Glomus mosseae enhances root growth and Cu and Pb acquisition of upland rice (*Oryza sativa* L.) in contaminated soils

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Abstract A pot culture experiment was carried out to investigate the roles of *Glomus mosseae* in Cu and Pb acquisition by upland rice (*Oryza sativa* L.) and the interactions between Cu and Pb. The soil was treated with three Cu levels (0, 100 and 200 mg kg⁻¹) and three Pb levels (0, 300, and 600 mg kg⁻¹). All treatments were designed with (+M) or without (–M) *G. mosseae* inoculation in a randomized block design. The addition of Cu and Pb significantly decreased root mycorrhizal colonization. Compared with –M, +M significantly increased root biomass in almost all treatments, and also significantly increased shoot biomass in the Pb₀Cu₂₀₀, Pb₃₀₀Cu₀, and all Pb₆₀₀ treatments. AM fungi enhanced plant Cu acquisition, but decreased plant Cu concentrations with all Cu plus Pb treatments, except for shoot in the Cu₂₀₀Pb₆₀₀ treatment. Irrespective of Cu and Pb levels, +M plants had higher Pb uptakes than –M plants, but had lower root Pb and higher shoot Pb concentrations than those of –M plants. Another interpretation for the higher shoot Pb concentration in +M plants relied on Cu–Pb interactions. The study provided further evidences for the protective effects of AM fungi on upland rice against Cu and Pb contamination, and uncovered the phenomenon that Cu addition could promote Pb

uptake and Pb partitioning to shoot. The possible mechanisms by which AM fungi can alleviate the toxicity induced by Cu and Pb are also discussed.

Keywords Arbuscular mycorrhizal colonization · *Oryza sativa* L. · Metal uptake

Introduction

Due to increasing environmental problems and human health risks, some novel and effective techniques have been developed for remediation of heavy metal contamination. Arbuscular mycorrhizal fungi (AMF), a group of special microorganisms connecting the soils and plants directly, play an important role in environmental remediation (Smith and Read 1997; Christie et al. 2004; Liao et al. 2003). The mycelium of AMF can colonize the roots and form a symbiosis with the roots, termed mycorrhiza (Smith and Read 1997), which offers an effective way for the exchange of nutrient substances between AMF and the plant. The extra-radical mycelium of AMF can increase the volume of soil explored by plant roots for the uptake of mineral nutrients (Bolan 1991; Marschner et al. 1998). For low mobility nutrients such as Zn, Cu or Fe, their short supply in soil is common and the formation of mycorrhizal association can increase the uptake of these nutrients by plants (Kothari et al. 1991; Li et al. 1991; Liu et al. 2000). On the other hand, when the plant is under heavy metal stress, mycorrhizal association can enhance the resistance of plants to the metals (Leyval et al. 1997).

Evidence for intracellular sequestration of metals in some mycorrhizal fungi is largely indirect and metal-specific (Blaudez et al. 2000; Frey et al. 2000). The metal uptake greatly varied among the AMF of different species

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and distinctive geographic origins (Zhang et al. 2005). Metal uptake can also be influenced by the concentrations of these metals in soils. AMF often improved plant Cu nutrition in low-Cu conditions (Kucey and Janzen 1987; Kothari et al. 1990). It was reported that heavy metal uptake by mycorrhizal maize (*Zey mays* L.) with soils contaminated by Cd, Zn, Cu, Pb, and Mn. Zhang et al. (2006) also discussed their observation that AMF increased Cu, Zn, Pb and Cd uptake by *Vicia faba* in the roots and decreased Pb uptake in the shoots compared with non-mycorrhizal treatments. Chen et al. (2007) reported Cu uptake by four kinds of mycorrhizal plants including two native plant species, *Coreopsis drummondii* and *Pteris vittata*, together with a turf grass, *Lolium perenne* and a leguminous plant *Trifolium repens*. Vogel-Mikuš et al. (2005) reported that Zn, Cd, and Pb uptake could be changed by a mycorrhizal hyporaccumulator *Thlaspi praecox* Wulfen and found that the mycorrhizal associations decreased Cd and Zn uptake. As a comprehensive consideration, the species of plants can affect metal uptake by mycorrhizae.

