.2 Changing of heavy metal speciation in the rhizosphere of AM maize

In comparison to the bulked soil, speciations of Cu, Zn and Pb changed significantly in the rhizosphere of AM-infected and non-infected maize (Table 2). The greatest change was exchangeable Cu that increased by 26% and 43% in non-infected and AM-infected rhizosphere, respectively, then in the bulked soil. With the exception of organic bound Cu in AM, other speciations were stable in the rhizosphere of AM and non-AM treatments. These results might suggest that Cu was activated by inducing roots in the rhizosphere, that is conformed to the published work of Tao *et al*. (Tao, 2003). The organic bound Zn and Pb increased significantly in the rhizosphere in comparison to those in the bulked soil. In contrast, carbonate and Fe-Mn oxides of Zn and Pb did not exhibit significant changes. These results are consistent with the notion that in rhizosphere, Zn and Pb were presented in relatively stable forms, i.e, bound to the organic matter.

Table 2 Biomass and speciations of Cu, Zn and Pb in soil of rhizosphere of AM-infected and non-infected maize (mg/kg; $n = 8$)

	Non-infected			Infected		
	Cu	Zn	Pb	Cu	Zn	Pb
Exch	0.80 ± 0.034*	ud	ud	0.82 ± 0.03*	ud	ud
Carb	5.49 ± 0.28	23.59 ± 0.59*	19.88 ± 0.37	4.58 ± 0.19	22.88 ± 0.10*	18.94 ± 0.47
Ferr	21.15 ± 0.20	59.64 ± 0.42	41.57 ± 0.13	20.16 ± 0.63	54.00 ± 2.55#	40.93 ± 0.09#
Orgn	50.29 ± 0.72	23.28 ± 0.11	33.74 ± 0.17	52.16 ± 0.10*	29.62 ± 0.08*	35.32 ± 0.11*
Residual	45.67 ± 0.82	227.51 ± 2.1	58.66 ± 0.23	38.09 ± 0.23	229.24 ± 2.48	58.01 ± 0.21
Biomass		4.24 ± 0.21			6.43 ± 0.30	

Notes: *. Positive significant under $P < 0.1$; #. negative significant under $P < 0.1$; ns. non significant under $P < 0.1$; ud. undetectable

However, the influence of AM roots on speciation of Cu, Zn and Pb occurred mainly in the organic fraction. When comparing with the bulked soil, organic bound Cu, Zn and Pb decreased slightly in the non-infected rhizosphere and

significantly increased in the infected rhizosphere (Table 2). We calculated the relative changes as the percentages of the speciation concentration difference between bulked soil and rhizosphere to the concentration of bulked soil. Results showed that the relative changes of organic bound Cu, Zn and Pb were, respectively, + 5%, + 23%, + 3% in the infected rhizosphere, and 0.8%, − 3%, − 2% in the non-infected rhizosphere (Fig. 1). Thus, significant amounts of Cu, Zn and Pb were bounded by organic matter in the infected rhizosphere.

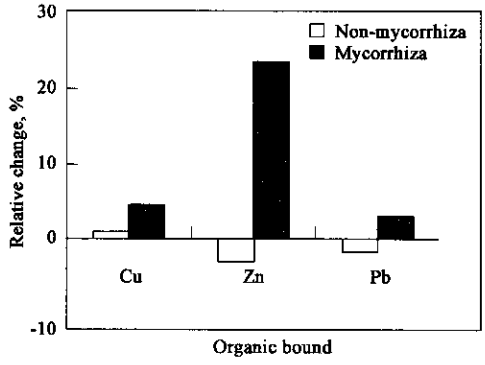

Fig. 1 Organic bound Cu, Zn and Pb in the rhizosphere of mycorrhizal and non-mycorrhizal roots

