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ARBUSCULAR MYCORRHIZAL SYMBIOSIS OF *VIOLA BAOSHANENSIS* AT BAOSHAN PB/ZN MINE IN CHINA

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Highlights:
- arbuscular mycorrhizal fungi (AMF) associated phytoremediation by hyperaccumulators;
- high level of AM colonization observed in naturally-occurring *Viola baoshanensis*;
- relative abundance of AMF species in the root zone determined by a morphological method.

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Abstract. Despite great potential for arbuscular mycorrhizal fungi (AMF) in restoration of heavy metals (HMs) polluted lands, limited information is available about the arbuscular mycorrhizal (AM) symbiosis of naturally-occurring hyperaccumulators. A preliminary survey was conducted to investigate the AM symbiosis of *Viola baoshanensis*, a Cadmium (Cd) hyperaccumulator, growing at an abandoned mine. Shoot/root ratios of 1.78 for Cd, and 2.57 for zinc (Zn) indicate that these two metals were preferentially transported from roots to shoots, whereas the ratio of 0.32 for lead (Pb) shows that most Pb was stored in roots. High level of colonization was found in the roots of *V. baoshanensis* with relative mycorrhizal root length of 69.1%, relative arbuscular richness of 46.9% and relative vesicular richness of 1.7%. Fifteen AMF species were identified from the root zone soil of *V. baoshanensis*. The dominant AMF genus was *Glomus,* and the most abundant species were *Glomus ambisporum* and *Claroideglomus etunicatum*.

Keywords: hyperaccumulator, arbuscular mycorrhizal fungi, heavy metals, phytoremediation, mycorrhizal colonization.

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1. Introduction

Heavy metal (HM)-contaminated soil is characterized by great HM toxicity, and poor nutrients and soil structure leading to reduced vegetation covering (Wong, 2003; Orłowska et al., 2005). Restoration of these sites and inhibition of the contamination in the surrounding soil via erosion and runoff are of great urgency (Vieira et al., 2018). Many plants, called metallophytes, grow at metal contaminated sites develop HM tolerance (Perrier et al., 2006; Antosiewicz et al., 2008). The utility of metallophytes to restore the HM-contaminated soils, known as phytoremediation, is an eco-friendly and cost-effective approach to remedy HM pollution compared with conventional methods (McGrath & Zhao, 2003; Rajkumar et al., 2012).

Hyperaccumulators, a rare group of metallophytes, are capable of accumulating HMs in the shoots at extraordinary high level without any symptoms of toxicity and can be used in phytoextraction, a key process of phytoremediation (Yang et al., 2019; Raklami et al., 2022). Thresholds for hyperaccumulation were set at 100 mg kg^{-1} Cd, 10000 mg kg^{-1} Zn and 1000 mg kg^{-1} Pb based on the measurement of shoot dry weight (Baker & Brooks, 1989). Bioconcentration factor (BCF) and translocation factor (TF) are regarded as important factors in determining whether a plant is a hyperaccumulator, and both factors should be greater than 1 (Baker et al., 1994; Tang et al., 2009), which may be appropriate for phytoextraction. An ideal candidate for efficient restoration requires fast growth and big shoot biomass (Wei et al., 2014), while most hyperaccumulating species grow slowly with small shoots (Maestri et al., 2010). To improve remediation efficiency, researchers have focused on studying the interaction between hyperaccumulators and soil microbes, especially the microbes that colonize the roots (Bandyopadhyay, 2022; Mei et al., 2022).

One of the most important plant symbiotic partners is arbuscular mycorrhizal fungi (AMF), as they can form symbiosis with most higher plants, connecting roots with soil (Smith & Read, 1997). AMF can efficiently improve water and minerals uptake of plants contributing to enhanced plant tolerance against HM stress. Not surprisingly plants are often colonized by AMF in many HM-contaminated soils (Whitfield et al., 2004; Sánchez-Castro et al., 2017). Initially, hyperaccumulators were thought to be non-mycorrhizal and likely due to the trade-off between hyperaccumulation and mycorrhization as both require carbon investment from plants (Leyval et al., 1997; Audet, 2013). In 2003, however, *Berkheya coddii* was found to be colonized by AMF (Turnau & Mesjasz-Przybylowicz, 2003). Since then, several mycorrhizal hyperaccumulators have been documented (e.g., Wu et al., 2007; Rashid et al., 2009; Wei et al., 2014; Lu et al., 2020; Guzmán-Cornejo et al., 2023). It has been demonstrated that AMF are able to enhance biomass and survival of hyperaccumulators, thus showing a great potential for restoration of polluted lands (Al Agely et al., 2005; Orłowska et al., 2013; Li et al., 2017).

