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# The Role of Mycorrhizae Associated with Vetiver Grown in Pb-/Zn-Contaminated Soils: Greenhouse Study

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## Abstract

The effects of mycorrhizae on growth and uptake of N, P, Zn, and Pb by plants were investigated in a greenhouse trial using vetiver grass (*Vetiveria zizanioides*) as host. Inoculation of the host plants with arbuscular mycorrhizal fungi (AMF), *Glomus mosseae* and *G. intraradices* spores, significantly increased the growth and P uptake. Mycorrhizal colonization increased Pb and Zn uptake by plants under low soil metal concentrations (at 0 and 10 mg/kg of Pb or Zn), whereas under higher concentrations (at 100 and 1,000 mg/kg of Pb or Zn), it decreased Pb and Zn uptake. P concentration in soil was

negatively correlated with mycorrhizal colonization as well as Zn or Pb concentrations. The results showed that inoculation of the host plants with AMF protects them from the potential toxicity caused by increased uptake of Pb and Zn, but the degree of protection varied according to the fungus and host plant combination. The potential of arbuscular mycorrhizae in phytoremediation of the Zn- or the Pb-contaminated soils is discussed in this article.

**Key words:** arbuscular mycorrhiza, lead, nitrogen, phosphorus, vetiver grass, zinc.

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## Introduction

Phytoremediation technology is emerging as a promising environmentally friendly method for large-scale cleanup of contaminated waters and soil (Brooks 1998; Chaudhry et al. 1998). It is important to select an appropriate pioneer plant species for successful site reclamation and in phytoremediation efforts to ensure a self-sustainable vegetative cover. Recently, Shu et al. (2002) showed successful establishment and colonization of vetiver grass as pioneering plant species on Pb/Zn mine spoils in China and concluded that this plant should be considered as one of the plants to be used for mine site revegetation. The vetiver plant (*Vetiveria zizanioides* L. Nansh), a common wetland grass species from the tribe Andropogoneae native to tropical and subtropical areas, has been cultivated commercially for many industrial applications and for the production of the medicinally valued volatile essential oil that can be distilled from its roots (Maffei 2002). Once established, it is not affected by droughts or floods. It is also highly tolerant to frost, heat, extreme soil pH, sodicity, salinity, and alkalinity as well as to a range of potential toxic elements such as As, Cd, Cu, Cr, Pb, Se, Zn, Ni, Al,

and Mn in the soil (Truong & Claridge 1996). Due to its unique physiological and morphological characteristics such as higher biomass; fast growth; higher metal tolerance, uptake, and accumulation; and strong ecological adaptability, the vetiver plant can play an important role in all subsets of phytoremediation of heavy metal-contaminated soils and water, that is, phytostabilization, phytoextraction, and phytofiltration of heavy metal contaminants (Mucciarelli et al. 1998; Khan et al. 2000; Lavania & Lavania 2000; Shu et al. 2002). Another benefit of using vetiver grass for phytoremediation is its foliage, which can be used as a mulch to improve soil physical properties.

Many plants growing on metal-contaminated soils possess mycorrhizae (Chaudhry et al. 1998), indicating that these fungi have evolved a tolerance to heavy metals and that they play an important role in the phytoremediation of contaminated soils (Khan et al. 2000; Khan 2001). Therefore, metal-tolerant mycorrhizal inoculants are promising for the phytoremediation of metal-contaminated soils.

Arbuscular mycorrhizal fungi (AMF) are known to improve plant growth on nutrient-poor soils and enhance their uptake of P, Cu, Ni, Pb, and Zn (Khan et al. 2000; Zhu et al. 2001). Despite the importance of the role that AMF play in plant interactions with the soil environment in general, and heavy metals in particular, relatively few studies have focused on their effect on metal-remediation efforts. Previous phytoremediation studies have focused on the predominantly nonmycorrhizal plant families, e.g., Brassicaceae or Caryophyllaceae, and arbuscular mycorrhizae (AM) have not been considered as important component of phytoremediation practices (Pawlowska et al.

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2000). It is possible to improve the phytoremediation capabilities of plants by inoculating them with appropriate AMF. The term “mycorrhizo-remediation” (Jamal et al. 2002) means the use of mycorrhizal plants in the phytoremediation of heavy metal-contaminated soils. The interaction between AM and minerals other than P, particularly heavy metals, has been the subject of a number of recent studies because of the possibility of the beneficial effect of mycorrhizae in improving the tolerance of plants against toxicity (Karagiannidis & Nikolaou 2000; Khan et al. 2000; Hayes et al. 2003). The uptake of heavy metals by mycorrhizal plants depends on several factors such as physicochemical properties of the soil, particularly its fertility level and pH; the host plants and the fungi involved; and, above all, the concentration of the metals in the soil (Smith & Read 1997).

