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Seedling growth and metal accumulation of selected woody species in copper and lead/zinc mine tailings

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Abstract

A greenhouse pot experiment was conducted to evaluate the potential of selected woody plants for revegetation in copper (Cu) and lead/zinc (Pb/Zn) mine tailing areas. Five woody species (*Amorpha fruticosa* Linn, *Vitex trifolia* Linn. var. *simplicifolia* Cham, *Glochidion puberum* (Linn.) Hutch, *Broussonetia papyrifera*, and *Styrax tonkinensis*) and one herbaceous species (*Sesbania cannabina* Pers) were planted in Cu and Pb/Zn tailings to assess their growth, root morphology, nutrition uptake, metal accumulation, and translocation in plants. *Amorpha fruticosa* maintained normal growth, while the other species demonstrated stress related growth and root development. *Sesbania cannabina* showed the highest biomass among the plants, although it decreased by 30% in Cu tailings and 40% in Pb/Zn tailings. Calculated tolerance index (TI) values suggested that *A. fruticosa*, an N-fixing shrub, was the most tolerant species to both tailings (TI values 0.92–1.01), while *S. cannabina* had a moderate TI of 0.65–0.81 and *B. papyrifera* was the most sensitive species, especially to Pb/Zn tailings (TI values 0.15–0.19). Despite the high concentrations of heavy metals in the mine tailings and plants roots, only a small transfer of these elements to the aboveground parts of the woody plants was evident from the low translocation factor (TF) values. Among the woody plants, *V. trifolia* var. *simplicifolia* had the highest TF values for Zn (1.32), Cu (0.78), and Pb/Zn (0.78). The results suggested that *A. fruticosa* and *S. cannabina*, which have the highest tolerance and biomass production, respectively, demonstrated the potential for tailings revegetation in southern China.

Key words: woody plants; mine tailing; tolerance; revegetation; root

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Introduction

Most mine tailing disposal sites are devoid of vegetation (Mendez and Maier, 2008) and are considered environmentally harmful due to their high heavy metal concentrations (Conesa et al., 2007; Wang et al., 2009). To reduce the environmental risks of mining waste, revegetation has been recommended as the most promising approach (Bradshaw and Johnson, 1992). Mine tailing revegetation is, however, a difficult practice due to high heavy metal toxicity and a lack in nutrient elements (Ye et al., 2002). Hence, plant species with a high tolerance to denudation and excess metal concentration in the soil are required.

Plant species screening is a prerequisite for successful revegetation. Recent studies have reported that herbaceous species, such as *Isocoma veneta* (Kunth.) Greene and *Teloxys graveolens* (Willd.) (Gonzalez and Gonzalez-Chavez, 2006), *Bidens humilis* (Bech et al., 2002), *Atriplex lentiformis* (Torr.) (Mendez et al., 2007), and *Lygeum spartum* and *Piptatherum miliaceum* (Conesa et al., 2006), can grow normally in copper or lead/zinc mine tailing areas, suggesting that these herbaceous species could be potentially utilized in the revegetation of tailing areas or in soils polluted by mining.

To date, most studies have focused on the potential utilization of grasses in revegetation, with only a few studies evaluating the potential of fast-growing woody species for the revegetation and remediation of mine tailing areas (Mertens et al., 2004; Domínguez et al., 2008; Seo et al., 2008; Brunner et al., 2008). Compared to herbaceous species, woody species constitute most plant biomass in native forests and shrublands. Shrubs and trees can provide a more extensive canopy cover and establish a deeper root network for long-term erosion prevention. Shrubs and trees provide a high nutrient environment for grasses while reducing moisture stress and improving the

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physical characteristics of soil in arid and semiarid regions (Belsky et al., 1989; Tiedemann and Klemmedson, 2004; Mendez and Maier, 2008). Furthermore, trees can decrease metal mobility and toxicity by root growth. Thus, research on suitable woody plants and their metal accumulation patterns are critical for potential revegetation utilization.

In this study, six plant species, including five woody species (*Amorpha fruticosa* Linn, *Vitex trifolia* Linn. var. *simplicifolia* Cham, *Glochidion puberum* (Linn.) Hutch, *Broussonetia papyrifera* and *Styrax tonkinensis*) and one comparison herbaceous plant (*Sesbania cannabina* Pers) were tested as potential metal-tolerant plants. The selected plants were grown in copper tailings, lead/zinc tailings, and reference soil pots and plant growth, root characterization, mineral nutrients, metal accumulation, and translocation in plants were measured. The objectives of this study were to reveal the adaptation of the selected woody plants to mine tailings and the potential use of woody species for the revegetation of mine tailings areas in China.

1 Materials and methods

1.1 Mine tailings and soil

Lead and zinc mine tailings were collected from Fuyang City (30°126′N, 119°847′E) and copper mine tailings from Shaoxing City, China (29°897′N, 120°621′E). The red soil from the Pb/Zn mine area contained Cu, Pb, and Zn in concentrations of 8.68, 69.21, and 219.60 mg/kg, respectively. The average pH value was 7.80 and the total N and P content was 2.38 and 0.99 g/kg, respectively. The red soil of the Cu mine area contained Cu, Pb, and Zn in concentrations of 184.70, 176.43, and 383.30 mg/kg, respectively. The average pH value was 7.01 and the total N and P content was 0.15 and 0.01 g/kg, respectively. In addition, a reference clay soil uncontaminated by heavy metals or mining activities (paddy soil) was collected from Fuyang City (30°001′N, 119°443′E). All areas selected in this study possess a subtropical climate. Samples from the upper 30 cm of the mine tailings and paddy soil substrate were analyzed for physicochemical properties (Table 1). According to environmental quality standards for soils (GB 15618-1995), heavy metal concentrations in the tailings areas were much higher than the recommended environmental soil quality standards.

