Effects of arbuscular mycorrhizal inoculation on uranium and arsenic accumulation by Chinese brake fern (*Pteris vittata* L.) from a uranium mining-impacted soil

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Abstract

A glasshouse experiment was conducted to investigate U and As accumulation by Chinese brake fern, *Pteris vittata* L., in association with different arbuscular mycorrhizal fungi (AMF) from a U and As contaminated soil. The soil used contains 111 mg U kg⁻¹ and 106 mg As kg⁻¹. *P. vittata* L. was inoculated with each of three AMF, *Glomus mosseae*, *Glomus caledonium* and *Glomus intraradices*. Two harvests were made during plant growth (two and three months after transplanting). Mycorrhizal colonization depressed plant growth particularly at the early stages. TF (transfer factor) values for As from soil to fronds were higher than 1.0, while those for roots were much lower. Despite the growth depressions, AM colonization had no effect on tissue As concentrations. Conversely, TF values for U were much higher for roots than for fronds, indicating that only very small fraction of U was translocated to fronds (less than 2%), regardless of mycorrhizal colonization. Mycorrhizal colonization significantly increased root U concentrations at both harvests. Root colonization with *G. mosseae* or *G. intraradices* led to an increase in TF values for U from 7 (non-inoculation control) to 14 at the first harvest. The highest U concentration of 1574 mg kg⁻¹ was recorded in roots colonized by *G. mosseae* at the second harvest. The results suggested that *P. vittata* in combination with appropriate AMF would play very important roles in bioremediation of contaminated environments characterized by a multi-pollution.

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1. Introduction

Uranium is a common radioactive contaminant in U mining and processing areas in many parts of the world including China (Ebbs et al., 1998; Xu et al., 2002), and

it is highly toxic to a broad range of organisms, particularly mammals in terrestrial ecosystems (Fellows et al., 1998). Remediation of U-contaminated soils has thus become an urgent need. Traditional remediation approaches for U-contaminated soils include physical (excavation and disposal) and chemical (extraction/washing, immobilization) methods (Francis and Dodge, 1998; Zhu and Shaw, 2000). However, these approaches are often very costly, and may significantly reduce soil

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quality and damage the local ecosystem. In recent years, phytoextraction has been proposed as a promising technique for remediating U-contaminated soils (Huang et al., 1998; Zhu and Shaw, 2000; Shahandeh et al., 2001). However, the success of phytoremediation of U-contaminated soils relies on efficient U uptake and translocation in plants and bioavailability of U in the soils.

The role of AMF in plant uptake of heavy metals has been extensively studied. It is generally agreed that AMF often protect plants against high concentrations of non-essential metals in their shoots by enhancing metal retention in the roots (Leyval et al., 2002). Consequently, AMF could possibly help in revegetation or phytostabilization of metal contaminated sites (Khan et al., 2000; Leyval et al., 2002). It was recently shown that the extra-radical AM fungal mycelium took up and translocated U towards roots of in vitro-cultivated mycorrhizas (Rufyikiri et al., 2002, 2003), but U transfer from root to shoot could not be quantified by this model system. In a pot experiment, Rufyikiri et al. (2004) also showed that AMF influenced shoot U concentrations of clover plants but only at high level of U addition (87 mg U kg⁻¹ soil). Obviously, the impacts of AM fungi on U uptake by plants, especially those having the potential to remediate U-contaminated soils, still need further investigation in order to determine the importance of mycorrhizal fungi in remediation of U-contaminated environments.

On the other hand, arsenic is a ubiquitous contaminant of global concern, and is also a highly toxic element. Arsenic contamination of groundwater and soils (thus food crops) is receiving increasing attention, particularly in Southeast Asia (Meharg and Hartley-Whitaker, 2002). Arsenic in the environment is often associated with other elements, such as Au, Ag, Cu and U. U mining and processing activities could cause significant As contamination in the vicinity of mining sites (Pichler et al., 2001; Moldovan et al., 2003). Arsenic concentrations in U mining tailings can be as high as 6 g kg⁻¹ (Moldovan et al., 2003).