Rice (*Oryza sativa* L.) is an important crop in Asia, especially in China. However, contamination of paddy soils with heavy metals is not uncommon (Lin et al. 2007) and several studies on the effects of mycorrhizal association on the uptake of heavy metals by upland rice have been performed (Zhang et al. 2005; 2009). Based on evidence for other heavy metals, mycorrhizal associations with rice could potentially reduce Cu and Pb concentrations in upland rice compared with non-mycorrhizal plants exposed to a mixture of Cu, Zn, Pb and Cd (Zhang et al. 2005). Regardless upon the results, the factors that can affect the heavy metal uptake by plants are complex, since these metals may interfere with each other. However, the role of AMF in Cu and Pb uptake by upland rice has not been precisely illuminated. Considering the above aspects, our objectives for the present study were (1) to test the effects of inoculation with AMF on the growth and Cu and Pb uptake of upland rice in soils with different Cu and Pb levels, and (2) to determine whether *G. mosseae* can be applied in changing Cu and Pb uptake by upland rice.

Materials and methods

Soil mixture

Paddy soils with the texture of Stagnic Anthrosols were sampled from the surface layer (0–20 cm) of cultivated field in Huzhou, Zhejiang province, China. The soil was sieved to pass a 2 mm mesh, autoclaved (121 °C, 2 h) to eliminate indigenous AMF, and then air-dried. Soil pH (soil:water 1:2.5) was 6.3 before sterilization. Olsen P was

extracted by NaHCO_3 (0.5 mol L^{-1}) and determined colorimetrically by the vanadomolybdate method. The soil contained 11.75 mg Olsen P kg^{-1} soil. Soil Cu and Pb concentrations were 40.0 and 45.0 mg kg^{-1} , respectively, determined by digesting the soil samples with aqua regia perchloric acid and analyzing with ICP-AES. In all treatments, basal nutrients in solution were mixed in the soil at rates of 342 mg N (NH_4NO_3), 150 mg K (K_2SO_4), 90 mg Mg (MgSO_4), and 150 mg Ca ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), kg^{-1} soil.

Mycorrhizal inoculum and host plant

The inoculum of *G. mosseae* (BGC, XJ01) from Professor Y. S. Wang (Beijing Academy of Agriculture and Forestry, China) contained dried roots of grain sorghum, hyphae and spores. Seeds of upland rice (*O. sativa* cv. 91 B 3) were surface sterilized with 10 % (v/v) peroxide (H_2O_2) for 10 min and immersed in deionized water for 24 h. They were then germinated on moist filter paper until radicals appeared and were selected for uniformity before sowing.

Experimental procedure

Three Pb addition levels of 0, 300 and 600 mg kg^{-1} (Pb_0 , Pb_{300} and Pb_{600}) were applied by mixing analytical grade $\text{Pb}(\text{NO}_3)_2$ solution thoroughly with the soil and equilibrating for 2 weeks. Three Cu application levels of 0, 100 and 200 mg kg^{-1} (Cu_0 , Cu_{100} and Cu_{200}) were designed and achieved by adding analytical grade $\text{Cu}(\text{NO}_3)_2$ solution and then equilibrating for 2 weeks. The solutions were prepared in deionized water and mixed with the soil manually. Upland rice plants were either inoculated with the AM fungus *G. mosseae* (+M) or uninoculated controls (−M) under all Cu addition and Pb application levels. Thus, there were 18 treatments in total with 3 replicates, giving a total of 54 pots in a randomized block design. Plants were grown in round plastic pots containing 420 g of soil plus 80 g of inoculum for the +M treatments, or 420 g of soil plus 80 g of inoculum which had been sterilized beforehand to eliminate the fungus for the −M treatments. The inoculum was mixed thoroughly with the soil. Three pre-germinated seeds were sown into each pot. The pots were regularly watered to weight with deionized water to maintain soil moisture at 70 % of field water holding capacity. The pots were randomly arranged in an environment-controlled growth chamber with 14 h photoperiod at 280 μmol of photons $\text{m}^{-2} \text{s}^{-1}$.