Although vetiver grass is regarded to be a suitable pioneering candidate for phytoremediation of heavy metal-contaminated sites and for rehabilitation of abandoned metalliferous mine wastelands due to its high growth rate and tolerance to various unfavorable soil conditions (Truong & Baker 1998; Truong 1999, 2002; Shu et al. 2002), no record of its mycorrhizal status exists in literature. Vietmeyer (2002) suggested that areas that still require investigations include the symbiosis of vetiver grass roots with mycorrhizal fungi and N-fixing bacteria. As far as we are aware, the first record of occurrence of AM in vetiver grass was reported in plants growing in the South China Botanical Gardens in Guangzhou, PRC, in soil containing moderate amounts of basic nutrients and trace elements (Wong 2003).

The objectives of this greenhouse investigation were to (1) confirm the presence of AM associations in *Vetiveria zizanioides*; (2) determine the extent of its dependency on AMF when grown on heavy metal-contaminated soil; (3) study the effect of soil Pb and Zn concentrations on the growth and mycorrhization of plants inoculated with *Glomus mosseae* or *G. intraradices*; and (4) evaluate the influence of AM infection on the uptake of Pb and Zn by mycorrhizal plants.

## Methods

### Soil Preparation

The garden soil (loamy sand) used in this study was obtained from a commercial company in Hong Kong. The soil was sieved (4 mm) and steam sterilized (100°C for 1 hour for 3 consecutive days) to eliminate naturally occurring AMF propagules. The different metal contents at the rates of 10, 100, and 1,000 mg Zn or Pb/kg soil were obtained by adding appropriate amounts of aqueous solution of zinc sulphate or lead nitrate, respectively. The soil without metal addition was maintained as a control. The metal solutions were sterilized by passing through a 0.45- $\mu$ m filter paper (GN-6 Metrical Grid 47 mm; Gelman Laboratory, Pall Corporation, East Hills, NY, U.S.A.) to

get rid of unwanted microbes. After mixing the soil with the added chemical solution, soil moisture was adjusted to a field capacity (approximately 70% of water-holding capacity) by adding deionized water. The soils were then stored in a plastic box at  $20 \pm 4^\circ$  for 15 days with frequent mixing (once every 3 days) to allow thorough equilibration (Diaz et al. 1996).

### Plant and AMF Inoculum

Uniform-sized young slips of *Vetiveria zizanioides* collected from South China Botanical Gardens in Guangzhou, China, were used in this study. The inocula of the two different AMF species, *Glomus mosseae* and *G. intraradices*, were purchased from Biorize Sarl Co., Dijon, France. They were sand-based mycorrhizal inocula containing abundant chopped mycorrhizal root pieces, spores, and hyphae (Wu et al. 2005). About 70 g of the inoculum was added to each pot containing 2 kg of soil 3-cm deep and mixed with adjacent soil. The experiment consisted of mycorrhizal and nonmycorrhizal treatments for all four different levels of Zn/Pb concentrations. There were four replicates for each treatment. The pots were organized in a greenhouse (14-hour, light intensity  $250 \mu\text{mol m}^{-2} \text{second}^{-1}$  photon flux density day; 10-hour, dark photoperiod; and temperature range of 25–30°C) under a randomized block. After a growth period of 4 months, plants were harvested.

### Chemical Analyses

**Soil and Plant.** Soil samples were air-dried for 7 days and grounded and sieved through a 200- $\mu$ m mesh. The following soil properties were tested: pH (pH meter; soil: distilled water = 1:2), electrical conductivity (EC) (conductivity meter), total N and P (Kjeldahl method), water-soluble P (molybdenum blue method), total Zn and Pb (microwave digestion—digested with concentrated hydrochloric acid, concentrated nitric acid, and hydrofluoric acid at the ratio of 3:9:2), and DTPA-extractable (extracted with 1.9 g diethylenetriamine-pentaacetic acid and 14.9 g triethanolamine in 1 L deionized water, pH 7.3) Zn and Pb (tested by inductively coupled plasma-atomic emission spectroscopy [ICP-AES]). Analytical procedures were based on the methods described by Sparks et al. (1996). A standard reference material, Montana soil (SRM 2711) from U.S. Department of Commerce National Bureau of Standard, was used to verify the accuracy of metal determination, and the recovery rates were within  $90 \pm 10\%$ .

All plant samples were dried at 105°C for 24 hours. Dry weights separated into root and shoot portions were recorded. About 100 1-cm long segments of fine lateral root were removed from each harvested plant before drying and were stored in 50% glycerol for mycorrhizal infection assessment. The dried plant material was analyzed for the following parameters: total N and P (Kjeldahl method) and total Zn and Pb (perchloric acid digestion—digested with concentrated nitric acid and perchloric acid at the ratio of 4:1), which were determined

by ICP-AES. All the procedures were based on the methods described by Sparks et al. (1996). A standard reference material, Tomato leaves (SRM 1573a) from U.S. Department of Commerce National Bureau of Standard, was used to verify the accuracy of metal determination, and the recovery rates were within  $90 \pm 10\%$ .

