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Behavior of mercury in a soil–plant system as affected by inoculation with the arbuscular mycorrhizal fungus *Glomus mosseae*

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Abstract Effects of inoculation with the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* on the behavior of Hg in soil–plant system were investigated using an artificially contaminated soil at the concentrations of 0, 1.0, 2.0, and 4.0 mg Hg kg^{-1}. Mercury accumulation was lower in mycorrhizal roots than in nonmycorrhizal roots when Hg was added at the rates of 2.0 and 4.0 mg kg^{-1}, while no obvious difference in shoot Hg concentration was found between mycorrhizal and nonmycorrhizal treatments. Mycorrhizal inoculation significantly decreased the total and extractable Hg concentrations in soil as well as the ratio of extractable to total Hg in soil. Equilibration sorption of Hg by soil was investigated, and the results indicated that mycorrhizal treatment enhanced Hg sorption on soil. The uptake of Hg was lower by mycorrhizal roots than by nonmycorrhizal roots. These experiments provide further evidence for the role of mycorrhizal inoculation in increasing immobilization of Hg in soil and reducing the uptake of Hg by roots. Calculation on mass balance of Hg in soil suggests the presence of Hg loss from soil presumably through evaporation, and AM inoculation enhanced Hg evaporation. This was evidenced by a chamber study to detect the Hg evaporated from soil.

Keywords Arbuscular mycorrhizal fungus · Mercury · Soil · Uptake · Maize

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Introduction

Hg, a worldwide hazard, is increasingly released to the environment by anthropogenic activities such as coal burning and mining (Han et al. 2002). Mercury in soil can enter and accumulate in the food chain through plant uptake, posing a potential danger to human health and welfare (Gnamuš et al. 2000). Therefore, understanding the behavior and fate of Hg in soil–plant system is very important to assess its environmental risk.

Mercury in soil can be taken up by plants, and bioavailability of Hg in soil is to a large extent determined by its interactions with soil. Most of Hg in soil is either bound to minerals or adsorbed onto solid inorganic and organic surfaces, and only a small part of Hg in soil (e.g., water soluble and exchangeable fractions) is considered to be bioavailable to plants (Schuster 1991; Millán et al. 2006). Mercury sorption/desorption on soil is subjected to a wide array of chemical and biological transformation processes such as oxidation/reduction, methylation, and complexation. Therefore, it depends on a complex of factors including soil properties such as soil pH, texture and organic matter, soil temperature, and complex ligands (e.g., OH^- and Cl^-) in soil (Schuster 1991). Soil organic matter exhibits a great affinity for Hg; therefore, its content and composition would have a significant influence on Hg sorption/desorption behavior in soil (Yin et al. 1997; Grigal 2003). In addition, Hg in soil can evaporate into air as the forms of either organic Hg or elemental mercury (Hg(0)). Transformation of divalent inorganic Hg (Hg(II)) to organic Hg and/or Hg(0) occurring in the uppermost soil layers is the rate-limiting process of Hg evaporation (Schlüter 2000). Several abiotic and biotic factors contribute to the conversion of Hg species in soil. Soil organic matter, in particular dissolved organic matter, has been demonstrated to mediate

the nonbiological process and affect Hg speciation (Schlüter 2000), while soil microbe is considered to play a key role in the biological process of Hg reduction and methylation in soil (Gabriel and Williamson 2004).

Arbuscular mycorrhizal (AM) fungi are ubiquitous in soil, forming symbiotic associations with roots of the majority of plant species (Smith and Read 1997). It has been addressed that mycorrhizal inoculation can affect the accumulation of metals such as Cu, Cd, Zn, and As by plants and enhance the tolerance of host plants to contamination of these metals in soil (González-Chávez et al. 2002; Janoušková et al. 2006; Marques et al. 2006; Yu et al. 2009). However, the interaction between AM fungi and Hg in soil–plant system has not been studied so far. AM inoculation can influence soil properties and soil microbial community (Smith and Read 1997; Li and Christie 2001). Qualitative changes in root exudation and rhizodeposition following AM colonization have also been reported (Jones et al. 2004). All of these are speculated to change the speciation, mobility, and bioavailability of Hg in soil, therefore influencing its behavior in soil–plant system.

