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RESEARCH ARTICLE

Assessment of arbuscular mycorrhizal fungi status and heavy metal accumulation characteristics of tree species in a lead–zinc mine area: potential applications for phytoremediation

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Abstract To select suitable tree species associated with arbuscular mycorrhizal fungi (AMF) for phytoremediation of heavy metal (HM) contaminated area, we measured the AMF status and heavy metal accumulation in plant tissues in a lead–zinc mine area, Northwest China. All 15 tree species were colonized by AM fungi in our investigation. The mycorrhizal frequency (F%), mycorrhizal colonization intensity (M%) and spore density (SP) reduced concomitantly with increasing Pb and Zn levels; however, positive correlations were found between arbuscule density (A%) and soil total/DTPA-extractable Pb concentrations. The average concentrations of Pb, Zn, Cu and Cd in plant samples were 168.21, 96.61, 41.06, and 0.79 mg/kg, respectively. *Populus purdomii* Rehd. accumulated the highest concentrations of Zn (432.08 mg/kg) and Cu (140.85 mg/kg) in its leaves. Considerable amount of Pb (712.37 mg/kg) and Cd (3.86 mg/kg) were concentrated in the roots of *Robinia pseudoacacia* Linn. and *Populus simonii* Carr., respectively. Plants developed different strategies to survive in HM stress environment: translocating more essential metals (Zn and Cu) into the aerial parts, while retaining more toxic heavy metals (Pb and Cd) in the roots to protect the above-ground parts from damage. According to the translocation factor (TF), bioconcentration factor (BCF), growth rate and biomass production, five tree species (*Ailanthus altissima* (Mill.) Swingle, *Cotinus coggygria* Scop., *P. simonii*, *P. purdomii*, and *R. pseudoacacia*) were considered to be the most suitable candidates for phytoextraction and/or phytostabilization purposes. Redundancy analysis (RDA) showed that the efficiency of phytoremediation was enhanced by AM symbioses, and soil pH, Pb, Zn, and Cd levels were the main factors influencing the HM accumulation characteristics of plants.

Keywords Phytoremediation · Arbuscular mycorrhizal fungi · Heavy metal accumulation · Tree species

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Introduction

Soil contamination with toxic heavy metals (HMs) is a serious and widespread issue that resulted from both natural and anthropogenic activities. It has aroused a lot of attention because HMs impose many negative effects on ecosystems and natural resources and thereby pose a danger to human health by contaminating the food chain or water supply (Peuke and Rennenberg 2005; Ren et al. 2014). Heavy metals in soils are derived from the soil parent material and various anthropogenic sources (Alloway 2013). With the development of society, a variety of human activities such as mining, smelting, electroplating, etc. induce serious HM pollution in soils and bring about hazards to the whole ecological environment, including terrestrial, aquatic, and atmosphere ecosystems. Heavy metals cannot be degraded easily like organic pollutants and can therefore constitute a persistent environmental hazard (Farrell et al. 2010).

A variety of methods have been developed for heavy metal remediation in contaminated areas, and they can be roughly divided into physical, chemical, and biological approaches based on processing techniques. The physical remediation can be mainly classified to replacement method and thermal desorption (Yao et al. 2012). The soil replacement method refers to dilution of the heavy metal concentrations and increasing the soil environmental capacity via replacing or partly replacing the polluted soils, while the thermal desorption method means to make the pollutant volatile using steam, microwave, and infrared radiation technologies. In the chemical processes, different types of chemicals are used to change the chemical structure of the pollutants in order to reduce the amount of toxic compounds. However, both of the physical and chemical treatments are expensive and might cause serious destruction of soil structure and reduce bioactivity and fertility of the soil (Wang et al. 2010).

As a cost-effective, environmental friendly method, the biological remediation, especially the phytoremediation, has attracted more attentions from society during the last decade (De Moor et al. 2013). Two main subgroups of phytoremediation have been widely used to remediate the polluted soils: phytoextraction and phytostabilization (Vangronsveld et al. 1995). Phytoextraction refers to the use of plants for extraction and accumulation of pollutants in their tissues, followed by harvesting of the above-ground plant material (Pilon-Smits 2005). Phytostabilization focuses on the use of plants for sequestration of heavy metals in rhizosphere to reduce HM bioavailability (Mendez et al. 2007). Both phytoextraction and phytostabilization show promising future in ecological restoration of mine tailings and remediation of HM polluted soils (Pilon-Smits 2005).

Grasses are thought to be excellent candidates for phytomediation because of their heavy metal tolerance, high biomasses, and fast growth characteristics (Kulakow et al. 2000). However, compared to grass, woody plants live longer and have a higher tolerance to poor nutrient conditions. Woody plants might be more suitable candidates for phytoremediation also because of their deep root systems, high transpiration rate, and high metal tolerance (Hu et al. 2013).

Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that develop mutual symbiotic association with most terrestrial plants, and they provide a direct physical link between soil and plant roots (Bothe et al. 2010). Numerous studies have indicated that AMF can enhance the host plants ability to grow in HM contaminated soils (Miransari 2011; Curaqueo et al. 2014) via strategies such as: (1) enhancing heavy metals sequestration or accumulation (Singh 2012), (2) improving nutrition uptake (Morgan and Connolly 2013), (3) improving soil enzyme activities (Qian et al. 2012), (4) influencing microorganism community of rhizosphere (Xu et al. 2012), and (5) regulating root exudates of host plants (Aggarwal et al. 2011). Using plants engaged in symbiosis with AMF and with the ability to sequestering or accumulating high amounts of heavy metals may be a promising way for bioremediation in heavy metal-polluted areas (Miransari 2010). However, the status of AMF in heavy metal-contaminated area varies, depending on plant and AMF species, soil conditions such as nutrient status, and climate (Smith and Read 1996).

Although numerous studies aimed at identifying suitable plants for phytoremediation in heavy metal-contaminated soils (García-Salgado et al. 2012), there are very few studies focusing on woody plants. Furthermore, our study area is located in Qinling Mountain of northwestern China with unique and special geography and climate conditions. As ideal phytoremediation materials, woody plants should adapt to local environment, such as climate, soil, and vegetation characteristics, so it is important to select suitable woody plants in symbiosis with suitable AMF species for phytoremediation because both woody plants and AMF species should adapt to the native soil properties, toxicity levels, and climate conditions (Xue et al. 2014). The objects of our study were (1) to assess the AMF status in heavy metal-contaminated area; (2) to evaluate the heavy metal tolerance, accumulation, and translocation characteristics of different tree species in study sites; and (3) to select suitable woody plants in symbiosis with AMF for phytoremediation. We determined the concentrations and enrichment capacity of Pb, Zn, Cu, and Cd in different tissues of 15 tree species in four lead–zinc mine sites. Our study could provide experimental evidence for using suitable woody plants engaged in symbiosis with AMF to remediate HM-polluted area.

Materials and methods

Study area

The study was conducted in Feng County (106° 24′ 54″ E–107° 7′ 30″ E, 33° 34′ 57″ N–34° 18′ 21″ N), which is located at the south foot of Qinling Mountain, Northwest China. The total area of Feng County is 3187 Km^2, with a population of approximately 110,000. The county was in the warm temperate semiarid, and the annual average temperature is 11.4 °C. The annual average rainfall and frost-free period of this county are 613.2 mm and 188 days, respectively. The main soil types are cinnamon and brunisolic soil according to the traditional soil genesis classification in China, and the soil texture is from light to heavy (Xu et al. 2012). The mineral resources are very abundant, and the mining industry has become an important economic mainstay in Shaanxi Province. Specifically, mineral resources of Pb–Zn are mostly distributed in Feng County-Taibai region, and its annual output accounts for 72 % of the entire provincial output. Feng County, with an annual 100,000 tons of zinc (Zn) concentrates, 30,000 tons of lead (Pb) concentrates, 10,000 tons of electrolytic lead, and 5000 tons of lead alloy production capacity, has become one of the four largest Pb–Zn bases in China. However, due to the traditional development model, which focuses more on economic growth using less advanced technologies and neglects environmental protection, a large amount of waste from factories has caused serious environmental pollution.