Mycorrhizal colonization and plant analysis

Plants were harvested after 8 weeks growth. Shoots were cut off, and roots were carefully washed with tap water,

and then washed with 1 mM CaCl_2 for 30 min and rinsed in deionized water. The clean roots were cut into segments about 1 cm long. A randomly selected subsample of fresh root was taken for assessment of root colonization. The subsample roots were assessed for AMF colonization according to Phillips and Hayman (1970) with some modifications. Roots were cleared in 10 % KOH at 90 °C for 30 min in a hot-water bath. Then the roots were stained with acid fuchsin at 90 °C for 30 min in a hot water bath. Percent colonization was determined by the grid intersection method (Giovannetti and Mosse 1980). Shoots and the rest of roots used for metal and P accumulation analysis were dried at 70 °C for 72 h. After the dry weights were determined, shoots and roots samples were ground to pass a 0.5-mm sieve and digested at 160 °C using concentrated nitric acid (HNO_3) for elemental analysis by ICP-AES (Andrade et al. 2004).

Statistical analysis

Results are presented as the mean $\pm$ SE. Data were calculated and analyzed statistically using the analytical tools of Microsoft Excel 2003. Multiple comparisons between treatments were carried out using SAS software.

Results

Mycorrhizal colonization and the biomass of shoot and root of upland rice

No root colonization was detected in the non-inoculated plants while the inoculated plants had relatively high colonization rates (Table 1). Mycorrhizal colonization exceeded 60 % without Cu and Pb added; and Cu and Pb addition remarkably inhibited the root colonization. A significant effect of Cu and Pb interaction on the root colonization was detected. Neither shoot nor root dry matter yield was inhibited by 300 mg kg^{-1} Pb addition, while 600 mg kg^{-1} Pb addition significantly decreased the growth of upland rice (Table 1). For all Pb levels, plant dry weight was inhibited by Cu application rates except for shoots being increased significantly by 100 mg kg^{-1} Cu application under 300 mg kg^{-1} Pb treatments (Table 1). Plant growth was generally enhanced by mycorrhizal colonization, especially the mycorrhizal roots, which showed significantly larger dry weights than uninoculated roots did under 300 and 600 mg kg^{-1} Pb treatments (Table 1). However, with a few specific exceptions, there were no significant differences in shoot dry weight between mycorrhizal and non-mycorrhizal treatments.

Cu uptake and partitioning in the shoot and root of upland rice

Cu concentration in the shoot increased with increasing soil Cu concentrations (Table 2). Compared with the zero Pb addition level, the 300 mg kg^{-1} Pb addition level did not affect the shoot Cu concentrations under Cu100 levels, while it increased the shoot Cu concentrations under Cu200 levels. For 600 mg kg^{-1} Pb application rates, the shoot Cu concentrations were enhanced significantly. In the roots (Table 2), Pb treatments decreased the Cu concentrations markedly. Compared with $-M$ treatments, mycorrhizal colonization showed different effects on Cu concentrations of upland rice plants depending on the experimental treatments (Table 2). Mycorrhizal plants showed higher shoot and root Cu concentrations under 200 mg kg^{-1} application levels when no Pb was added. The addition of 300 mg kg^{-1} and mycorrhizal colonization significantly increased the shoot Cu concentration for Cu100 while it significantly decreased the shoot and root Cu concentrations for the Cu200 level. The shoot and root Cu concentrations was decreased by mycorrhizal colonization and addition of 600 mg Pb Kg^{-1} for Cu100 addition, while Cu200 addition increased the shoot but decreased the root Cu concentrations markedly in the mycorrhizal plants. Cu uptake in the shoots and roots was enhanced with increasing soil Cu concentration (Table 2). Compared with the zero Pb addition level, Pb addition decreased root Cu uptake significantly while slightly affecting shoot Cu uptake under all Cu addition levels except for Cu100. Compared with $-M$ treatments, mycorrhizal colonization showed different effects on the Cu uptake of upland rice plants depending on the experimental treatments Table 2. Mycorrhizal plants had higher Cu uptake in the shoots and roots for Cu100 addition when zero Pb and 300 mg kg^{-1} Pb was added into the soils, however, mycorrhizal colonization decreased shoot and root Cu uptake several-fold under Cu200 treatments when the soil Pb concentration was 300 mg kg^{-1} .