The amount of organic fraction in contaminated soil is often related to amount of organic matter (Grezbisz, 1997; Pichtel, 2000; Lu, 2003), it was considered that organic fraction of metals were those element bounded with various forms of organic matter in soil (Tessier, 1979; Hamon, 1995). Furthermore, mycorrhizal fungal slim is consisted with organic acids and had strong capacity to restrain heavy metals (Denny, 1995; Huang, 2000). The AM-infected root system is consisted with plant root and hypha of mycorrhizal fungi (Harlay, 1983), and fungus slim must have mixed with the root secretion. The increment of organic bound Cu, Zn and Pb in the soil of rhizosphere of AM-infected maize provides an indication of AM induced change in the amount. As the organic bound metal is not bioavailable (Vulkan, 2000; Tao, 2003), the result suggested that mycorrhiza limited metal bioavailablity and prohibited the absorption of heavy metals by host plant under excessive heavy-metal stresses. That may be caused by the changing of ingredients of root secretion induced by mycorrhiza infection. Further research shall be carried out in analysis of ingredient of mycorrhizal root exudates under heavy metal stresses.

2.3 Accumulation of heavy metals in AM infected maize

A marked variation was observed in the heavy metal concentrations between AM-infected and non-infected maize. The average concentration of measured heavy metals and the *t*-test results are listed in Table 3. The results showed that more heavy metals were accumulated in the roots than those in the shoots, except Zn in AM-infected maize. The amount of Cu was significantly lower in the AM-infected roots (11%) than that in the non-infected roots. There was insignificant difference in the shoots. The Zn content of the infected seedlings was lower in the root (50%) and in the shoot (15%) in comparison with the non-infected plants. These results indicated that mycorrhiza could have restricted the plants to absorb excessive Cu and Zn from the soil into the root system; analogous to our previous discussion on the function of AM in changing heavy metal speciations in the rhizosphere from bio-available to unavailable form. Considering that the biomass of AM-infected maize was 50% higher than non-infected plants in previous research (Table 2), AM have effectively impaired the phytotoxicity of excessive Cu and Zn or other heavy metals in the soil to the host plant. This is consistent with the findings reported by Gildon and Tinker (Gildon, 1983) and Chen *et al*. (Chen, 2003). Li and Christie believed that mycorrhiza improved the biomass accumulation of its host plant by increasing the absorption of P from soil (Li, 2001).

Table 3 Biomass and concentration of Cu, Zn, and Pb in maize and the *t*-test (mg/kg; $n = 8$)

	Shoots		*P*	Roots		*P*
	Non-infected	Infected		Non-infected	Infected	
Cu	17.05 ± 1.327	17.74 ± 0.130	0.324	91.58 ± 0.429	82.46 ± 1.050	0.001**
Zn	246.57 ± 7.801	210.26 ± 2.089	0.009*	252.37 ± 3.622	167.39 ± 4.317	0.001**
Pb	3.32 ± 0.164	5.29 ± 0.053	0.001**	24.46 ± 6.614	18.20 ± 1.607	0.023*

In the infected roots, the Pb content was 25% lower than that in the non-infected roots. But, the Pb content was 60% higher in the infected shoots than that in the non-infected shoots. These results would indicate that mycorrhiza aided the host plant to hold excessive Pb in the shoots. Working with Cd, Steffens (Steffens, 1990) indicated that in order to adapt the stress of high concentration of metals, pytochelatins would form to chelate the excessive metal in the plant cell. In addition, plants that were tolerant to high concentration of metal formed more pytochelatins than the heavy metal sensitive plants (Steffens, 1990). Though Steffens did not work with Pb, Pb may have the similar mechanism for retention in plants to Cd as both elements take the same form of bivalence cation in plant metabolism process (Kabata-Pendias, 1985). The excessive heavy metal in plant would severely destroy activity of key enzymes of C and N metabolism and photosynthesis that lead to great loss of biomass (Huang, 2001; 2004). In this study, the results of relative higher Pb in shoots of AM maize which did not influence growth might explain that AM had stimulated the plant cells to form pytochelatins to chelate the excessive Pb to alleviate the phototoxicity of Pb in shoots. However, there is no affirmative evidence to support the existence of pytochelatins in this study. Thus, future research should

investigate the type and the amount of pytochelatins in AM-infected plant.