Qiandongshan lead and zinc region is the largest and the most typical five national nonferrous metal planning mines and accounts for 25.3 % of the total reserves of Feng County (Yao et al. 2004). The predominant pollution sources in this region are mine wastewater, beneficiation wastewater, and mine tailings (Hou et al. 2003). According to our previous study (Xu et al. 2012), four sites were chosen for the collection of plant and soil samples in August and September in 2011 (Fig. 1): Site 1 (S1) was a new mine-tailing pond, site 2 (S2) was an old mine-tailing pond, site 3 (S3) was a mine area, and site 4 (S4) was an abandoned smelter chimney. At each site, five subquadrates with dimension of 15×15 m were selected randomly, and all tree species in the subsample were determined (Fig. 1).

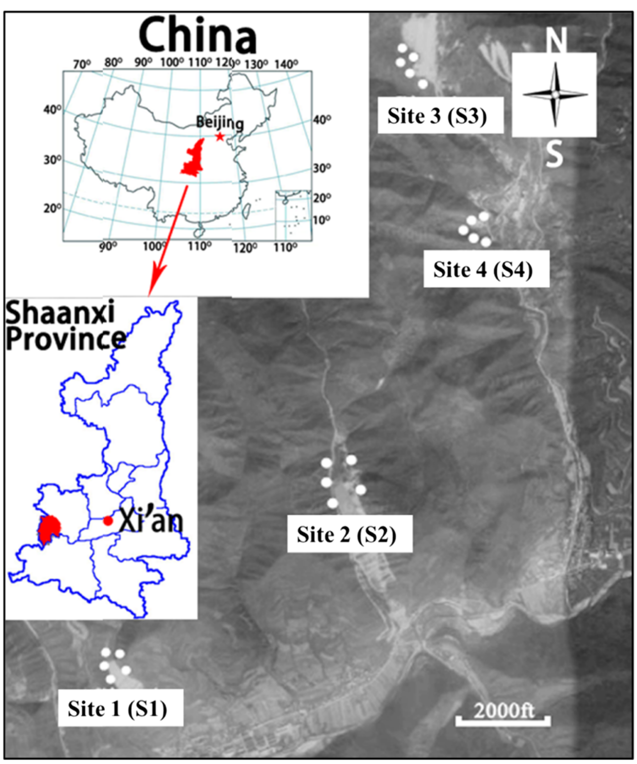

Fig. 1 Map of Qiandongshan lead and zinc region with the localization of four study sites

Collection of plant and soil samples

All trees growing in the study area were recorded, and the relative abundance of each species was estimated visually and then described as dominant, frequent, occasional, or rare (Yang et al. 2014) (Table S1). According to the vegetative state and cover area, a total of 15 tree species were recorded (13 from S1, 11 from S2, 9 from S3, and 6 from S4, respectively) and the plant samples included roots, woods, barks, branches, and leaves were collected from four study sites. Portions of each composite root samples were contained in centrifuge tubes filled with formaldehyde-acetic acid alcohol (FAA) for mycorrhizal colonization (MC) analysis. The soil samples (0–20 cm) from four different directions where the plants were grown were also collected in every subsample site using a stainless steel spade and then mixed in self-sealing plastic bags. The spade was washed with deionized water and wiped dry with paper towels between each use (Guo et al. 2012).

Plant and soil samples analysis

Different plant samples were washed thoroughly with tap water and rinsed with deionized water for five times to remove surface dust and soil, then dried at 80 °C in an oven for 48 h until constant weight. The samples were crushed by microphyte disintegrator (FZ102, Tianjing, China), and then grounded into fine powder in an agate mortar. The concentrations of Pb, Zn, Cu, and Cd in plant samples were determined according to the method described by Allen (1989). The samples were completely digested with concentrated HNO_3 (16 mol/L) and $HClO_4$ (12 mol/L) at the rate of 5:1 (*v/v*). The metal concentrations were determined with flame atomic absorption spectrometer (FAAS, Hitachi Z-2000, Tokyo, Japan).

The collected soil samples were transported to the laboratory, air-dried at room temperature, ground to a fine powder in an agate mortar, and then sieved through a 10 mesh (<2 mm) and an 80 mesh (<180 μm). The finely grounded powder (<180 μm) was used to determine the chemical property, and the powder <2 mm was used to measure the pH and electrical conductivity (EC). All handling procedures were carried out without contacting any metals to avoid potential cross-contamination of the samples. The soil pH was determined according to the international analysis method of ISO 10390:2005 (Leici PHS-3D, Shanghai, China), and the EC was determined by conductivity meter (DDSJ-308A, Zhejiang, China). Organic matter (OM) content was measured by dichromate oxidation and titration with ferrous sulfate (Nelson and Sommers 1982). Total nitrogen (TN) content was determined according to the semi-micro Kjeldahl method (Bremner and Mulvaney 1982). Soils were digested by HF-$HClO_4$ (Jackson and Barak 2005), and then the total P (TP) and K (TK) contents were analyzed according to molybdenum-blue colorimetry and flame photometry methods, respectively. The total and DTPA-extractable heavy metal concentrations were determined by flame atomic absorption spectrometry (FAAS, AA-7003A, Beijing, China), following the digestion of a 0.5-g soil sample with aqua regia (HNO_3/HCl=1:3) and $HClO_4$, DTPA solution (0.005 mol L^{-1} diethylene triamine penta-acetic acid (DTPA)+0.01 mol L^{-1} $CaCl_2$+0.1 mol L^{-1} triethanolamine, pH=7.3). The blank reagent and standard reference soils were analyzed for quality assurance and quality control. All of the results were calculated from the triplicate of the analytical data.

AMF colonization and spore density analysis

To evaluate AMF colonization, the root samples were washed with tap water and then cut into about 1-cm length segments. The method modified from Koske and Gemma (1989) was used to clean and stain root samples. The segments were first softened in 2.5 % KOH at 90 °C for 1 h, bleached in alkaline hydrogen peroxide at room temperature for 30 min, acidified in 1 % HCl at room temperature for 1 h, and then stained with trypan blue (0.05 %) at 90 °C for 20 min. The AMF root colonization was estimated according to Trouvelot and Gianinazzi-Pearson (1986). Thirty root fragments per plant specimen were used to determine mycorrhizal frequency (F%), mycorrhizal intensity (M%), and arbuscular density (A%) by using MYCOCALC software.

AMF spores were extracted from the collected soil samples using wet sieving and decanting method to obtain viable and debris-free AMF spores (Gerdemann and Nicolson 1963). The soil sample (100 g) was mixed into a beaker with 1 L water, and the mixture was swirled. After the soil particles settled down the bottom of the beaker, the suspension was washed through 710, 250, and 45 μm pore sieves with running water respectively. The same procedure was repeated for four times, and the residues from the last two sieves were filtered through filter paper using a vacuum pump. The filter paper containing the residues was then placed on the Petri plates, and the number of AMF spores was counted under light dissecting microscope using a hand tally counter.

Bioconcentration and translocation factors

The bioconcentration factor indicates the efficiency of a plant species in accumulating a metal into its tissues from the surrounding environment (Ladislas et al. 2012). It is calculated as follows:

$$\text{Bioconcentration Factor(BCF)} = \left(C_{\text{plant tissue}}\right)/\left(C_{\text{soil}}\right) \quad (1)$$

where $C_{\text{plant tissue}}$ is the concentration of the target metal in the plant tissue and C_{soil} is the concentration of the same metal in the soil.