Pb uptake and partitioning in the shoot and root of upland rice

Compared with the zero Pb treatments, Pb concentration in the shoots and roots increased with Pb addition in the soils under all Cu levels (Table 3). Cu addition in the soil clearly increased Pb concentration in the roots (Table 3). However, the Pb concentration in the shoots and roots under all Cu addition levels was decreased compared with that of Pb treatments without Cu. Mycorrhizal colonization generally enhanced shoot Pb concentrations, while it reduced root Pb concentrations for all the Pb levels compared with $-M$ treatments. Compared with the zero Pb treatments, Pb

Table 1 Root mycorrhizal colonization and the biomass of shoot and root of upland rice

Pb treatments (mg kg ⁻¹)	Cu treatments (mg kg ⁻¹)	Inoculation treatments	Mycorrhizal colonization (%)	Shoot biomass (g pot ⁻¹)	Root biomass (g pot ⁻¹)
0	0	−M	−	3.06 ± 0.11	0.86 ± 0.10
		+M	69.0 ± 5.0 a ^a	2.84 ± 0.04	1.04 ± 0.05
	100	−M	−	2.52 ± 0.06	0.56 ± 0.08
		+M	54.9 ± 0.8 a	2.70 ± 0.14	0.94 ± 0.09
	200	−M	−	2.53 ± 0.05	0.82 ± 0.05
		+M	35.5 ± 2.7 a	3.00 ± 0.26	1.10 ± 0.14
300	0	−M	−	3.06 ± 0.11	0.86 ± 0.10
		+M	44.6 ± 3.1 b	2.51 ± 0.15	0.56 ± 0.06
	100	−M	−	3.02 ± 0.27	0.98 ± 0.04
		+M	26.0 ± 2.4 c	3.07 ± 0.16	0.42 ± 0.04
	200	−M	−	2.91 ± 0.11	1.35 ± 0.14
		+M	29.0 ± 2.0 b	2.75 ± 0.08	0.50 ± 0.11
600	0	−M	−	2.15 ± 0.03	0.44 ± 0.01
		+M	24.6 ± 3.2 c	2.56 ± 0.03	0.83 ± 0.04
	100	−M	−	2.18 ± 0.15	0.28 ± 0.08
		+M	35.1 ± 4.5 b	2.72 ± 0.03	0.79 ± 0.06
	200	−M	−	1.87 ± 0.13	0.45 ± 0.08
		+M	15.9 ± 1.3 c	2.56 ± 0.18	0.81 ± 0.09
Significance ^b of					
Inoculation			*** ^b	**	**
Pb addition level			***	**	*
Cu addition level			***	NS	NS
Pb × inoculation			***	**	**
Cu × inoculation			***	**	**
Pb × Cu			**	**	**
Pb × Cu × inoculation			**	**	**

NS no significance

*** $p < 0.001$; ** $p < 0.01$;* $p < 0.05$ ^a In each column, means followed by the same letter are not significantly different by LSD at the 5 % level^b By analysis of variance

uptake in the shoots and roots increased with Pb addition in the soils under all the Cu levels (Table 3). Cu addition in the soil clearly reduced Pb uptake in the shoots and roots under all Cu addition levels, except for that in the shoots of Cu200 under the 300 mg kg⁻¹ Pb level, compared with Pb treatments alone. Mycorrhizal colonization generally enhanced shoot and root Pb uptake under all Pb levels compared with –M treatments.

