Increase of multi-metal tolerance of three leguminous plants by arbuscular mycorrhizal fungi colonization

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Abstract A greenhouse pot experiment was conducted to investigate the effects of the colonization of arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* on the growth and metal uptake of three leguminous plants (*Sesbania rostrata*, *Sesbania cannabina*, *Medicago sativa*) grown in multi-metal contaminated soil. AMF colonization increased the growth of the

legumes, indicating that AMF colonization increased the plant's resistance to heavy metals. It also significantly stimulated the formation of root nodules and increased the N and P uptake of all of the tested leguminous plants, which might be one of the tolerance mechanisms conferred by AMF. Compared with the control, colonization by *G. mosseae* decreased the concentration of metals, such as Cu, in the shoots of the three legumes, indicating that the decreased heavy metals uptake and growth dilution were induced by AMF treatment, thereby reducing the heavy metal toxicity to the plants. The root/shoot ratios of Cu in the three legumes and Zn in *M. sativa* were significantly increased ($P < 0.05$) with AMF colonization, indicating that heavy metals were immobilized by the mycorrhiza and the heavy metal translocations to the shoot were decreased.

Keywords *Sesbania* · *Medicago sativa* · *Glomus mosseae* · *A. caulinodans* · Heavy metals

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Introduction

Metal contamination of soils and the subsequent uptake by plants constitute a major environmental problem in some areas of China. Soil contamination by heavy metals is derived from various sources, such as atmospheric deposition, the use of sewage sludge in agricultural land, and the exploitation of mineral resources. An important part of China's sus-

tainable development strategy is the ecological restoration of contaminated soils, especially those contaminated by mine tailings (Gao et al. 1998), and re-vegetation is a key measure in this restoration (Tordoff et al. 2000; Wong 2003). However, plant establishment and growth in metal-contaminated sites are inhibited by adverse soil factors, such as acidity, poor physical structure, toxic metals, and nutrient deficiencies (Pichtel and Salt 1998; Wong 2003).

Nitrogen (N) is one of the most important nutrients for plant growth. The lack of N and inhibition of N uptake induced by heavy metals have a strong impact on plant growth. Therefore, plants that can accumulate more N may grow better in contaminated soil. Leguminous plants have advantages in N₂-fixing by formation of root nodules (Ma et al. 2006). However, their root nodules may be inhibited by heavy metals in the soil, and thus the N₂-fixing ability of leguminous plants may be hindered. *Medicago sativa* (Alfalfa) was found to grow in soils contaminated by heavy metals since it can accumulate metal concentrations above the tolerance levels of most plants (Tiemann et al. 1999). *Sesbania* species such as *Sesbania rostrata* and *Sesbania cannabina* can also grow in soil contaminated by heavy metals (Ye et al. 2001b). *S. rostrata*, native to tropical West Africa, is an annual species that bears stem as well as root nodules and is associated with *Azorhizobium caulinodans*, a specialized fast-growing strain of *Rhizobia* that can fix N₂ in its free-living form (Ye et al. 2001b). Unlike root nodules, stem nodules do not make direct contact with the soil and are therefore less susceptible to the stresses imposed on root nodules by toxic mine tailings, thus providing more N for seedling growth (Yang et al. 1997). For this reason, *S. rostrata* has been used as green manure in crop rotation systems and for the phytoremediation of metal-contaminated soils (Yang et al. 1997; Ye et al. 2001a). Chan et al. (2003) reported that *Sesbania* plants, such as *S. rostrata*, *S. sesban*, *S. cannabina*, and *S. grandiflora*, can be used in the remediation of Pb/Zn and Cu mine tailings. Yang et al. (1997) and Ye et al. (2001b) concluded that *S. rostrata* is effective in reclaiming soils contaminated with Cu, Zn, Pb, and Cd. However, their studies did not investigate possible roles of arbuscular mycorrhizal fungi (AMF). AMF can enhance plants' tolerance to metal and are often used in the restoration of

metal-contaminated sites (Liu et al. 2005; Vogel-Mikuš et al. 2005). A number of studies have indicated that mycorrhizas can assist pioneer plant species to colonize metal-contaminated sites by improving the plants' phosphorus (P) uptake and subsequently enhancing their growth (Smith and Read 1997; Karandashov and Bucher 2005; Vogel-Mikuš et al. 2005).

It was reported that P is a critical element in forming nodules (Toro et al. 1998). Therefore, the study was aimed to investigate whether AMF colonization could enhance the formation of nodules, increase P and N uptake, and increase multi-metal tolerance of leguminous plants (including *S. rostrata*, *S. cannabina*, and *M. sativa*).