The translocation factor indicates the efficiency of a plant to translocate the metal from its root to shoot (Padmavathiamma and Li 2007). It is calculated as follows:

$$\text{Translocation Factor(TF)} = \left(C_{\text{aerialtissue}}\right)/\left(C_{\text{root}}\right) \quad (2)$$

where $C_{\text{aerial tissue}}$ is the concentration of the metal in plant aerial tissues and C_{root} is concentration of the same metal in plant root.

Statistical analysis

Statistical analysis were performed using SPSS for Windows 7, version 16.0. Significant differences were detected by employing a one-way analysis of variance (ANOVA) ($P<0.05$). Significant differences between means were determined by Duncan's test ($P<0.05$). To determine the effects of plant species and plant tissue on heavy metal uptake, the TF and BCF were tested with a two-way analysis of variance (ANOVA). Pearson's correlation coefficients were calculated to determine the relationships between variables for different parameters. Redundancy analyses (RDA) were conducted to determine the multivariate relationship between HM concentrations in different plant tissues and environment factors using the software Canoco (version 4.5, Centre for Biometry, Wageningen, The Netherlands).

Results

Soil chemical properties

The main characteristics of the soil samples collected from the four study sites are presented in Table 1. The pH of four sites ranged from 7.90 to 8.17, indicating slightly alkaline. The S4 had the highest EC (0.93 dS/m), whereas the lowest value was found in S1 (0.74 dS/m). The OM content presented a significant difference among four study sites. The highest value of OM appeared in S4 (14.22 g/kg), whereas the lowest value was found in S1 (7.66 g/kg). The S4 had much higher TN (1.07 mg/kg), TP (1.20 mg/kg), and TK (9.18 mg/kg) contents compared with other sites. The lowest TN (0.82 mg/kg), TP (0.83 mg/kg), and TK (8.20 mg/kg) contents appeared in S3, S1, and S1, respectively. However, no difference could be found in TN and TP contents between S1, S2, and S3 (Table 1). The concentrations of the metals showed a large variability among different sites. The S4 suffered from the most serious heavy metal pollution, and the total concentrations of Pb, Zn, Cu, and Cd in this site were 4.50, 1.51, 1.55, and 4.46 times more than the environmental quality standard (grade II) in soils of China (GB 15618–1995). S2 was slightly polluted with Pb (480.35 mg/kg), Zn (301.19 mg/kg), and Cd (2.54 mg/kg), whereas S4 was greatly polluted that the total

Table 1 Soil properties of four study sites

Soil property	S1	S2	S3	S4
pH	8.17±0.36a	8.00±0.39a	7.90±0.41a	8.01±0.68a
EC (dS/m)	0.74±0.13b	0.82±0.07ab	0.81±0.18ab	0.93±0.18a
OM (g/kg)	7.66±1.00d	10.00±1.17c	12.36±1.51b	14.22±2.45a
TN (g/kg)	0.85±0.13b	0.87±0.16b	0.82±0.20b	1.07±0.16a
TP (g/kg)	0.83±0.16b	0.92±0.18b	0.87±0.17b	1.20±0.17a
TK (g/kg)	8.20±1.36a	8.56±1.94a	8.71±1.77a	9.18±1.93a
TPb (mg/kg)	226.41±98.28d	480.35±153.04c	1027.26±260.82b	1575.17±553.42a
DPb (mg/kg)	17.61±7.44c	39.43±20.68c	96.42±26.45b	178.47±64.20a
TZn (mg/kg)	130.45±85.03c	301.19±65.70b	349.21±110.98b	454.10±151.30a
DZn (mg/kg)	2.80±2.44c	6.75±1.56b	7.73±2.83b	12.91±3.71a
TCu (mg/kg)	30.64±11.02c	94.72±23.77b	54.04±20.41c	155.06±49.62a
DCu (mg/kg)	1.13±0.48c	4.01±1.02b	2.23±0.80c	6.90±2.46a
TCd (mg/kg)	0.62±0.38c	2.54±0.81b	2.00±0.96b	4.46±1.38a
DCd (mg/kg)	0.10±0.06c	0.47±0.13b	0.38±0.18b	0.87±0.28a

The same letter within each column indicates no significant difference

OM organic matter, *EC* electrical conductivity, *TP* total phosphorus, *TN* total nitrogen, *TK* total potassium, *TPb* total Pb, *TZn* total Zn, *TCu* total Cu, *TCd* total Cd, *DPb* DTPA-extractable Pb, *DZn* DTPA-extractable Zn, *DCu* DTPA-extractable Cu, *DCd* DTPA-extractable Cd

Pb, Zn, and Cd concentrations of S4 were 3.28, 1.51, and 1.76 times than that of S2; the DTPA-extractable Pb, Zn, Cu, and Cd presented the same pattern as total Pb, Zn, Cu, and Cd (Table 1). However, only the Zn and Cu concentrations of S1 were under the level of the environmental quality standard (grade II, GB 15618–1995) in soils of China. In our study, the heavy metal concentrations of soils were found to decrease in the order Pb>Zn>Cu>Cd. The increasing order of total/DTPA-extractable concentrations of Pb and Zn at four study sites was S1<S2<S3<S4, whereas the increasing order of total/DTPA-extractable concentrations of Cu and Cd at four study sites changed to S1<S3<S2<S4 (Table 1). Besides, the concentration of DTPA-extractable heavy metals in relation to total concentration was as follows: Pb 8.86, Zn 2.24, Cu 4.05, and Cd 18.21 % when all soil samples were taken into consideration.

AMF status

It can be confirmed that all plant species were colonized by AMF in all surveyed sites because at least one of the typical structures of vesicles or arbuscules was found in the root (Fig. S1). Furthermore, some other structures, such as intra- and intercellular hyphae, and hyphal coils of AMF were abundant in most of plant roots and sometimes the intraradical spores could be observed. There were significant differences in mycorrhizal colonization parameters (F%, M%, and A%) and spore density (SP) among tree species and study sites. The value of F% ranged from 9.96 % (for *Platycladus orientalis* (Linn.) Franco) in S4 to 42.51 % (for *Populus purdomii*) in S1. The *Robinia pseudoacacia* had the highest M% compared with other woody trees in S1 (21.92 %) and S4 (9.59 %), whereas the lowest value appeared in the roots of *P. orientalis* (3.42 %) in S1. The value of A% was quite low in all surveyed sites, ranging from 1.88 % (for *Acer davidii* Franch.) in S1 to 10.88 % (for *Populus simonii*) in S3. However, the spore density (SP) of AMF in rhizospheric zone soils varied greatly, ranging from 46 to 670 per 100 g dry soil. The maximum SP was found in the rhizosphere of *P. simonii* in S2, and the minimum spore density appeared in the rhizosphere of *Cotinus coggygria* in S3 (Fig. 2).