P uptake and partitioning in the shoot and root of upland rice

Cu addition levels slightly affected the P concentration in the shoots and roots at the zero Pb addition level (Table 4), while Pb application observably decreased the root P concentration compared with the zero Pb treatments (Table 4). At the 600 mg kg⁻¹ Pb addition level, the P concentration in the shoots decreased with increasing Cu addition. Mycorrhizal colonization was associated with a large difference in P concentration in the shoots and roots

of upland rice depending on the experimental design. When no Pb was added to the soil, mycorrhizal plants showed higher concentration in the shoots, but a lower one in the roots under Cu100 and Cu200 levels compared with –M treatments. For 300 mg kg⁻¹ Pb application, mycorrhizal colonization increased the shoot P concentration of the plants at the Cu100 level, while it decreased the shoot P concentration of the plants at the Cu200 level. Cu addition levels slightly affected the P uptake in the shoots and roots at the zero Pb addition level (Table 4), while Pb application observably decreased root and shoot P uptake compared with zero Pb treatments (Table 4). Under the 300 and 600 mg kg⁻¹ Pb mg kg⁻¹ addition levels, P uptake in the roots and shoots decreased with increasing Cu addition. Mycorrhizal colonization was associated with a large difference in P uptake in the shoots and roots of upland rice depending on the experimental treatments. When there was no Pb added to the soil, mycorrhizal plants showed higher uptake in the shoots and roots for the Cu100 levels compared with –M treatments. For 300 and 600 mg kg⁻¹ Pb

Table 2 The concentrations and accumulations of Cu in the shoot and root of upland rice

Pb treatments (mg kg ^{−1})	Cu treatments (mg kg ^{−1})	Inoculation treatments	Shoot Cu concentration (mg kg ^{−1})	Shoot Cu uptake (μg pot ^{−1})	Root Cu concentration (mg kg ^{−1})	Root Cu accumulation (μg pot ^{−1})
0	0	−M	17.1 ± 1.1	52.0 ± 2.1	220.4 ± 13.4	143.9 ± 15.4
		+M	16.6 ± 1.9	44.6 ± 6.0	108.9 ± 4.3	115.0 ± 2.5
	100	−M	26.3 ± 2.6	63.5 ± 5.2	283.5 ± 1.3	124.9 ± 13.7
		+M	24.0 ± 1.0	100.2 ± 8.8	171.4 ± 13.8	140.2 ± 23.5
	200	−M	35.6 ± 6.4	66.9 ± 8.4	342.8 ± 6.4	235.2 ± 13.1
		+M	67.5 ± 5.6	193.0 ± 42.2	409.4 ± 4.0	446.4 ± 56.6
300	0	−M	15.1 ± 0.5	37.9 ± 2.8	142.4 ± 2.4	80.0 ± 7.8
		+M	20.6 ± 0.4	62.5 ± 6.4	117.2 ± 1.5	115.0 ± 6.2
	100	vM	26.4 ± 3.0	98.2 ± 7.0	197.6 ± 25.7	97.6 ± 18.5
		+M	52.2 ± 7.9	163.0 ± 17.6	182.1 ± 26.7	232.6 ± 26.5
	200	−M	194.7 ± 12.1	568.5 ± 32.6	211.7 ± 18.1	106.8 ± 26.5
		+M	55.4 ± 11.0	142.5 ± 30.7	124.0 ± 12.7	143.8 ± 25.1
600	0	−M	14.1 ± 0.5	30.3 ± 0.9	113.7 ± 1.8	50.2 ± 0.8
		+M	16.6 ± 1.9	42.5 ± 4.3	85.6 ± 1.0	71.2 ± 3.1
	100	−M	92.1 ± 3.5	202.2 ± 21.1	235.8 ± 39.5	123.0 ± 6.9
		+M	33.0 ± 5.5	71.7 ± 7.1	156.8 ± 1.9	145.9 ± 14.9
	200	−M	87.5 ± 9.9	180.9 ± 33.5	220.5 ± 27.9	84.3 ± 12.7
		+M	145.6 ± 8.4	268.8 ± 14.8	133.5 ± 30.7	124.1 ± 26.0
Significance of						
Inoculation			**	*	**	**
Pb addition level			**	**	**	**
Cu addition level			**	**	**	**
Pb × inoculation			**	**	**	**
Cu × inoculation			**	**	**	**
Pb × Cu			**	**	**	**
Pb × Cu × inoculation			**	**	**	*

NS no significance

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

application, mycorrhizal colonization increased the shoot and root P uptake of the plants at all Cu levels significantly compared with –M treatments.

