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RESEARCH ARTICLE

Comparative of *Quercus* spp. and *Salix* spp. for phytoremediation of Pb/Zn mine tailings

Xiang Shi[1,2] · **Shufeng Wang**[1] · **Haijing Sun**[1] · **Yitai Chen**[1] · **Dongxue Wang**[1,3] · **Hongwei Pan**[1] · **Yazhu Zou**[1] · **Jianfeng Liu**[2] · **Linyu Zheng**[2] · **Xiulian Zhao**[2] · **Zeping Jiang**[2]

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Abstract A pot experiment was conducted to evaluate the feasibility of using tree seedlings for the phytoremediation of lead/zinc (Pb/Zn) mine tailings. Seedlings of three *Quercus* spp. (*Q. shumardii*, *Q. phellos*, and *Q. virginiana*) and rooted cuttings of two *Salix* spp. (*S. matsudana* and *S. integra*) were transplanted into pots containing 50 and 100 % Pb/Zn mine tailings to evaluate their tolerance of heavy metals. The five species showed different tolerance levels to the Pb/Zn tailings treatments. *Q. virginiana* was highly tolerant to heavy metals and grew normally in the Pb/Zn tailings. The root systems showed marked differences between the *Quercus* spp. and *Salix* spp., indicating that different mechanisms operated to confer tolerance of heavy metals. The maximum efficiency of photosystem II photochemistry value of the five species showed no differences among the treatments, except for *Q. shumardii*. All species showed low metal translocation factors (TFs). However, *S. integra* had significantly higher TF values for Zn (1.42–2.18) and cadmium (1.03–1.45) than did the other species. In this respect, *Q. virginiana* showed the highest tolerance and a low TF, implying that it is a candidate for phytostabilization of mine tailings in southern China. *S. integra* may be useful for phytoextraction of tailings in temperate regions.

Keywords Mine tailing · Phytoremediation · Tolerance · *Quercus* spp. · *Salix* spp. · Root

Introduction

Heavy metals are environmental contaminants that are hazardous to humans and other biota (Marques et al. 2009). Heavy metal contamination of soils has become a serious environmental problem in industrial and agricultural areas (Bhargava et al. 2012). Mining is one of the most important sources of heavy metal contamination (Li et al. 2014). For example, overburden and waste materials from mining, with their consequent environmental risks, are contaminants in surrounding areas (Acosta et al. 2011; Parra et al. 2014). Furthermore, mine tailings are difficult to revegetate because of high concentrations of metals, low nutrient levels, abnormal pH values, and low water retention capacity (Cano-Reséndiz et al. 2011; Solís-Dominguez et al. 2012). In most cases, the tailings also have steep slopes (Cano-Reséndiz et al. 2011). In China, mining activities have generated about 1.5 million ha of wasteland (Zhuang et al. 2009), and the area is increasing at 46,700 ha annually (Li et al. 2007). Thus, there is an urgent demand for effective management of mine tailings.

There is a variety of techniques for in situ reclamation of mine wastes, many with high maintenance costs and secondary pollution risk (Ucisik and Trapp 2008). Phytoremediation is a green technology that uses plants to mitigate environmental problems without the need for excavation and disposal of the contaminating material (Zhang et al. 2014). However, most tailing disposal sites are devoid of vegetation cover, principally as a result of the elevated concentration and

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✉ Zeping Jiang
jiangzp@caf.ac.cn

[1] Research Institute of Subtropical Forestry, Key Laboratory of Tree Breeding of Zhejiang Province, Chinese Academy of Forestry, Hangzhou 311400, China

[2] Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China

[3] Forestry College of Inner Mongolia Agricultural University, Huhhot 010019, China

bioavailability of heavy metals combined with adverse growth conditions (Mendez and Maier 2008a; Monterroso et al. 2014). Therefore, a crucial consideration in phytoremediation technology is the selection of plant species to meet the objectives of phytoremediation (Solís-Dominguez et al. 2012; Suchkova et al. 2014; Zhang et al. 2014). The ideal plant for phytoremediation should grow rapidly, produce high biomass, develop a deep root system, grow on poor soils, and show metal stress tolerance (Pérez-Esteban et al. 2013; Pottier et al. 2015). Relatively few plants possess all these attributes. However, woody species constitute the majority of plant biomass in native forests and shrublands, which can provide extensive canopy cover and establish a deep root network to prevent erosion of mine wastes in the long term (Mendez and Maier 2008a). Furthermore, trees can decrease metal mobility and toxicity through root growth (Brunner et al. 2008). Thus, research on suitable woody plants, and understanding their metal accumulation patterns, is critically needed to assess their potential use in phytoremediation in the field.

Few studies have evaluated the utility of woody species for phytoremediation of mine tailings or contaminated soil (Mertens et al. 2004; Domínguez et al. 2008; Seo et al. 2008; de Souza et al. 2012; Pottier et al. 2015). In recent years, willow species (*Salix* L. spp.), which show rapid growth and produce extensive root systems, have been suggested as suitable for use in the remediation of metal-contaminated soils. Willows typically accumulate the highest contents of heavy metal in wood compared with other tree species (Algreen et al. 2014; Evlard et al. 2014). A number of willow species have been studied for their ability to tolerate and accumulate heavy metals (Zacchini et al. 2009, 2011; Pietrini et al. 2010; Evlard et al. 2014; Wang et al. 2014). The genus *Quercus* L. (oak species) is a keystone taxon from both ecological and economic perspectives (Aldrich and Cavender-Bares 2011). Compared with willow species, knowledge of the physiological responses of oak trees to heavy metal stress is incomplete (Gogorcena et al. 2011). Oak trees have been reported as more tolerant than willow trees to growth in metal-contaminated soils (Evangelou et al. 2012). Moreover, oak trees have proven suitable for reforestation in areas contaminated with heavy metals (Prasad and Freitas 2000; Domínguez et al. 2009; Zhao et al. 2015). The red oak species Shumard oak (*Q. shumardii* Buckley) and willow oak (*Q. phellos* L.), and the white oak species southern live oak (*Q. virginiana* Mill.), from southern USA, were introduced to China for the establishment of plantations in different sites and soil types in the Yangzi River Delta in 2002. These oak species show good adaptability to natural conditions in the delta, and trees began to bear fruit at age 6–9 years. *S. matsudana* Koidz. and *S. integra* Thunb. are native willow species in China and are widely planted throughout China. These five tree species were tested as candidates for phytoremediation by growing seedlings in lead/zinc (Pb/Zn) mine tailings. The growth, tolerance index, root traits, chlorophyll parameters, and metal uptake of seedlings were assessed. The objectives of this study were to elucidate the adaptive capabilities of the selected oak and willow species for growth on mine tailings and their potential use for phytoremediation of mine tailings in China.