Heavy metal concentrations in plant tissues

Figure 3 summarizes the metal concentrations of Pb, Zn, Cu, and Cd in different plant tissues collected from four study sites. In our study, the plants growing in polluted area accumulated the highest concentration of Pb and the lowest concentration of Cd, and metals accumulated in plants followed the decreasing order of Pb>Zn>Cu>Cd. Great variations of metal concentrations could be found in both plant species and within the same species. The concentrations of heavy metals varied widely from 5.52 to 712.37 mg/kg for Pb, 2.15–432.08 mg/kg for Zn, 2.86–135.33 mg/kg for Cu, and 0.04–3.86 mg/kg for Cd, taking all the plant samples into account. In general, the roots (106.02–712.37 mg/kg) accumulated higher concentration of Pb compared with other tissues within the same species. The highest concentration of Pb was found in the roots of *R. pseudoacacia* in S4, whereas the lowest value appeared in the woods of *A. davidii* in S1. Moreover, relatively high Pb concentration was also found in the roots of *Amygdalus davidiana* (Carr.) C. de Vos (S4), *P. simonii* (S4),

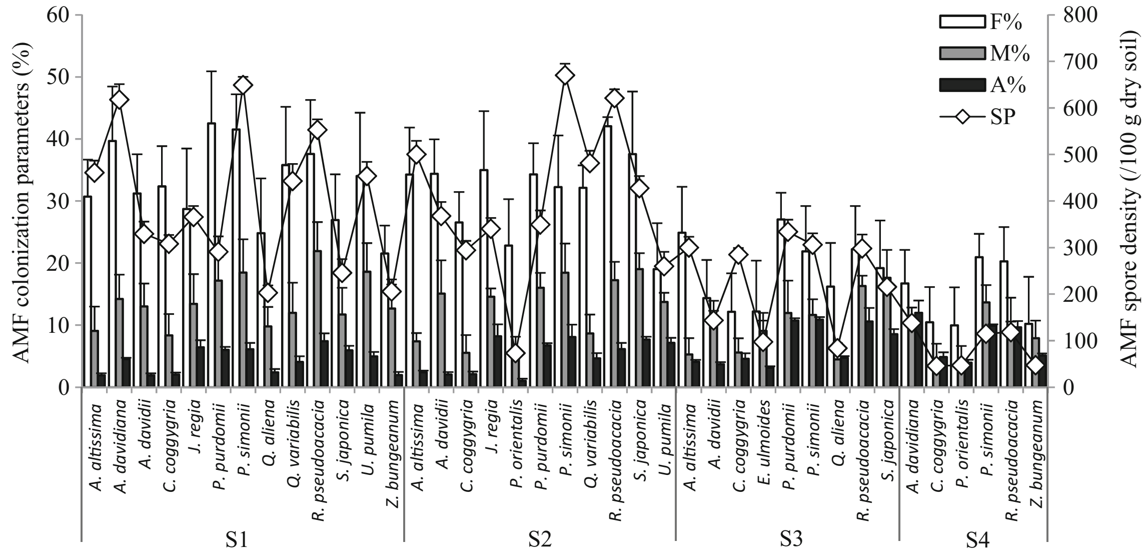

Fig. 2 AMF colonization parameters (F%, M%, and A%) and spore density (SP) in roots or rhizosphere zone soils of woody plants grown in four study sites. Data are presented as mean±SE from three replicates

and *R. pseudoacacia* (S3 and S4), in the leaves of *P. purdomii* (S3) and *R. pseudoacacia* (S3) (>440 mg/kg) (Fig. 3a).

Unlike Pb, most of Zn was accumulated in the leaves of woody plants. The concentration of Zn in the plant samples collected from four study sites ranged from 26.39 to 388.48 mg/kg in roots, 2.15–35.87 mg/kg in woods, 16.41–198.36 mg/kg in barks, 22.64–255.44 mg/kg in branches, and 30.80–432.08 mg/kg in leaves. The highest Zn concentration appeared in the leaves of *P. purdomii* (432.08 mg/kg) in S2, whereas the lowest value was found in the woods of *Eucommia ulmoides* Oliver (2.05 mg/kg) in S3. Meanwhile, only the roots of *Ailanthus altissima* (S2) and *P. simonii* (S2) and the leaves of *A. altissima* (S1 and S3), *P. purdomii* (S3), and *R. pseudoacacia* (S3) concentrated large amount of Zn (>300 mg/kg) (Fig. 3b).

The concentration of Cu in all plant samples was much lower compared with Pb and Zn, ranging from 17.26 to 110.46 mg/kg in roots, 2.86–37.53 mg/kg in woods, 11.82–97.32 mg/kg in barks, 10.28–105.95 mg/kg in branches, and 9.07–140.85 mg/kg in leaves. The highest concentration of Cu was accumulated in the leaves of *P. purdomii* in S2, and the lowest value was found in the woods of *E. ulmoides* in S3. Furthermore, the roots and leaves of *A. davidiana* and *P. purdomii* in S2 and S4, and the branches and leaves of *R. pseudoacacia* in S4 accumulated high Cu concentrations (>100 mg/kg) (Fig. 3c).

The concentration of Cd showed to be the lowest compared with Pb, Zn, and Cu metals in plant tissues, only ranging from 0.16 to 3.86 mg/kg in roots, 0.03–0.62 mg/kg in woods, 0.13–1.17 mg/kg in barks, 0.13–2.51 mg/kg in branches, and 0.14–2.86 mg/kg in leaves, with the maximum being in the roots of *P. simonii* (3.86 mg/kg) in S4 and the minimum being in the woods of *Sophora japonica* Linn. (0.03 mg/kg) in S2. Furthermore, the roots of *A. davidiana* (S4), *C. coggygria* (S3), *P. simonii* (S3 and S4) and *R. pseudoacacia* (S3), and the branches and leaves of *A. davidiana* (S4) could uptake Cd exceeding 2.5 mg/kg (Fig. 3d). Figure 3 also showed that Pb and Cd concentrations in roots were much higher than that in leaves, whereas Zn and Cu were mainly accumulated in leaves instead of other tissues of woody plants.

Phytoremediation efficiency

The translocation factors (TFs) and bioconcentration factors (BCFs) of Pb, Zn, Cu, and Cd were various among plant species, tissues, and study sites, and most of the TFs and BCFs of HMs in the current study were lower than 1 (Fig. 4, Tables S2 and S3). However, no significant difference could be found in TFs of Pb and Zn among study sites (Table S4). TF of Cu in wood and TF of Cd in bark presented much higher value in S1 compared with that in other three sites. The average BCFs of Pb, Zn, Cu, and Cd in plant tissues in S1 were much larger than that in S4 (Table S4).

The average TF of Cu was the highest (0.68), followed by Zn (0.60), Pb (0.51), and Cd (0.44) when all plant samples were taken into consideration. The TFs of Pb, Zn, Cu, and Cd presented to be significantly different among plant tissues (Fig. 4, Table S2). The largest TF of Pb was found in leaves of *S. japonica* (1.51) in S1. Leaves accumulated much higher amount of Zn from soils compared with other plant tissues, and the maximum TF of Zn was found in leaves of *R. pseudoacacia* (2.89) in S3. The TF of Cu was relatively high only in the leaves of *A. davidiana*, *C. coggygria*, and *R. pseudoacacia* (1.13–1.81) collected from all study sites. However, only the TF of Cd in branches and leaves of