The aim of the present study was to investigate the behavior of Hg in soil–plant system as affected by inoculation with AM fungus. Maize was used as the model plant and the soil was spiked with Hg at different concentrations. The uptake of Hg by roots and sorption of Hg on soil were characterized and compared between mycorrhizal and nonmycorrhizal treatments in order to understand the contribution of AM inoculation to the uptake of Hg by plants and the immobilization of Hg in soil. A chamber study was carried out to monitor the evaporation of Hg from soil for mycorrhizal and non-mycorrhizal treatments.

Materials and methods

Inoculum and soil preparation

Inoculum of the AM fungus (*Glomus mosseae*, BGC XJ01), isolated from a noncontaminated soil in Sinkiang, China, was propagated for 10 weeks in pot culture on broomcorn (*Sorghum vulgare* Pers.) plants grown in a soil–sand media in a greenhouse. The inoculum, which was air-dried and sieved (<2 mm), consisted of spores, mycelium, sandy soil, and root fragments and had approximately 180 propagules per gram of dry soil based on the most probable number estimation (Porter 1979).

A sandy soil (Alfisol) collected from the surface horizon (0–15 cm depth) of a field in a farm near Beijing was air-dried, ground, and passed through a 2-mm sieve. The soil has the following properties (on a dry weight soil basis): pH (1:2.5 soil to water), 8.3; organic matter, 0.39%; cation exchange capacity, 65.8 cmol kg^{-1}; total Hg, 0.04 mg kg^{-1}; and 0.5 mol L^{-1} $NaHCO_3$-extractable P, 2.75 mg kg^{-1}. The soil was sterilized by γ-irradiation (10 kGy, 10 MeV γ ray) for the elimination of native AM fungi. An application of 200 mg kg^{-1} N (NH_4NO_3), 60 mg kg^{-1} P (KH_2PO_4), and 150 mg kg^{-1} K (K_2SO_4) was added in the soil as a basal fertilizer.

Plant growth and harvest

Mercury was added as $HgCl_2$ in solution and mixed with the soil by shaking for 24 h to obtain the homogeneous artificially contaminated soils at the concentrations of 1.0, 2.0, and 4.0 mg kg^{-1}, respectively. Soil without addition of Hg was used as blank control. The soils were equilibrated for a period of 4 weeks by undergoing four cycles of saturation with deionized water and air-dried in a greenhouse. Triplicate pots were prepared for each treatment. Pots received a mixture of 650 g soil and 50 g inoculum for mycorrhizal treatment or sterilized inoculum plus 15 mL of inoculum washings filtered through a 37-μm filter paper for nonmycorrhizal treatment. Seeds of maize (*Zea mays* L. cv. ND108; from China Agricultural University, Beijing, China) were surface sterilized in a 10% (*v*/*v*) solution of hydrogen peroxide and then pregerminated on a moist filter paper for 2 days. Four pregerminated maize seeds were then sown in each pot, and two seedlings were left after emergence. Plants grew in a controlled environment glasshouse with a photoperiod of 14 h at a light intensity of 250 μmol $m^{-2}s^{-1}$ provided by supplementary illumination. The temperature was 25°C at daytime and 18°C at night. Deionized water was added as required to maintain moisture content at about 50% of water holding capacity by regular weighing.

After growth for 8 weeks, shoots and roots were harvested separately. Root fragments were collected by sieving the soil and adding them to the root samples. Soils were sampled from each pot after mixed thoroughly. Roots were first carefully washed with tap water to remove any adhering soil particles. Then roots and shoots were rinsed thoroughly with deionized water, blotted dry, and weighed. Mycorrhizal colonization of roots was determined by the grid line intersect method (Giovannetti and Mosse 1980). The remaining plant and soils samples were then freeze-dried, ground, weighted, and stored at 4°C. The fresh to dry root ratio was used to estimate the total dry mass of roots.

Chemical analysis of Hg

Subsamples of 0.1 g dry plant materials were added with 3 mL HNO_3 in closed Teflon vessels and kept overnight and then were added with 3 mL H_2O_2 and heated at 120°C for 2 h. Soil samples (0.1 g) were digested with 3 mL

HNO_3 and 1 mL $HClO_4$ in closed Teflon vessels which were heated at 180°C for 4 h after kept overnight. Reagent blank and certified reference materials of NBS 1572 (citrus leaves) and NIST SRM 2709 (soil) were included to verify the accuracy and precision of the digestion procedure and subsequent analysis. The recovery rates were within 95± 10%. Available Hg in soil was extracted with 1 mol L^{-1} $CaCl_2$ at pH7.0 for 1 h at room temperature. The solutions were then centrifuged at 2,000 rpm for 10 min and filtered. Mercury concentration was determined by a hydride generation atomic fluorescence spectrometer (HG-AFS; AF-610A, Beijing RuiLi Instrumental Company, Beijing, China).