Materials and methods

Site description and soil samples

Tailings from a Pb/Zn mine were collected from the city of Hangzhou (30° 126′ N, 119° 847′ E), China. The control medium (river sand), which was unaffected by heavy metal contamination or mining activities, was collected from Hangzhou (30° 057′ N, 119° 956′ E). River sand was chosen because its texture is similar to that of the mine tailings. All areas where materials were collected for this study experience a subtropical climate. The physicochemical properties of samples from the uppermost 30 cm of the mine tailings and river sand were analyzed (Table 1). According to the Chinese Environmental Quality Standard for Soils (GB 15618-1995), the Class 3 standards of maximum concentrations of Pb, Zn, and cadmium (Cd) permitted in soils are 500, 500, and 1.0 mg kg^{-1}, respectively. However, heavy metal concentrations in the mine tailings were much higher than the recommended limits.

Plant materials and growth conditions

Seeds of *Q. shumardii*, *Q. phellos*, and *Q. virginiana* were collected from plantations in Shanghai, China. The seeds were

Table 1 Characteristics of three types of medium

	Control	Tailing (50 %)	Tailing (100 %)
Lead (mg kg^{-1})	22	3260	6090
Available lead (mg kg^{-1})	nd	200	336
Zinc (mg kg^{-1})	38.4	1610	2975
Available zinc (mg kg^{-1})	nd	6.3	12.6
Cadmium (mg kg^{-1})	1.5	15.9	28.5
Available cadmium (mg kg^{-1})	nd	nd	nd
Hydrolysable nitrogen (mg kg^{-1})	0.64	3.21	6.40
Available phosphorus (mg kg^{-1})	10.76	11.24	8.46
Rapidly available potassium (mg kg^{-1})	21.0	22.4	25.0
Organic matter (g kg^{-1})	2.53	29.35	62.40
pH	7.90	7.66	7.66

nd not detection

sown in a pot made of non-woven fabric (diameter 6 cm × height 10 cm) that contained growing nutrient medium comprising perlite/peat (1:3; *v*/*v*). When they reached the stage of eight to ten leaves, uniform seedlings were selected and transplanted into black cylindrical plastic pots (diameter 18 cm × height 23 cm) without fertilizer for the experiment. Stem cuttings of *S. integra* and *S. matsudana* were obtained from native habitats in Shandong and Zhejiang provinces. Healthy willow stem cuttings of uniform length (8 cm) were rooted in pots made of non-woven fabric (diameter 6 cm × height 10 cm). After growth for 2 months, uniform rooted cuttings of similar height and weight were selected for the experiment.

Experimental method

The experiment was performed in the greenhouse of the Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, China. The river sand or tailings were air-dried, blended, sieved, and then 3-kg samples were placed in the black cylindrical plastic pots without fertilizer (diameter 18 cm × height 23 cm). Uniform seedlings/rooted cuttings (*S. integra*, *S. matsudana*, *Q. shumardii*, *Q. phellos*, and *Q. virginiana*) with their nutrient medium were transplanted into the pots in April 2011. Each pot contained 2 seedlings or rooted cuttings. Each species was grown in control medium, 50 % tailings substrate, and 100 % tailing substrate (river sand/tailings at 1:0, 1:1, and 0:1, *v*/*v*, respectively), resulting in 15 treatments each comprising 5 pots. Each of the 15 treatments was an experimental unit, and the 3 experimental units were arranged in a randomized block design. The plants were cultivated for 150 days with natural light, day/night conditions of 25–35/20–25 °C, and relative humidity of 70/85 %. During the growth period, irrigation was scheduled according to tensiometer measurements to maintain the soil moisture content at approximately field capacity. Metal-related phytotoxicity symptoms were recorded during the experiment. All seedlings were harvested after 150 days.

Biomass measurements

After harvesting, the leaves, stems, cuttings, roots in nutrient medium (inner roots), and roots in river sand or tailings (external roots) were excised and separated. The samples were washed thoroughly with distilled water. The roots were immersed in 20 mmol L^{-1} Na_2-EDTA for 15 min to remove metals adhering to the root surface (Wang et al. 2014). The dry weights of the samples were measured after drying at 75 °C for 3 days to a constant weight. To compensate for the marked differences in initial plant development between species, relative growth rate (RGR) was adopted for calculation of total plant dry weight. The RGR from initiation of the experiment to final harvest was calculated in accordance with the formula of Dimitriou et al. (2006):

$$\mathrm{RGR} = \frac{(\ln A_{\mathrm{f}} - \ln A_{\mathrm{i}})}{t_{\mathrm{f}} - t_{\mathrm{i}}} \qquad (1)$$

where A_f is measured plant dry biomass at the final harvest calculated as the mean value of three replicates per species ($n = 3$), A_i is measured plant dry biomass at the initial harvest calculated as the mean of six plants' dry biomass per species ($n = 6$), and t is time in weeks.

To assess the tolerance of the six species to mine tailings, the tolerance index (TI) based on biomass was calculated for each treatment as TI = B_t/B_c (Metwally et al. 2005), where B_t (g $plant^{-1}$) is treatment biomass and B_c (g $plant^{-1}$) is control biomass. High values indicate high tolerance by plants.