**Mycorrhizae.** The preserved root segments from each treatment were washed with deionized water to remove all particles adhering to the root surface, cleared for 20 minutes at  $100^\circ\text{C}$  in 10% KOH, and stained with lactophenol cotton blue (Phillip & Hayman 1970). The stained root segments were mounted on glass slides (five pieces of root per slide) for examination under a compound microscope ( $\times 100$ – $\times 400$ ) with an eyepiece equipped with a crosshair that could be moved to randomly select positions. Mycorrhizal colonization was estimated for each sample by examining the hundred 1-cm stained pieces of the roots (Brundrett et al. 1996).

**Mycorrhizal Dependency.** For each plant species used in this study that formed association with mycorrhizae, a mycorrhizal dependency value, “relative mycorrhizal dependency,” was calculated. This referred to the difference between dry shoot mass of mycorrhizal plants and nonmycorrhizal plants, expressed as a percentage of dry mass of the mycorrhizal plant (Plenchette et al. 1983).

### Statistical Analyses

Data analyses were preformed using SPSS statistical program 8.0 version (SPSS, Inc., Chicago, IL, U.S.A.). Analysis of variance was used to test whether treatment effects existed, followed by Duncan’s multiple range test to identify means which differed significantly (at the 5% level) in mycorrhizal treatments and nonmycorrhizal control at each metal concentration (Little & Hills 1978). The correlation coefficient between mycorrhizal dependency and metal concentration was also analyzed.

## Results

### Soil Properties

The chemical properties of the soil samples before mixing with the different concentrations of Pb and Zn are listed in Table 1. In general, the soil contained moderate amounts of basic nutrients (N and P) and lower concentrations of total and DTPA-extractable Pb and Zn. There was no significant difference ( $p < 0.05$ ) for soils added with different concentrations of both Pb and Zn in terms of pH and EC values between soils inoculated with the two mycorrhizal inocula and the control. Total N concentrations were lower ( $p < 0.05$ ) in soils inoculated with *Glomus mosseae* for both metal amendments. There were no

**Table 1.** Physicochemical properties of the artificially metal-contaminated soil after treatment with different mycorrhizal inoculations.

Metal Added (mg/kg)	Mycorrhizal Fungus	pH	EC ( $\mu\text{s}/\text{cm}$ )	Total N (g/kg)	Total P (mg/kg)	Available P (mg/kg)	
Original soil		7.3	0.84	1.79	358	7.60	
Pb (mg/kg)	0	Control	7.1a	0.79a	1.97a	459c	7.34b
		<i>Glomus mosseae</i>	7.0a	0.80a	1.58b	654b	8.56a
		<i>G. intraradices</i>	7.2a	0.85a	2.04a	753a	9.01a
	10	Control	7.1a	0.82a	2.01a	393c	6.15c
		<i>G. mosseae</i>	7.1a	0.85a	1.61b	681b	8.17b
		<i>G. intraradices</i>	7.2a	0.87a	1.90a	703a	8.98a
	100	Control	7.2a	0.83a	1.95a	315a	6.06b
		<i>G. mosseae</i>	7.4a	0.91a	1.56b	329a	6.29b
		<i>G. intraradices</i>	7.3a	0.87a	1.88a	414a	7.05a
1,000	Control	7.3a	0.85a	1.88a	299a	5.22b	
	<i>G. mosseae</i>	7.5a	0.91a	1.56b	218a	5.35b	
	<i>G. intraradices</i>	7.4a	0.90a	2.02a	308a	6.17a	
Zn (mg/kg)	0	Control	7.2a	0.85a	1.97a	387a	7.21a
		<i>G. mosseae</i>	7.2a	0.79a	1.67b	435a	7.34a
		<i>G. intraradices</i>	7.2a	0.89a	2.01a	454a	7.87a
	10	Control	7.4a	0.86a	2.02a	351b	6.96c
		<i>G. mosseae</i>	7.2a	0.84a	1.67b	509a	8.85a
		<i>G. intraradices</i>	7.3a	0.86a	1.78b	410b	7.64b
	100	Control	7.3a	0.83a	1.85a	291c	6.05c
		<i>G. mosseae</i>	7.4a	0.90a	1.59b	492a	7.77a
		<i>G. intraradices</i>	7.3a	0.91a	1.81a	387b	6.83b
	1,000	Control	7.3a	0.88a	1.90a	245b	5.88b
		<i>G. mosseae</i>	7.4a	0.90a	1.57b	417a	6.95a
		<i>G. intraradices</i>	7.3a	0.89a	1.85a	294b	6.10b

The different letters in the same column of a certain metal concentration indicate a significant difference between different mycorrhizal inoculation treatments at  $p < 0.05$  level according to Duncan’s multiple range test.

significant differences in terms of total K concentrations in all soils with Zn amendments (ranging from 0.48 to 0.53%), whereas slightly lower ( $p < 0.05$ ) concentrations were observed in soils inoculated with *G. mosseae* in Pb amendments. Total as well as water-soluble P decreased according to the increase of metal additions in all three sets of soils with both Pb and Zn amendments. For soils with Pb amendments (Table 2), total as well as DTPA-extractable Pb increased according to the increase in Pb additions. No significant difference was found in total and DTPA-extractable Zn in soils with different Pb amendments.