1.2 Plant species

Two trees, *B. papyrifera* and *S. tonkinensis*, three shrub species, *A. fruticosa*, *V. trifolia* var. *simplicifolia*, and *G. puberum*, and a herbaceous plant, *S. cannabina*, were chosen as potential metal tolerant species. *Amorpha fruticosa* is a N-fixing shrub, which has long been used to revegetate degraded agricultural and mining land (Seo et al., 2008). *Vitex trifolia* var. *simplicifolia* is a rapidly growing non N-fixing shrub and *G. puberum* is one of the most officinal species in southern China (Zhang et al., 2008). The herbaceous plant, *S. cannabina*, was chosen as a non-tolerant species. Seeds of these species were all collected from Fuyang City.

1.3 Pot experiment

The experiments were performed in the open-top chamber of the Research Institute of Subtropical Forestry, Chinese Academy of Forestry. The Institute is situated in Fuyang City at an altitude of 90 m above sea level, and has a subtropical climate with a mean annual temperature of 16.2°C and 1452 mm of annual rainfall.

Each plant species was sown in the Cu and Pb/Zn tailings and the paddy soil. There were three replicates for each treatment and five pots were used for each replicate, totaling 270 pots in a randomized block design. After the growth media were air dried, blended, and sieved, 3000 g samples were collected and placed into cylindrical plastic pots (diameter 15 cm × height 15 cm). The seeds of the six species were sown directly into the pots and covered with a layer of paddy soil (1–1.5 cm) to ensure germination percentage. After germination, six seedlings were cultured in each pot. The experiment was conducted for 16 hr at 25–35°C during the day and 8 hr at 10–18°C during the night. The plants grew for 120 days from 1 April to 30 July, 2008. During the vegetation period, controlled irrigation was scheduled according to tensiometer measurements to keep soil moisture content around field capacity. At the end of the experiment period, all plants were harvested to determine biomass, root morphology, total N, P and K, and heavy metal content.

1.4 Harvest and chemical analysis

After the plants were harvested, the shoots and roots were cut off and separated from each other. The root samples were firstly washed with tap water three times followed by deionized water to remove any soil/tailings particles attached to the root surfaces, and were then washed with 0.1 mol/L of HCl and with de-mineralized water. The dry weights of the shoots and roots were measured after drying at 75°C for three days. The subsamples were ground into a powder and were digested (0.2 g) with a 4 mL of HNO_3 and 1 mL of $HClO_4$ mixture. Metal concentrations (Cu, Pb, and Zn) and nutrients (P and K) were determined using flame atomic absorption spectroscopy (AAS, Solaar M6, Thermo Fisher Scientific Inc, USA). Plant material was analyzed for N by Kjeldahl

Table 1 Physical and chemical characteristics of paddy soil and two mixed metals tailing areas

Type	TN (g/kg)	TP (g/kg)	K (g/kg)	pH	Heavy metal concentration (mg/kg soil dw)		
					Zn	Pb	Cu
Pb/Zn tailing	0.03 ± 0.004	0.37 ± 0.003	1.82 ± 0.08	7.72 ± 0.12	1328.10 ± 265.25	1217.94 ± 231.81	163.10 ± 44.76
Cu tailing	0.32 ± 0.01	4.46 ± 0.29	11.16 ± 0.11	8.26 ± 0.10	1149.84 ± 65.87	113.80 ± 14.00	642.05 ± 61.82
Paddy soil	2.38 ± 0.15	0.99 ± 0.10	2.35 ± 0.09	7.80 ± 0.15	219.60 ± 16.88	69.21± 5.26	8.68 ± 0.65

TN: total nitrogen; TP: total phosphor.

digestion (Madejon et al., 2003). In this study, the samples of *B. papyrifera* and *S. tonkinensis* are no enough for elemental analysis in tissues.

Soil and tailing samples were air dried and sieved (< 2 mm) after sampling from the mine tailing areas. Samples were then analyzed for total metals (Pb, Zn, Cu, and K) and total N and P content. Total N was determined by the Berthelot reaction method and total P by Molybdenum Blue method after digestion with 1.0 g of K_2SO_4 and 5 mL of concentrated H_2SO_4 (Page et al., 1982; Deng et al., 2004). Total Pb, Zn, Cu, and K content were determined using AAS after extraction with 5 mL of a 4:1 mixture (V/V) of 65% HNO_3 and 70% $HClO_4$ (Shu et al., 2001). The pH values (solid:distilled water of 1:2.5; V/V) of soil/tailings samples were measured. Certified reference materials (peach leaves, GBW 08501, China) were used to ensure the quality of analyses. Good agreement was obtained between our method and certified values.

The fine roots were freshly scanned by a root positioning system/STD4800 scanner (Regent Instruments Inc, Canada). From the pictures, the characteristics of the fine roots (total length, surface, volume, number of root tips, and length of root at different diameters) were analyzed with the WinRHIZO Pro 2005b (Regent Instruments Inc., Canada).