Materials and methods

Growth medium

The field soil (paddy soil) used in the present study was collected from Fuyang, Zhejiang Province, China. The soil was sampled from the surface layer (0–20 cm) of cultivated fields that were contaminated by wastewater from local smelting factories containing high concentrations of Cd, Cu, Zn, and Pb. The soil was sieved to pass through a 2-mm mesh, autoclaved (121°C, 2 h) to eliminate indigenous AMF, and air-dried. The soil pH (soil:water = 1:2.5) was 6.3 before sterilization. The soil was digested with conc. HNO₃ and conc. HCl (1:3, v/v) at 160°C for 48 h, and the concentrations of Cu, Zn, Pb, Cd, and P in the solutions were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Cui et al. 2004). A standard soil material (NIST 2711 Montana Soil) was used to verify the acid digestion method used in this study. The recovery for all trace metals in the standard material was within 100 ± 10%. Bioavailable fractions of Cu, Zn, Pb, and Cd in soils were extracted using a solution containing 0.05 mol l⁻¹ DTPA (diethylene triamine pentaacetic acid), 0.01 mol l⁻¹ CaCl₂, and 0.1 mol l⁻¹ TEA (triethanolamine) at pH 7.30 (Gupta and Sinha 2006) and were determined by ICP-AES. Plant available P was extracted by 0.5 mol l⁻¹ NaHCO₃ solution and determined colorimetrically by the vanadomolybdate method. In all treatments, basal nutrients in solution were mixed with the soil. The nutrients were added to

the soil at rates of 684 mg N (NH_4NO_3), 300 mg K (K_2SO_4), 180 mg Mg (MgSO_4), and 300 mg Ca (CaCl_2) kg^{-1} .

AMF isolates

The leguminous plants were inoculated with *Glomus mosseae* (BGC, XJ01) isolates (+AMF treatment) or left uninoculated (–AMF treatment). The inoculum of *G. mosseae*, obtained from the Beijing Academy of Agriculture and Forestry, China, contained dried roots of grain sorghum, hyphae, and spores.

Seeds of *S. rostrata*, *S. cannabina*, *M. sativa* and their inoculants

Strains of *A. caulinodans* (ACCC 19502) and seeds of *M. sativa* and its inoculant (*Rhizobium meliloti*) were obtained from the Chinese Academy of Agricultural Sciences (CAAS). Seeds of *S. rostrata* were purchased from AgroForestor (UK). Seeds of *S. cannabina* were collected from Zhengzhou, Henan Province, China, and its inoculant (*Rhizobium* spp.) was obtained from CAAS.

Plant growth conditions

Seeds of *Sesbania rostrata*, *Sesbania cannabina*, and *Medicago sativa* were surface-sterilized with 10% (v/v) H_2O_2 for 10 min and washed with tap water and then deionized water. After being immersed in deionized water for 24 h, the seeds were allowed to germinate on moist filter paper. When the radicles appeared, uniform seedlings were selected for the experiment. The seedlings were grown in 500 g plastic pots containing 460 g of soil plus 40 g of inoculum for the +AMF treatment, or 460 g of soil plus 40 g of sterilized inoculum for the –AMF treatment. The AMF inocula were fully mixed with the soil. The plastic pots of both treatments of +AMF and –AMF for *Sesbania rostrata*, *Sesbania cannabina*, and *Medicago sativa* were inoculated with *A. caulinodans*, *Rhizobium* spp., and *Rhizobium meliloti*, respectively. The soil moisture was maintained at 70% of field water holding capacity by adding appropriate amounts of deionized water. Each treatment had four replicates. The containers were arranged using a random block design in a temperature-controlled ($20 \pm 5^\circ\text{C}$) growth chamber for 6 weeks with a 14 h

photoperiod at $280 \mu\text{mol m}^{-2} \text{s}^{-1}$ and relative humidity of 70%.

Plant harvest

The plants were harvested after 6 weeks of growth. After the shoots were cut off, the roots were carefully washed free of soil with tap water, fully rinsed in deionized water, and then washed with 1 mmol l^{-1} CaCl_2 solution for 30 min to remove all the particles that had adhered to the root surface and to desorb metals associated with cell walls. The root nodules were visually counted.

Root colonization

The clean roots were cut into segments of about 1 cm in length. A randomly selected fresh root subsample of about 1 g was taken for the assessment of root colonization. The roots were cleaned in 10% KOH, stained with 0.05% trypan blue (Phillips and Hayman 1970). The percent colonization was then determined by the grid intersect method (Giovannetti and Mosse 1980).