3 Conclusions

Comparing with the bulked soil, speciations of Cu, Zn and Pb greatly changed in the rhizosphere of maize cultivated in heavy metal contaminated soil(Table 1 and Table 2). The exchangeable Cu increased significantly, but speciations of carbonate, Fe-Mn oxide and organic bound Cu remained constant. This might suggest that plant roots induced Cu to change from the stable to the unstable form. While exchangeable Zn and Pb were undetectable, the organic bound Zn and Pb increased significantly in the rhizosphere. Speciations of Cu, Zn and Pb in the rhizosphere of AM infected and non-infected maize demonstrated that large amounts of Cu, Zn, and Pb were bound by organic matter in the infected rhizosphere.

The results also indicated that Cu and Zn accumulation in the roots and shoots of AM infected plants were significantly lower than those in the non-infected plants, that might also suggest that AM efficiently restricted excessive Cu and Zn absorptions into the host plants. Compared to the non-infected seedlings, the Pb content of infected seedlings was 60% higher in shoots. This might illustrate that AM have a different mechanism for protecting its host from excessive Pb phytotoxicity by chelating Pb in the shoots.

It is conceivable that AM could protect the host plants from the phytotoxicity of excessive Cu, Zn and Pb by changing their speciation from the bio-available to the unavailable form.

References:

Atkinson D, Watson C A, 2000. The beneficial rhizosphere: a dynamic entity [J]. Applied Soil Ecology, 15: 99—104.

Bradley R, Burt A J, Read D J, 1981. Mycorrhizal infection and resistance to heavy metal toxicity in *Calluna vulgaris*[J]. Nature, 292: 335—337.

Bradley R, Burt A J, Read D J, 1982. The biology of mycorrhiza in the *Ricacacea* VIII, the role of mycorrhizal infection in heavy metal resistance [J]. New Phytol, 91: 197—209.

Chen B D, Li X L, Tao H Q *et al*., 2003. The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc[J]. Chemosphere, 50: 839—846.

Chen N C, Chen H M, 1992. Heavy metals pollution in soil-plant system[M]. Beijing: Science Press. 309—333.

Chinese Environ. Monitoring Center, 1990. Soil background values of elements in China[M]. Beijing: China Environmental Science Press.

Davies F T, Puryear J D, Newton R J, 2001. Mycorrhizal fungi enhance accumulation and tolerance of chromium in sunflower (*Helianthus annuus*) [J]. J Plant Physiol, 158(6): 777—786.

Denny H J, Ridge I, 1995. Fungal slime and its role in the mycorrhizal amelioration of zinc toxicity to higher plants[J]. New Phytol, 130: 252—257.

Duck T H A, Visser P, Ernst W H O *et al*., 1986. Vesicular-arbuscular mycorrhizae decrease zinc toxicity to grasses growing in zinc polluted soil[J]. Soil Biol Biochem, 18: 331—333.

Gildon A, Tinker P B, 1983. Interactions of vesicular-arbuscular mycorrhizal infections and heavy metals in plants[J]. New Phytol, 95: 263—268.

Grezbisz W, Kocialkowski W Z, Chudzinski B, 1997. Copper geochemistry and availability in cultivated soils contaminated by a copper smelter [J]. J Geochemical Exploration, 58: 301—307.

Hamon A, Lorenz S E, Holm P E *et al*., 1995. Changes in trace metal species and other components of the rhizosphere during growth of radish[J]. Plant Cell and Environment, 18: 749—756.

Harlay J L, Smith S E, 1983. Mycorrhizal symbiosis[M]. London and New York: Academic Press. 32.

Hartley J, Carmey J, Sanders F E *et al*., 1997. Toxic interactions of metals ions (Cd, Pb, Zn and Sb^{3+}) on *in vitro* biomass production of ectomycorrhizal fungi[J]. New Phytal, 137: 551—562.

Heggo A, Angle J S, Chaney R L, 1990. Effects of viesicular-arbuscular mycorrhizal fungi on heavy metal uptake by soybeans[J]. Soil Biol Biochem, 22: 865—869.

Hetrick B A K, Wilson G W T, Gigge D A H, 1994. The influence of mycorrhizal symbiosis and fertilizer amendments on establishment of vegetation in heavy metal mine spoil[J]. Environ Pollut, 86: 171—179.