For soils with Zn amendments (Table 1), a similar trend as for soils with Pb amendments was observed in terms of total and water-soluble P. In general, both concentrations decreased as Zn additions increased (except for soils inoculated with *G. mosseae*). The total as well as DTPA-extractable Zn increased according to the increase of Zn additions in all three sets of soils. The Pb concentrations did not alter on Zn additions with or without inoculation of either mycorrhizal fungus.

#### Mycorrhizal Colonization of the Roots

Figures 1 and 2 show the mycorrhizal colonization of vetiver under different Pb and Zn concentrations, respectively. For vetiver growing in different Pb additions, on inoculation with *G. mosseae*, the infection increased from 29.8 to 61.9% at 100 mg/kg Pb but dropped to 28.7% at 1,000 mg/kg Pb. On inoculation with *G. intraradices*, the infection increased from 33.8 to 71.5% at 100 mg/kg Pb addition but dropped to 45.5% at 1,000 mg/kg Pb (Fig. 1). For vetiver growing in soils with different concentrations of Zn, on inoculation with *G. mosseae*, mycorrhizal infection increased in low Zn additions from 0 to 10 mg/kg, reaching a maximum of 35.0% but dropped to 17.6% at 1,000 mg/kg Zn. When inoculated with *G. intraradices*, a similar trend was obtained, in which root infection ratio

**Table 2.** DTPA-extractable fraction in soil amended with Pb or Zn.

Metal Added (mg/kg)	Mycorrhizal Fungus	Pb (mg/kg)	Zn (mg/kg)
0	Control	40.3a	69.2a
	<i>Glomus mosseae</i>	43.2a	69.9a
	<i>G. intraradices</i>	50.1a	70.5a
10	Control	45.2a	76.1a
	<i>G. mosseae</i>	50.3a	85.8a
	<i>G. intraradices</i>	49.8a	77.8a
100	Control	88.2a	154a
	<i>G. mosseae</i>	103a	97.5b
	<i>G. intraradices</i>	99.2a	110a
1,000	Control	495b	498a
	<i>G. mosseae</i>	608a	505a
	<i>G. intraradices</i>	597a	551a

The different letters in the same column of a certain metal concentration indicate a significant difference between different mycorrhizal inoculation treatments at  $p < 0.05$  level according to Duncan's multiple range test.

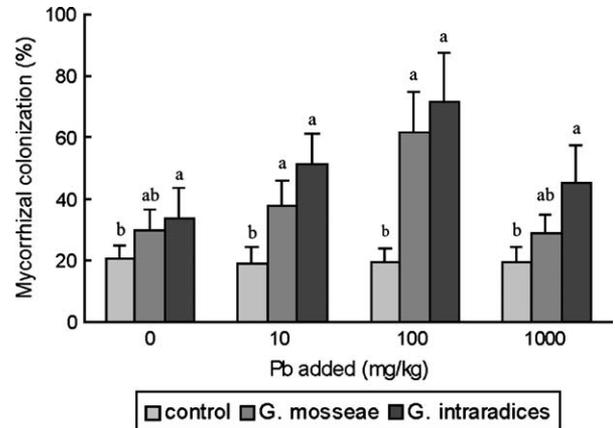


Figure 1. Root infection ratio of *Vetiveria zizanioides* by different mycorrhizal fungi under different Pb concentrations ( $\bar{X} \pm SD$ ,  $n = 4$ ). Different letters above the bars indicate a significant difference at  $p < 0.05$  level between different mycorrhizal inoculations under same metal stress according to Duncan's multiple range test.

increased from 21.3 to 33.8% but dropped to 24.9% at 1,000 mg/kg Zn (Fig. 2).

#### Plant Analyses

**Dry Weights.** The shoot dry weights of vetiver are shown in Figure 3. In general, shoot weights decreased as metal concentrations increased. The shoot dry weights were significantly lower ( $p < 0.05$ ) in control than in mycorrhizal plants at high Pb (100 and 1,000 mg/kg) and Zn (100 and 1,000 mg/kg) concentrations.