Plant biomass determination and HMs analysis

After being dried at 70°C for 72 h, the dry weights of the shoots and roots were determined, and the samples were ground with a stainless steel mill. The plant materials were then digested using conc. $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ with an open-vessel method at 300°C , and the concentrations of Cu, Zn, Pb, Cd, and P were determined by ICP-AES, while the N concentration was determined by the Kjeldahl nitrogen method (Lowther 1980). A standard plant material (GBW07602) from the Department of Earth and Mines of China was used to verify the accuracy of metal determination. The recovery rates for all heavy metals (Cu, Zn, Pb, Cd), N, and P were within $90 \pm 10\%$.

Data analysis

Results are presented as mean $\pm$ SE, and all data were subjected to multi-comparisons by the least significant difference (LSD) at the 5% level using GenStat for PC/Windows 6.1 GenStat (Payne 2002).

Table 1 Root colonization (%) and nodule numbers of three leguminous plants (mean $\pm$ SE, $n = 4$)

Plants	Root colonization		Nodule numbers	
	–AMF	+AMF	–AMF	+AMF
<i>M. sativa</i>	0 c	35 $\pm$ 2 b	0 d	1 $\pm$ 2 bc
<i>S. rostrata</i>	0 c	66 $\pm$ 2 a	2 $\pm$ 1 c	6 $\pm$ 2 b
<i>S. cannabina</i>	0 c	42 $\pm$ 5 b	0 d	12 $\pm$ 1 a

–AMF and +AMF represent uninoculated treatment and inoculation with *Glomus mosseae*, respectively. Different letters in the same group (root colonization or nodule numbers) indicate significant differences between inoculation treatments and uninoculated treatment by LSD multi-comparison at the 5% level

Results

Concentrations of heavy metals in the soil

The concentrations of Cu, Zn, Pb, and Cd in the soil were 1,481, 2,286, 388, and 1.6 mg kg^{–1}, respectively, and 150, 93, 45 mg kg^{–1} of Cu, Zn, and Pb in the soil was bioavailable (DTPA-extractable), indicating that the soil was highly contaminated by these metals.

Root colonization and root nodule number of different leguminous plants

No root colonization by *G. mosseae* was detected in the –AMF treatments, and under the +AMF treatments *S. rostrata* had the highest root colonization among the three legumes ($P < 0.05$) (Table 1). Root nodules were only detected in *S. rostrata* under the –AMF treatments. The +AMF treatments significantly enhanced the development of root nodules compared with the –AMF treatments ($P < 0.05$), and *S. rostrata* developed more nodules than the other leguminous plants (Table 1).

Plant biomass

Compared with the –AMF treatments, the +AMF treatments significantly enhanced the shoot and root biomass of all plants ($P < 0.05$). The shoot and root biomasses of *S. rostrata* were the highest among the three plants irrespective of the inoculation by *G. mosseae* (Fig. 1).

Fig. 1 The effects of AMF on shoot and root dry weights of three leguminous plants with and without AMF. –AMF and +AMF represent uninoculated treatment and inoculation treatment with *Glomus mosseae*, respectively. By analysis of variance, dry weights and inoculation were significant ($P < 0.05$) for both shoot and roots of all plants. The letters above the columns indicate significant differences between inoculation and uninoculated treatments by LSD multi-comparison at the 5% level

Concentrations of N and P in plant tissues

The concentrations of N and P in the plants after 6 weeks varied among plant species and treatments (Table 2). In the –AMF and +AMF plants, the N concentrations in both shoots and roots increased in the following order: *S. cannabina* < *S. rostrata* < *M. sativa*. In the –AMF plants, the P concentrations in the roots increased in the same order, but the P concentrations in the shoots of *S. rostrata* were higher than those in *M. sativa* and *S. cannabina* ($P < 0.05$). Compared with the –AMF treatments, colonization by *G. mosseae* significantly decreased the N concentrations in the shoots and the P concentrations in the roots of *M. sativa*. The concentrations of N and P in the shoots and roots of the *Sesbania* plants were increased by the colonization of AMF ($P < 0.05$).