Due to the co-existence of U and As contamination in soils impacted by U mining, the development of remediation approaches for both contaminants becomes necessary. *Pteris vittata*, Chinese brake fern, has recently been identified as an As hyperaccumulator (Ma et al., 2001). It can accumulate up to 22630 mg As kg⁻¹ frond dry weight with a bio-concentration factor of over 10. After the discovery of *P. vittata*, several other fern species have also been reported to be able to hyperaccumulate As (Zhao et al., 2002). Many studies have demonstrated that phytoextraction of As-contaminated soils using these ferns may be a promising remediation technique. However, so far there is little information available on whether *P. vittata* can also significantly accumulate other metals or metalloids that co-exist with As. If this is the case, then phytoremediation using *P. vittata* may have wide application.

P. vittata forms arbuscular mycorrhizas (Fitz and Wenzel, 2002), but the role of AMF in phytoremediation of As-contaminated soil using this fern plant has received only little attention. Isolates of AMF from an As/Cu mine site were shown to be able to maintain a relatively low As concentration in the host plant via efflux (Sharples et al., 2000). However, the enlarged plant biomass due to improved mineral nutrition may still lend advantages to mycorrhizal plants for remediation of As contaminated soils (Liu et al., 2005).

In this study, a soil contaminated by a mixture of U and As was collected from a U mining area and was used to grow *P. vittata* in association with different AMF. The aim was to identify (1) whether *P. vittata* has the potential to extract U from U contaminated environments; (2) the As and U accumulation pattern by *P. vittata* under combined contaminations; (3) the effects of AM colonization on U and As accumulation, and (4) the feasibility to screen effective AMF strains for remediation purposes.

2. Materials and methods

2.1. Host plants and AM fungi

Spores of Chinese brake fern (*P. vittata* L.) were collected from Chenzhou, Hu'nan province, China (N25°35.360'; E113°00.346"). Seedlings were raised from spores in sterilized growth medium (sandy soil mixed with vermiculite at a mixing ratio of 2:1 v/v) in two-litter round plastic pots. Usually it took two weeks for the emergence of tiny gametocytes, and two more weeks for appearance of sporophytes. Till the young sporophytic seedlings had grown up to a height of about 1 cm, they were carefully separated into singles and transplanted immediately into seedling trays for further cultivation in the same growth medium. When the seedlings had two fronds and were about 3 cm in height, they were ready for transplanting and establishing the experiment.

The AM fungi used were *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe (BEG167), *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerdemann and *Glomus intraradices* Schenck & Smith (BEG 87). The original inoculum of *G. caledonium* (lab No. 90036) was kindly provided by Professor X.G. Lin of the Institute of Soil Science, Chinese Academy of Sciences. Each fungus was propagated in pot culture with maize plants grown in a sandy soil for 10 weeks. Inoculum from pot culture was a mixture of spores, mycelium, sandy soil and root fragments containing approximately 1000 spores per 100 g.

2.2. Growth medium

The growth medium was a sandy soil collected from a U mining site in Shangrao County, Jiangxi province,

China. Before the experiment, the soil was passed through a 2 mm sieve, partially sterilized (10 kGy, 10 MeV electron beam) and amended with basal nutrients without P as recommended by Pearson and Jakobsen (1993). Soil pH was 8.23 (1:2.5 soil to water). The soil had a final extractable P content of 4.23 mg kg^{-1} (extracted by 0.5 M NaHCO_3 using the methods established by Olsen et al., 1954) and CaCO_3 equivalent content of 77.8 g kg^{-1} . Total U content was 111 mg U kg^{-1} , and exchangeable and carbonate-U (following the methods described by Shahandeh and Hossner, 2002) were 8.9 and 29.3 mg kg^{-1} soil, respectively. Total As content was $106 \text{ mg As kg}^{-1}$, and water extractable As (soil: water = 1:10) was 5.9 mg kg^{-1} soil.