**Mycorrhizal Dependency.** In general, the dependency of vetiver indicated by shoot dry weight on both AMF increased as metal concentrations increased ( $p < 0.05$ ) (Fig. 3). Data analyses showed that there was a positive

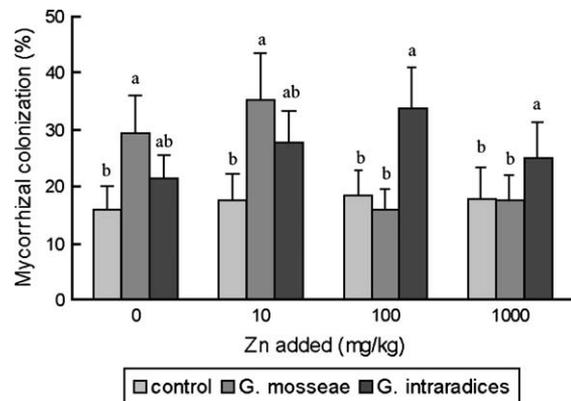


Figure 2. Root infection ratio of *Vetiveria zizanioides* by different mycorrhizal fungi under different Zn concentrations ( $\bar{X} \pm SD$ ,  $n = 4$ ). Different letters above the bars indicate a significant difference at  $p < 0.05$  level between different mycorrhizal inoculations under same metal stress according to Duncan's multiple range test.

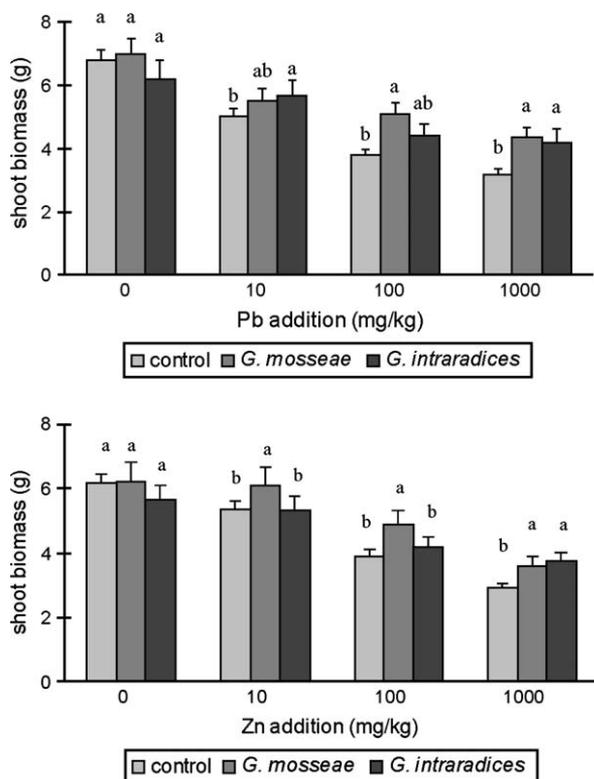


Figure 3. Effects of mycorrhizal inoculation on shoot biomass of *Vetiveria zizanioides* under different metal concentrations. Different letters above the bars indicate a significant difference at  $p < 0.05$  level between different mycorrhizal inoculations under same metal stress according to Duncan's multiple range test.

and linear correlation between mycorrhizal dependency and metal concentrations, with  $r = 0.8991$  for Pb and  $r = 0.9174$  for Zn (both with  $p < 0.001$ ) when inoculated with *G. mosseae* and  $r = 0.7469$  for Pb and  $r = 0.7558$  for Zn (both with  $p < 0.05$ ) when inoculated with *G. intraradices*.

**Table 3.** Metal (Pb, Zn) distribution in roots and shoots of *Vetiveria zizanioides* when treated with different mycorrhizal inoculations (mg/kg).

Metal Concentration (mg/kg)	Mycorrhizal Fungus	Pb		Zn	
		Root	Shoot	Root	Shoot
0	Control	19.4 ± 1.82b	6.98 ± 1.22a	107 ± 44.5ab	64.7 ± 12.2a
	<i>Glomus mosseae</i>	23.8 ± 4.07b	4.84 ± 0.16b	76.6 ± 29.b	69.2 ± 23.1a
	<i>G. intraradices</i>	55.1 ± 7.82a	1.21 ± 0.29c	178 ± 57.6a	48.5 ± 0.87b
10	Control	48.8 ± 4.87b	7.53 ± 1.08a	110 ± 32.4a	27.9 ± 6.05b
	<i>G. mosseae</i>	29.5 ± 15.5b	5.84 ± 0.33a	91.4 ± 5.98a	39.2 ± 12.9b
	<i>G. intraradices</i>	78.8 ± 8.46a	2.73 ± 0.71b	128 ± 36.9a	77.8 ± 24.1a
100	Control	66.3 ± 20.8a	11.1 ± 0.88a	256 ± 24.3a	50.3 ± 8.15b
	<i>G. mosseae</i>	56.2 ± 20.8a	5.35 ± 0.32b	113 ± 24.3b	48.5 ± 8.15b
	<i>G. intraradices</i>	79.4 ± 8.14a	9.10 ± 3.48a	270 ± 14.1a	80.3 ± 10.9a
1,000	Control	449 ± 4.93a	17.4 ± 0.89b	477 ± 0.73a	154 ± 11.3a
	<i>G. mosseae</i>	117 ± 53.0c	12.9 ± 0.71c	310 ± 35.4b	72.2 ± 23.7b
	<i>G. intraradices</i>	213 ± 8.24b	29.4 ± 1.10a	495 ± 62.4a	85.5 ± 8.73b

The different letters in the same column of a certain metal concentration indicate a significant difference between different mycorrhizal inoculation treatments at  $p < 0.05$  level according to Duncan's multiple range test.