Total N and P uptakes by three leguminous plants

Both *Sesbania* species showed higher capacity for N and P uptakes than *M. sativa* (Table 2), which was largely due to the fact that the biomasses of the *Sesbania* plants were larger than those of *M. sativa*. The N uptakes in the shoots and roots of *S. rostrata* were higher than those of *S. cannabina* ($P < 0.05$). The

Table 2 The effects of AMF on the concentrations (mg kg^{-1}) and uptake (mg pot^{-1}) of N and P in three leguminous plants (mean $\pm$ SE, $n = 4$)

Plant species		Treatment	Nutrient concentration		Nutrient uptake	
			N	P	N	P
Shoot	<i>M. sativa</i>	–AMF	4.06 ± 0.16 a	1,215.4 ± 23.4 g	14.07 ± 0.64 f	497.5 ± 94.4 g
		+AMF	3.26 ± 0.08 b	1,081.0 ± 37.3 g	35.06 ± 4.23 d	1,338.8 ± 119.1 e
	<i>S. rostrata</i>	–AMF	1.76 ± 0.06 e	1,297.1 ± 2.6 f	62.80 ± 1.48 b	4,415.6 ± 233.5 b
		+AMF	2.54 ± 0.06 c	2,494.5 ± 51.0 b	108.96 ± 1.04 a	5,865.0 ± 355.6 a
	<i>S. cannabina</i>	–AMF	1.40 ± 0.04 f	1,139.8 ± 15.1 g	37.40 ± 0.92 d	2,989.1 ± 166.6 c
		+AMF	1.68 ± 0.07 e	1,350.6 ± 98.3 ef	58.09 ± 0.87 c	4,803.8 ± 374.9 b
Root	<i>M. sativa</i>	–AMF	2.84 ± 0.36 b	3,238.4 ± 40.6 a	3.84 ± 0.42 h	421.4 ± 46.1 g
		+AMF	3.16 ± 0.26 b	2,152.8 ± 26.8 c	13.21 ± 1.32 f	898.8 ± 39.5 f
	<i>S. rostrata</i>	–AMF	1.34 ± 0.06 g	2,161.0 ± 24.5 c	12.49 ± 0.72 f	1,237.1 ± 62.2 e
		+AMF	2.07 ± 0.04 c	2,618.1 ± 74.2 a	26.75 ± 1.13 e	1,727.7 ± 91.3 d
	<i>S. cannabina</i>	–AMF	1.07 ± 0.07 h	1,563.5 ± 104.1 e	7.50 ± 0.50 g	967.8 ± 117.9 f
		+AMF	1.33 ± 0.02 g	2,000.2 ± 59.8 d	12.83 ± 0.14 f	1,647.3 ± 189.4 de

–AMF and +AMF represent uninoculated treatment and inoculation with *Glomus mosseae*, respectively. Different letters in the same column indicate significant differences between inoculation treatments and uninoculated treatment by LSD multi-comparison at the 5% level

colonization by *G. mosseae* significantly increased N and P uptakes of the roots and shoots of the three plants ($P < 0.05$).

Concentrations of Cu, Zn, Pb, and Cd in the plant tissues

The three leguminous plants showed significant differences in heavy metal concentrations in their tissue (Table 3). The concentrations of Cu, Zn, Pb, and Cd in the roots were significantly higher ($P < 0.05$) than those in the shoots of all three plants. In the –AMF plants, the concentrations of Cu and Cd in the shoots of *S. rostrata* were significantly higher ($P < 0.05$) than those of *S. cannabina* and *M. sativa*. The Zn concentrations in both *Sesbania* plants were significantly higher than those of *M. sativa* ($P < 0.05$), and the Pb concentrations in the shoots of *S. cannabina* were significantly higher than those of *S. rostrata* and *M. sativa* ($P < 0.05$). Compared with the –AMF treatments, colonization by *G. mosseae* decreased the Cu concentrations in the shoots of all species tested and the Pb concentrations in the shoots of *M. sativa* and *S. cannabina* ($P < 0.05$), but increased the Cd concentrations in the shoots of *M. sativa* and *S. cannabina* ($P < 0.05$). Compared with the –AMF treatments, colonization by *G. mosseae* significantly increased the Cu and Zn concentrations in the roots of *S. cannabina*

and *M. sativa*; however, it decreased the Cu and Zn concentrations in the roots of *S. rostrata* ($P < 0.05$). The +AMF treatments decreased the Pb and Cd concentrations in the roots of *M. sativa* compared with those of the –AMF treatments ($P < 0.05$), while the Cu and Zn concentrations in the roots of *S. rostrata* were significantly increased ($P < 0.05$).

Cu, Zn, Pb, and Cd uptake by the leguminous plants

Under both the –AMF and +AMF treatments, the accumulation of Cu, Zn, Pb, and Cd in the shoots of both *Sesbania* plants was significantly higher than that in the shoots of *M. sativa* ($P < 0.05$) (Table 4). Under the –AMF treatments, the Cu accumulation in the shoots of *S. rostrata* was higher than that in the shoots of *S. cannabina*, while the Pb accumulation in the shoots of *S. rostrata* was less than that in the shoots of *S. cannabina* ($P < 0.05$). The AMF colonization increased the uptakes of Cu, Zn, Pb, and Cd in the roots of the three plants except for Cu in *S. rostrata* and Pb in *S. cannabina* ($P < 0.05$) compared with –AMF treatments (Table 4).