Discussion

Root mycorrhizal colonization and plant biomass of upland rice

It has been suggested that mycorrhizal status is related to environmental elements, especially contamination levels in soil (Smith and Read 1997; Lins et al. 2006). Cu and Pb addition decreased the percent of root colonization in the present study and root colonization decreased with increasing soil Cu and Pb concentrations. These results

demonstrated that high levels of soil Cu and Pb inhibited the growth of AMF and then inhibited the formation of mycorrhizae (Table 1). Andrade et al. (2004) reported that the increase of soil Pb levels decreased the root colonization and the spore numbers, which is insistent with the present study. However, Andrade et al. (2010) also reported that Cu addition did not alter the growth of *Glomus etunicatum* and the formation of mycorrhizae, which is contrary to the present study. The differences in the tolerance toward Cu addition of the fungi may contribute to the contrary results. Several studies (Leyval et al. 1997; Leyval and Joner 2001) demonstrated that the tolerance of fungus separated from a contaminated environment was less affected compared with that from uncontaminated soils. In the present study, under the 200 mg kg⁻¹ Cu treatment, the root colonization was still maintained at

Table 3 The concentrations and accumulations of Pb in the shoot and root of upland rice

Pb treatments (mg kg ⁻¹)	Cu treatments (mg kg ⁻¹)	Inoculation treatments	Shoot Pb concentration (mg kg ⁻¹)	Shoot Pb uptake (μg pot ⁻¹)	Root Pb concentration (mg kg ⁻¹)	Root Pb accumulation (μg pot ⁻¹)
0	0	−M	21.4 ± 1.3	81.2 ± 3.2	51.0 ± 1.8	30.5 ± 4.4
		+M	17.5 ± 1.1	57.6 ± 4.1	72.0 ± 2.7	77.8 ± 3.2
	100	−M	16.0 ± 0.0	4.9 ± 0.0	30.8 ± 0.6	3.7 ± 0.3
		+M	18.3 ± 0.1	5.5 ± 0.0	30.1 ± 0.6	4.6 ± 0.3
	200	−M	16.7 ± 0.2	5.1 ± 0.0	29.1 ± 0.4	3.5 ± 0.6
		+M	17.2 ± 0.4	5.0 ± 0.1	29.3 ± 0.7	5.1 ± 0.3
300	0	−M	34.1 ± 2.4	65.5 ± 3.7	176.8 ± 3.7	80.5 ± 7.7
		+M	45.4 ± 1.1	122.6 ± 13.1	147.3 ± 6.1	156.3 ± 15.2
	100	−M	20.3 ± 1.6	57.0 ± 3.5	334.0 ± 19.0	38.8 ± 4.2
		+M	59.0 ± 2.0	165.2 ± 3.0	227.2 ± 4.3	121.0 ± 4.8
	200	−M	36.7 ± 0.9	113.2 ± 5.4	160.9 ± 21.0	76.0 ± 11.7
		+M	52.7 ± 1.3	145.5 ± 7.5	104.4 ± 7.5	99.5 ± 9.8
600	0	−M	80.3 ± 8.0	135.0 ± 3.2	334.0 ± 19.0	147.3 ± 7.6
		+M	51.9 ± 3.5	166.1 ± 2.0	227.2 ± 4.3	177.5 ± 14.0
	100	−M	48.6 ± 0.7	115.3 ± 13.0	261.9 ± 24.4	70.8 ± 12.3
		+M	54.0 ± 3.2	153.7 ± 12.7	221.0 ± 5.6	181.8 ± 4.8
	200	−M	44.3 ± 2.7	84.3 ± 3.6	287.1 ± 22.2	115.7 ± 23.3
		+M	74.9 ± 4.3	188.1 ± 12.0	208.1 ± 13.8	174.2 ± 25.3
Significance of						
Inoculation			**	**	**	**
Pb addition level			**	**	**	**
Cu addition level			**	**	**	**
Pb × inoculation			**	**	**	**
Cu × inoculation			**	**	**	**
Pb × Cu			**	**	**	**
Pb × Cu × inoculation			**	**	**	**

NS no significance

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

16–30 %, which indicated that *G. mosseae* had a degree of tolerance toward Cu.