However, the mechanisms of arbuscular mycorrhizal (AM) symbiosis involved in hyperaccumulation of HMs remain unraveled, as the effects of AMF on heavy metals uptake by hyperaccumulating plants are not consistent (Alford et al., 2010). Some AMF were found to reduce (Liu et al., 2005; Jankong et al., 2007), or increase HM accumulation by plant (Wu et al., 2009; Orłowska et al., 2013), whereas others have mixed effects (Vogel-Mikuš et al., 2006) or no effects (Liu et al., 2009) on plant uptake. Up to date, only 4% of over 700 reported hyperaccumulators have been studied regarding AM symbiosis (Wang et al., 2021). Thus, the knowledge about interactions between plants, AMF and HMs is still limited (Dietterich et al., 2017). An equilibrium have been reached between AMF, host plants, and HMs over time in nature (Zarei et al., 2008), therefore investigations of AMF diversity at mining sites could provide a deeper interpretation of the interaction between AMF and hyperaccumulators in the process of restoration (Sánchez-Castro et al., 2017; Kushwaha et al., 2022).

Viola baoshanensis Shu, Liu and Lan has been found as a Cd hyperaccumulator in a field survey (Liu et al., 2004), and also a strong Zn and Pb accumulator by hydroponic and pot experiments (Wu et al., 2010). Cd and Zn was extracted with high efficiency in a field study in this species (Zhuang et al., 2007) and AM colonization was observed by pot experiments (Zhong et al., 2012). However, the AM status of native *V. baoshanensis* at the mining site remains unclear. The objectives of the present survey include (1) determining the uptake of Cd, Zn and Pb by *V. baoshanensis* in relation to soil HM concentrations; (2) examining the AM colonization status of *V. baoshanensis* in relation to soil chemo-physical properties; (3) investigating the diversity of AMF in the root zone of *V. baoshanensis*.

2. Material and methods

2.1. Site description and sampling

Field sampling was done at Baoshan Pb/Zn Mine (25°42′N, 112°35′E), Guiyang County, China, with a long history of mining and being abandoned in 1996 (Liu et al., 2004). The annual mean temperature is 17.2 °C and rainfall is 1385 mm in Guiyang County. *V. baoshanensis* is a perennial herb and only found at the Baoshan Pb/Zn mine with an elevation of 400–650 m (Liu et al., 2004). The sampling site was a slope (about 10 m high and 50 m long) near the edge of the open-pit mine, where the vegetation covering was scarce. *V. baoshanensis* grew separately as a pioneer plant and there was no overlapping leaves and roots with other plants. Plants together with the root zone soils were sampled at the flowering stage of *V. baoshanensis* in May, 2018. In total, 35 plants of *V. baoshanensis* were randomly collected, and about 500 g soil from root zone was collected per plant at a depth of 0–20 cm and stored at 4 °C for analysis in the laboratory.

2.2. Chemical analyses

Fresh soil was sieved with 2 mm mesh. One part was stored at 4 °C for the spore counting and identification of AMF, while the remaining part was air-dried for the chemical analyses. 0.5 g air-dried soil was dissolved with HNO_3 + HCl + $HClO_4$ (1:3:1) to determine the total Cd, Zn and Pb concentrations. Diethylenetriamine- pentaacetic acid (DTPA) was adopted to measure the extractable Cd, Zn and Pb concentration with 10.0 g air-dried soil (Lindsay & Norvell, 1978). The Cd, Zn and Pb concentrations were measured by Inductively-coupled optical emission spectrometry (ICP-OES, iCAP6300, Thermo Electron, Waltham, MA, USA). To determine soil phosphor concentration, 0.5 g air-dried soil was dissolved by $HClO_4$ and measured by vanadium-molybdenum method. Soil pH was determined in a suspension (5:1, deionized water:soil), and measured by a pH electrode.