Root/shoot ratios of Cu, Zn, Pb, and Cd in plants

There were significant differences in the root/shoot ratios of Cu, Zn, Pb, and Cd in the three leguminous

Table 3 The effects of AMF on the concentrations (mg kg⁻¹) of Cu, Zn, Pb, and Cd in three leguminous plants (mean ± SE, *n* = 4)

Plant species		Treatment	Cu	Zn	Cd	Pb
<i>M. sativa</i>	Shoot	–AMF	21.7 ± 2.5 h	64.4 ± 9.1 g	5.0 ± 0.1 h	7.9 ± 0.1 f
		+AMF	9.8 ± 0.2 j	78.1 ± 3.9 g	5.4 ± 0.2 g	7.3 ± 0.1 g
	Root	–AMF	247 ± 5.8 d	394 ± 51.0 b	26.1 ± 2.5 a	95.7 ± 6.5 a
		+AMF	785 ± 9.1 a	717 ± 51.4 a	19.7 ± 2.6 b	62.4 ± 1.9 b
<i>S. rostrata</i>	Shoot	–AMF	43.6 ± 1.5 g	108.2 ± 7.3 d	7.2 ± 0.2 f	7.0 ± 0.1 h
		+AMF	24.6 ± 0.7 h	93.4 ± 2.4 f	7.2 ± 0.1 f	7.4 ± 0.1 g
	Root	–AMF	377 ± 5.6 b	418 ± 1.7 b	13.1 ± 0.3 d	36.4 ± 0.5 d
		+AMF	307 ± 3.1 c	351 ± 3.1 c	14.5 ± 0.6 c	41.4 ± 0.3 c
<i>S. cannabina</i>	Shoot	–AMF	24.8 ± 1.7 h	105 ± 3.1 d	5.7 ± 0.1 g	9.1 ± 0.8 f
		+AMF	12.5 ± 0.7 i	104 ± 2.2 f	6.7 ± 0.5 f	7.5 ± 0.1 g
	Root	–AMF	124 ± 4.0 f	189 ± 0.8 d	9.2 ± 0.1 e	22.2 ± 0.1 e
		+AMF	213 ± 7.6 e	229 ± 19.9 d	11.9 ± 1.3 d	20.8 ± 0.5 e

–AMF and +AMF represent uninoculated treatment and inoculation with *Glomus mosseae*, respectively. Different letters in the same column indicate significant differences between inoculation treatments and uninoculated treatment by LSD multi-comparison at the 5% level

Table 4 The effects of AMF on uptake (μg pot⁻¹) of Cu, Zn, Pb, and Cd by three leguminous plants (mean ± SE, *n* = 4)

Plant species		Treatment	Cu	Zn	Cd	Pb
<i>M. sativa</i>	Shoot	–AMF	7.4 ± 0.6 i	28.0 ± 3.2 j	2.1 ± 0.3 i	3.1 ± 0.5 g
		+AMF	10.5 ± 1.1 h	73.3 ± 9.1 h	5.2 ± 0.6 g	8.7 ± 0.8 f
	Root	–AMF	39.6 ± 11.4 g	45.5 ± 6.0 i	3.3 ± 0.4 h	10.8 ± 1.0 e
		+AMF	339 ± 56.2 a	309 ± 47.1 bc	10.9 ± 0.8 ce	31.8 ± 5.3 a
<i>S. rostrata</i>	Shoot	–AMF	156.2 ± 7.9 c	284 ± 31.2 cd	16.9 ± 0.8 b	17.0 ± 1.5 c
		+AMF	105.3 ± 1.7 d	311 ± 8.6 bd	22.0 ± 1.5 a	23.4 ± 1.3 b
	Root	–AMF	353 ± 24.0 a	161 ± 8.5 f	5.8 ± 0.4 g	17.4 ± 1.1 c
		+AMF	399 ± 21.3 a	209 ± 6.9 e	8.9 ± 0.3 d	25.8 ± 0.4 ab
<i>S. cannabina</i>	Shoot	–AMF	67.0 ± 6.7 f	285 ± 1.3 c	15.5 ± 0.3 b	28.6 ± 0.8 a
		+AMF	43.2 ± 1.7 g	373 ± 19.4 a	23.1 ± 1.7 a	24.4 ± 0.7 b
	Root	–AMF	87.8 ± 7.7 e	121 ± 11.4 g	6.8 ± 0.1 f	16.4 ± 0.1 d
		+AMF	205 ± 10.1 b	174 ± 20.7 f	8.8 ± 1.4 de	13.9 ± 1.6 e