Sorption of Hg on soil

Sorption of Hg on soil was carried out in batch equilibration experiment using the Hg-free control soils collected after maize growth for both the mycorrhizal and nonmycorrhizal treatments. Experiments were carried out in triplicate by using the soils collected from separate pots without further mixing. Portions of 0.5 g soil were transferred into 50-mL polypropylene centrifuge tubes, and 25 mL of 0.01 mol L^{-1} $CaCl_2$ (pH7.8) solution containing different levels of Hg (0, 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00, 2.00, and 5.00 mg kg^{-1}, used as $HgCl_2$) was added to each tube. The tubes were shaken at 100 rpm for 5.0 h at 20°C. Then the suspensions were centrifuged at 2,000 rpm for 10 min followed by filtering. Hg concentration in the filtered solutions was determined by HG-AFS after appropriate dilution.

Kinetic uptake of Hg by roots

Mycorrhizal and nonmycorrhizal roots were prepared separately for the test of Hg uptake. Methods for inoculation and plant growth in noncontaminated soil were the same as described above. Experimental design for Hg uptake by roots was adopted from the method of Esteban et al. (2008). In brief, roots were excised at the basal node after carefully washed by soaking in water to remove soil particles. Triplicate root samples (0.2–0.5 g fresh weight) were incubated in aerated test solution (containing 5.0 mM morpholinoethanesulfonic acid and 0.5 mM $Ca(NO_3)_2$, adjusted to pH5.0 using KOH) for 30 min at 20°C in a water base. Then the roots were incubated in aerated test solutions with different concentrations of Hg (0, 0.2, 0.5, 1.0, and 2.0 mg L^{-1}, used as $HgCl_2$) for 20 min. After incubation in the test solution, the roots were then rinsed in ice-cold phosphate solution containing 1 mM K_2HPO_4 to remove any adsorbed Hg from the root-free space and stop further root activity. The roots were freeze-dried and weighed. Digestion and analysis of the samples were conducted following the methods described above.

Determination of Hg evaporation

Evaporation of Hg from soil was monitored separately using soil spiked with 4.0 mg kg^{-1} Hg. Triplicate pots were prepared for mycorrhizal and nonmycorrhizal treatments with and without plant growth. After 1 month of plant growth, each pot was enclosed within an acrylic chamber (10.5 L volume). Air Hg was monitored by the method of Moreno et al. (2005) with some modifications. In brief, a continuous air flow was supplied using a small air pump, and the flow rate was held constant at 200 mL min^{-1}. Evaporated Hg was captured in 50 mL acid trap solution containing 5% $KMnO_4$ dissolved in 1 mol L^{-1} H_2SO_4 for a 1-day period. The trap solution was then neutralized by the addition of oxammonium hydrochloride solution (100 gL^{-1}) and subjected to analysis of Hg with HG-AFS.

Statistical analyses

All data are presented as the average of triplicates. Significant ($P \leq 0.05$) treatment effects were determined by one-way analysis of variance using SPSS 11.0 software.

Results

Colonization rate, biomass, and concentration of Hg in maize

Roots of inoculated plants were extensively colonized by *G. mosseae*, while noninoculated controls remained nonmycorrhizal. The percentages of root length colonized were 77%, 78%, 74%, and 75% at Hg application rates of 0, 1.0, 2.0, and 4.0 mg kg^{-1}, respectively. Addition of Hg in soil did not significantly influence root colonization rate.

Table 1 displays the maize biomass and concentration of Hg in maize roots and shoots. Neither Hg addition nor mycorrhizal treatment significantly influenced the biomass. The accumulation of Hg in roots increased consistently with increasing Hg concentration in soil irrespective of inoculation treatment. Shoot Hg concentration was in the range of 0.030–0.045 mg kg^{-1}, which was much lower than root concentration and did not change obviously with increasing soil Hg concentration. Mycorrhizal inoculation significantly reduced Hg concentration in maize roots when Hg was applied at the rates of 2.0 and 4.0 mg kg^{-1}, while the concentration of Hg in shoots was not significantly different between nonmycorrhizal and mycorrhizal plants.