It is well known that the association of plants with AMF is beneficial to their resistance of metal-induced stress, increasing plants' tolerance in metal-contaminated soils (Zhang et al. 2005, 2006, 2009; Lin et al. 2007; Khan et al. 2000). In the present study, plant growth was inhibited by Cu treatments, while on the contrary, it was enhanced by mycorrhizal colonization; especially the mycorrhizal roots, which showed significantly larger dry weights than uninoculated roots did under 300 and 600 mg kg⁻¹ Pb treatments. It was demonstrated that the formation of mycorrhizae alleviated the Cu toxicity toward the plants. Andrade et al. (2010) reported that mycorrhizae increased jack bean biomass under 450 mg kg⁻¹ Cu treatments. Chen et al. (2007) also reported

that mycorrhizae promoted the biomass of *T. repens*, *P. vittata* and *C. drummondii* planted in Cu mine tailings. A significant decrease in plant biomass was observed as Pb concentration increased in soil, suggesting that metabolic processes might be impaired by high Pb concentrations in soil. Mycorrhizae promoted the plant biomass accumulation, which also indicated that the formation of mycorrhizal association between plants and fungus was a beneficial symbiosis. The promotion of mycorrhizal plants' roots was significantly several-fold larger than that without inoculating fungus under combined Cu and Pb treatments in the present study. Many other studies showed similar results (Zhang et al. 2005, 2006; Chen et al. 2004, 2007; Wu et al. 2010). The function of mycorrhizae may attribute to the Cu, Pb uptake and P uptake.

Table 4 The concentrations and accumulations of P in the shoot and root of upland rice

Pb treatments (mg kg ^{−1})	Cu treatments (mg kg ^{−1})	Inoculation treatments	Shoot P concentration (%)	Shoot P uptake (mg pot ^{−1})	Root P concentration (%)	Root P accumulation (mg pot ^{−1})
0	0	−M	0.15 ± 0.01	4.83 ± 0.3	0.11 ± 0.01	1.01 ± 0.08
		+M	0.16 ± 0.00	4.40 ± 0.3	0.10 ± 0.00	1.04 ± 0.03
	100	−M	0.15 ± 0.01	3.68 ± 0.2	0.10 ± 0.01	0.37 ± 0.09
		+M	0.22 ± 0.00	5.72 ± 0.3	0.09 ± 0.00	0.88 ± 0.05
	200	−M	0.17 ± 0.01	4.73 ± 0.2	0.10 ± 0.00	0.88 ± 0.06
		+M	0.20 ± 0.03	4.25 ± 0.2	0.08 ± 0.00	0.86 ± 0.10
300	0	−M	0.14 ± 0.00	3.17 ± 0.1	0.08 ± 0.00	0.46 ± 0.03
		+M	0.15 ± 0.01	3.96 ± 0.2	0.10 ± 0.02	0.94 ± 0.15
	100	−M	0.11 ± 0.00	3.30 ± 0.1	0.07 ± 0.00	0.32 ± 0.04
		+M	0.13 ± 0.00	4.54 ± 0.3	0.06 ± 0.01	0.81 ± 0.06
	200	−M	0.15 ± 0.00	3.09 ± 0.4	0.07 ± 0.00	0.16 ± 0.01
		+M	0.11 ± 0.02	4.05 ± 0.2	0.07 ± 0.00	0.64 ± 0.06
600	0	−M	0.15 ± 0.00	3.17 ± 0.1	0.09 ± 0.00	0.41 ± 0.00
		+M	0.14 ± 0.00	3.76 ± 0.2	0.06 ± 0.00	0.53 ± 0.00
	100	−M	0.11 ± 0.01	2.90 ± 0.3	0.08 ± 0.01	0.30 ± 0.04
		+M	0.13 ± 0.01	3.50 ± 0.0	0.06 ± 0.00	0.57 ± 0.07
	200	−M	0.07 ± 0.01	1.16 ± 0.2	0.06 ± 0.00	0.29 ± 0.05
		+M	0.05 ± 0.01	1.18 ± 0.0	0.08 ± 0.00	0.59 ± 0.07
Significance of						
Inoculation			NS	*	**	**
Pb addition level			**	**	**	**
Cu addition level			**	**	**	**
Pb × inoculation			**	**	**	**
Cu × inoculation			**	*	*	**
Pb × Cu			**	*	**	**
Pb × Cu × inoculation			**	**	**	**