Plant shoots and roots were separated and about 1 g fresh fine roots was left for observation of root AM colonization. The shoots and remaining roots were dried at 80 °C for 48 hr, and 0.2 dried material was taken to determine the Cd, Zn and Pb concentration with ICP-OES as described above. The accumulation and transfer of Cd, Zn and Pb by *V. baoshanensis* was described by the translocation factor and the bioconcentration factor.

2.3. Root AM colonization

Fine roots of *V. baoshanensis* were prepared as 1 cm fragments, treated with 10% potassium hydroxide and then 0.01% trypan blue with the method modified from Koske and Gemma (1989). Fifty root segments per plant were examined by a light microscope (Nikon, ANTI-MOULD, Japan) with 200×magnification to measure the colonization rate by the magnified intersection method (McGonigle et al., 1990). Mycorrhizal parameters, such as mycorrhizal frequency (F%), relative mycorrhizal root length (M%: hyphae, vesicles, and arbuscules), relative arbuscular richness (A%) and relative vesicular richness (V%) were used to assess the mycorrhizal status of *V. baoshanensis*.

2.4. Spore extraction and identification

Spores were extracted from soils by wet sieving and decanting, then centrifuged with sucrose density gradient

(Tommerup & Kidby, 1979). The isolated spores were identified with polyvinyl alcohol-lactic acid glycerol (PVLG) and Melzer's reagent by a light microscope (Nikon, RX50, Japan) at 400-fold magnification. Spore identification was based mainly on morphological features including spore size and color, wall structures and subtending hyphae. Broken spores were not counted in the total number of AMF spores, while the remaining spores were taken pictures for further identification of AMF species and counted in total number of spores. Classification and taxonomic identification of AMF is according to identification reports (Oehl et al., 2011; Redecker et al., 2013; Bills & Morton, 2015), species description from INVAM homepage (http://www.invam.caf.wvu.edu), and other institutional collection of original species. Relative abundance of each species in one sample was determined by the percentage of spores of a species in relation to those of all species (Del Val et al., 1999).

2.5. Statistical analysis

The SPSS software package (Version 17.0) were used to conduct all statistical analyses. Correlation analysis was performed by Pearson's test ($P < 0.05$). Variables of HM concentrations in plants and relative abundance of AMF species were analyzed by one-way analysis of variance (ANOVA). Homogeneity of variance test was conducted followed by Post Hoc Multiple Comparisons using the Duncan test ($P < 0.05$). Data sets of root colonization, HM concentrations in soil and plant shoots were analyzed by a principal components analysis (PCA).

3. Results and discussion

3.1. Soil chemical properties

The root zone soil of *V. baoshanensis* was slightly alkaline (pH = 7.60) and heavily polluted by Cd, Zn and Pb with maximal concentrations of 990 mg kg^{-1} Cd, 16 548 mg kg^{-1} Zn, and 20111 mg kg^{-1} Pb. A wide variability in metal concentrations from 35 soil samples was observed, which was also observed by Liu et al. (2004). Heavy metal concentrations in root zone soil were shown in Table 1. Total soil phosphorous concentration was 857±57 mg kg^{-1}.

Table 1. Heavy metal concentrations in the root zone soil at Baoshan Pb/Zn mine (Mean ± S.E., n = 35)

Total Metal (mg kg^{-1})			Extractable metal[a] (mg kg^{-1})		
Cadmium	Zinc	Lead	Cadmium	Zinc	Lead
147±30	7290±720	7239±694	36±7	421±42	506±57

Note: [a] Extractable metal = Diethylenetriaminepentaacetic acid (DTPA) extractable metal.