–AMF and +AMF represent uninoculated treatment and inoculation with *Glomus mosseae*, respectively. Different letters in the same column indicate significant differences between inoculation treatments and uninoculated treatment by LSD multi-comparison at the 5% level

plants (Table 5). Under both treatments (+AMF and –AMF), *M. sativa* translocated less Cu, Zn, Pb, and Cd than *S. rostrata* and *S. cannabina* ($P < 0.05$). Compared with the –AMF treatments, the root/shoot ratios of Cu and Zn in *M. sativa* were significantly increased by the colonization of *G. mosseae* ($P < 0.05$). The +AMF treatments increased the root/shoot ratios of Cu in both *Sesbania* plants ($P < 0.05$), and no significant differences in the root/shoot ratios of Zn, Pb, and Cd were found between the +AMF and –AMF treatments.

Discussion

Effects of AMF colonization on nutrient accumulation by different species of legumes

Under +AMF and –AMF treatments, the biomass of *S. rostrata* was significantly higher than that of *S. cannabina* and *M. sativa*, which might be attributed to the higher capacity of N accumulation. On one hand, the capacity for N₂-fixation of *S. rostrata* was higher and the stem nodules might be efficient in N₂-fixation

Table 5 Root/shoot ratios of Cu, Zn, Pb and Cd in the three leguminous plants (mean $\pm$ SE, $n = 4$)

Plants	Treatments	Cu	Zn	Pb	Cd
<i>M. sativa</i>	–AMF	5.2 $\pm$ 1.2 b	1.7 $\pm$ 0.3 b	3.6 $\pm$ 0.5 a	1.6 $\pm$ 0.2 a
	+AMF	31.9 $\pm$ 2.4 a	4.4 $\pm$ 1.0 a	3.6 $\pm$ 0.3 a	2.2 $\pm$ 0.3 a
<i>S. rostrata</i>	–AMF	2.3 $\pm$ 0.3 d	0.6 $\pm$ 0.0 c	1.0 $\pm$ 0.1 b	0.3 $\pm$ 0.0 b
	+AMF	3.8 $\pm$ 0.2 c	0.7 $\pm$ 0.0 c	1.1 $\pm$ 0.1 b	0.4 $\pm$ 0.0 b
<i>S. cannabina</i>	–AMF	1.3 $\pm$ 0.1 d	0.4 $\pm$ 0.0 c	0.6 $\pm$ 0.0 c	0.4 $\pm$ 0.0 b
	+AMF	4.8 $\pm$ 0.2 bc	0.5 $\pm$ 0.1 c	0.6 $\pm$ 0.1 c	0.4 $\pm$ 0.1 b

–AMF and +AMF represent uninoculated treatment and inoculation with *Glomus mosseae*, respectively. Different letters in the same column indicate significant differences between inoculation treatments and uninoculated treatment by LSD multi-comparison at the 5% level

(Ye et al. 2001b; Chan et al. 2003; Ma et al. 2006). On the other hand, the soil was contaminated by heavy metals, which could inhibit the development of root nodules directly exposed to the soil, making the stem nodules more important. This is similar to previous results reported by Ye et al. (2001b) who reported that stem nodules significantly increased N accumulation, especially under stress from metal contamination.

The growth of all of the plants in the present study was increased by AMF colonization, indicating that AMF colonization increased the plants' tolerance to metals. In addition to the increased P accumulation, AMF colonization stimulated the formation of root nodules, especially in *S. rostrata* and *S. cannabina*, indicating that better P nutrition in *S. rostrata* could also contribute to better N nutrition by stimulating the development of root nodules of *S. rostrata* (Toro et al. 1998). The higher numbers of root nodules under the +AMF treatments led to higher N accumulation in the plants compared with the –AMF treatments. Andrade et al. (2004) also reported that inoculation by *Glomus macrocarpum* increased root nodule numbers of soybean plants, which then attributed to better P nutrition in mycorrhizal plants. P is a key element in forming root nodules, and therefore the AMF colonization is beneficial to the development of root nodules (Barea et al. 2005). It was also reported by Chen et al. (1999) that AMF could increase N accumulation in mycorrhizal plants because of higher nitrogenase-fixation activity in mycorrhizal plants and consequently increased efficiency of N_2 -fixation.