1.5 Data analysis

Tolerance index (TI) (data not shown) based on biomass was used to assess the tolerance of the six species in the same tailings. The tolerance index for different treatments was calculated as:

$$\mathrm{TI} = \frac{B_\mathrm{t}}{B_\mathrm{c}} \tag{1}$$

where, B_t (g/tree) means treatment biomass, and B_c (g/tree) means control biomass.

A higher value represented a higher tolerance in the plants. In addition, bioconcentration factors (BCF) was used to estimate the plant's ability to accumulate metals from soils. It is defined as the ratio of metal concentration in the aboveground plant tissues to that in the soil (Liu et al., 2008). Translocation factors (TF) was used to estimate the plant's ability to translocate metals from roots to shoots. The TF of each species was calculated as:

$$\mathrm{TF} = \frac{A_\mathrm{s}}{A_\mathrm{r}} \tag{2}$$

where, A_s (mg/kg) means total heavy metal accumulated in shoots and A_r (mg/kg) means total heavy metal accumulated in roots.

All statistical analyses (ANOVA and LSD test for mean comparisons) were conducted with SPSS 13.0. Differences at $p < 0.05$ were considered significant.

2 Results

2.1 Plant growth

The biomass of plants grown in the paddy soil and the two tailings is shown in Fig. 1. Results suggest that plant growth for all species was inhibited by the tailings except for *A. fruticosa*, which demonstrated the highest tolerance to tailings stress by maintaining normal growth. Compared to the *A. fruticosa* seedlings grown in paddy soil, *A. fruticosa* seedlings grown in Cu tailings increased biomass by 11.1%, while those grown in Pb/Zn tailings decreased biomass by only 6.43%. Compared to paddy soil, shoot biomass was reduced by 41.9% and 30.8% and root biomass was reduced by 40.4% and 33.7% for *S. cannabina* in Pb/Zn and Cu tailings, respectively, although *S. cannabina* still had the highest biomass (Fig. 1) in all media. The shoot biomass of *S. cannabina* was four times that of *A. fruticosa* and more than thirty times that of the other four species in Pb/Zn and Cu tailings. The biomass of the other four species grown in the two tailings was reduced by 60% compared to that grown in the paddy soil.

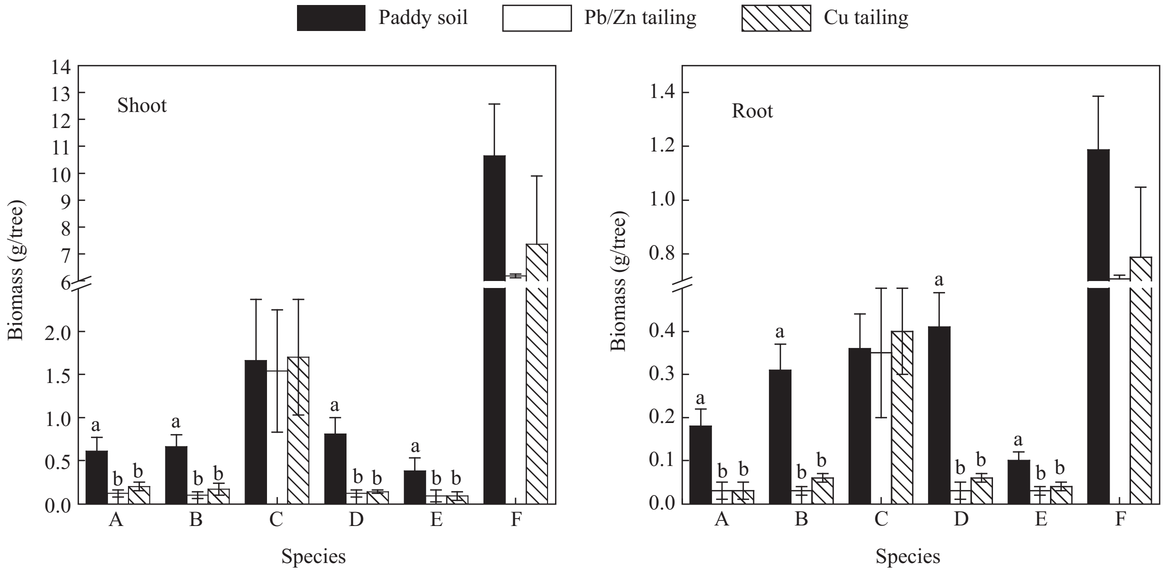

Fig. 1 Seedling biomass after 120 days in the three media. A: *V. trifolia* var. *simplicifolia*; B: *G. puberum*; C: *A. fruticosa*; D: *B. papyrifera*; E: *S. tonkinensis*; F: *S. cannabina*; Data represent mean ± SE. Different letters denote statistically significant differences at $p < 0.05$ among treatments for each species.

Figure 1 shows the better growth of all species in Cu tailing than in Pb/Zn tailing.

According to tolerance index values, the N-fixing shrub, *A. fruticosa*, was the most tolerant species to both tailings (TI values ranged from 0.92 to 1.01), while the N-fixing species, *S. cannabina*, had a moderate TI values of 0.65 to 0.81 and *B. papyrifera* was the most sensitive species to both tailings, with the TI values 0.19 for Cu and 0.15 Pb/Zn.