Measurement of root traits

All roots from an individual plant were scanned while fresh using a root positioning system/STD4800 scanner (Regent Instruments Inc., Québec, Canada) at the end of the experiment. From the images obtained, the characteristics of roots (mean total length, mean surface area, mean volume, mean diameter, and mean number of root tips) were analyzed using WinRHIZO Pro 2005b software (Regent Instruments).

Estimation of chlorophyll content

We used an Opti-Sciences CCM-200 chlorophyll content meter (Opti-Sciences Inc., Hudson, NH, USA) to non-destructively estimate the chlorophyll content in leaves by recording the chlorophyll concentration index (CCI).

Estimation of chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters for mature leaves (third–fourth leaf from the apex) from plants of the control and each treatment was measured after 150 days, with measurement performed during 8:30–11:00 a.m. In vivo chlorophyll fluorescence was measured on the upper surface of mature leaves at room temperature after dark adaptation for 25 min using a portable fluorometer (Mini-PAM, Walz, Effeltrich, Germany). The minimal fluorescence level in the dark-adapted state (F_0) was measured when the modulated light was sufficiently low (<0.1 μmol m^{-2} s^{-1}) to avoid inducing significant variable fluorescence. The maximal fluorescence level in the dark-adapted (F_m) and light-adapted ($F_{m'}$) states was determined before or after addition of the actinic light by 0.8 s of saturating white light (160 μmol m^{-2} s^{-1}) to close all reaction centers and drive photochemical quenching to zero. The steady-state value of fluorescence (F_s) under actinic light was also recorded. Using both light- and dark-adapted fluorescence parameters, we calculated the maximum efficiency of photosystem II (PSII) photochemistry (F_v/fm) in the dark-adapted state, the quantum yield of PSII electron transport as $\Phi_{PSII} = (F_{m'} - F_s)/fm'$, photochemical quenching as $qP = (F_m - F_s)/(F_{m'} - F_0)$, and non-photochemical quenching as $NPQ = (F_{m'} - F_m)/fm'$ (Kumar and Prasad 2015).

Chemical analysis

Dried plant samples (shoots and roots) were ground into powder, and 0.2 g of each sample was digested with a mixture of 4 mL of nitric acid (HNO_3) and 1 mL of perchloric acid ($HClO_4$). Metal concentrations (Cd, Pb, and Zn) were determined using atomic absorption spectroscopy (AAS) (SOLAAR M6, Thermo Fisher Scientific Inc., Waltham, MA, USA). Tailings and soil samples were air-dried and then sieved through a mesh (<2 mm) to yield a homogeneous mixture. Total Pb, Zn, and Cd concentrations were determined using flame AAS after extraction with 5 mL of a 4:1 (*v*/*v*) mixture of 65 % HNO_3 and 70 % $HClO_4$.

River sand and tailing samples were air-dried and sieved (<2 mm) after sampling from the mine tailing areas. Samples were then analyzed for total and available metals (Pb, Zn, and Cd) and hydrolysable nitrogen, available phosphorus, and rapidly available potassium content. Hydrolyzable nitrogen was determined by the Berthelot reaction method and available phosphorus by molybdenum blue method after digestion with 1.0 g of potassium sulfate and 5 mL of concentrated sulfuric acid (Deng et al. 2004). Total Pb, Zn, Cu, and K contents 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 river sand/tailing samples were measured. Organic matter was determined according to Paniz et al. (2001). Certified reference materials (mixed shoots of shrubs from Pb/Zn mine tailings; GBW 07602, China) were used to ensure the quality of analyses. Analyses of the element concentrations in the plant material were performed at the Quality Testing Center for Edible Forest Products of State Forestry Administration (Hangzhou, China).

The bioconcentration factor (BCF) was used to estimate a plant's ability to accumulate metals from soils. BCF was defined as the ratio of metal concentration in plant roots or shoots to that in soil. BCF at the end of the experiment was calculated as $BCF = A_{tissues}/A_{soil}$ (Deng et al. 2004), where $A_{tissues}$ (mg kg^{-1}) is the total heavy metal accumulated in roots or shoots, and A_{soil} (mg kg^{-1}) is the heavy metal concentration in the soil.

The translocation factor (TF) can be used to estimate a plant's ability to translocate metals from the roots to the shoots. The TF at the end of the experiment was calculated as $TF = A_s/A_r$ (Deng et al. 2004), where A_s and A_r are total heavy metals accumulated in shoots and in roots, respectively (both in mg kg^{-1}).

Statistical analysis

The data are expressed as the mean of at least three replications ± standard error. All statistical tests were performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The fitting of the data to a normal distribution for all parameters determined was checked with the Kolmogorov–Smirnov test. When necessary, analytical data were transformed using logarithms to assure normal distribution (Parra et al. 2014). For all experimental variables, two-way analysis of variance was applied with tailings and species as factors and the least significant difference test was used for comparisons of means. Differences were considered significant when the analysis of variance *F*-test achieved $p < 0.05$.

Results

Biomass

The biomass of plants grown in each treatment is shown in Table 2. The growth of all species, except *Q. virginiana*, was inhibited by the tailings. Compared with the control, the biomass of *Q. virginiana* grown in 100 % tailings increased slightly, but the biomass in different treatments showed no differences. *Q. shumardii* produced the highest leaf, root, and total biomass among all species. However, compared with the control, the biomass of leaves, stems, and external roots in 100 % tailings decreased significantly ($p < 0.05$). The performance of *S. matsudana* was similar to that of *Q. shumardii*; the biomass of *S. matsudana* was reduced by 36.6 % in 100 % tailings, which was a greater reduction than for the other species. Relative to controls, the biomass of *Q. phellos* and *S. integra* grown in 50 and 100 % tailings was reduced slightly, but the biomass of *Q. phellos* did not significantly differ for the different treatments. RGR showed the same change trends as described above for dry biomass.