Effects of AMF colonization on accumulation of Cu, Zn, Pb, and Cd by different species of legumes

Not only were the legumes nutrient uptakes affected by the AMF colonization, but their metal accumula-

tions were also affected, which changed the plants' tolerance to metal contamination. In addition to the better nutrient uptake, Ibekwe et al. (1995) suggested that the tolerance of *S. rostrata* to heavy metals was significantly enhanced via development of a symbiotic relationship between the roots and rhizobium since rhizobium showed less sensitivity to Zn and Cd than plants. In the present experiment, the biomasses of the three legumes and root nodules developed were increased by AMF colonization, possibly due to the fact that the microbial cooperation in the rhizosphere could increase the resistance of the host plant to the toxicity of heavy metals. Rhizobium infected the cells and adsorbed heavy metals, decreasing the influx of heavy metals into protoplasts of roots and thereby enhancing the tolerance of the host plant to metals (Kotrba et al. 1999).

Heavy metal accumulation was different among the legume species, and inoculation by *G. mosseae* yielded different effects on the accumulations of Cu, Zn, Pb, and Cd by the three legumes in the present study. Compared with the –AMF treatment, the +AMF treatments significantly increased the total metal accumulation in the shoots of *S. rostrata* except for Cu, but no significant increases in the concentrations of the heavy metals except for Pb were found. Compared with the –AMF treatments, +AMF significantly enhanced the biomass of *M. sativa*, indicating that AMF could also decrease toxicity of heavy metals in *M. sativa* by dilution. In contrast, AMF decreased the Cu accumulation in *S. rostrata* as well as the Cu concentrations, indicating that AMF could alleviate the Cu toxicity to *S. rostrata* in a different way from that of Pb and Cd by reducing the Cu uptake. Therefore, in this study, the mechanisms by which AMF improved the resistance of the plants to different metals were different.

It was reported that AMF could immobilize heavy metals in special parts of the roots or in the hyphal structure to reduce the toxicity of metal to plants (Zhang et al. 2005). Compared with the –AMF treatments, the root/shoot ratios of Cu in the three legumes and root/shoot ratios of Zn in *M. sativa* were significantly increased under the +AMF treatments, indicating that compartmentation was also one of the resistance mechanisms of *M. sativa* to heavy metals. According to Andrade (2002), acidic polysaccharides found in the cell wall of algae have been associated with the capacity of these organisms to accumulate heavy metals when exposed to high concentrations of heavy metals, indicating that cell walls play an important role in alleviating metal toxicity. AMF might change the components of root cell walls, thus increasing the adsorption capacity to metals, such as Cu (data not shown). The mechanisms to resist heavy metals induced by the same AMF were different because of the different plant species and different metals.

Conclusion

We found that the interaction between AMF and rhizobium increased plant growth in soil contaminated by heavy metals by increasing both the plant nutrient uptake and tolerance to multi-metals. AMF colonization enabled the plants to accumulate more P, consequently stimulating root nodules and increasing N accumulation. Besides plant nutrient improvement, AMF increased plant tolerance to metals via such mechanisms as dilution effects, increased tolerance to metals, decreased metal uptake, and translocation from root to shoot. These mechanisms could be utilized in phytoremediation or ecological restoration.