Huang Y, Tao S, Chen Y J *et al*., 2000. Accumulation and distribution of Cu Zn in mycorrhizal *Pinus sylvestris* and its associated ectomycorrhizal fungus *Suillus bovinus* exposed to elevated Cu Zn concentrations in cultivating substrate[J]. Environmental Science, 21(2): 1—6.

Huang Y, Tao S, 2001. Excessive Cu and Zn affecting on distribution of the metals and activities of glycolytic and nitrogen incorporating key enzymes in mycelia of ectomycorrhizal fungi *Suillus bovines*[J]. Journal of Environmental Sciences, 13(3): 337—341.

Huang Y, Tao S, 2004. Influences of excessive Cu on photosynthesis and growth in ectomycorrhizal *Pinus sylvestris* seedlings[J]. Journal of Environmental Sciences, 16(3): 414—419.

Kabala C, Singh R R, 2001. Fractionation and mobility of copper, lead, and zinc in soil profiles in the vicinity of a copper smelter[J]. J Environ Qual, 30: 485—492.

Kabata-Pendias A, Pendias H, 1985. Trace elements in soils and plants[M]. Boca Raton, Florida: CRC Press.

Kuchenbuch R, Jungk A, 1982. A method for determining concentration profiles at the soil-root interface by thin slicing rhizospheric soil[J]. Plant Soil, 68: 391—394.

Li X L, Christie P, 2001. Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil [J]. Chemosphere, 42: 201—207.

Liu A, Hamel C, Hamilton R I *et al*., 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels[J]. Mycorrhiza, 9 (6): 331—336.

Lu Y, Gong Z T, Zhang G L *et al*., 2003. Concentration and chemical speciations of Cu, Zn, Pb and Cr of urban soils in Nanjing, China[J]. Geoderma, 115: 101—111.

Nyamangara J, 1998. Use of sequential extrction to evaluate zinc and copper in a soil amended with sewage sludge and inorganic metal salts[J]. Agri Ecosyst Environ, 69: 135—141.

Onyatta J O, Huang J O, 1999. Chemical speciation and bioavailability index of cadmium for selected tropical soils in Kenya[J]. Geoderma, 91: 87—101.

Pichtel J, Kuroiwa K, Sawyer H T, 2000. Distribution of Pb, Cd and Ba in soils and plants of two contaminated sites[J]. Environmental Pollution, 110: 171—178.

Schramel O, Michalke B, Kettrup A, 2000. Study of the copper distribution in contaiminated soils of hop fields by single and sequential extraction procedures [J]. Sci Toptal Environ, 263: 11—22.

Sparks D L, 1983. Ion activities: an historical and theoretical overviw[J]. Soil Science Society of America Journal, 48: 514—518.

Steffens J C, 1990. The Heavy metal-binding peptides of plants[J]. Annu Rev Plant Physiol Plant Mol Biol, 41: 553—575.

Tao S, Chen Y J, Xu F L *et al*., 2003, Changes of copper speciation in maize rhizosphere soil[J]. Environmental Pollution, 122: 447—454.

Tessier A, Campbell P G C, Bisson M, 1979. Sequential extraction procedure for the speciation of particulate trace metals[J]. Analytical Chemistry, 51: 844—851.

Vierheilig H, Coughlan A P, Wyss U *et al*., 1998. Ink and vinegar, a simple staining technique for arbusculur-mycorrhizal fungi [J]. Applied and Environmental Microbiology, 64(12): 5004—5007.

Vulkan R, Zhao F J, Barbosa-Jefferson V *et al*., 2000. Copper speciation and impacts on bacterial biosensors in the pore water of copper-contaminated soils [J]. Environmental Science & Technology, 34(24): 5115—5121.

Zhang M, Gong Z T, 1996. Content and distribution of some heavy metal elements in vegetable cultivated soil in China[J]. Acta Pedologica Sinica, 33: 85—93.

Zoysa A K N, Loganathan P, Hedley M J, 1997. A technique for studying rhizosphere processes in tree crops: soil phosphorus depletion around camellia (*Camellia japonica* L.) roots[J]. Plant Soil, 190: 253—265.

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