2.2 Root characterization

The characteristics of root architecture, including root length, root surface, root volume and number of root tips, are listed in Table 2. The results show that root characteristics for all species grown in the tailings varied remarkably depending on the species and tailing. All root parameters of *A. fruticosa* increased when it was grown in both tailings, while all values for the other plant species were significantly reduced (except root length of *S. cannabina* in Cu tailings). Root length, root surface, and root volume of *G. puberum* grown in Pb/Zn tailings were reduced by 70%, 80%, and 86%, respectively, compared to that grown in paddy soil. In addition, *S. cannabina* developed a much longer and finer root network than the other species, which agrees with their larger dry mass (Table 2 and Fig. 1).

To further evaluate root adaptation to mine tailings, root lengths with different diameters were analyzed. Table 3 shows the length of roots in different diameter classes. All species had significantly greater root length in the smaller-diameter root classes (< 0.5 mm) than in the bigger diameter root classes (> 0.5 mm). Root length in four of the determined root diameters for *V. trifolia* var. *simplicifolia*, *G. puberum*, *B. papyrifera*, and *S. tonkinensis* grown in tailings was significantly reduced compared to that grown in reference soil. Root lengths of treated plants were significantly inhibited in the bigger-diameter root classes of 1.0–2.0 mm and > 2.0 mm for non-N-fixing species. Plants grown in Cu tailings had much longer root length in all root classes than that grown in Pb/Zn tailings.

Table 2 Root parameters for the six species in three media after 120 days

Species	Medium	Length (cm)	Surface (cm^2)	Volume (cm^3)	Number of root tips
V. trifolia var. *simplicifolia*	Paddy soil	121.00 ± 14.46 a	18.24 ± 2.87 a	0.22 ± 0.04 a	372 ± 68.27 a
	Pb/Zn tailing	77.97 ± 16.61 b	6.76 ± 1.25 b	0.05 ± 0.01 b	334 ± 57.51 a
	Cu tailing	82.86 ± 22.76 b	7.42 ± 1.93 b	0.06 ± 0.02 b	329 ± 70.36 a
G. puberum	Paddy soil	757.17 ± 37.48 a	70.89 ± 21.29 a	0.54 ± 0.23 a	1244 ± 87.88 a
	Pb/Zn tailing	224.53 ± 161.36 b	14.49 ± 2.20 ab	0.07 ± 0.01 b	574 ± 50.97 b
	Cu tailing	257.58 ± 74.03 b	22.05 ± 3.28 b	0.15 ± 0.01 b	980 ± 372.60 ab
A. fruticosa	Paddy soil	345.80 ± 164.70 a	35.28 ± 5.6 a	0.29 ± 0.05 a	1855 ± 200.08 a
	Pb/Zn tailing	350.21 ± 78.06 a	36.36 ± 14.13 a	0.31 ± 0.09 a	1784± 682.69 a
	Cu tailing	428.70 ± 228.04 a	40.06 ± 11.16 a	0.31 ± 0.02 a	2361 ± 1147.76 a
B. papyrifera	Paddy soil	368.80 ± 37.17 a	39.56 ± 5.23 a	0.35 ± 0.01 a	1870 ± 614.56 a
	Pb/Zn tailing	111.19 ± 81.87 b	10.50 ± 4.58 b	0.09 ± 0.05 b	492 ± 157.63 b
	Cu tailing	284.96 ± 154.03 a	19.71 ± 9.54 b	0.11 ± 0.05 b	1340 ± 369.74 ab
S. tonkinensis	Paddy soil	335.53 ± 86.28 a	45.39 ± 14.36 a	0.50 ± 0.22 a	566 ± 105.94 a
	Pb/Zn tailing	231.44 ± 10.12 a	24.63 ± 2.92 a	0.21 ± 0.04 b	443 ± 14.03 a
	Cu tailing	312.35 ± 25.45 a	36.73 ± 4.31 a	0.35 ± 0.07 ab	480 ± 48.25 a
S. cannabina	Paddy soil	848.34 ± 42.87 ab	95.14 ± 21.62 a	0.87 ± 0.29 a	6038 ± 600.19 a
	Pb/Zn tailing	696.10 ± 109.19 b	71.58 ± 3.74 b	0.60 ± 0.05 bc	4429 ± 504.38 b
	Cu tailing	891.43 ± 23.37 a	88.82 ± 23.87 ab	0.75 ± 0.40 ab	5449 ± 563.36 a

Data represent mean ± SD. Different letters denote statistically significant differences at $p < 0.05$ among treatments for each species.

Table 3 Root length in different diameter classes of the six species in the three media after 120 days