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References

- Andrade, M. R., Farina, M., & Amado Filho, G. M. (2002). Role of *Padina gymnospora* (Dictyotales, Phaeophyceae) call walls in cadmium accumulation. *Phycologia*, 41, 39–48.
- Andrade, S. A. L., Abreu, C. A., de Abreu, M. F., & Silveira, A. P. D. (2004). Influence of lead additions on arbuscular mycorrhiza and rhizobium symbioses under soybean plants. *Applied Soil Ecology*, 26, 123–131.
- Barea, J. M., Pozo, M. J., Azcón, R., & Azcón A. (2005). Microbial co-operation in the rhizosphere. *Journal of Experimental Botany*, 56, 1761–1778.
- Chan, Y. S. G., Ye, Z. H., & Wong, M. H. (2003). Comparison of four *Sesbania* species to remediate Pb/Zn and Cu mine tailings. *Environmental Management*, 32, 246–251.
- Chen, W., Bruhlmann, F., Richias, R. D., & Mulchandani, A. (1999). Engineering of improved microbes and enzymes for bioremediation. *Current Opinion in Biotechnology*, 10, 137–141.
- Cui, Y. J., Zhu, Y.-G., Zhai, R. H., Chen, D. Y., Huang, Y. Z., Qiu, Y., & Liang, J. Z. (2004). Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. *Environment International*, 30, 785–791.
- Gao, L., Miao, Z., Bai, Z., Zhou, X., Zhao, J., & Zhu, Y. (1998). A case study of ecological restoration at the Xiaoyi Bauxite Mine, Shanxi Province, China. *Ecological Engineering*, 11, 221–229.
- Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist*, 84, 489–500.
- Gupta, A. K., & Sinha, S. (2006). Chemical fractionation and heavy metal accumulation in the plant of *Sesamum indicum* (L.) var. T55 grown on soil amended with tannery sludge: Selection of single extractants. *Chemosphere*, 64, 161–173.
- Ibekwe, A. M., Angle, J. S., Chaney, R. L., & Van-Berkum, P. (1995). Sewage sludge and heavy metal effects on nodulation and nitrogen fixation of legumes. *Journal of Environmental Quality*, 24, 1199–1204.
- Karandashov, V., & Bucher, M. (2005). Symbiotic phosphate transport in arbuscular mycorrhizas. *Trends in Plant Science*, 10, 22–29.
- Kotrba, P., Doleckova, L., Lorenzo, V., & Rumi, T. (1999). Enhanced bioaccumulation of heavy metal ions by bacterial cells due to surface display of short metal binding peptides. *Applied and Environmental Microbiology*, 65, 1092–1098.
- Liu, Y., Zhu, Y.-G., Chen, B. D., Christie, P., & Li, X. L. (2005). Influence of the arbuscular mycorrhizal fungus *Glomus mosseae* on uptake of arsenate by the As hyperaccumulator fern *Pteris vittata* L. *Mycorrhiza*, 15, 187–192.
- Lowther, J. R. (1980). Use of a single sulfuric acid–hydrogen peroxide digest for the analysis of *Pinus radiata* needles. *Communications in Soil Science and Plant Analysis*, 11, 175–188.
- Ma, Y., Dickinson, N. M., & Wong, M. H. (2006). Beneficial effects of earthworms and arbuscular mycorrhizal fungi on establishment of leguminous trees on Pb/Zn mine tailings. *Soil Biology & Biochemistry*, 38, 1403–1412.
- Payne, R. W., (Ed.), (2002). *The guide to GenStat Release 6.1—Part 1: Syntax and data management*. GenStat Committee. Hemel Hempstead, UK: VSN International.
- Phillips, J. M., & Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of British Mycological Society*, 55, 158–160.

- Pichtel, J., & Salt, C. A. (1998). Vegetative growth and trace metal accumulation on metalliferous wastes. *Journal of Environmental Quality*, 27, 618–642.
- Smith, S. E., & Read, D. J. (1997). *Mycorrhizal Symbiosis*. London: Academic Press.
- Tiemann, K. J., Gardea-Torresdey, J. L., Gamez, G., Dokken, K., Sias, S., Renner, M. W., Furenlid, L. R. (1999). Use of X-ray absorption spectroscopy and esterification to investigate Cr(III) and Ni(II) ligands in Alfalfa biomass. *Environmental Science & Technology*, 33, 150–154.
- Tordoff, G. M., Baker, A. J. M., & Willis, A. J. (2000). Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere*, 41, 219–228.
- Toro, M., Azcón, R., & Barea, J. M. (1998). The use of isotopic dilution techniques to evaluate the interactive effects of Rhizobium genotype, mycorrhiza fungi, phosphate-solubilizing Rhizobacteria and rock phosphate on nitrogen and phosphorus acquisition by *Medicago sativa*. *New Phytologist*, 138, 265–273.
- Vogel-Mikuš, K., Drobne, D., & Regvar, M. (2005). Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. *Environmental Pollution*, 133, 233–242.
- Wong, M. H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere*, 50, 775–780.
- Yang, Z. Y., Yuan, J. G., Xin, G. R., Chang, H. T., & Wong, M. H. (1997). Germination, growth and nodulation of *Sesbania rostrata* grown in Pb/Zn tailings. *Environmental Management*, 21, 1–6.
- Ye, Z. H., Wong J. W. C., Wong, M. H., Lan, C. Y., & Baker, A. J. M. (2001a). Lime and pig manure as ameliorants for the revegetation on lead/zinc mine tailings, a greenhouse study. *Bioresource Technology*, 69, 35–45.
- Ye, Z. H., Yang, Z. Y., Chan, G. Y. S., & Wong, M. H. (2001b). Growth response of *Sesbania rostrata* and *S. cannabina* to sludge-amended lead/zinc mine tailings. *Environment International*, 26, 449–455.
- Zhang, X. H., Zhu Y.-G., Chen, B. D., Lin, A. J., Smith, S. E., & Smith, A. E. (2005). Arbuscular mycorrhizal fungi contribute to the resistance of upland rice to combined metal contamination of soil. *Journal of Plant Nutrition*, 28, 2065–2077.