Table 1 Dry biomass and concentrations of Hg in maize roots and shoots for nonmycorrhizal and mycorrhizal treatments (mean±SE, n=3)

Hg addition (mgkg^{-1})	Inoculation treatment	Dry biomass (gpot^{-1})		Hg concentration (mgkg^{-1})	
		Root	Shoot	Root	Shoot
0	NM	6.4±0.3	11.2±0.1	0.05±0.01	0.035±0.006
	M	6.0±0.1	11.9±0.4	0.04±0.01	0.032±0.007
1.0	NM	6.5±0.3	10.7±0.5	0.26±0.06	0.037±0.004
	M	7.0±0.1	10.5±0.1	0.30±0.04	0.030±0.007
2.0	NM	6.0±0.4	10.9±0.1	1.05±0.10[a]	0.035±0.003
	M	6.2±0.6	10.9±0.4	0.80±0.09	0.040±0.016
4.0	NM	6.9±0.5	10.9±0.5	1.83±0.08[a]	0.040±0.012
	M	6.7±0.2	11.3±0.3	1.33±0.14	0.045±0.011

NM nonmycorrhizal treatment, *M* mycorrhizal treatment

[a] Means difference at significant level of 5% between inoculation treatments in each Hg addition level

Concentrations of Hg in soil and Hg evaporated from soil

Concentrations of the total and extractable Hg in soil after plant harvest are given in Table 2. The residual Hg concentrations in soils decreased by 16.5–34.5% compared with their initial concentrations. Mycorrhizal treatment led to a lower Hg concentration in soil than the nonmycorrhizal treatment. $CaCl_2$-extractable Hg has been shown to correlate with its accumulation in plants and is considered to represent the bioavailable fraction of Hg in soil (Panda et al. 1992; Zagury et al. 2006). The $CaCl_2$-extractable Hg concentration in soil was very low, ranged from 0.12% to 0.31% of the total Hg in soil. Mycorrhizal inoculation significantly reduced the extractable Hg concentration as well as the ratio of extractable to the total Hg in soil when Hg was added at the levels of 2.0 and 4.0 mg kg^{-1}.

Evaporated Hg detected for each treatment in a 1-day period was plotted in Fig. 1. Pots with plant growth had higher Hg evaporation (4.8±0.9 μg pot^{-1}day^{-1}) than those of plant-free control (3.1±0.6 μg pot^{-1}day^{-1}). Mycorrhizal inoculation significantly enhanced Hg evaporation from soil.

Table 2 The residual and extractable Hg concentrations in the soils and the calculated proportion of evaporated Hg after plant cultivation (mean±SE, n=3)

Hg addition (mgkg^{-1})	Plant treatment	Inoculation treatment	Residual concentration (mgkg^{-1})	Extractable concentration		Calculated proportion of evaporated Hg (%)
				μgkg^{-1}	%[b]	
0	No plant	NM	0.03±0.01	0.07±0.02	0.20±0.03	16.7±14.4
		M	0.03±0.01	0.07±0.02	0.22±0.06	16.2±12.4
	Plant	NM	0.03±0.01	0.05±0.03	0.12±0.04	18.0±15.0
		M	0.03±0.01	0.05±0.04	0.13±0.05	22.6±20.3
1	No plant	NM	0.90±0.03	2.94±0.62	0.33±0.08	13.8±2.9
		M	0.88±0.06	2.62±0.37	0.30±0.06	15.4±5.4
	Plant	NM	0.79±0.07	1.21±0.32	0.15±0.03	21.0±7.3
		M	0.73±0.08	1.16±0.3	0.16±0.03	26.1±7.6
2	No plant	NM	1.73±0.15	9.74±0.93	0.57±0.10	15.0±7.5
		M	1.70±0.10	10.00±1.00	0.59±0.09	16.7±4.9
	Plant	NM	1.51±0.11[a]	3.36±0.74[a]	0.31±0.05[a]	24.0±5.6[a]
		M	1.31±0.06	1.76±0.45	0.19±0.05	33.8±3.2
4	No plant	NM	3.60±0.17	23.33±2.89	0.65±0.10	10.9±4.3
		M	3.55±0.17	21.67±1.53	0.61±0.03	12.1±4.3
	Plant	NM	3.34±0.16[a]	4.49±1.18[a]	0.27±0.06[a]	15.8±4.1[a]
		M	2.90±0.14	2.12±0.18	0.15±0.01	27.0±3.5