2.3. Experimental procedures

Round plastic pots were used, each containing 940 g of the soil. Inoculated treatments received 60 g of the fungal inoculum, which was carefully mixed into the growth medium, while non-inoculated plants received equivalent amount of autoclaved soil–sand mixture. Each pot was transplanted with one seedling. The resulting four treatments had eight replicates and the 32 pots were arranged in a randomized block design. The experiment was established on 16 June 2003 and conducted in a controlled environment glass house with 16 h – 25°C day and 8 h – 18°C night and natural light. De-ionized water was added as required to maintain moisture content at 55% of water holding capacity by regular weighing. Nitrogen (as NH_4NO_3) was added to the pots 30 and 45 d after transplanting to provide a total of 120 mg N per pot. The first harvest was made two months after transplanting on 15 August 2003. Four replicates of each treatment were harvested and the remaining pots were harvested on 10 September 2003.

2.4. Harvest and chemical analysis

Fronds and roots were harvested separately. Soil samples were collected for determination of hyphal length density. Root samples were then carefully washed with tap water followed by deionised water to remove adhering soil particles. Sub-samples of fresh roots were collected for the determination of AM colonization as described below. The dry weights of fronds and roots were determined after oven drying at 70°C for 48 h. Oven-dried sub-samples were milled and digested by acid digestion using a mixture of HNO_3 and HClO_4 (4:1 v/v) at 225°C . The dissolved samples were analyzed for uranium (^{238}U) on inductively coupled plasma-mass spectroscopy (ICP-MS) (Finnigan Mat, Germany) without any radiochemical treatment. The uranium isotope ^{233}U was used as an internal standard. Arsenic concentrations were determined using an atomic fluorescence spectrometer (Model AF-610A, Beijing Rayleigh Ana-

lytical Instrument Co., China), while P was analyzed using the molybdo-phosphovanado-molybdate method (Hanson, 1950).

Sub-samples of fresh roots were cleared in 10% KOH and stained with Trypan blue by the procedure of Phillips and Hayman (1970), modified to omit phenol from solutions and HCl from the rinse. Percentage root colonization and root length were determined by the grid-intersect method (Giovanetti and Mosse, 1980).

2.5. Data analysis

The transfer factor (TF) was defined as the ratios of elemental concentrations in plant tissues (mg kg^{-1} d.w.) to those in the soil (mg kg^{-1} d.w.); this parameter was used to evaluate the phytoextraction efficiency.

All data were subjected to two-way ANOVA to compare harvest and inoculation treatment using GenStat for PC/Windows 6.1 (GenStat Committee, 2002). Multi-comparisons by least significant difference (LSD) at the 5% level were applied to compare means of inoculation treatments across the two harvests, where harvest and inoculation interacted, or within each harvest where there were no significant interactions.

3. Results

3.1. Colonization of roots by different AM fungi

Table 1 shows that the root colonization with three AMF ranged from 22% to 63% at the first harvest. There was a general decrease in the colonization rates from the first to the second harvest ($P < 0.001$) for *G. mosseae* and *G. caledonium*. At the first harvest (Table 1),

Table 1

Root colonization and extraradical hyphal length density of Chinese brake fern inoculated with different AM fungi

Inoculation treatment	Root colonization (%)	
	First harvest	Second harvest
-M	0	0
Glm	63	27
Glc	41	28
Gli	22	23
LSD	11	
Significance ^a of		
Harvest		***
Inoculation		***
Interaction		***

-M, non-mycorrhizal; Glm, *G. mosseae*; Glc, *G. caledonium*; Gli, *G. intraradices*.

^a By analysis of variance. *** $P < 0.001$.

G. mosseae produced significantly higher colonization than the other two fungi ($P < 0.001$), while the differences in colonization among the three AMF treatments were not noted at the second harvest.