Species	Medium	Root length (cm)			
		< 0.5 mm	0.5–1.0 mm	1.0–2.0 mm	> 2.0 mm
V. trifolia var. *simplicifolia*	Paddy soil	91.68 ± 11.78 a	12.38 ± 5.09 a	11.05 ± 1.18 a	5.67 ± 1.78 a
	Pb/Zn tailing	65.66 ± 12.13 a	7.99 ± 1.50 a	3.51 ± 1.70 b	0 ± 0 b
	Cu tailing	70.84 ± 21.26 a	6.76 ± 1.21 a	5.03 ± 1.54 b	0.04 ± 0.03 b
G. puberum	Paddy soil	681.75 ± 138.76 a	49.44 ± 23.10 a	16.82 ± 7.28 a	7.86 ± 2.63 a
	Pb/Zn tailing	209.70 ± 39.46 b	10.61 ± 0.51 b	3.55 ± 1.42 b	0.03 ± 0.04 b
	Cu tailing	237.29 ± 76.07 b	13.04 ± 1.65 b	5.82 ± 1.04 b	0.82 ± 0.45 b
A. fruticosa	Paddy soil	308.70 ± 74.86	18.82 ± 4.45 a	7.10 ± 0.75 a	9.85 ± 1.78 a
	Pb/Zn tailing	310.04 ± 153.55	21.26 ± 8.19 a	8.87 ± 1.70 a	9.03 ± 2.76 a
	Cu tailing	383.92 ± 216.50	25.53 ± 10.70 a	8.88 ± 3.56 a	8.58 ± 3.28 a
B. papyrifera	Paddy soil	328.67 ± 72.84 a	19.36 ± 6.02 a	7.48 ± 3.25 a	11.47 ± 1.08 a
	Pb/Zn tailing	96.63 ± 31.19 b	7.02 ± 2.78 a	5.04 ± 2.30 a	1.42 ± 1.29 b
	Cu tailing	266.79 ± 147.92 a	10.02 ± 5.18 a	5.21 ± 0.97 a	1.66 ± 0.99 b
S. tonkinensis	Paddy soil	247.06 ± 70.13 a	75.56 ± 43.47 a	11.01 ± 7.04 a	1.55 ± 0.69 a
	Pb/Zn tailing	196.19 ± 14.52 a	30.50 ± 9.21 b	5.52 ± 4.98 a	0.54 ± 0.66 b
	Cu tailing	248.49 ± 29.04 a	54.30 ± 11.46 a	8.78 ± 1.17 a	0.46 ± 0.23 b
S. cannabina	Paddy soil	731.26 ± 77.22 ab	70.69 ± 19.10 a	24.73 ± 9.57 ab	18.40 ± 5.20 a
	Pb/Zn tailing	610.94 ± 31.23 b	51.22 ± 13.50 a	19.51 ± 1.35 b	11.76 ± 0.41 b
	Cu tailing	779.27 ± 5.38 a	66.91 ± 16.92 a	28.36 ± 14.24 a	13.73 ± 8.29 b

Data are represented as mean ± SD. Different letters denote statistically significant differences at $p < 0.05$ among treatments for each species.

2.3 Nutrition in tissues

Total N, P, and K content in plant tissues varied significantly among species ($p < 0.05$, Fig. 2). In the shoots and roots of N-fixing shrubs, the N content in *A. fruticosa* was significantly higher than that in other species (especially in roots). In addition, the nutrient content in plant tissues varied among tailings. Generally, the concentrations of N, P, and K in plant tissues grown in Cu tailings were much higher than that grown in Pb/Zn tailings.

2.4 Accumulation and translocation of metals in plants

Metal content in plants grown in the tailings was measured to evaluate their ability to uptake heavy metals. Table 4 compares Cu, Pb, and Zn content in roots with that in shoots of the selected four species. Generally, the four species grown in Cu and Pb/Zn tailings contained higher metal concentrations in their tissues than those grown in paddy soil. Higher metal content was also observed in the roots than in the shoots of plants grown in different media, except Zn concentrations in *V. trifolia* var. *simplicifolia* grown in paddy soil and Cu tailings, and Pb concentrations in *G. puberum* grown in paddy soil. In addition, plants grown in Pb/Zn tailings contained higher Zn concentrations in their tissues than those grown in Cu tailings. The highest Zn content was found in the shoots of *V. trifolia* var. *simplicifolia* and the roots of *G. puberum*, both grown in Pb/Zn tailings. The highest Pb content was found in the roots of *V. trifolia* var. *simplicifolia* and the shoots of *S. cannabina* grown in Pb/Zn tailings. The highest Cu content was observed in the shoots and roots of *G. puberum* grown in the Cu tailings. In addition, metal concentrations in plant tissues differed among species grown in the same tailing. Both *G. puberum* and *A. fruticosa* accumulated significantly higher Zn (208.75 and 201.91 mg/kg, respectively) in their roots than other species grown in the Pb/Zn tailings. In Cu tailings, *V. trifolia* var. *simplicifolia* had an average Zn concentration of 91.40 mg/kg in the aboveground tissues, which was much higher than for other species. Variation in concentration was greater in roots than in shoots. For instance, the Pb concentration in roots was more than thirty times that in the shoots of *S. cannabina* grown in the Pb/Zn tailings.

The TF values of the four selected species subjected to the tailings are listed in Table 5. In general, the TF