Tolerance of the five species in tailings were assessed by calculating TI values (Fig. 1), which differed significantly between treatments ($p < 0.05$). In general, the TI values of the five species were similar in 50 % tailings. However, the low TI value of *S. matsudana* indicated greater sensitivity than did the other species in 100 % tailings ($p < 0.05$), and TI of 0.97–1.04 of *Q. virginiana* was the most tolerant of the tested species.

Root traits

After exposure to heavy metals, the roots of all plants exhibited different levels of blackening, and the symptoms became

Table 2 Dry weight (DW) (g) of root and aboveground tissues of five woody species grown in mine tailings for 150 days

Species	Treatment	Leaves	Stems	Cuts	Roots (inner)	Roots (external)	Plants	RGR
S. matsudana	Control	0.78 ± 0.19a	2.93 ± 0.27a	1.51 ± 0.11a	0.45 ± 0.03a	0.45 ± 0.07a	6.26 ± 0.54a	0.18 ± 0.02a
	Tailing (50 %)	0.69 ± 0.03a	2.84 ± 0.03a	1.18 ± 0.06b	0.44 ± 0.01a	0.51 ± 0.19a	5.64 ± 0.22a	0.17 ± 0.01a
	Tailing (100 %)	0.41 ± 0.04b	2.03 ± 0.09b	1.14 ± 0.21b	0.33 ± 0.01b	0.09 ± 0.09b	3.97 ± 0.49b	0.13 ± 0.01b
S. integra	Control	0.81 ± 0.06a	1.92 ± 0.09a	2.82 ± 0.09a	0.66 ± 0.03a	0.46 ± 0.09a	6.67 ± 0.55a	0.18 ± 0.01a
	Tailing (50 %)	0.76 ± 0.07a	1.48 ± 0.10b	2.40 ± 0.13b	0.38 ± 0.03b	0.43 ± 0.07a	5.39 ± 0.27b	0.17 ± 0.02a
	Tailing (100 %)	0.68 ± 0.15a	1.31 ± 0.24b	2.41 ± 0.19b	0.47 ± 0.07b	0.27 ± 0.02b	5.13 ± 0.55b	0.15 ± 0.03a
Q. shumardii	Control	1.84 ± 0.29a	2.01 ± 0.03a		4.34 ± 0.46a	0.60 ± 0.12b	8.78 ± 0.14a	0.22 ± 0.04a
	Tailing (50 %)	1.65 ± 0.05ab	1.55 ± 0.13b		4.63 ± 0.56a	0.89 ± 0.13a	8.72 ± 0.62a	0.19 ± 0.04ab
	Tailing (100 %)	1.44 ± 0.09b	1.28 ± 0.14c		3.99 ± 0.69a	0.28 ± 0.01c	6.99 ± 0.87b	0.12 ± 0.03b
Q. phellos	Control	0.87 ± 0.18a	1.02 ± 0.39a		1.14 ± 0.35a	0.25 ± 0.17a	3.28 ± 1.32a	0.13 ± 0.01a
	Tailing (50 %)	0.70 ± 0.01a	0.78 ± 0.12a		1.07 ± c 0.11a	0.19 ± 0.02a	2.74 ± 0.09a	0.11 ± 0.01a
	Tailing (100 %)	0.75 ± 0.03a	0.73 ± 0.10a		1.05 ± 0.12a	0.19 ± 0.02a	2.71 ± 0.04a	0.11 ± 0.03a
Q. virginiana	Control	1.16 ± 0.05a	0.93 ± 0.11a		1.58 ± 0.06a	0.14 ± 0.03a	3.80 ± 0.20a	0.14 ± 0.01a
	Tailing (50 %)	1.06 ± 0.12a	0.80 ± 0.09a		1.69 ± 0.11a	0.14 ± 0.02a	3.69 ± 0.28a	0.13 ± 0.02a
	Tailing (100 %)	1.22 ± 0.06a	0.85 ± 0.04a		1.79 ± 0.15a	0.12 ± 0.01a	3.97 ± 0.12a	0.14 ± 0.01a
p value	Species	****	****	****	****	****	****	**
	Treatments	***	****	***	ns	****	****	**
	Species × treatments	*	***	ns	ns	****	**	ns

Each value represents the mean of three ($n = 3$) replicates ± SD. Different letters for the same species indicate significant difference between the treatments ($p < 0.05$). *P* values of the ANOVAs of treatments, species, and heir interactions were also shown

ns not significant

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$

more severe with increasing heavy metal concentrations. The root systems of *Quercus* spp. and *Salix* spp. showed marked differences among all treatments because of the different propagation materials. In general, *Quercus* spp. had well-developed taproots but variable lateral root development. For example, roots of *Q. shumardii* developed throughout the whole container, whereas roots of *Q. virginiana* and *Q. phellos* only partially filled the container (Table 3). The root systems of the two *Salix* spp. filled the whole container owing to the development of adventitious roots in control and 50 % tailings treatment. The root characteristics of all species grown in tailings varied significantly (Tables 2 and 3, $p < 0.05$) depending on species and tailings concentration. The root biomass of *Salix* spp. and *Q. shumardii* grown in 100 % tailings decreased significantly compared with controls. In addition, the external root morphological parameters of *Salix* spp. and *Q. shumardii* were reduced significantly by 100 % tailings (except for average root diameter). In general, reductions in root biomass and morphological parameters were more pronounced in external than inner roots.