3.2. Plant growth

Plant dry weights increased markedly from the first to the second harvest ($P < 0.001$) (Fig. 1). At the first harvest, mycorrhizal colonization generally decreased plant dry weights ($P < 0.001$), and plants inoculated

with *G. intraradices* had the smallest dry weight. At the second harvest, only the inoculation with *G. intraradices* produced a small growth depression compared with non-inoculated controls, and no significant differences was observed among inoculation with *G. mosseae*, *G. caledonium* and the control.

3.3. Plant phosphorus nutrition

Mycorrhizal colonization led to an overall increase in P concentrations at the first harvest but the beneficial

Fig. 1. Frond (a) and root (b) dry weight of Chinese brake fern inoculated with different AM fungi. -M, Glm, Glc and Gli represent non-inoculation control and inoculation with *G. mosseae*, *G. caledonium* and *G. intraradices*, respectively. By analysis of variance, harvest and inoculation were highly significant ($P < 0.001$) for both fronds and roots. Different letters above columns indicate significant differences among inoculation treatments at each harvest by LSD multi-comparison at the 5% level.

Table 2

P concentration and uptake in Chinese brake fern inoculated with different AM fungi

	Inoculation treatment	P concentration (mg g ⁻¹)		P uptake (mg pot ⁻¹)	
		First harvest	Second harvest	First harvest	Second harvest
Frond	-M	1.54	1.49	6.81	12.26
	Glm	1.84	1.52	5.54	12.21
	Glc	1.91	1.59	5.22	12.39
	Gli	2.02	1.66	4.03	11.10
LSD		0.13		1.20	
Significance ^a of					
Harvest		***		***	
Inoculation		***		*	
Interaction		*		NS	
Root	-M	1.56	1.60	3.15	5.77
	Glm	1.71	1.61	2.05	5.42
	Glc	1.75	1.69	1.72	5.18
	Gli	1.81	1.70	1.24	4.89
LSD		0.08		0.59	
Significance ^a of					
Harvest		*		***	
Inoculation		***		***	
Interaction		NS		NS	

-M, non-mycorrhizal; Glm, *G. mosseae*; Glc, *G. caledonium*; Gli, *G. intraradices*.

^a By analysis of variance. *** $P < 0.001$; * $P < 0.05$; NS, not significant.

effect weakened at the second harvest (Table 2). At the first harvest, mycorrhizal colonization significantly reduced total P uptake ($P < 0.001$). At the second harvest, no difference in total P uptake was observed between the treatments.

3.4. Arsenic uptake and partitioning

Mycorrhizal colonization had no effect on As concentration (Table 3). At the first harvest, mycorrhizal colonization significantly reduced As uptake into fronds and roots ($P < 0.01$), but the difference was not noted at the second harvest. Transfer factors (TF) for As from soil to fronds were higher than 1, while those for roots were much lower. TF for the first harvest were higher than for the second harvest and mycorrhizal colonization had no significant effect. At the first harvest, average TF for fronds was 1.83 and that for roots was 0.79; while at the second harvest, average TF for fronds was 1.34 and that for roots was 0.59.

P/As ratios in both fronds ($P < 0.001$) and roots ($P < 0.01$) were higher at the second harvest than at the first harvest (Fig. 2). Inoculation treatment affected only P/As ratios in fronds ($P < 0.05$); at the first harvest, the ratios were significantly increased by inoculation with *G. mosseae* and *G. caledonium*.

3.5. Uranium uptake and partitioning

Frond U concentrations were significantly higher at the second harvest than at the first harvest ($P < 0.001$) except for the plants colonized by *G. caledonium*, but in general root U concentrations did not differ between the two harvests (Table 4). At the first harvest mycorrhizal colonization increased frond U concentrations ($P < 0.01$). At the second harvest plants colonized with *G. intraradices* had significantly higher frond U concentrations compared to non-inoculated plants, and plants colonized with *G. caledonium* had significantly lower frond U concentrations than non-inoculated plants. Mycorrhizal colonization significantly increased root U concentrations at both harvests ($P < 0.01$) except for plants colonized by *G. caledonium* at the second harvest.