DTPA-extractable fractions of totals ranged from 6.1 to 33.6% for Cd, from 0.9 to 9.7% for Zn, and from 4.5 to 13.1% for Pb, while the means for extractable Cd, Zn, and Pb were 25%, 6% and 7%, respectively. Total and DTPA-extractable concentrations correlated significantly as follows: Cd ($r = 0.990$, $p < 0.01$), Zn ($r = 0.767$, $p < 0.01$), and Pb ($r = 0.871$, $p < 0.01$). As the soil was slightly alkaline, it is reasonable that the bioavailability of these metals was low, which is consistent with the previous results at the same site (Wu et al., 2010). The DTPA-extractable Cd, Zn, and Pb can be considered as the bioavailable fraction, and they significantly correlated with the total concentrations in this survey, which is consistent with the results of previous studies (Xu et al., 2019; Luo et al., 2022). As the bioavailable HMs reduced soil microbial diversity and activity, soil fertility was decreased and the vegetation covering was scarce at HM mining sites (Luo et al., 2022).

3.2. Cd, Zn, Pb and Phosphate accumulation in *V. baoshanensis*

As shown in Table 2, the shoot Cd concentration (250 mg kg^{-1}) was > 100 mg kg^{-1}, and the BCF (3.06) and TF (1.78) were both >1, confirming the hyperaccumulation of Cd by *V. baoshanensis*. The BCF and TF for Cd observed in this study are comparable to the results of Liu et al. (2004) at the same site, which were 2.38 and 1.32, respectively. Significant correlations were found between shoot Cd concentration and total Cd ($r = 0.484$, $p < 0.01$), extractable Cd ($r = 0.499$, $p < 0.01$) concentration in soil. Additionally, significant correlations were also observed between root Cd concentration and total Cd ($r = 0.753$, $p < 0.01$), or extractable Cd ($r = 0.747$, $p < 0.01$) concentration in soil.

In contrast, there was no significant correlation between Zn concentration of plants and total soil Zn concentration ($r = 0.153$, $p > 0.05$) or extractable Zn concentration ($r = 0.007$, $p > 0.05$). Although the TF of Zn was 2.57 indicating very efficient transport from roots to shoots in *V. baoshanensis*, the shoot Zn concentration was much lower than the threshold (10,000 mg kg^{-1}), thus no hyperaccumulation of Zn was observed, which was also verified by the low BCF value (0.15).

In addition, the TF of Pb for *V. baoshanensis* was 0.32, indicating that most of the Pb accumulated was immobilized in roots. There were significant correlations between shoot Pb concentration and total soil Pb concentration ($r = 0.398$, $p < 0.05$) or extractable soil Pb concentration ($r = 0.361$, $p < 0.05$), while root Pb concentration significantly correlated with total soil Pb concentration ($r = 0.621$, $p < 0.01$), and extractable soil Pb concentration ($r = 0.673$, $p < 0.01$). The BCF value for Pb was much lower than those for Cd and Zn.

Accumulation and exclusion are two main approaches taken by metallophytes growing on sites with high concentrations of HMs, which are commonly determined by BCF and TF (McGrath & Zhao, 2003). TF over 1 in metal accumulator species are normal, whereas those in metal excluder species are often less than 1 (Baker, 1981). In this survey, the shoot Cd concentration exceeded the threshold, and the BCF and TF for Cd were both greater than 1, thus confirming hyperaccumulation of Cd in *V. baoshanensis* (Liu et al., 2004) and has the potential application for remediation of polluted soils (Zhuang et al., 2007).

Table 2. Heavy metal concentrations (mg kg^{-1}, DW), Bioconcentration factor (BCF) and Translocation factor (TF) of *V. baoshanensis* growing at Baoshan Pb/Zn mine (Mean ± S.E., n = 35)

Metal	Shoot	Root	BCF	TF
Cadmium	250±19 a	165±17 a	3.06±0.43 a	1.78±0.13 a
Zinc	719±32 b	705±134 b	0.15±0.02 b	2.57±0.46 b
Lead	159±12 c	546±44 b	0.03±0.004 b	0.32±0.02 c

Values denoted with the same letters are not statistically different by the Duncan test at the level of 5% significance.