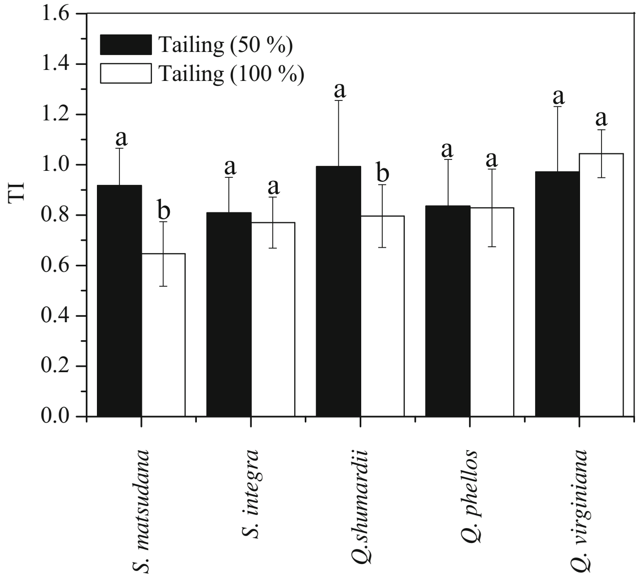

Fig. 1 Tolerance index (TI) of five woody species grown in Pb/Zn mine tailings. Each value represents the mean of three ($n = 3$) replicates ± SE. Different *letters* on the bars for the same species indicate significant difference between the treatments ($p < 0.05$). TI values of five species, species ****; tailings ****; species × tailings ***. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$

Chlorophyll concentration index

After 150 days of heavy metal exposure, foliage of *S. matsudana* and *Q. shumardii* treated with 100 % tailings had begun to yellow, whereas leaves of other species showed no pronounced toxicity symptoms. The CCI values at harvest were decreased by

Table 3 Root characteristics of five woody species grown in mine tailings for 150 days

species	Treatment	Mean root total length (cm)		Mean root surface area (cm^2)		Mean root average diameter (mm)		Mean root volume (cm^3)		Mean number of root tip	
		External	Inner	External	Inner	External	Inner	External	Inner	External	Inner
S. matsudana	Control	1492 ± 711a	1820 ± 191ab	109.02 ± 51.08a	120.35 ± 16.99a	0.31 ± 0.13a	0.24 ± 0.05a	0.57 ± 0.06a	0.63 ± 0.12a	15,599 ± 7334a	18,193 ± 7804a
	Tailing (50 %)	1453 ± 409ab	1988 ± 194a	107.97 ± 38.59a	130.15 ± 16.30a	0.27 ± 0.06a	0.25 ± 0.05a	0.65 ± 0.37a	0.68 ± 0.07a	13,484 ± 3643a	17,294 ± 5843ab
	Tailing (100 %)	518 ± 182b	1672 ± 21b	9.91 ± 5.30b	103.80 ± 1.91a	0.18 ± 0.01a	0.20 ± 0.01a	0.15 ± 0.13b	0.51 ± 0.01b	8376 ± 180b	13,739 ± 1534b
S. integra	Control	1127 ± 220a	896 ± 86a	89.45 ± 18.35a	69.57 ± 2.95a	0.28 ± 0.03a	0.25 ± 0.01a	0.57 ± 0.12a	0.44 ± 0.02a	9314 ± 1650a	8317 ± 2809a
	Tailing (50 %)	818 ± 112b	742 ± 13a	69.41 ± 9.47ab	51.03 ± 3.57b	0.30 ± 0.07a	0.22 ± 0.01b	0.47 ± 0.03ab	0.28 ± 0.03c	7083 ± 1272ab	7040 ± 1389a
	Tailing (100 %)	538 ± 89b	856 ± 159a	46.86 ± 4.45b	61.66 ± 5.26a	0.30 ± 0.04a	0.24 ± 0.02ab	0.35 ± 0.02b	0.36 ± 0.02b	4981 ± 1172b	8364 ± 2707a
Q. shumardii	Control	1506 ± 140a	756 ± 71a	112.03 ± 3.61a	89.51 ± 1.65a	0.36 ± 0.03a	0.41 ± 0.02b	0.68 ± 0.04a	0.88 ± 0.06b	14,339 ± 134a	7490 ± 2033a
	Tailing (50 %)	1213 ± 227ab	523 ± 71b	88.72 ± 17.86a	76.76 ± 4.70b	0.31 ± 0.07a	0.52 ± 0.09a	0.52 ± 0.11b	0.93 ± 0.05ab	12,570 ± 1394a	6336 ± 2490b
	Tailing (100 %)	843 ± 183b	473 ± 82b	59.24 ± 9.62b	74.32 ± 0.35b	0.31 ± 0.02a	0.54 ± 0.03a	0.34 ± 0.02c	0.99 ± 0.05a	8616 ± 1190b	5605 ± 1635b
Q. phellos	Control	548 ± 71a	337 ± 25a	42.49 ± 13.01a	40.62 ± 0.37a	0.23 ± 0.02a	0.39 ± 0.03ab	0.27 ± 0.14a	0.40 ± 0.05a	6151 ± 496a	4987 ± 963a
	Tailing (50 %)	655 ± 58a	251 ± 28b	43.92 ± 2.30a	33.15 ± 3.43b	0.21 ± 0.01a	0.43 ± 0.01a	0.24 ± 0.02a	0.36 ± 0.06a	7036 ± 403a	4213 ± 690b
	Tailing (100 %)	580 ± 61a	336 ± 8a	39.67 ± 2.63a	38.78 ± 1.66a	0.22 ± 0.01a	0.37 ± 0.01b	0.22 ± 0.01a	0.36 ± 0.03a	6637 ± 769a	5353 ± 880a
Q. virginiana	Control	277 ± 31a	171 ± 13c	21.76 ± 2.10a	31.61 ± 2.26a	0.24 ± 0.01a	0.62 ± 0.06a	0.12 ± 0.01a	0.49 ± 0.08a	3379 ± 156a	2539 ± 724b
	Tailing (50 %)	272 ± 20a	198 ± 5b	21.85 ± 2.42a	33.75 ± 1.86a	0.25 ± 0.01a	0.55 ± 0.04ab	0.14 ± 0.03a	0.47 ± 0.04a	3106 ± 174b	2891 ± 622a
	Tailing (100 %)	217 ± 22b	244 ± 11a	17.94 ± 0.41b	35.35 ± 2.24a	0.26 ± 0.01a	0.47 ± 0.02b	0.12 ± 0.01a	0.41 ± 0.02a	2645 ± 30c	2846 ± 282a
p value	species	****	****	****	****	***	****	****	****	****	****
	Treatments	****	ns	****	ns	ns	**	****	ns	***	ns
	Species × treatments	*	***	***	***	ns	***	ns	**	ns	**

Each value represents the mean of three ($n = 3$) replicates ± SD. Different letters for the same species indicate significant difference between the treatments ($p < 0.05$). *P* values of the ANOVAs of tailings, species, and heir interactions were also shown

ns not significant

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$

tailings treatment for all species, and there were significant differences among all species ($p < 0.05$; Fig. 2).