Total U uptake was significantly higher at the second harvest than at the first harvest ($P < 0.001$, Table 4). Mycorrhizal colonization did not affect U accumulation in fern fronds and roots at the first harvest; while at the second harvest, colonization with *G. caledonium* decreased U accumulation in fronds and did not affect U accumulation in roots. In contrast, colonization by *G. mosseae* or *G. intraradices* did not affect U accumulation in fronds but significantly increased U accumula-

Table 3

Arsenic concentration and uptake in Chinese brake fern inoculated with different AM fungi

	Inoculation treatment	As concentration (mg kg ⁻¹)		As uptake (mg pot ⁻¹)	
		First harvest	Second harvest	First harvest	Second harvest
Frond	-M	191	146	0.843	1.208
	Glm	192	131	0.572	1.049
	Glc	175	140	0.464	1.089
	Gli	218	151	0.451	1.012
LSD		18		0.168	
Significance ^a of					
Harvest		***		***	
Inoculation		NS		**	
Interaction		NS		NS	
Root	-M	81	65	0.164	0.238
	Glm	80	59	0.097	0.202
	Glc	84	60	0.081	0.185
	Gli	88	65	0.059	0.187
LSD		8		0.043	
Significance of					
Harvest		***		***	
Inoculation		NS		**	
Interaction		NS		NS	

-M, non-mycorrhizal; Glm, *G. mosseae*; Glc, *G. caledonium*; Gli, *G. intraradices*.

^a By analysis of variance. *** $P < 0.001$; ** $P < 0.01$; NS, not significant.

Fig. 2. P/As ratios in frond (a) and root (b) of Chinese brake fern inoculated with different AM fungi. -M, Gln, Glc and Gli represent non-inoculation control and inoculation with *G. mosseae*, *G. caledonum* and *G. intraradices*, respectively. By analysis of variance, harvest was highly significant for both fronds ($P < 0.01$) and roots ($P < 0.001$), while inoculation was significant for only fronds ($P < 0.05$). Different letters above columns indicate significant differences among inoculation treatments at each harvest by LSD multi-comparison at the 5% level.

Table 4

^{238}U concentration and uptake in Chinese brake fern inoculated with different AM fungi

	Inoculation treatment	^{238}U concentration (mg kg^{-1})		^{238}U uptake ($\mu\text{g pot}^{-1}$)	
		First harvest	Second harvest	First harvest	Second harvest
Frond	-M	2.08	6.85	9.36	56.30
	Gln	2.69	6.17	8.45	49.77
	Glc	4.16	4.09	9.55	31.45
	Gli	4.97	9.03	9.83	60.86
	LSD	2.31		14.83	
Significance ^a of	Harvest	***		***	
	Inoculation	**		*	
	Interaction	*		*	
Root	-M	776	1027	1525	3722
	Gln	1040	1574	1298	5259
	Glc	1453	1128	1387	3420
	Gli	1534	1511	963	4321
	LSD	436		843	
Significance of	Harvest	NS		***	
	Inoculation	**		*	
	Interaction	*		**	

-M, non-mycorrhizal; Gln, *G. mosseae*; Glc, *G. caledonum*; Gli, *G. intraradices*.

^a By analysis of variance. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS, not significant.

tion in roots compared with non-mycorrhizal plants and those colonized by *G. caledonum*.