The TF for Cd (1.78) of *V. baoshanensis* is comparable to that of a Cd, Zn hyperaccumulator, *Sedum alfredii*, naturally growing at a mining site with a TF of 1.87 (Yang et al., 2019). In addition, *Thlaspi praecox*, Cd and Zn hyperaccumulator from the surrounding area of a mine and smelter, had a TF of 1.4 for Cd (Vogel-Mikuš et al., 2005). Since only 10 species have been confirmed as Cd hyperaccumulators across the world (Wang et al., 2021), and Cd lists first in the pollution caused by heavy metals and metalloids in China (Riaz et al., 2021), the potential utilization of *V. baoshanensis* is vital for remediation of soils contaminated by Cadmium.

As shoot concentration of Zn was lower than 10000 mg kg^{-1}, *V. baoshanensis* was not a Zn hyperaccumulator. However, the TF of *V. baoshanensis* for Zn was 2.57, indicating there was efficient transfer of Zn from roots to shoots and it is comparable to those for Zn of *S. alfredii* and *T. praecox*, which was 1.24 and 3.2, respectively (Vogel-Mikuš et al., 2005; Yang et al., 2019). Additionally, the TF of *V. baoshanensis* for Pb was 0.32, indicating that most of Pb uptake were stored in roots, which is consistent with previous findings, as the TF (0.30) for Pb were commonly observed in *Thlaspi* species (Vogel-Mikuš et al., 2005), and that of *S. alfredii* was 0.28 (Yang et al., 2019).

3.3. Root colonization by AMF

Despite the harsh soil conditions, high level of AM colonization was observed for *V. baoshanensis* growing at the abandoned mine with mean F% of 94.5%, and mean M% of 69.1% ranging from 51.3 to 83.8%. The roots of *V. baoshanensis* were extensively colonized by inter-cellular hyphae, forming well-developed arbuscules within the cortical cells, exhibiting typical Arum type mycorrhizal colonization. The average A% was 46.9%, while the average V% was only 1.7%. The result indicated that the symbiosis between AMF and *V. baoshanensis* was well established regardless of high HM concentrations.

Although negative effects of HMs on root colonization were observed, correlations were not significant between AM colonization (M%) and extractable Cd (r = –0.135, p > 0.05), extractable Zn (r = –0.039, p > 0.05) and extractable Pb (r = –0.100, p > 0.05) in the soil. The results of PCA indicated that three first components accounted 73% of the total variance. PC1 explained 49%, where concentrations of shoot Cd, total soil Cd, extractable Cd were the most relevant parameters. PC2 contributed 12% and was associated mainly with concentrations of Shoot Pb, total soil Zn, and extractable Zn, while PC3 accumulated 12% and most related parameters were shoot P concentration and mycorrhizal colonization (%M).

The developmental stage of the plants is one of the factors influencing AM colonization. Flowering is a development reference point for the determination of AM status in calamine plants (Pawlowska et al., 1996), as the enhanced elemental demand during flowering favors mycorrhizal colonization, thus offering adequate essential elements (Pongrac et al., 2007). Therefore, the plants of *V. baoshanensis* in this survey were collected at the flowering stage.

It has been suggested that selective pressure would decrease colonization unless the symbiosis supplies some benefits to the host (Whitfield et al., 2004). There is a mechanism for AMF to alleviate toxicity of heavy metals to plants by storing them in mycorrhizal structures including hyphae, arbuscules, vesicles, etc., where large quantities of heavy metals are concentrated (Dhalaria et al., 2020). The colonization rate of *V. baoshanensis* (M% = 69.1%) is well above 30%, which is considered as the threshold of a functional symbiosis (Zarei et al., 2010). In addition, there is evidence that inhibition of AM development reduced the tolerance of apple to Cd stress (Huang et al., 2021), suggesting that the colonization status may correlate with HM tolerance to some extent. In contrast, several hyperaccumulators belonging to the genus *Thlaspi* are poorly colonized by AMF (Regvar et al., 2003; Vogel-Mikuš et al., 2005). However, *Viola tricolor* L. growing on a calamine spoil mound was also found to be well colonized by AMF with colonization rate above 60% (Pawlowska et al., 1996), showing that the colonization status may depend on the plant species.