Chlorophyll fluorescence

The leaf F_0 value after 150 days was lower in tailing-treated *Salix* spp. compared with controls (Fig. 3). However, F_0 for *Quercus* spp. showed an opposite trend. Furthermore, the tailing treatment did not significantly affect the F_m value (Fig. 3). F_v/fm reflects the potential quantum efficiency of PSII. The F_v/fm value of *Q. shumardii* showed a linear decrease with tailing treatment ($p < 0.05$; Fig. 3), with significant decreases in F_v/fm under treatment with 50 % (1.5 %) and 100 % tailings (2.8 %). However, F_v/fm for the other species showed no significant difference in all treatments. The ΦPSII values for *S. integra* and *Q. phellos* did not significantly differ among treatments. In contrast, ΦPSII values of *S. matsudana*, *Q. shumardii*, and *Q. virginiana* were significantly lower in tailing treatments compared with controls. qP gives an indication of the proportion of PSII reaction centers that are open, and the trend for qP (Fig. 3) was very similar to that of ΦPSII. In contrast, for 100 % tailings, NPQ values changed slightly in *S. matsudana* and *Q. virginiana* and increased notably in all other species; only *Q. phellos* also increased for 50 % tailings (Fig. 3). In addition, *S. matsudana* had significantly higher NPQ values than did the other species.

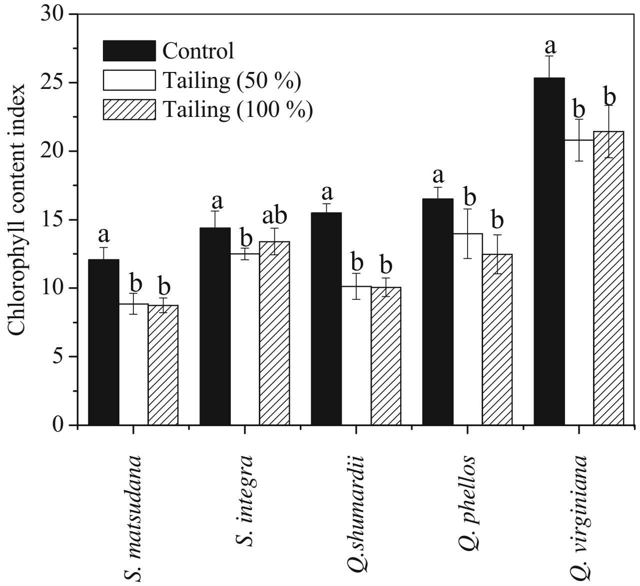

Fig. 2 Chlorophyll concentration index (CCI) of leaves (third to fourth leaf from the apex) of 5 wood species grown in mine tailings for 150 days. Each value represents the mean of 15 ($n = 15$) replicates ± SE. Different *letters* on the bars for the same species indicate significant difference between the treatments ($p < 0.05$). CCI values of five species, species ****; tailings *; species × tailings ***. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$

Accumulation and translocation of heavy metals in plants

The concentrations of Pb, Zn, and Cd in tissues of plants grown in tailings are shown in Fig. 4. Generally, tissues of all plant species grown in tailings contained higher metal concentrations than did those grown in river sand ($p < 0.05$). There were also higher metal concentrations in roots than in shoots for all species grown in the same tailing treatment, except for Cd and Zn concentrations in *S. integra* ($p < 0.05$). The Cd concentrations in shoots of *Quercus* spp. were below the limit of detection. Plants grown in tailings contained higher Pb and Zn concentrations in their tissues than Cd. Furthermore, the Pb and Zn concentrations in roots were higher for 100 than 50 % tailings, except for *Q. shumardii*. However, Pb and Zn concentrations in *S. integra* shoots showed an opposite trend to that for roots. The Cd concentrations in tissues were higher in 50 than 100 % tailings, except for *Q. virginiana* (Fig. 4).

The BCF and TF were used to further evaluate the ability of the five species to accumulate heavy metals (Fig. 5). In general, the BCF and TF values of the five species were <1, except for *S. integra* grown in tailings, which had the highest TF values for Zn (TF = 1.42–2.18) and Cd (TF = 1.03–1.45). For all metals, BCF values of roots were higher for 50 than 100 % tailings in all species, except for *Q. virginiana* and the BCF value of Zn for *Q. phellos*. BCF of shoots showed a similar trend to BCF of roots, except for Pb in *Q. shumardii*; however, BCF for Cd could only be calculated for *Salix* spp. The BCF values of Zn were higher than those of Pb ($p < 0.05$). Finally, TF for Zn showed a trend similar to BCF, with lower values in 100 % tailings. The TF values ranged around 0.5 with the exception of TF >1 for *S. integra*. In contrast, TF values were generally lower for Pb than Zn and decreased with increasing proportion of tailings for *S. integra* and *Q. virginiana* but increased for *S. matsudana* and *Q. shumardii*.