The frond to root ratios of total U uptake indicated that only a very small fraction of U was translocated to fronds (less than 2%, Fig. 3). The ratios were generally higher at the second than the first harvest ($P < 0.01$), not significantly affected by mycorrhizal colonization. TF values of U were much higher for roots than for fronds. For fronds the TF values were higher at the sec-

ond than at the first harvest ($P < 0.001$), but for roots there were no obvious differences (Fig. 4). There were significant interactions between harvest and inoculation recorded ($P < 0.05$). At the first harvest, mycorrhizal colonization tended to increase TF values for both the fronds and roots. At the second harvest, colonization with *G. intraradices* increased TF values for both fronds and roots, and colonization with *G. mosseae* increased the TF value for the roots.

Fig. 3. Frond to root ratio of total U uptake by Chinese brake fern inoculated with different AM fungi. -M, Glm, Glc and Gli represent non-inoculation control and inoculation with *G. mosseae*, *G. caledonium* and *G. intraradices*, respectively. By analysis of variance, U translocation ratios were only influenced by harvest ($P < 0.001$). Different letters above columns indicate significant differences among inoculation treatments at each harvest by LSD multi-comparison at the 5% level.

4. Discussion

The Chinese brake fern, *P. vittata* can hyperaccumulate As with a TF value greater than 10 (Ma et al., 2001). However, the TF values can be influenced by various soil and plant factors, such as As speciation in soils (Tu and Ma, 2002). TF values of *P. vittata* grown in field soils in China were between 0.07 and 7.42, decreasing with increasing soil As concentrations (Chen et al., 2002), and were generally lower than those reported by Ma et al. (2001) and Tu and Ma (2002). The ecotype of *P. vittata* was the same used in the present study as in the study by Chen et al. (2002), and the TF values we observed were similar to their report. *P. vittata* is a

mycorrhizal plant, and in the present study roots of *P. vittata* were highly colonized by different strains of AMF. However, mycorrhizal colonization depressed plant growth under our experimental conditions, particularly at the early stages. Growth depressions are not uncommon in mycorrhizal plants (Peng et al., 1993; Graham and Abbott, 2000), and are usually believed to result from carbon drain to the AM fungus that exceeds growth benefits from improved mineral nutrition (Johnson et al., 1997). In addition, U is known to be highly toxic to plants (Fellows et al., 1998), and the predicted no-effect concentration for chemical toxicity of U to terrestrial plants was proposed as 250 mg U kg^{-1} dry soil (Sheppard et al., 2005). Although soil U concentration was lower than this value in present study, U accumulated in roots of *P. vittata* to such high concentration could also possibly lead to U phytotoxicity, and thus other explanation of growth depression by AMF could possibly be U toxicity due to AM-mediated enhanced U concentrations in roots.

Despite the growth depression, mycorrhizal colonization tended to increase P concentration (especially at the first harvest) but had no effect on As concentration. This has resulted in increased P/As ratios and may enhance arsenic resistance of the brake fern. Sharples et al. (2000) reported that *Calluna vulgaris* formed an ericoid mycorrhizal association and the mycorrhizal fungus, *Hymenoscyphus ericae*, played a major role in plant resistance to As by selectively accumulating phosphate over arsenate. The work of Gonzalez-Chavez et al. (2002) demonstrated that AMF on mine spoils have evolved arsenate resistance, and conferred enhanced resistance on *Holcus lanatus*. The AMF strains used in present study were initially not isolated from contaminated areas, but still indicated the potential to facilitate P uptake under experimental conditions, which might help host plants surviving As contaminations. It's

Fig. 4. U transfer factor (TF) of Chinese brake fern from soil to fronds (a) and roots (b). -M, Glm, Glc and Gli represent non-inoculation control and inoculation with *G. mosseae*, *G. caledonium* and *G. intraradices*, respectively. By analysis of variance, harvest was highly significant for only fronds ($P < 0.001$), while inoculation was highly significant ($P < 0.01$) for both fronds and roots. Significant interaction ($P < 0.05$) of harvest with inoculation was observed for both fronds and roots. Different letters above columns indicate significant differences among inoculation treatments across harvests by LSD multi-comparison at the 5% level.

noticeable that under experimental conditions, due to growth depression by mycorrhizal colonization AMF did not increase total As uptake by Chinese brake fern, which was not in accordance with the findings by Liu et al. (2005). Quite obviously further investigations are needed to optimize the mycorrhizal associations, and the results also suggested that cautious considerations should be taken when introducing AMF into the phytoremediation strategies.