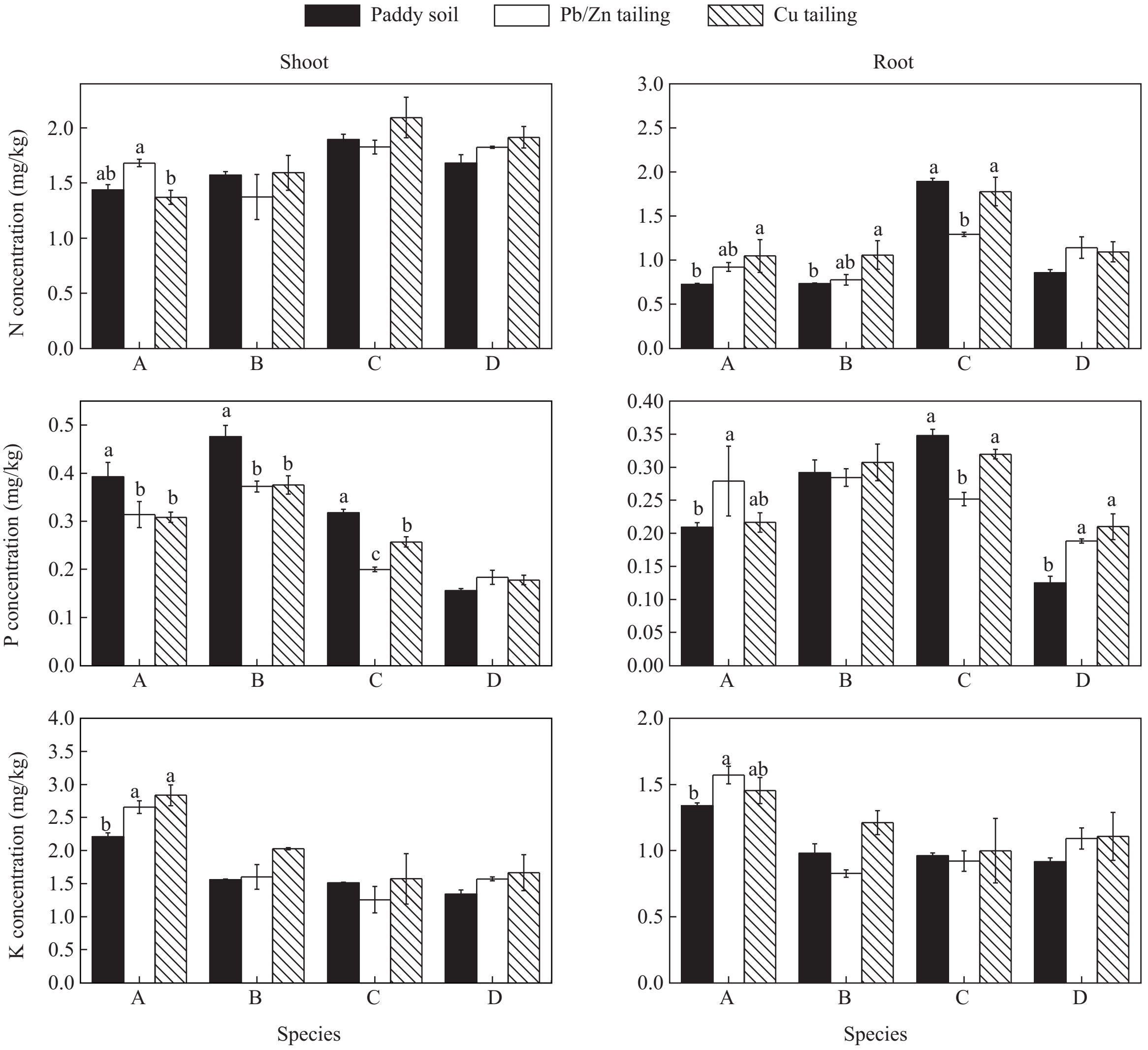

Fig. 2 Content of N, P and K in four species grown in the three media after 120 days. A: *V. trifolia* var. *simplicifolia*; B: *G. puberum*; C: *A. fruticosa*; D: *S. cannabina*. Data are represented as mean ± SE. Different letters denote statistically significant differences at $p < 0.05$ among treatments for each species.

Table 4 Heavy metal concentrations in the shoot and root tissue of four species grown in different types of soil after 120 days

Species	Medium	Zn concentration (mg/kg)		Pb concentration (mg/kg)		Cu concentration (mg/kg)	
		Shoot	Root	Shoot	Root	Shoot	Root
V. trifolia var. *simplicifolia*	Paddy soil	78.67 ± 1.1 c	69.98 ± 7.8 ef	0.44 ± 0.2 b	1.11 ± 0.2 c	10.32 ± 0.7 bc	31.19 ± 1.1 b
	Pb/Zn tailing	130.85 ± 10.5 a	166.74 ± 8.6 bc	3.57 ± 0.6 a	18.76 ± 14.1 b	nd	nd
	Cu tailing	91.40 ± 0.8 b	71.51 ± 14.2 ef	nd	nd	13.38 ± 3.2 ab	36.84 ± 11.7 ab
G. puberum	Paddy soil	67.98 ± 2.4 d	73.08 ± 2.4 ef	1.56 ± 0.7 b	0.72 ± 0.2 c	8.61 ± 1.0 bc	24.92 ± 1.5 b
	Pb/Zn tailing	83.66 ± 2.8 bc	208.75 ± 24.2 a	1.73 ± 2.4 b	9.00 ± 2.4 c	nd	nd
	Cu tailing	79.85 ± 5.0 c	126.93 ± 18.5 cd	nd	nd	19.53 ± 11.8 a	60.57 ± 11.7 a
A. fruticosa	Paddy soil	38.41 ± 6.6 f	102.59 ± 2.4 de	0.85 ± 0.1 b	2.27 ± 0.5 c	5.11 ± 2.4 c	25.28 ± 1.8 b
	Pb/Zn tailing	49.56 ± 5.5 e	201.91 ± 36.9 ab	1.23 ± 0.3 b	4.11 ± 1.5 c	nd	nd
	Cu tailing	38.79 ± 10.9 f	161.86 ± 57.0 bc	nd	nd	4.65 ± 1.2 c	60.35 ± 37.8 a
S. cannabina	Paddy soil	37.22 ± 2.4 f	47.49 ± 4.5 f	0.36 ± 0.1 b	1.05 ± 0.2 c	4.65 ± 0.5 c	18.46 ± 1.98 b
	Pb/Zn tailing	56.47 ± 6.5 e	161.10 ± 15.9 bc	0.70 ± 0.5 b	34.82 ± 4.1 a	nd	nd
	Cu tailing	37.41 ± 3.5 f	88.71 ± 23.6 def	nd	nd	7.84 ± 0.4 bc	31.99 ± 9.7 b

Data are represented as mean ± SD; nd stands for not determined in this study; different letters denote statistically significant differences at $p < 0.05$ among treatments for species.