NS no significance

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Cu, Pb and P uptake by upland rice

Under adverse conditions, the supply of nutrients plays an important role in the growth of plants. Better mineral nutrition, such as P uptake and lower heavy metal uptake and transport, may be the possible mechanisms for the success of mycorrhizal plants growing in soils with excessive levels of metals (Liao et al. 2003; Weissenhorn and Leyval 1995; Zhang et al. 2005; Ouziad et al. 2005). In the present study, under low Cu and Pb treatments, a sharp increase of P uptake in mycorrhizal plants was observed along with an increase in biomass, which suggested that mycorrhiza was beneficial for upland rice in taking up P nutrition and also enhanced its capability against Cu and Pb toxicity. However, under high Cu and Pb treatments, P uptake in the mycorrhizal shoots showed no difference

from that of the nonmycorrhizal shoots. This is related to the dilution effect, because mycorrhizal association enhanced biomass while it decreased the P concentrations in the shoots.

The mechanisms of the resistance of mycorrhizal plants against heavy metals are related to the uptake of heavy metals (Wu et al. 2010; Chen et al. 2007; Wang et al. 2007; Ma et al. 2006). In the present study, under Pb and Cu additions, the concentration of Pb in mycorrhizal shoots was increased significantly compared with that of –M treatments. One part of the cause may be attributed to the dilution effect because mycorrhizae enhanced the root biomass, while the other part may be the selective uptake of elements by mycorrhizae. Reports have demonstrated that mycorrhizae can change the bioavailability of heavy metals in the soil (Leyval and Joner 2001; Joner and Leyval

1997; Joner et al. 2000) and then change the heavy metal supply to the plants. It was suggested that mycorrhizae increased the Cu uptake of clover under low Cu addition to the soil while it was inhibited for high Cu addition treatments. Zhang et al. (2006) indicated that *G. mosseae* decreased the Pb uptake of *V. faba* in soil contaminated by multiple metals. Transportation of the metals from root to shoot affected the uptake of plants. Irrespective of mycorrhizae, Pb showed a tendency to fix more Pb in the roots with increasing Pb treatments. On the contrary, Cu showed a tendency to transport more Cu in the shoots with increasing Cu treatments. The difference in Pb and Cu resulted in the diversity of uptake by upland rice.

The hyphae of AMF possessed a higher ability to absorb multiple metals. Therefore, heavy metals can be immobilized in the structure of external hyphae or internal hyphae, which then alleviates the metal toxicity to the plants (Ouziad et al. 2005; Zhang et al. 2006). However, the formation of mycorrhizae increased the Pb transportation from root to shoot, which may be attributed to the fact that Pb treatments destroyed the internal mycorrhizal structure and thus inhibited the growth of *G. mosseae*. Andrade et al. (2004) reported that the arbuscular structure decreased noticeably under 600 mg kg⁻¹Pb addition. On the other hand, the partitioning of metals in the plants is affected by multiple factors, such as the species of fungus, the variety of plants, the plants' ability of metal transportation, and the concentration of metals in the soil. There is no doubt that there is an urgent need to conduct further investigations to understand the involvement of AMF in plant interactions and encouraging concentration of metals, and to reveal the underlying mechanisms.

Conclusions

Mycorrhizal colonization exceeded 60 % when no Cu and Pb was added, however, Cu and Pb addition remarkably inhibited the root colonization as the root colonization rate decreased to around 16 %. Mycorrhizal colonization might improve the root growth markedly compared with -M treatments under some all the metal treatments. Cu and Pb showed different effects on the plant growth. Under all the metal treatments, compared with -M treatments, mycorrhizal colonization caused changes in Cu and Pb uptake. Mycorrhizal colonization increased the root P uptake compared with -M treatments under all the treatments.

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Conflict of interest The authors declare that they have no conflict of interest.

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