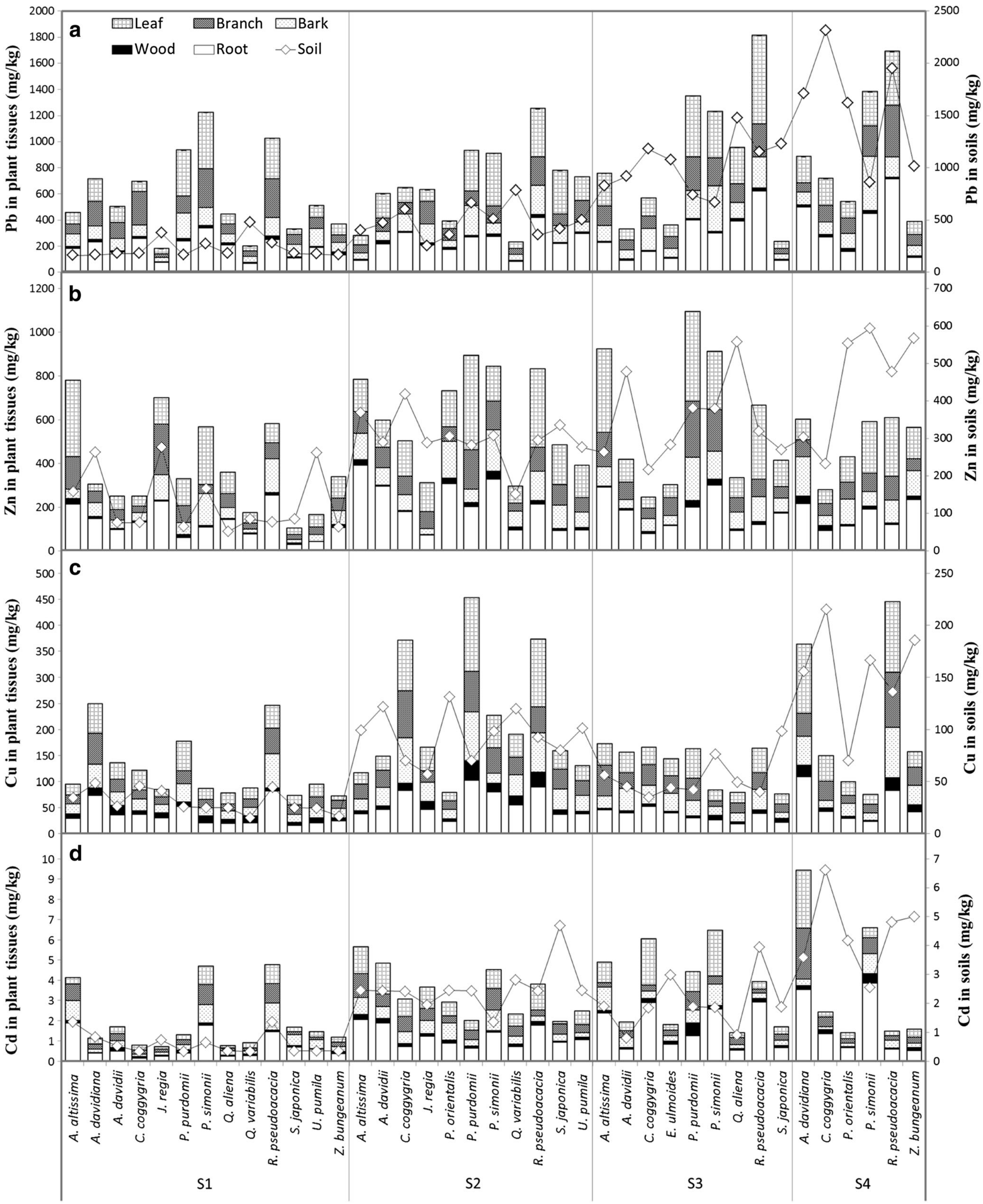

Fig. 3 Concentrations of Pb (**a**), Zn (**b**), Cu (**c**), and Cd (**d**) in plant tissues and soils collected from four study sites in Pb–Zn mine area

C. coggygria was greater than 1, ranging from 1.04 to 1.41 in S1 and S2.

Plants concentrated much more Pb in their roots, rather than in other tissues. The highest BCF of Pb was found in

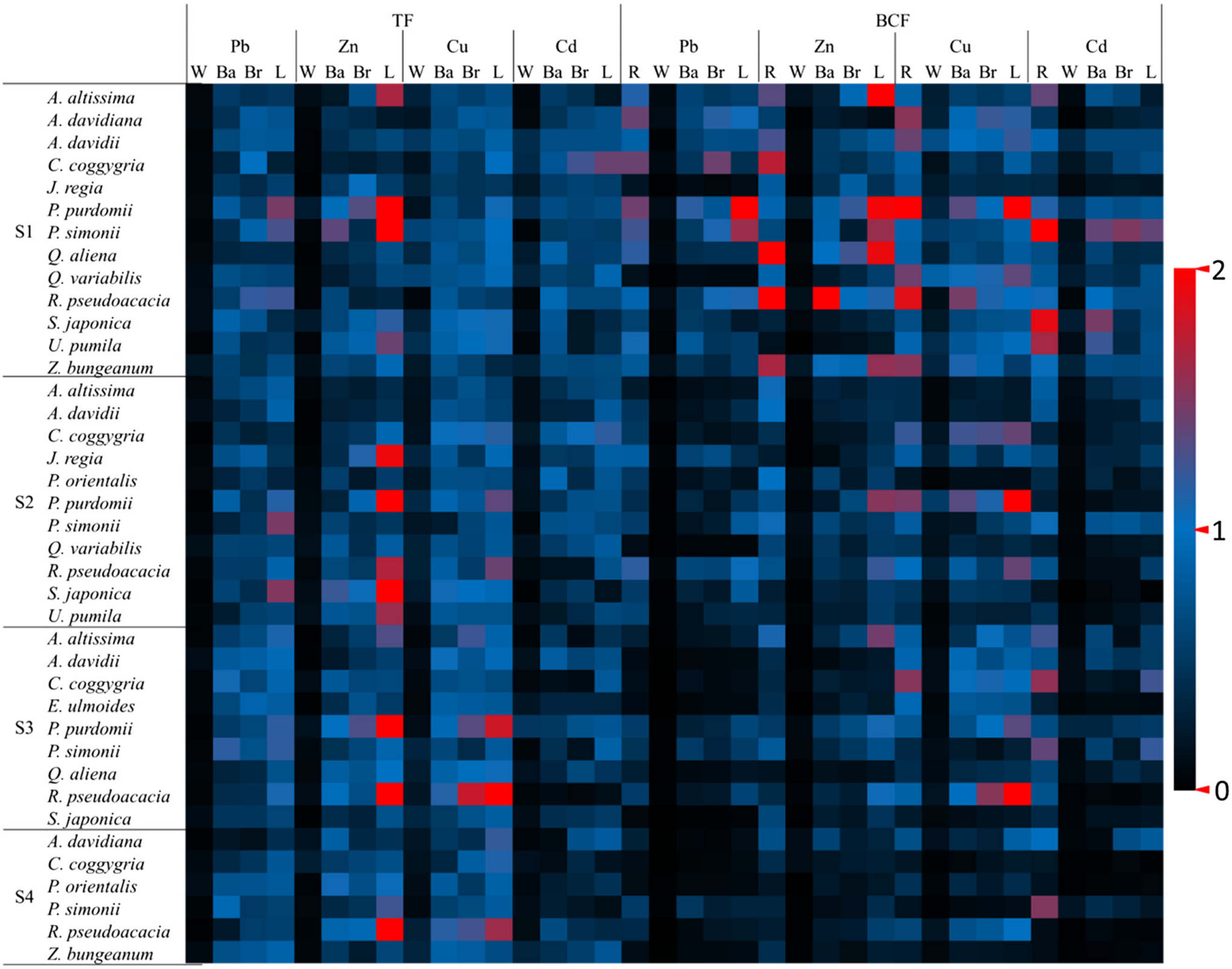

Fig. 4 Translocation factors (TFs) and biological concentration factors (BCFs) of Pb, Zn, Cu, and Cd in plant tissues collected from four study sites. *R* root, *W* wood, *Ba* bark, *Br* branch, *L* leaf

leaves of *P. purdomii* in S1 (2.11), whereas the lowest value appeared in the woods of *E. ulmoides* in S3 (0.006). Unlike Pb, the largest BCF of Cu was observed in the leaves of *P. purdomii* in S3 (2.21), and the lowest value was found in the woods of *P. simonii* in S4 (0.013). Large amount of Zn was transformed from soil to leaves of woody plants, and the highest BCF of Zn appeared in leaves of *A. altissima* in S1 (2.23). Interestingly, most of the plants seems preferred to stabilize soil Cd in their roots, and the highest BCF of Cd was found in roots of *P. simonii* in S1 (2.72). Obviously high BCF of Cd was also found in roots of *C. coggygria* (S3), *P. simonii* (S4), *S. japonica* (S1), and *Ulmus pumila* Linn. (S1) (>1.50) (Fig. 4, Table S3).