Table 5 Translocation factor (TF) and bioconcentration factor (BCF) of Pb, Cu, and Zn in four species

Species	TF or BCF of Pb/Zn tailing		TF or BCF of Cu tailing	
	Zn	Pb	Zn	Cu
	TF			
V. trifolia var. simplicifolia	0.78 ± 0.07 a	0.28 ± 0.20 a	1.32 ± 0.28 a	0.37 ± 0.04 a
G. puberum	0.40 ± 0.04 b	0.17 ± 0.22 ab	0.63 ± 0.09 b	0.32 ± 0.15 a
A. fruticosa	0.25 ± 0.02 c	0.31 ± 0.03 a	0.24 ± 0.06 c	0.10 ± 0.04 b
S. cannabina	0.35 ± 0.08 bc	0.02 ± 0.01 b	0.44 ± 0.15 bc	0.26 ± 0.09 a
	BCF			
V. trifolia var. simplicifolia	0.22 ± 0.01 a	0.02 ± 0.01 b	0.12 ± 0.01 ab	0.08 ± 0.02
G. puberum	0.22 ± 0.02 a	0.01 ± 0.001 bc	0.16 ± 0.01 a	0.12 ± 0.03
A. fruticosa	0.18 ± 0.03 bc	0.005 ± 0.01 c	0.15 ± 0.03 a	0.10 ± 0.06
S. cannabina	0.16 ± 0.01 c	0.03 ± 0.001 a	0.09 ± 0.03 b	0.06 ± 0.02

Data are represented as mean ± SD; different letters denote statistically significant differences at $p < 0.05$ among treatments for each species.

values of the four species were lower than 1, except for *V. trifolia* var *simplicifolia* grown in Cu tailings, which had the highest TF values of Zn (TF = 1.32). For all plant species studied, BCF values ranged from 0.09–0.22 for Zn, 0.005–0.03 for Pb, and 0.06–0.12 for Cu (Table 5).

3 Discussion

3.1 Plant growth and tolerance

Previous studies on heavy metal tolerance confirm that populations surviving in metal contaminated areas are differentiated from the same species growing in non-contaminated areas by possessing genetically based tolerances (Yang and Chen, 2009). In this study, the development of five of the six species (excluding *A. fruticosa*) grown in tailings was seriously inhibited. One of the main factors affecting this growth was the abundance of Pb, Zn and Cu in the tailings. While these elements play an important role in several metabolic processes in plants, excess Zn, Cu, and Pb content in soil may retard plant growth (Kamal et al., 2004). Kabata-Pendias and Pendias (1984) reported that 100–400 mg/kg of Pb, 70–400 mg/kg of Zn, and 60–125 mg/kg of Cu in soil would be considered toxic to plants. The content of Cu, Pb, and Zn in the selected mine tailings greatly exceeded these ranges. Although the selected species in this experiment can survive in tailings, their growth and root development were inhibited to varying degrees. The different ability of each plant to maintain normal growth in the tailings reflected their difference in resistance or tolerance to metal toxicity. Levy et al. (1999) also reported that normal and phytotoxic concentrations of Pb, Zn, and Cu ranged from 0.5–10 and 30–300 mg/kg for Pb, 3–30 and 20–100 mg/kg for Cu, and 10–150 and >100 mg/kg for Zn, respectively. The metal concentrations in all species in this study were higher than the above limits. Our results indicate that the species grown in the mine tailings were tolerant to these metals by varying degrees.

Roots are necessary for growth and development, and root modification will have an effect on other plant parts (Biernacki and Lovett-Doust, 2002). The present work showed that the root development of all species grown in the tailings, except for *A. fruticosa*, were significantly inhibited and led to small plant biomass. As seen in Fig. 1, *A. fruticosa* showed normal growth, suggesting its high tolerance to Pb, Zn and Cu.

Mine tailings contain little nutrients (Mendez and Maier, 2008), which is a limiting factor for revegetation. The N-fixing species, *A. fruticosa* and *S. cannabina*, retained a high biomass when grown in the tailings, suggesting that N-fixing species, which fix nitrogen from the air and improve soil nutrition, increase a plant's adaptive ability to tailings stress. It seems, therefore, that N-fixing species are promising plant species for the revegetation of mine tailings areas. With the exception of *A. fruticosa*, the remaining studied species exhibited growth inhibition in the tailings to varying degrees. Plants grown in Cu tailings had higher biomass production than that of Pb/Zn tailings, which is likely related to the higher content of N, P, and K in Cu tailings. This result agrees with findings obtained by Clemente et al. (2005).