NM nonmycorrhizal treatment, *M* mycorrhizal treatment

[a] Means difference at significant level of 5% between inoculation treatments in each Hg addition level

[b] Ratio of the extractable Hg to the total Hg in soil after plant harvest

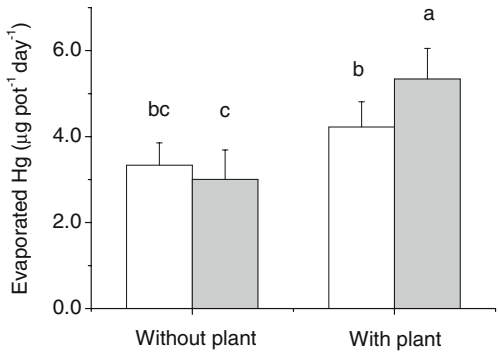

Fig. 1 Amount of evaporated Hg from soil for the treatments with and without plant growth, with (*closed bar*) and without (*open bar*) mycorrhizal inoculation. Soil was spiked with Hg at 4.0 mg kg^{-1}. Data represent mean±SE, n=3. *Means with the same letter* are not significantly different at the 5% level

Sorption of Hg on soil

In order to determine the influence of mycorrhizal inoculation on Hg immobilization in soil, sorption of Hg was investigated using the soils collected after plant harvest with or without mycorrhizal treatment, and the results are given in Fig. 2. The soil with previous mycorrhizal treatment showed a higher sorption capacity for Hg than did the soil without mycorrhizal treatment ($P<0.01$). Mercury sorption on soil can be well described by Langmuir $(C/X = C/X_m + 1/(X_m \times K))$ sorption model (Table 3). The correlation coefficients (R^2) were 0.9997 and 0.9977 for mycorrhizal and nonmycorrhizal treatments, respectively. The monolayer maximum sorption (X_m) obtained from Langmuir equation is frequently used to compare the potential sorption capacity of different soils or soil components. A higher X_m value (257.3 mg kg^{-1}) was obtained for mycorrhizal treatment compared with the nonmycorrhizal treatment (235.8 mg kg^{-1}). The physical meaning of K is usually related to the binding energy of an ion adsorbed. The greater the K value is, the more tightly the adsorbed ion is bonded. The K values were 1.99 and 1.75 for mycorrhizal and nonmycorrhizal treatments, respectively.

Table 3 Isothermal characteristics of Langmuir equation for Hg sorption on soils for nonmycorrhizal and mycorrhizal treatments

Inoculation treatment	X_m (mgkg^{-1})	K	R^2
NM	235.8±7.9	1.75±0.14	0.9977
M	257.3±2.8	1.99±0.05	0.9997

NM nonmycorrhizal treatment, *M* mycorrhizal treatment

Kinetic uptake of Hg

Figure 3 shows the kinetic uptake of Hg by mycorrhizal and nonmycorrhizal roots. The uptake of Hg by roots showed a hyperbolic increase with increasing Hg concentration in the test solution. The mycorrhizal roots took up significantly less Hg than the nonmycorrhizal roots. The uptake kinetics can be adequately described by the Michaelis–Menten function (Table 4). There was no significant difference in V_{max} values (the maximum velocity) between the mycorrhizal and nonmycorrhizal treatments, while mycorrhizal inoculation obviously increased the K_m values (Michaelis–Menten constant, which is the substrate concentration needed to achieve a half-maximum velocity of uptake).

Discussion

All interactions of Hg with soil, roots, and mycelium influence its uptake by plants. Therefore, a detailed interpretation of each individual effect is difficult. Both

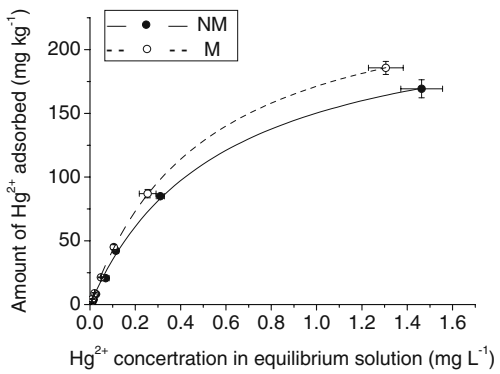

Fig. 2 Isotherms of Hg sorption on soils after cultivation of mycorrhizal (*M*) and nonmycorrhizal (*NM*) maize plants. Means and standard errors (n=3) on a dry matter basis are presented