Physiochemical and biological factors

To further analyze the effect of environmental variables on the heavy metal accumulation characteristics of plants, 14 physiochemical factors (soil properties) and four biological factors (F%, M%, A%, and SP) were analyzed using redundancy analysis (RDA). The length of the red arrows indicate the relative importance of each environmental factor in explaining variation of heavy metal accumulation characteristics, while the angles between the arrows and axis indicate the degree to which they are correlated (Fig. 5). For heavy metal concentrations in plants, more than 84 % of the variance in HM concentrations could be explained by the two canonical axes (Fig. 5a). The first canonical axis explained 70.7 % of the variables of heavy metal concentrations in plant tissues and was negatively correlated with soil pH. The second axis represented 13.4 % of variance and showed greatly negative correlations with soil total/DTPA-extractable Pb concentrations. A%, soil total Zn, Cd, and DTPA-extractable Pb and Cd concentrations had a strong influence on the heavy metal accumulation characteristics of plants, but still, a large proportion of the variance remained unexplained. Overall, soil heavy metal concentration could enhance accumulation of HMs in plant tissues and F%, SP presented positive correlations with Zn, Cd concentrations in plant tissues, while M% and A%

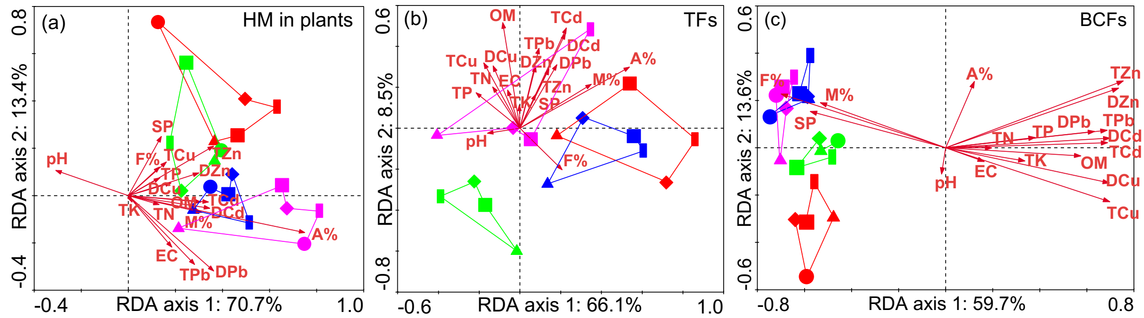

Fig. 5 Redundancy analysis (*RDA*) of the correlations between environment variables (physiochemical and biological factors) and HM accumulation (**a**), TFs (**b**), and BCFs (**c**) of plants. *Pink square* (■) represents Pb, *red square* (■) represents Zn, *blue square* (■) represents Cu, and *green square* (■) represents Cd. Circle (●) represents root samples, *triangle* (▲) represents wood samples, *square* (■) represents bark samples, *diamond* (◆) represents branch samples, and *box* (▌) represents leaf samples. The *red arrows* represent environment variables. *F%* mycorrhizal frequency, *M%* mycorrhizal intensity, *A%* arbuscular density, *SP* spore density. Other abbreviations are presented in Table 1

had positive relationships with Pb and Cu uptake in plant samples (Table S5). For TFs of HMs in plant samples, a total of 74.6 % of the cumulative variance in the TFs data set was explained by the first two canonical RDA axes (Fig. 5b). The first canonical axis explained 66.1 % of TFs in plant samples and was positively correlated with F%, M%, and A%, soil total/DTPA-extractable Cd concentrations, while the second axis explained 8.5 % of variance and had greatly positive correlations with soil OM, total Pb concentration, and total/DTPA-extractable Cd concentrations. The TFs of both Zn and Cu in plant tissues were strongly influenced by F%, M%, and A%, while the TFs of Zn in plant samples showed significantly negative correlations with all the soil heavy metal concentrations. Eight of the 18 environmental variables fitted as vectors onto the RDA plot were significantly correlated with the TFs of heavy metals in plant samples; of these, soil OM content, M% and A%, and total/DTPA-extractable Cd concentrations were most strongly related to the TFs of HMs (Table S6). For BCFs of HMs in plant tissues, more than 73 % of the variance in BCFs of HMs could be explained by the two canonical axes. The first axis represented 59.7 % of variance and showed greatly positive correlations with soil total/DTPA-extractable Pb, Zn, Cu, and Cd concentrations, but was negatively correlated with F% (Fig. 5c). The second canonical axis explained 13.6 % of the variables of BCFs in plants and was negatively correlated with soil total Zn concentration and A%. The BCFs of Pb and Cu showed significantly positive correlations with F%, M%, and SP, but no correlations could be found between them and soil pH and A% (Table S7).

Discussion

Soil properties and correlations with AMF status

According to the China Environmental Quality Standard for Soils (GB15618-1995, grade II for soil pH>7.5: Pb≤350, Zn≤300, Cu≤100, and Cd≤1.0 mg/kg, indicating a pollution warning threshold), soil Cd levels exceeded grade II quality in all study sites except for S1, and both Pb and Zn levels of the soil only satisfied grade II quality in the same study site (S1). Unlike Pb and Zn, soil Cu levels showed to be below grade II quality at all study sites, except for S4. In general, potentially available metal content is probably more important than total amount of heavy metal because the former allows for prediction of the risk of metal uptake by plants and its mobility in the system (Li et al. 2007). In our study, the average DTPA-extractable percentage of total Pb, Zn, Cu, and Cd was 8.86, 2.24, 4.05, and 18.21 %, respectively, reflecting a lower phytotoxic potential than in South China (Shu et al. 2005).

The mine-degraded soil usually has low pH and reduced concentrations of important nutrients like OM, N, and P due to topsoil loss (Sheoran et al. 2010). However, in the current study, the soil samples did not show low levels of pH and nutrient contents in HM-polluted soils. The main reason is that all tree species and soil samples were not collected from central mine soils, but around the mine area because the central of study sites was always seriously damaged by human activities (deforested) and only few species of annual grass could survive in the real meaning of mine soil for several months per year. Secondly, in industrial processes, the solid and liquid wastes are usually treated with lime to adjust the pH value and reduce extractable HM concentrations. This may cause the soil to be lightly alkaline and the measured total Pb and Zn concentrations to be only 1575.17 mg/kg (Pb) and 454.10 mg/kg (Zn) at the highest even in S4 with the heaviest pollution level (Table 1). Thirdly, the toxic metals can adversely affect the number, diversity, and activity of soil organisms and thus inhibit soil organic matter decomposition and N and P mineralization processes, resulting in an accumulation of OM, N, and P in soils (Dai et al. 2004).

Soil pH, OM, and TP contents were suggested to be the most important soil properties affecting speciation, movement, and final or actual availability of metals (Zeng et al. 2011). In the current study, no significant correlations were found among pH value (7.90–8.17), TN content, and availability of Pb, Zn, Cu, and Cd (P>0.05) in all study sites (Table S8). However, soil OM showed significantly negative correlation with Pb availability in S2, whereas soil TP content had obviously negative correlations with availability of Pb (in S1 and S2) and Cu (in S4) (P<0.05) (Table S8). The results were consistent with Miretzky and Fernandez-Cirelli (2008) who reported that phosphate effectively immobilizes Pb from contaminated soils, but was different from the results of Liu et al. (2009) who observed that OM could reduce the availability of heavy metals in soils by adsorption or forming stable complexes with humic substances. The conflicting results might come from the fact that the effects of OM on heavy metal fractionation in soils were pH-dependent, and the addition of OM could also result in the release of metals from solids to the soil solution (Gregson and Alloway 1984; Zeng et al. 2011), especially for soils with high pH values. High solubility of heavy metals in soil with alkaline pH could be attributed to enhanced formation of organic matter metal complexes, in which case most of the dissolved heavy metals in soils presented as metal soluble organic ligand complexes (Sauve et al. 1998).