Discussion

Heavy metal tolerance of woody species

Previous studies confirmed that plant populations surviving in areas contaminated by heavy metals are differentiated from other populations of the same species by a genetically based tolerance (Haliru et al. 2009). The *Quercus* spp. and *Salix* spp. in this experiment were able to survive in mine tailings. However, the five species responded differently to high concentrations of heavy metals in tailings. The different abilities to maintain normal growth in tailings reflect differences in resistance or tolerance of plants to metal toxicity (Wang et al. 2014). Growth inhibition resulting in reduction in biomass production is common in plants exposed to high metal concentrations (de Souza et al. 2012). In the present study,

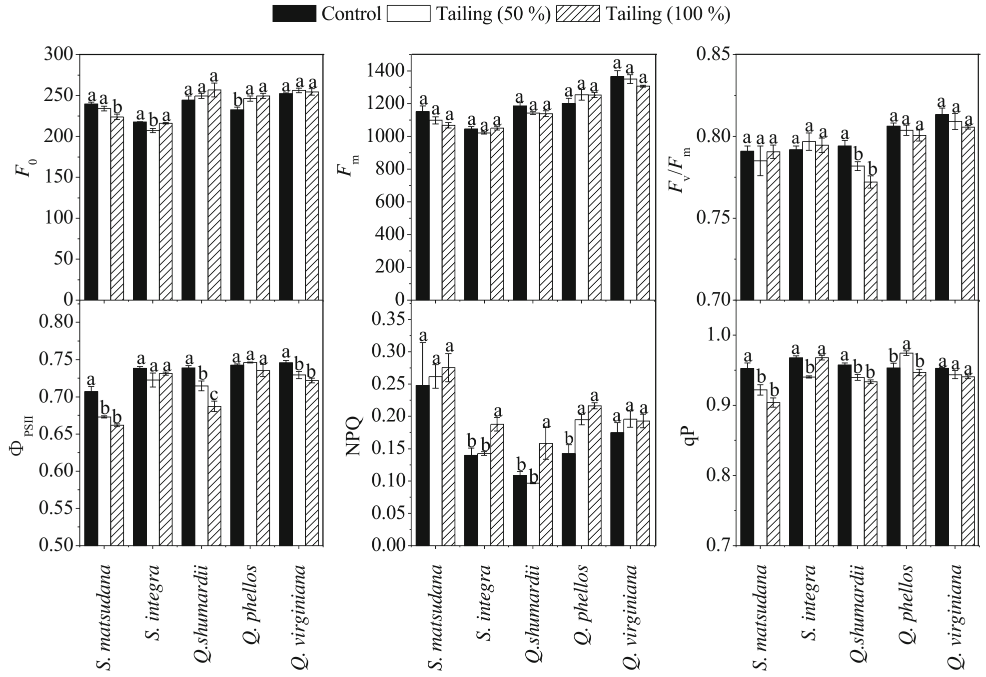

Fig. 3 Chlorophyll fluorescence parameters in the leaves (third to fourth leaf from the apex) of five species grown in mine tailings for 150 days. Each value represents the mean of nine ($n = 9$) biological replicates ± SE. Different *letters* on the bars for the same species indicate significant difference between the treatments ($p < 0.05$). F_0 of five species, species ****; tailings ns; species × tailings **. *F*m of five species, species ****; tailings ns; species × tailings ns. *F*v/*F*m of five species, species ****; tailings ns; species × tailings ns. ΦPSII of five species, species ****; tailings ****; species × tailings ***. NPQ of five species, species ****; tailings ****; species × tailings ***. qP of five species, species ****; tailings ****; species × tailings ****. *ns* not significant; $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$; $****p \leq 0.0001$

Q. virginiana grew well in tailings, with no apparent effect on measured plant growth parameters. The other four species showed reductions in biomass production when grown in tailings compared with river sand. These findings indicate that

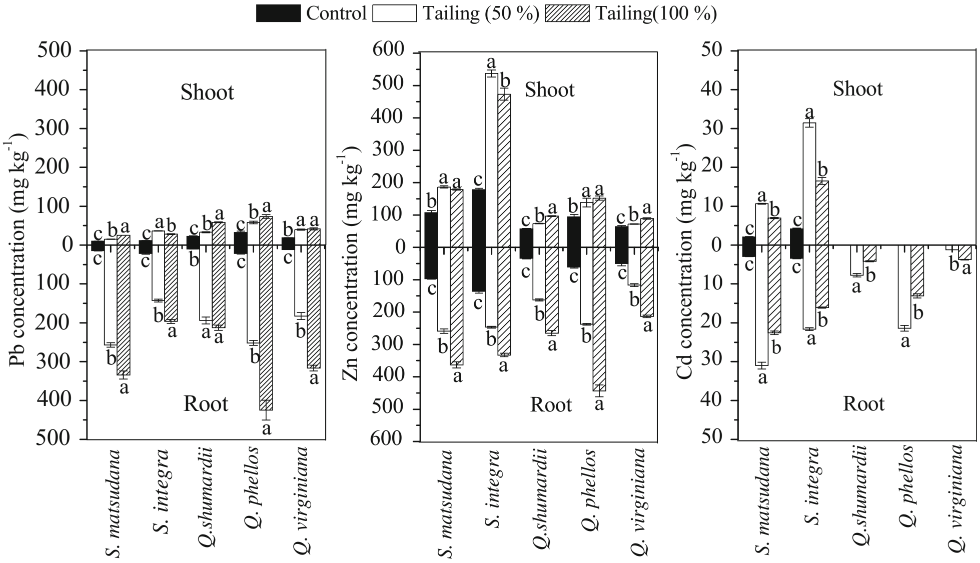

Fig. 4 Average Pb, Zn, and Cd concentrations (mg kg^{-1}) in dry plant tissues of five species exposed to mine tailings for 150 days. Each value represents the mean of three ($n = 3$) replicates ± SE. Different *letters* on the bars for the same species of roots and shoots indicate significant difference between the treatments respectively ($p < 0.05$). Pb concentration of shoots, species ****; tailings ****; species × tailings ****. Pb concentration of roots, species ****; tailings ****; species × tailings ****. Zn concentration of shoots, species ****; tailings ****; species × tailings ****. Zn concentration of roots, species ****; tailings ****; species × tailings ****. Cd concentration of shoots, species ****; tailings ****; species × tailings ****. Cd concentration of roots, species ****; tailings ****; species × tailings ****. $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$; $****p \leq 0.0001$