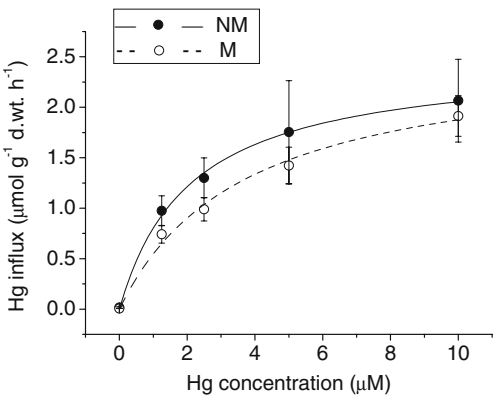

Fig. 3 Concentration-dependent kinetics for Hg uptake by roots of mycorrhizal (*M*) and nonmycorrhizal (*NM*) maize. Means and standard errors (n=3) on a dry matter basis are presented

Table 4 Kinetic parameters for Hg influx into maize roots for nonmycorrhizal and mycorrhizal treatments

Inoculation treatment	V_{max} (μmolg^{-1} d.wt.h^{-1})	K_m (μM)	R^2
NM	2.48±0.58	2.09±0.59	0.9287
M	2.58±0.39	3.68±0.67	0.8832

NM nonmycorrhizal treatment, *M* mycorrhizal treatment

positive and negative effects of mycorrhizal inoculation on metal accumulation have been observed in previous works (Chen et al. 2005; Ahmed et al. 2006). Mercury concentration was significantly lower in mycorrhizal maize roots than in nonmycorrhizal roots when Hg was applied at the rates of 2.0 and 4.0 mg kg^{-1}, which could be attributed to the decrease of bioavailable Hg in soil as the result of AM inoculation. Plant uptake should remove a part of bioavailable Hg in soil, and the removal fraction should be higher for nonmycorrhizal treatment because Hg accumulation was higher in nonmycorrhizal maize. Nevertheless, the bioavailable Hg in soil extracted by $CaCl_2$ after maize harvest was lower for mycorrhizal treatment than nonmycorrhizal treatment. Bioavailability of an element in soil is highly controlled by its interactions with soil matrixes. The lower extractable Hg in soil for mycorrhizal treatment suggested a stronger interaction of Hg with soil compared with the nonmycorrhizal treatment. A batch equilibration experiment was further conducted to exam the influence of mycorrhizal inoculation on the sorption of Hg on soil. The results indicated that mycorrhizal inoculation remarkably enhanced Hg sorption on soil. Several aspects could be expected to influence Hg sorption on soil. AM fungi can influence soil pH value and soil aggregation (Li and Christie 2001; Solaiman and Abbott 2004) which are among the key factors influencing Hg sorption/desorption on soil. In addition, the abundant extraradical mycelium produced by AM fungi has a high cation exchange capacity (up to several hundred centimoles per kilogram) and has been confirmed to have high sorption capacities for Cu, Zn, and Cd (Joner et al. 2000; Chen et al. 2001; González-Chávez et al. 2002). Mycorrhizal fungi also secrete a gooey protein known as glomalin, which can immobilize heavy metals such as Cu, Cd, Zn, Pb, and Mn in soils and decrease their availability to soil microorganisms and plants (González-Chávez et al. 2004). It can be speculated that mycelium and glomalin might also have a strong sequestering ability toward Hg. Enhanced immobilization of soil Hg by mycorrhizal inoculation is important to reduce its potential risks to transfer into the food chain and contaminate surface and ground waters.

Kinetic uptake of Hg by roots was studied and the results showed that mycorrhizal roots had a lower uptake of Hg compared with nonmycorrhizal roots. K_m in Michaelis–Menten equation approximately describes the affinity of the substrate for uptake carriers. The higher K_m value for the mycorrhizal treatment indicated that the uptake carriers of mycorrhizal roots had a lower affinity toward Hg ion compared with that of nonmycorrhizal roots. Furthermore, hyphae of AM fungi, which contain free amino, hydroxyl, carboxyl, and other groups (Smith and Read 1997), could enter inside plant cells and change cell wall components, possibly enhancing the binding of Hg on hyphae and root surface. As a result, Hg translocation into roots could be inhibited.