It is important to understand how AM fungus itself and the establishment of the symbiosis are affected by contaminated soils in order to use the indigenous AM symbiosis for phytoremediation. In the current study, the F% and SP strongly decreased with increasing soil OM and TP contents, but the M% had a negative correlation with soil OM content (P<0.05) (Table S9). The results agreed with Kahiluoto et al. (2001) who demonstrated that AMF colonization of roots and spore density in soils reduced with increase of P content. Even moderate contents of P fertilizer (45 kg ha^{-1} year^{-1} P) could reduce spore density of AMF (Mårtensson and Carlgren 1994). Our results showed that the F% and SP had significantly negative correlations with soil total/DTPA-extractable concentrations of Pb, Zn, Cu, and Cd (P<0.05) (Table S9). These were similar to Del Val et al. (1999) and Meier et al. (2012) who reported that high metal levels could inhibit AMF spore germination, extraradical mycelium growth, and root colonization. However, it is interesting to notice that the A% of AMF presented positively correlated with soil total/DTPA-extractable Pb concentrations, indicating that high level of A% might be attributed to alleviation of heavy metal toxicity in plants growing on heavy metal-polluted soils, although further studies on this topic is required.

Heavy metal concentrations in plant tissues

The concentrations of Pb, Zn, Cu, and Cd in plant samples were showed in Fig. 3, and the wide variations of heavy metal concentrations were found both among plant species and within plant tissues of the same species (Zhang et al. 2014). Besides, the phytotoxic concentrations of heavy metals in plants were considered to be 30–300 mg/kg for Pb, 500–1500 mg/kg for Zn, 25–40 mg/kg for Cu, and 5–30 mg/kg for Cd (Chaney 1989; Kabata-Pendias 2010). Based on this, the Pb and Cu concentrations in most plant samples collected from the four study sites presented in phytotoxic ranges, whereas the Zn and Cd concentrations were approximately normal (Fig. 3). Significant variation of heavy metal concentrations in tissues was found for the same tree species collected from different sites (Fig. 3), indicating that different levels of heavy metals in the soils selected species in a short/long-term effect (Xue et al. 2014).

In the current study, most tree species accumulated more Zn and Cu in leaves than in other tissues, while the concentrations of Pb and Cd in roots were much higher compared with the other parts of woody plants (Fig. 3), indicating that different types of metals had different patterns of behavior and mobility within plants: Pb and Cd tended to be immobilized and held in roots, whereas Zn and Cu were generally translocated to leaf tissues (Pulford and Watson 2003). Zn and Cu are essential metals for plant growth and play an important role in photosynthesis and enzyme composition for protein synthesis (Påhlsson 1989; Bonanno and Lo Giudice 2010), while Pb and Cd were non-essential elements and toxic to plant growth (resulting in membrane damage and oxidative stress) (Małecka et al. 2009; Castagna et al. 2014; Yang and Ye 2014; Rodriguez et al. 2015). Therefore, plants may develop their own strategy to survive in HM stress environments: translocating more essential metals (Zn and Cu) into the aerial parts, while retaining more toxic heavy metals (Pb and Cd) in the roots to protect the above-ground parts from damage.

The concentrations of Pb, Zn, Cu, and Cd varied among plant species. In our study, the highest concentration of Cu was found in the leaves of *P. purdomii* (140.85 mg/kg) in S2 followed by the leaves of *R. pseudoacacia* (135.33 mg/kg) in S4 (Fig. 3). The concentrations of Zn in the leaves of *P. purdomii* (432.08 mg/kg) in S2 and *R. pseudoacacia* (358.91 mg/kg) in S3 were much higher compared with other plant species (Fig. 3). Serbula et al. (2012) reported that *R. pseudoacacia* could accumulate high concentrations of Cu (6418.2 mg/kg) and Cd (4699.8 mg/kg). Chang et al. (2005) indicated that *P. purdomii* had potential for phytoremediation, not only because of its high tolerance to heavy metals but also because of its capacity for easy establishment and fast growth in polluted area. However, in the current study, the concentrations of Cu and Zn were not as high as the previous reported data even for the same tree species. Two reasons may explain the differences. Firstly, in the current study, the neutral or alkaline soils reduced the mobility of heavy metals to move from soil to plants. Secondly, different plant growth stages and soil conditions might influence the accumulation characteristics of heavy metals in plants (Kabata-Pendias 2010). The highest concentration of Pb was found in the roots of

R. pseudoacacia (712.37 mg/kg) in S4, whereas much higher level of Cd was detected in the roots of *P. simonii* (3.86 mg/kg) in S4 and *A. davidiana* (3.54 mg/kg) in S4 compared with other plant species (Fig. 3). However, the Pb and Cd concentrations in these plants were much lower than in other plants measured by Zu et al. (2005) in Pb–Zn mining area in Yunnan, China, but higher than the plants growing in Hunan Province, China, reported by Zhang et al. (2014). These differences suggested that plant species, growth stage, soil physical and chemical properties, metal immobility/mobility characteristic, and other environmental factors jointly influence the HM concentrations in plant samples.

Candidate tree species for phytoremediation

The evaluation and selection of plants for phytoremediation purposes depend on bioconcentration factor (BCF) and translocation factor (TF) values (Wu et al. 2011). The plant species with higher BCF and lower TF could be considered as candidates for phytostabilization, while only the plant species with both BCF and TF greater than one have the potential to be used for phytoextraction (Yoon et al. 2006). According to these basic criteria, *A. altissima* for Zn, *P. simonii* for Pb and Cu, *P. purdomii*, and *R. pseudoacacia* for Pb, Zn, and Cu presented to be potential species for phytoextraction in HM-polluted soils. No plant species was found with BCF and TF of Cd higher than one at the same time in the current study (Fig. 4, Tables S2 and S3). The high root to shoot translocation of metals indicated that these tree species have important characteristics to be used in phytoextraction of the correspondent metals (Malik et al. 2010). Our results were consist with Gatti (2008), who reported that *A. altissima* was a fast-growing and contamination-resistant species and also had the potential for phytoremediation in areas polluted by HMs. Poplar (in this study, *P. purdomii* and *P. simonii*) had been studied as a possible candidate in phytoremediation approaches to clean up soil with heavy metal pollution (Atangana et al. 2014). Seo et al. (2008) reported that *R. pseudoacacia* was quite efficient in removing Pb, Zn, Cu, and Cd metals from mine spoils. Our results also showed that a total of ten species with BCF >1 and TF <1 might be useful for phytostabilization of one, two, or three metals in contaminated sites. These species included *A. altissima* for Pb, Zn, and Cd; *C. coggygria* for Zn, Cu, and Cd; *A. davidiana* for Pb and Zn; *Zanthoxylum simulans* Hance for Zn and Cu; *A. davidii* and *Quercus variabilis* Blume for Zn; *Q. variabilis* for Cu; *P. simonii*, *S. japonica*, and *U. pumila* for Cd (Fig. 4, Tables S2 and S3). However, we should notice that the same plant species may have different potential for phytoextraction and/or phytostabilization grown in different sites, indicating that the growth stage and other environmental factors might affect the performance and HM accumulation characteristics of plants. Successful establishment and colonization of these metal tolerant species would effectively reduce surface erosion due to binding of the substrate to plant roots and reduce the mobility of metals to leach into ground water or spread by air, thereby reduce risks of further environmental degradation (Peng et al. 2006).