**Metal Concentrations in Plant Tissues.** Concentrations of Pb and Zn in both root and shoot tissues of vetiver plants mycorrhizal with *G. mosseae* were lower ( $p < 0.05$ ) than in the nonmycorrhizal controls (Table 3), except that under low soil Zn (0 and 100 mg/kg) concentrations, higher shoot Zn concentrations were also observed in mycorrhizal plants.

When mycorrhizal with *G. intraradices*, root and shoot Pb contents were significantly lower ( $p < 0.05$ ) compared with the nonmycorrhizal controls (Table 3). At low levels of soil Zn, root Zn concentrations of mycorrhizal and the control were more or less the same. At higher levels of soil Zn (100 and 1,000 mg/kg), contents of Zn in mycorrhizal roots were significantly lower ( $p < 0.05$ ) than in the control. In the case of shoots, both Pb and Zn concentrations in mycorrhizal plants were lower ( $p < 0.05$ ) than in non-mycorrhizal plants at all levels of metal additions to soil.

**Nutrient Contents (N and P).** There was no significant difference regarding N concentrations among different treatments (data not shown). Figures 4 and 5 show the P concentrations in the shoot of vetiver under different metal treatments with or without AM inoculation. Additions of both metals resulted in substantial decreases of P concentrations in vetiver in all treatments, especially in nonmycorrhizal plants (the control). However, significantly higher ( $p < 0.05$ ) P concentrations were noted in mycorrhizal plants added with Pb and Zn compared with the control.

## Discussion

Vegetative slips of vetiver grass were able to produce young plantlets in Pb-/Zn-amended soils in the greenhouse. These observations are consistent with those of previous workers (Truong & Baker 1998; Zheng et al. 1998) who reported that vetiver is able to tolerate a variety of pollutants in soils and water.

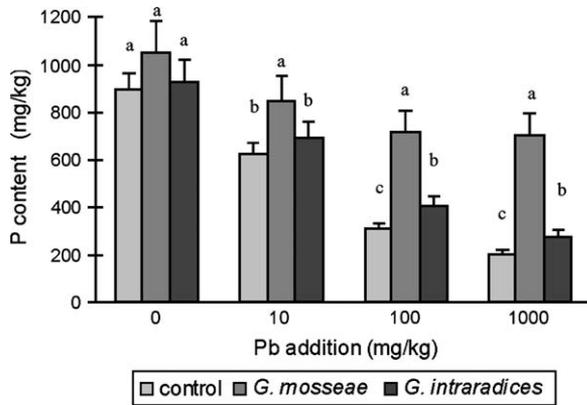


Figure 4. Effects of mycorrhizal inoculation on the P assimilation by *Vetiveria zizanioides* under different Pb concentrations ( $n = 4$ ). Different letters above the bars indicate a significant difference at  $p < 0.05$  level between different mycorrhizal inoculations under same metal stress according to Duncan's multiple range test.

The occurrence of AM endophyte was confirmed in vetiver roots growing in heavy metal-contaminated soils as first reported by Wong (2003). Vetiver grass is yet another plant that has been found to be mycorrhizal with AMF in heavy metal-contaminated soils (see Chaudhry et al. 1998 for references). *Vetiveria zizanioides* showed dependency on mycorrhizae when soil is contaminated with Pb or Zn.

One of the questions posed in this study is whether mycorrhizae of different AMF would behave differently in the presence of heavy metals in soil. The sensitivity of AM endophytes to high amounts of heavy metals, expressed as a reduction or delay in its colonizing ability, has been observed (Karagiannidis & Nikolaou 2000). This does not seem to be the case for the fungi used in this study because their ability to colonize increased when soil Pb and Zn concentrations were increased.

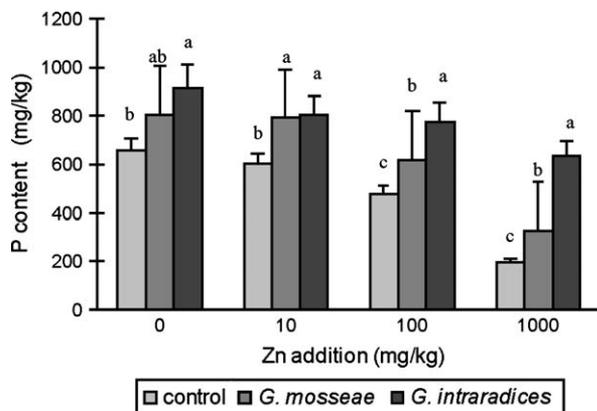


Figure 5. Effects of mycorrhizal inoculation on the P assimilation by *Vetiveria zizanioides* under different Zn concentrations ( $n = 4$ ). Different letters above the bars indicate a significant difference at  $p < 0.05$  level between different mycorrhizal inoculations under same metal stress according to Duncan's multiple range test.