Phytoextraction has been proposed as a possible technique to clean up U-contaminated soils. Successful phytoextraction depends on the identification of a plant species capable of both taking up and translocating U aboveground, and the development of agronomic and/or amendment strategies to improve plant growth and the bioavailability of U in soils (Ebbs et al., 1998). Apart from chemical amendments, biological amendments, such as mycorrhizal symbiosis, have also been suggested to be included in phytoremediation (Khan et al., 2000; Leyval et al., 2002). To our knowledge, the present study is the first one addressing the effects of AMF on plant uptake of As and U from soil contaminated with a mixture of As and U. Our data demonstrated that the Chinese brake fern could highly accumulate U, with TF values for roots as high as 14, which were much higher than those reported so far (e.g., for sunflower and Indian mustard root bioaccumulation coefficients were 8.1 and 5.4, respectively; Shahandeh and Hossner, 2002). TF values for the roots were increased by the inoculation with AMF, especially for *G. intraradices*. However, translocation of U from the roots to the fronds was relatively low, and was also observed in many other plants (Saric et al., 1995; Shahandeh and Hossner, 2002). It is possible that the high TF values in mycorrhizal roots were the result of adsorption of U on or in mycorrhizal hyphae, and also likely U accumulation in intraradical hyphae due to efficient U translocation via hyphal pathway. It has been reported that U concentrations in mycorrhizal hyphae could be 5.5 and 9.6 times higher than for the mycorrhizal and non-mycorrhizal roots (Rufyikiri et al., 2003), respectively. Weiersbye et al. (1999) revealed that in mycorrhiza of *Cynodon dactylon* U accumulated in fungal vesicles, and decreased U translocation from plant roots to shoots were observed in very recent studies (Rufyikiri et al., 2004; Chen et al., 2005). These studies strongly supported the potential use of AMF to stabilize U contaminated environments.

Rhizofiltration, namely to remove contaminants using plants with extensive root systems and high accumulation capacity for contaminants, was proposed as one of the novel approaches for cleaning up of waste water. Some researchers noticed that sunflower could accumulate U in the roots with concentrations 5000–10000 times greater than that in the water (Entry et al., 1996). This plant also produces large biomass, and as a result was considered as best candidate for

treatment of U contaminated water (Dushenkov et al., 1995; Salt et al., 1995; Entry et al., 1996). Chinese brake fern is also reported as fast growing and high biomass plant with particular tolerance to As (Ma et al., 2001), and therefore it can be exploited for rhizofiltration purposes especially on As and U combined contaminated sites. The U sequestration in the fern would be further improved by introducing AMF into the system because of the high U accumulation in mycorrhizal roots. Thus mycorrhizal colonization may not only improve in situ retention of U by the Chinese brake fern, but may also increase U retention by the vast extraradical AMF hyphal network in the soil.

In conclusion, under U–As combined contamination TF values for As from soil to fronds of *P. vittata* were higher than 1.0, while AM colonization caused growth depressions but had no effect on tissue As concentrations. More importantly, to our knowledge the present study is the first one that addresses effects of AMF on plant uptake of As and U from the soil contaminated with a mixture of As and U. Our data demonstrated that the Chinese brake fern could highly accumulate U in roots. It could be planted on U mining tailings and near drainage sites for mining wastewater. The high levels of U sequestration in roots of the fern could retain U in situ, thus reduce the off-site impacts of U mining and processing activities. Due to the fact that symbiotic association to certain extent enhanced U accumulation in roots, U sequestration could be further improved by introducing AMF into the system. The variations in mycorrhizal effects on U accumulation supported the feasibility of screening effective AM fungal strains for remediation purposes.

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