As nutrient exchange mainly takes place in arbuscules (Smith & Read, 1997), the arbuscular richness is considered as the most useful parameter for measuring mycorrhizal status (Orłowska et al., 2005). The abundant arbuscules in the roots of *V. baoshanensis* (A% = 46.9%) also indicates a functional symbiosis. In contrast, A% of Cd hyperaccumulators *Desmostachya bipinnata* and *Dichanthium annulatum* were 7% and 6%, respectively (Rashid et al., 2009). On the other hand, the relative vesicular richness is quite low, which could be the result of seasonal sporulation of AMF or colonization of no-vesicle-producing species (Oehl et al., 2009; Melo et al., 2019), or the nutrient exchange still in function.

3.4. AMF spore abundance and diversity

Spores with intact walls were taken as healthy spores, while spores with decaying walls or without distinctive characters were not identified to species level but counted in the total number of AMF spores. In this study, only 81.7% of the extracted spores were identified to species level. Identified spores ranged from 63 to 237 per 100 g soil. A total of 15 AMF species from 7 genera and 3 families of Glomeromycota were identified in the root zone soil of *V. baoshanensis* (Table 3). Most of the species found belong to the family Glomeraceae (12 species), and most of spores identified belonged to this family (Table 3). In terms of species richness the most abundant genus was *Glomus* followed by the genus *Rhizophagus*, while the relative abundance of *Glomus* was 44.0% followed by *Claroideglomus* (18.5%) (Figure 1). In addition, *Glomus ambisproum* and *Claroideglomus etunicatum* were the most abundant species in the root zone soil of *V. baoshanensis* (Table 3).

The traditional way of AMF identification is based on spore structures, which may be flawed owing to limited discriminating features (Young, 2012). Recently molecular techniques have been developed for more accurate identification of AMF species.

Table 3. Relative abundance (mean, n = 35) of AMF species in the root zone of *Viola baoshanensis* at Baoshan Pb/Zn mine

Current names of species	Relative abundance (%)
Funneliformis mosseae C. walker & A. Schuessler	3.58 a–c
Glomus ambisporum G. S. Sm. & N. C. Schenck	14.20 h
Glomus brohultii R. A. Herrera, Ferrer & Sieverd.	6.44 b–g
Glomus formosanum C. G. Wu & Z. C. Chen	7.59 c–g
Glomus sp	9.80 f, g
Glomus hoi S. M. Berch & Trappe	6.00 a–g
Rhizophagus aggregatus C. Walker	4.89 a–e
Rhizophagus fasciculatus C. Walker & Schuessler	3.83 a–d
Rhizophagus invermiaum C. Walker	1.82 a
Sclerocystis rubiformis Gerd. & Trappe	7.92 d–g
Septoglomus constrictum Sieved., G. A. Silva & Oehl	8.86 e–g
Septoglomus deserticola G. A. Silva, Oehl & Sieverd.	2.39 a, b
Claroideglomus claroideum C. Walker & Schuessler	6.75 c–g
Claroideglomus etunicatum C. Walker & Schuessler	11.79 g, h
Ambispora leptoticha C. Walker, Vestberg & Schuessler	4.17 a–d

Note: values denoted with the same letters are not statistically different by the Duncan test at the level of 5% significance.

Most used genetic data were nuclear rRNA genes of small and large sub-units or nuclear internal transcribed spacer region (Krüger et al., 2012). The AMF in the rhizoshpere or the roots could be quite different from those in bulk soil and the sporulation of many species is seasonal

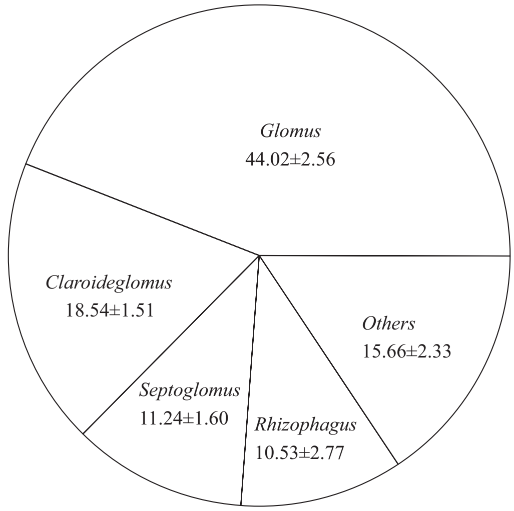

Figure 1. Percentage of genera ± S.E. (% of the total spores) in the root zone of *V. baoshanensis* at Baoshan Pb/Zn mine

(Oehl et al., 2009; Melo et al., 2019). In this survey, only the morphological method was adopted to identify AMF species in root zone soil of *V. baoshanensis* due to financial reasons and the samples were collected only once in May, so the results of species abundance should be treated with caution.