It seems that the soil P concentration and its availability are affected by metal concentrations. This in turn affects P uptake by plants as reflected by shoot P concentration. The present results showed that available P decreased according to the increase in soil metal concentrations, which could be attributed to the possible P precipitation with added metals (Ma et al. 1997). When comparing the control without mycorrhizal inoculation, two mycorrhizal endophytes, *Glomus mosseae* and *G. intraradices*, both stimulated P uptake by vetiver. However, the former had a better performance under Pb stress, whereas the latter under Zn stress. This implies that *G. mosseae* could endure higher Pb but more sensitive to Zn; on the contrary, *G. intraradices* seems to be more sensitive to Pb but tolerant to Zn. When mycorrhizal with either AMF, shoot P concentrations were significantly higher than in nonmycorrhizal controls. It is generally believed that high metal concentrations would lower the availability of P to host plant (McGnigle et al. 1999; Clark & Zeto 2000; Tang et al. 2001), thus favoring the colonization of the roots.

The present study indicated that Pb and Zn concentrations in shoots of vetiver can be modulated by mycorrhizae when growing in soil contaminated with these metals. Mycorrhizae of vetiver appear to be protective by way of reducing the amount of the heavy metals accumulating in the plant. It is also apparent that *G. mosseae* is different from *G. intraradices* in modulating movement of these heavy metals into the plant, with the former being better at excluding both Pb and Zn. These observations are in agreement with those of Joner and Leyval (2001), who concluded that mycorrhizae tend most often to lower heavy metal concentrations in the shoots of nonhyperaccumulator plants, and also with those of Diaz et al. (1996), who showed that metal uptake by mycorrhizal plants increases in soils with low metal concentrations but decreases in soils with high metal levels. Although the mechanisms of protection against heavy metals provided by mycorrhizae to their host plants are not clear, a possible retention of heavy metals by the fungal mycelium involving adsorption to cell wall and fixation by polyphosphate granules (Galli et al. 1994) could occur. The abundance of external mycelium produced by the AMF can be important for heavy metal-fixing ability of the fungi and consequently for their plant-protecting action. Thus, differences in the extrametrical development of the two fungal species, translocation of mineral elements, or their symbiosis efficiency under stress conditions could explain the situations observed in the present study.

## Conclusions

It is concluded that inoculation with AMF protects host plant from the potential toxicity caused by Pb and Zn, but the degree of protection varies according to the fungus and host plant combination. It appears that the choice of AMF is a factor in using mycorrhiza in rehabilitation of heavy metal-contaminated sites. Little evidence was

found for mycorrhizal vetiver in terms of phytoremediation, that is, mycorrhiza on vetiver does not increase accumulation of either Pb or Zn. However, it appears that mycorrhiza will be important nevertheless because it appears to reduce the accumulation of Pb and Zn in mycorrhizal plants, thus offering a protective effect.

#### Implications for Practice

- Mycorrhizal inoculation can improve the growth of host plant due to a better nutrient supply and effective alleviation of metal toxicity.
- Thus, it would be a promising approach for revegetation of phytostabilization. However, the role of mycorrhizae in phytoextraction cases remains uncertain, as mycorrhizae associated with vetiver did not increase the accumulation of either Pb or Zn in the aboveground plant tissues.