Plant species with fast growth, wide root system, and high biomass production are generally suitable for phytoremediation. In addition, the candidate plant species should have the advantage of growing in the semiarid and arid area. Legume plants are promising candidates for ecological restoration even in the central destroyed area with low nutrients due to their fast growth rate, high biomass, and nitrogen-fixing capacity. According to these criteria, *A. altissima*, *C. coggygria*, *P. simonii*, *P. purdomii*, and *R. pseudoacacia* could be considered the most suitable candidates for phytoextraction and/or phytostabilization purposes. Our screening data of woody plants presented here revealed that some poplar and legume species could accumulate large amount of HMs in different tissues. This is quite important for long-term management of polluted area as it implies that different kinds of HMs could be extracted or stabilized efficiently only when proper species were used (Ali et al. 2013).

Physiochemical and biological factors

Although distinct patterns of heavy metal uptake among the plant species were found, redundancy analysis (RDA) showed that heavy metal concentration was not the only soil parameter influencing heavy metal accumulation characteristics of plants. Soil pH showed negative correlations with Pb, Zn, Cu, and Cd concentrations in plant tissues (Fig. 5a). Negative correlations between soil pH and heavy metal mobility and availability to plants have been well documented in numerous studies (Wang et al. 2006; Rees et al. 2014). Lower soil pH would enhance the uptake of heavy metals by plants and thereby pose a threat to human health (Zeng et al. 2011; Durães et al. 2014). The BCFs of Pb, Zn, Cu, and Cd presented significantly negative correlations with soil total/DTPA-extractable heavy metal concentrations (Fig. 5c), indicating the excluding strategy in all plant species growing in HM-polluted soils (Wójcik et al. 2014). In contrast to the previous study (Jung 2008), no significant correlations could be found between total/DTPA-extractable Pb, Zn, Cu, and Cd concentrations in soils and corresponding HM concentrations in plant tissues except for Pb in roots (Table S10), indicating that Pb was a low availability and non-essential element and plants might develop a strategy to retain most of Pb in roots to prevent damage. However, the competition among Pb, Zn, Cu, and Cd was frequently observed in the current study. Our results showed that Pb and Cu could still be effectively uptaken from soil in the presence of Cd, but accumulation of the Cd and Zn was suppressed in the presence of Pb to a certain extent, especially Zn in roots and Cd in barks and leaves (Fig. 5a). The phenomenon was supported by

Mahamadi and Nharingo (2010) who observed similar results in binary and ternary systems. The large difference in TFs of Pb and Cd among plant tissues could be found in the current study, and the correlations of TFs of Pb, Zn, Cu, and Cd with soil HM concentrations followed the order of Pb>Cu=Zn>Cd (Fig. 5b). Qin et al. (2006) reported that the competitive ability of Pb, Cu, and Cd followed the same order Pb>Cu>Cd, indicating that when metals compete for the same adsorption sites of an adsorbent, metals with greater affinity could displace the others with lower affinity (Christophi and Axe 2000). However, more precise experiment is required to get a better understanding of the competition among Pb, Zn, Cu, and Cd under specific conditions.

Phytoremediation of heavy metals (HMs) by plants provides us a promising future for the remediation of contaminated sites. However, it always takes long time to clean up HMs in soils by using this technology. Various methods were tested to improve the efficiency of phytoextraction to reduce the remediation–time period (Robinson et al. 2000). One of these methods is the inoculation with mycorrhizal fungi (Joner and Leyval 2001). AMF play a significant role in the growth of host plants and can also affect their HM accumulation characteristics (Entry et al. 2002). Our results further suggested that the potential of phytoremediation of contaminated soil can be enhanced by AMF associations. The F% and SP increased the Zn and Cd concentrations in plant tissues, while M% and A% were positively correlated with Pb and Cu concentrations in plant samples (Fig. 5a). F%, M%, and A% enhanced the translocations of Pb and Cu from roots to above-ground parts of host plants, but did not show any effect on TFs of Cd (Fig. 5b). In addition, it is interesting to notice that the accumulations of Pb and Cu from soil were improved by F%, M%, and SP, but these positive effects could not be detected among A% and BCFs of Pb, Cu, and Cd (Fig. 5c). Various reports indicated that AMF could enhance plant accumulation and tolerance of Pb, Zn, Cu, and Cd in a number of plant species (Carvalho et al. 2006; Marques et al. 2007; Sudová and Vosátka 2007). However, the role of AM fungi in uptaking and transfering HMs to the plant is still poorly understood, and literature results are conflicting. The uptake of Pb, Zn, Cu, and Cd and their immobilization were found to be higher in roots of mycorrhizal than non-mycorrhizal plants (Vivas et al. 2003; Chen et al. 2003, 2005). Some researchers indicated that AM symbioses might have created a more balanced environment that ultimately allows roots to cope with higher HM concentrations, possibly by enriching HM at/in fungal structures (Göhre and Paszkowski 2006). It is still difficult to evaluate the influence of AM fungi on plant ability to tolerate and accumulate Pb, Zn, Cu, and Cd because the environmental variables were so complex. For example, the effects of mycorrhizal colonization on cleanup of contaminated soils depend on the plant-fungus–HM combination and are also influenced by soil conditions. On the other hand, plants growing in metal-contaminated soils harbor a diverse group of microorganisms (Zarei et al. 2010) that are capable of tolerating high concentrations of metals and providing a number of benefits to both the soil and the plant (Lombi et al. 2001). The rhizosphere bacteria colonized in the roots of plants can directly improve the phytoremediation process by changing the metal bioavailability through altering soil pH, release of chelators, and oxidation (Wenzel 2009). In the current investigation, the dark deptate endophytes (DSE) with low sensitivity to HM were also commonly found in the roots of plants growing in metal-polluted soils (Fig. S2). This mutual symbiosis might be another efficient strategy for host plants to survive in the HM stressful environments, although the knowledge about the roles of DSE in improving HM tolerance of their host plants is still lacking (Li et al. 2011). It has to be emphasized, however, that AMF are only one of the most common rhizosphere microorganisms associated with plants growing in metal-polluted soils. It is therefore difficult to identify precise roles of AMF in phytoremediation in this field study. It seems necessary to evaluate the factors influencing the roles of AMF in the phytoremediation of soils polluted by HMs in more comprehensive experiments (Meier et al. 2012) in order to select plants with specific AM fungal isolation and adapt to high concentrations of heavy metals in future research for phytoremediation projects.

Conclusions

To our knowledge, this is the first time study on selecting woody plants growing in lead–zinc-contaminated sites and analyzing their metal phytoremediation potential in northwest region of China. The results indicated that none of the plant species were identified as hyperaccumulator because none of the concentrations of heavy metals in plant tissues reached hyperaccumulating level (Pb, Cu>1000 mg/kg, Zn>10, 000 mg/kg, Cd>100 mg/kg dry weight). However, plant species could be identified which had the potential for phytostabilization and phytoextraction according to BCF and TF values, growth rate, and biomass production. *A. altissima*, *C. coggygria*, *P. simonii*, *P. purdomii*, and *R. pseudoacacia* were the most suitable candidates for phytoextraction and/or phytostabilization purposes. As a pioneer species, black locust (*R. pseudoacacia*) appeared to be the most promising tree for phytoremediation due to its fast growth and ability to fix atmospheric N in nutrient-poor area. The introduction of indigenous stress-adapted AMF into these heavy metal-contaminated areas could be a potential strategy for successful phytoremediation. Despite the commonly observed low AMF colonization and spore diversity in metal-enriched soils, the existing fungal colonizers were presumably the best suited to cope with the existing heavy metal-polluted environments

(Regvar and Vogel-Mikuš 2008). The efficiency of phytoremediation of contaminated soils was improved by AMF associated with HM-tolerant plants growing in study sites polluted by heavy metals. However, to explore and culture potential plants with specific AMF isolates adapted to high level of metal concentration will become one of the most important tasks in the future research. Furthermore, detailed studies are needed to investigate the phytoremediation potential (growth performance, biomass production, and heavy metal accumulation) of these tree species in both pot culture and field researches.

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