3.2 Metal accumulation and translocation

Metal accumulation in the roots and shoots of four studied species was much lower compared to other phytoaccumulators (Zu et al., 2005). Seo et al. (2008) reported that in unfertilized treatments, the leaf, stem, and roots of *A. fruticosa* contained Zn concentrations of 358.6, 67.3, and 644.3 mg/kg, Pb concentrations of 47.5, 51.2, and 323.0 mg/kg, and Cu concentrations of 18.2, 5.1, and 215.7 mg/kg, respectively. The metal concentrations in *A. fruticosa* were lower in this study mainly due to the relatively short growth period (4 months compared to 18 months. Another factor may be that smaller saplings and shrubs are affected more significantly by heavy metals than are adult trees.

Plant roots also play a crucial role in phytoremediation because they aggregate soil particles and can stabilize or take up heavy metals (Brunner et al., 2008). In this study, big roots with diameters > 1.0 mm were inhibited significantly, which was likely related to decreased heavy metal uptake. In the view of toxicology, however, this could be a desirable result as metals would not enter the food chain and potential environmental risks could be avoided (Deng et al., 2004).

The difference in metal uptake between species reflected the status of their associated tailings, which indicates that metal uptake could be affected by several factors. The plants growing in tailings were most efficient in taking up and translocating Zn. Low translocation of Pb from their roots to shoots may be due to Pb toxicity (Yoon et al., 2006). Soil pH was also a factor affecting metal bioavailability due to its determinant influence on metal solubility (Clemente et al., 2003; Burgos et al., 2006). Alkaline soil may play a role in limiting heavy metal bioavailability in soil (Rosselli et al., 2003), possibly explaining why woody plants growing in Pb/Zn and Cu tailings with pH of 7.72 and 8.26, respectively, had small BCF and TF values. In this study, P concentration was much higher in Cu tailings than in Pb/Zn tailings, but plants growing in Pb/Zn tailings accumulated much higher Zn in their tissues than those in Cu tailings. High P levels in soil may decrease Zn availability and uptake by plants due to chemical reactions in the rhizosphere where P has a strong tendency to absorb metals (Kabata-Pendias and Pendias, 1984). This is also supported by Deng (2004) who reported that phosphorus was negatively related to some metal uptake by plants.

The ability of plants to accumulate metals is often expressed as BCF. In our study, the BCF for all metals was far below 1 and metal translocation from roots to shoots appears to be very restricted in all species, suggesting that harvesting shoots will not remove metal effectively from mine tailing. Different tissues of the species exhibited wide variation in their ability to accumulate heavy metals. Table 4 shows that root tissues accumulated significantly greater concentrations of metals than shoot tissues, presenting high plant availability of the metals as well as its limited mobility once inside the plant (Fitzgerald et al., 2003; Deng et al., 2004).

3.3 Potential use of woody species in phytoremediation

There are two ways to practice phytoremediation: phytoextraction and phytostabilization (Tian et al., 2009). Phytoextraction is the extraction and translocation of metals from soils to aboveground plant parts (Elizabeth, 2005). The term of phytostabilization denotes the use of plants to stabilize pollutants in soil, either by preventing erosion, leaching, or runoff, or by converting pollutants to less bioavailable forms (Elizabeth, 2005). According to Mendez and Maier (2008), if the TF and BCF values of plant are lower than 1, it could be considered for use in phytostabilization. The ideal phytoremediation plant should be tolerant to high levels of the metal and be able to accumulate high levels of the metal in its harvestable parts (Deng et al., 2007). Present results showed that *A. fruticosa* and *S. cannabina* would be a good choice for phytostabilization in revegetation of mine tailings due to their higher biomass production and faster growth rates than other species. In addition, the amount of heavy metals in the shoots of *A. fruticosa* and *S. cannabina* was much higher than other species. For instance, *A. fruticosa* extracted 7.37 μg (Cu), 76.16 μg (Zn, Pb/Zn tailing), and 1.96 μg (Pb). However, although *S. cannabina* could uptake more heavy metal, it is an annual species and thus not a priority choice for phytostabilization of mine tailing. As *V. trifolia* var. *simplicifolia* was able to accumulate relatively high concentrations of Zn in its aboveground tissues and is a rapidly growing shrub, it could also be considered a good candidate for phytoextraction. The other species studied exhibited stress to heavy metal and are, therefore, more suitable as indicator plants. In addition, the N-fixing plants in this study grew well and quickly in mine tailings with low nutrient conditions, indicating that they would be efficient plant options for the revegetation of mine tailings without fertilizer amendments.

4 Conclusions

Both Pb/Zn and Cu tailings contain elevated concentrations of total Pb, Zn, and Cu, which impose high stress to species planted for revegetation. The highest tolerance to tailings stress was demonstrated by *A. fruticosa*, which maintained normal root development and biomass production. *S. cannabina* showed the highest biomass production among the tested plants, although biomass decreased by 30% and 40% in the Cu and Pb/Zn tailings, respectively. The remaining four species studied (*V. trifolia* var *simplicifolia*, *G. puberum*, *S. tonkinensis* and *B. papyrifera*) maintained growth in both tailings although their biomass production decreased.

Despite the high concentrations of heavy metals in the mine tailings, there was only a slight transfer of these elements to the aboveground plant parts. All species exhibited low TF and BCF values, except the TF value of Zn for *V. trifolia* var *simplicifolia*, which suggested that these species could be applied in mine tailing areas for revegetation. The N-fixing species, *A. fruticosa* and *S. cannabina*, which have the highest tolerance and biomass production, respectively, demonstrated the greatest potential for revegetation in tailings of southern China.

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