The primary sources of Hg in shoots include translocation of soil Hg through roots and foliar uptake from the air (Ericksen and Gustin 2004). Various studies have indicated that only a very small amount of Hg is translocated to plant shoot after root uptake and Hg in shoots mainly comes from the uptake of air Hg (Ericksen and Gustin 2004; Greger et al. 2005; Fay and Gustin 2007). In the present study, shoot Hg concentration was found much lower than that in roots and did not correlate with Hg concentration in either maize roots or soil. Furthermore, there was no significant difference in shoot Hg concentration among the treatments including Hg addition in soil and mycorrhizal inoculation. These evidences support the view that contribution of root uptake to shoot accumulation of Hg is very limited.

Supposing maize shoots took up Hg from the air, there should be Hg evaporation from soil as the source of air Hg. Furthermore, we noticed that the total Hg content in soil with maize growth was lower for mycorrhizal treatment than for nonmycorrhizal treatment, although mycorrhizal maize accumulated less Hg than nonmycorrhizal maize. Leaching of Hg from soil was avoided because there was no drain hole in the bottom of the pots. Therefore, Hg losses from soil might also contribute to its evaporation from soil to the air. Based on this hypothesis, the proportion of Hg evaporated (Q_v) was calculated by the following equation:

$$Q_v(\%) = \frac{Q_0 - Q_s - Q_p}{Q_0} \times 100\% \quad (1)$$

where, Q_0 (milligrams per pot), Q_s (milligrams per pot), and Q_p (milligrams per pot) are the amounts of Hg initially added to soil, retained in the soil after plant harvest, and accumulated in plants, respectively. Based on the evidence of extremely low Hg content in maize shoots (less than 0.07% of Hg added in soils), the contribution of leaf-to-air exchange of Hg (evaporation and/or sorption of Hg vapor) was ignored to the mass balance of Hg in soil–plant system. Mass balance calculation indicates that mycorrhizal treatment led to a markedly higher proportion of evaporated Hg than nonmycorrhizal treatment. A chamber study was further conducted to monitor the evaporated Hg. Evaporation of Hg from soil has been evidenced (Schlüter 2000). In this study,

it was observed that plants growth enhanced Hg evaporation from soil and mycorrhizal inoculation further promoted the enhancement. We speculate that the increase of Hg evaporation would contribute to the enhanced soil microbial activity since plant roots particularly inoculated roots can greatly increase soil microbial activity (Wu et al. 2008).

Mercury usually evaporates from soil to the air as the forms of organic Hg and Hg(0). A biological transformation of Hg(II) to organic Hg and/or Hg(0) can occur in the uppermost soil layer, which is the rate-limiting process of Hg evaporation from soil (Schlüter 2000). In this experiment, soils were extracted and analyzed for Hg speciation by high-performance liquid chromatography–inductively coupled argon plasma mass spectrometry following the method described by Cattani et al. (2008). Only Hg(II) was present in the soil, and neither methyl-Hg nor ethyl-Hg was detected (data not shown). It suggests that no transformation of Hg(II) to organic species occurred in soil. It has been expected that topsoil with high organic matter and high microbial activity is the major source of Hg reduction and evaporation (Schlüter 2000). Mycorrhizal fungi can secrete organic materials and regulate root exudation into soil (Jones et al. 2004), leading to a change in the quantity and composition of soil organic matter. Furthermore, mycorrhizal inoculation has been identified to result in an increase in soil microbial activity particularly in the rhizosphere soil (Wu et al. 2008; Huang et al. 2009), which can benefit the reduction of Hg(II) and Hg evaporation. Emission of Hg from soil is a significant source of Hg in the atmosphere, and comprehensive investigations are necessary to elucidate the role of mycorrhizal fungi in Hg circulation in biological environment.

To our knowledge, the present study is the first report about the effects of mycorrhizal inoculation on Hg behavior in soil–plant system. AM inoculation reduced bioavailable fraction of Hg in soil and Hg uptake by roots, thus leading to a lower accumulation in maize. In addition, evaporation of Hg from soil to atmosphere was promoted by mycorrhizal treatment. The findings of this study indicate that AM fungi play an important role in the transport and fate of Hg in the soil–plant system.

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