#### LITERATURE CITED

- Brooks, R. R., editor. 1998. Plants that accumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration, and phytomining. CAB International, Wallingford, Oxford, United Kingdom.
- Brundrett, M. C., N. Bougher, B. Dell, T. Grove, and N. Malajczuk, editors. 1996. Working with mycorrhizas in forestry and agriculture. 374p. ACIAR Monograph 32. Australian Centre for International Agricultural Research, Canberra, Australia.
- Chaudhry, T. M., W. J. Hayes, A. G. Khan, and C. S. Khoo. 1998. Phytoremediation—focusing on accumulator plants that remediate metal-contaminated soils. *Australasian Journal of Ecotoxicology* **4**:37–51.
- Clark, R. B., and S. K. Zeto. 2000. Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition* **23**:867–902.
- Diaz, G., C. Azcon-Aguilar, and M. Honrubia. 1996. Influence of arbuscular mycorrhizae on heavy metal (Zn & Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. *Plant and Soil* **180**: 241–249.
- Galli, U., H. Schuepp, and C. Brunold. 1994. Heavy metal binding by mycorrhizal fungi. *Physiologia Plantarum* **12**:364–368.
- Hayes, W. J., T. M. Chaudhry, R. T. Buckney, and A. G. Khan. 2003. Phytoaccumulation of trace metals at the Sunny Corner mine, New South Wales, with suggestions for a possible remediation strategy. *Australasian Journal of Ecotoxicology* **9**:69–82.
- Jamal, A., N. Ayub, M. Usman, and A. G. Khan. 2002. Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by soybean and lentil. *International Journal of Phytoremediation* **4**:205–221.
- Joner, E. J., and C. Leyval. 2001. Time-course of heavy metal uptake in maize and clover as affected by different mycorrhiza inoculation regimes. *Biology and Fertility of Soils* **33**:351–357.
- Karagiannidis, N., and N. Nikolaou. 2000. Influence of arbuscular mycorrhizae on heavy metal (Pb & Cd) uptake, growth, and chemical composition of *Vitis vinifera* L. (cv. Razaki). *American Journal of Enology and Viticulture* **51**:269–275.
- Khan, A. G. 2001. Relationships between chromium biomagnification ratio, accumulation factor, and mycorrhizae in plants growing on tannery effluent-polluted soil. *Environment International* **26**:417–423.
- Khan, A. G., C. Kuek, T. M. Chaudhry, C. S. Khoo, and W. J. Hayes. 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere* **41**:197–207.
- Lavania, U. C., and S. Lavania. 2000. Vetiver grass technology for environmental technology and sustainable development. *Current Science* **78**:944–946.
- Little, T. M., and J. J. Hills. 1978. *Agricultural experimentation: design and analysis*. John Wiley, New York.
- Ma, L. Q., A. L. Choate, and G. N. Rao. 1997. Effects of incubation and phosphate rock on lead extractability and speciation in contaminated soils. *Journal of Environmental Quality* **26**:801–807.
- Maffei, M., editor. 2002. *Vetiveria: the genus vetiveria*. Taylor & Francis, London, United Kingdom.
- McGnigle, T. P., D. Young, and M. H. Miller. 1999. Mycorrhizae, crop growth, and crop phosphorus nutrition in maize-soybean rotations given various tillage treatments. *Plant and Soil* **210**:33–42.
- Mucciarelli, M., C. M. Berteà, S. Scannerini, and M. Gallino. 1998. *Vetiveria zizanioides* as a tool for environmental engineering. *Acta Horticulturae* **457**:261–269.
- Pawlowska, T. E., R. L. Chaney, M. Chin, and I. Charvat. 2000. Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal-contaminated landfill. *Applied and Environmental Microbiology* **66**:2526–2530.
- Phillip, J. M., and D. S. Hayman. 1970. Improved procedure for clearing roots and staining parasites and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**:158–160.
- Plenchette, C., J. A. Fortin, and V. Furlan. 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. *Plant and Soil* **70**:199–209.
- Shu, W. S., H. P. Xia, Z. Q. Zhang, C. Y. Lan, and M. H. Wong. 2002. Use of vetiver and three other grasses for revegetation of Pb/Zn mine tailings: field experiment. *International Journal of Phytoremediation* **4**:47–57.
- Smith, S. E., and D. J. Read. 1997. *Mycorrhizal symbiosis*. 2<sup>nd</sup> edition. Academic Press, London, United Kingdom.
- Sparks, D. L., A. L. Page, P. A. Helmke, and R. H. Loeppert, editors. 1996. *Methods of soil analysis*. Part 3. Chemical methods. Soil Science Society of America, Madison, Wisconsin.
- Tang, F., J. A. White, and I. Charvat. 2001. The effect phosphorus available on arbuscular mycorrhizal colonization of *Typha angustifolia*. *Mycologia* **93**:1042–1047.
- Truong, P. 1999. Vetiver grass technology for mine rehabilitation. *Technical Bulletin 1999/2*. Pacific Rim Vetiver Network, Bangkok, Thailand.
- Truong, P. 2002. Vetiver grass technology. Pages 114–132 in M. Maffei, editor. *Vetiveria—the genus Vetiveria*. Taylor & Francis, London, United Kingdom.
- Truong, P., and D. Baker. 1998. Vetiver grass system for environmental protection. *Pacific Rim Vetiver Network: Technical Bulletin 1998/1*. Pacific Rim Vetiver Network, Bangkok, Thailand.
- Truong, P., and J. Claridge. 1996. Effects of heavy metal toxicities on vetiver growth. *Thailand Vetiver Network Newsletter* **15**.
- Vietmeyer, N. 2002. Beyond the vetiver hedge: organizing vetiver's next step to global acceptance. Pages 176–186 in M. Maffei, editor. *Vetiveria—the genus Vetiveria*. Taylor & Francis, London, United Kingdom.
- Wong, C. C. 2003. The role of mycorrhizae associated with *Vetiveria zizanioides* and *Cyperus polystachyos* in the remediation of metals (lead and zinc) contaminated soils. M.Phil. Thesis. Hong Kong Baptist University, Hong Kong, China.

- Wu, S. C., Z. H. Cao, Z. G. Li, K. C. Cheung, and M. H. Wong. 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma* **125**:155–166.
- Zheng, C. R., C. Tu, and H. M. Chen. 1998. Preliminary experiment on purification of eutrophic water with vetiver. Pages 81–84 in L. Y. Xu, editor. *Vetiver research development*. Agricultural Science and Technology Press, Beijing, China.
- Zhu, Y. G., P. Christie, and A. S. Laidlaw. 2001. Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil. *Chemosphere* **42**:193–199.