The dominant AMF were Glomeraceae species in HMs polluted areas such as mining tailings, which is a common observation worldwide (e.g., Zarei et al., 2010; Sun et al., 2016), showing that fungi in this family are more disturbance-tolerant than those in family Acaulosporaceae and Gigasporaceae (van der Heyde et al., 2017). At sites polluted by HMs, soil pH has been found as an important factor in determining the AMF community structure (Sun et al., 2016; Vieira et al., 2018). *Acaulospora* was widely observed in acid soils and *Gigaspora* are tolerant for acid pH (Clark, 1997; van der Heyde et al., 2017), while *Glomus* are normal in neutral and alkaline soils (da Silva et al., 2005). In consistent with the findings, the most abundant genus in the root zone soil of *V. banshanesis* was *Glomus*, as the pH of the soil is slightly alkaline at Baoshan Pb/Zn mine. In addition, species from genus *Claroideglomus* have been found to be tolerant to HMs (Cornejo et al., 2013; Park et al., 2016) and *Claroideglomus* was the second abundant genus in this survey.

Most of the AMF species identified in this survey have already been found in soils with high concentrations of HMs. Spores of *G. ambisporum* was found at a mine polluted by Zn, Cd and Pb in southeast Kansas (USA), where *S. constrictum* was abundantly found (Shetty et al., 1994). *C. etunicatum* was identified from five of six sites in a copper mine (da Silva et al., 2005), meanwhile *C. etunicatum* was the most abundant species at an abandoned mine, where *S. constrictum* and *C. claroideum* were also abundantly observed (Park et al., 2016). *F. mosseae* is normally observed in soils polluted by Zn and Pb (Turnau et al., 2001; Zarei et al., 2008), which is confirmed by the result of this study. The species identified in this survey have potential applications since indigenous AMF should be extracted and used in phytoremediation (Vieira et al., 2018).

Hyperaccumulators with associated AMF are promising for restoration of lands polluted by HMs (Wang et al., 2021; Raklami et al., 2022), and the appropriate combination of hyperaccumulators and AMF is very important (Vogel-Mikuš et al., 2006; Miransari, 2011). Indigenous AMF from long-term polluted sites have been found more suitable than non-native ones, therefore knowledge of native AMF is relevant for phytoremediation (Sánchez-Castro et al., 2017; Suárez et al., 2023). Therefore, roles of the dominant AMF in the remediation needs further investigation, and molecular techniques should be used to identify the AMF species colonized in the roots of *V. baoshanensis* for unraveling the mechanisms associated with restoration.

4. Conclusions

High level of Cd, Zn and Pb were found in root zone soil of *V. baoshanensis* growing naturally as a pioneer plant at Baoshan Pb/Zn mine. Hyperaccumulation of Cd by *V. baoshanensis* was confirmed in this survey. Efficient Zn transfer form roots to shoots was observed, while most of Pb were stored in the roots. The roots of *V. baoshanensis* were extensively colonized by AMF indicating that a functional symbiosis was established between plants and AMF regardless of high concentrations of HMs. The AMF in root zone of *V. baoshanensis* should be HM tolerant and enhance plant tolerance against HMs through the symbiosis. The results of this study show the potential application of AMF in restoration of HM-contaminated soils. Molecular techniques should be used to identify AMF species in the rhizosphere and roots of *V. baoshanensis* in future investigation.

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Zhong Weiliang is the only author of this manuscript and no other author contributes to the manuscript.

Disclosure statement

The author declares no competing interests.

Data availability statements

The data that support the findings of this study are not openly available due to sensitivity of the data and are available from the corresponding author upon reasonable request.

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