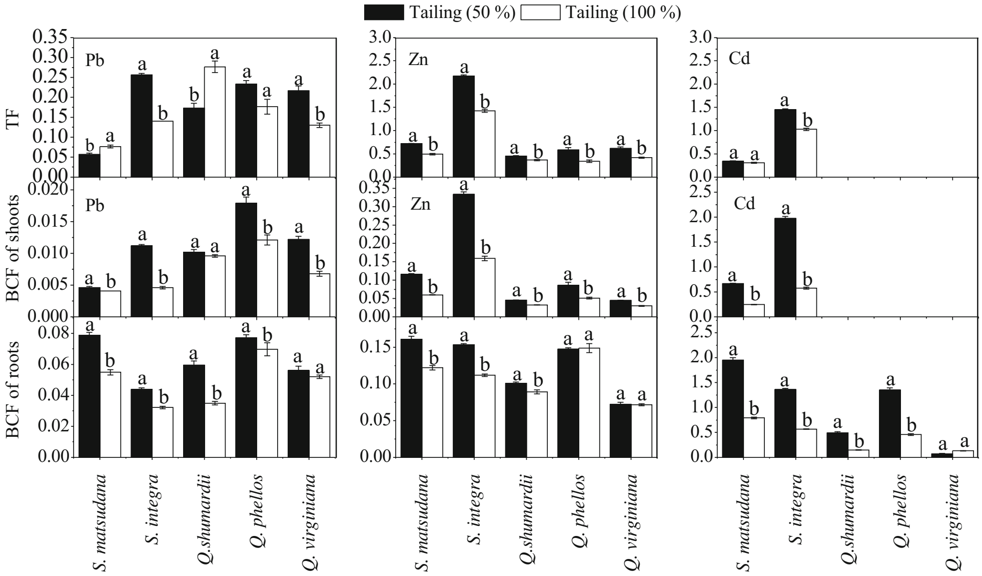

Fig. 5 Translocation factor (TF) and bioconcentration factors (BCF) of Pb, Zn, and Cd in five woody species. Each value represents the mean of three ($n = 3$) replicates ± SE. Different *letters* on the bars for the same species indicate significant difference between the treatments ($p < 0.05$). TF values of Pb, species ****; tailings ***; species × tailings ****. TF values of Zn, species ****; tailings ****; species × tailings ****. TF values of Cd, species ****; tailings ****; species × tailings ****. Shoot BCF values of Pb, species ****; tailings ****; species × tailings ****. Shoot BCF values of Zn, species ****; tailings ***; species × tailings ****. Shoot BCF values of Cd, species ****; tailings ***; species × tailings ****. $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$; $****p \leq 0.0001$; Root BCF values of Pb, species ***; tailings ***; species × tailings ***. Root BCF values of Zn, species ***; tailings ***; species × tailings ***. Root BCF values of Cd, species ****; tailings ***; species × tailings ****. $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$; $****p \leq 0.0001$

Q. virginiana had relatively high tolerance to heavy metals compared with the other four species, especially when grown in 100 % tailings. After exposure to tailings, particularly 100 %, *S. matsudana* and *Q. shumardii* plants were stunted compared with controls. After approximately 75 days of exposure to tailings, the foliage of these two species began to yellow and wilt from the leaf tip, with more severe symptoms at harvest (data not shown). The *Q. phellos* and *S. integra* showed no leaf wilting or sign of dehydration. The growth measures used in the present study indicated that *Q. virginiana* was better adapted to metal-contaminated soils.

Seedlings of *Quercus* spp. and rooted cuttings of *Salix* spp. showed relatively high tolerance to Pb, Zn, and Cd. Some chlorophyll fluorescence parameters (F_m and F_v/fm) remained unchanged in all species (except for decreases in *Q. shumardii*), with values of F_v/fm slightly lower than the optimum (0.83) measured for most plant species (Maxwell and Johnson 2000). This suggested that heavy metal accumulation in leaves caused a reduction but not complete failure of photosynthesis (He et al. 2011). In the present study, the decrease in F_v/F_m value of *Q. shumardii* can be explained by the negative effect of tailings on efficiency of the PSII photochemical reaction and electron transport chain (Kumar and Prasad 2015). The results also indicated that reoxidation of the primary electron acceptor was limited, which results in a decrease in electron transport from PSII to PSI (Mallick and Mohn 2003; Kumar and Prasad 2015). ΦPSII was similar in all substrates only for *S. integra* and *Q. phellos* and decreased in all other cases for substrates with tailings. The qP value decreased particularly in leaves of plants grown in 100 % tailings, indicating that the number of closed or inactive PSII reaction centers increased, with lower use of light in photochemical reactions and a higher NPQ. This response signifies that light absorption exceeded the capacity of photosynthetic electron transport rate and carbon dioxide fixation (Vassilev and Manolov 1999) with the use of NADP decreased as a result of heavy metal stress (Vernay et al. 2008), resulting in the decline in plant biomass production. Similar trends were observed in maize (Vernay et al. 2008) and *Talinum triangulare* (Kumar and Prasad 2015). However, among *Quercus* spp., *Q. virginiana* demonstrated that the best response to all treatments and *Q. shumardii* was the most sensitive. Both *Salix* spp. performed well, although in 100 % tailings, *S. integra* maintained ΦPSII and partially also qP, increasing the energy dissipation capacity (i.e., NPQ).

Root growth was used as a measure of heavy metal tolerance because roots are the first organs to contact metals and therefore are likely the first to display symptoms of metal toxicity (Ma et al. 2014; He et al. 2015). In general, plant roots rapidly respond to absorbed heavy metals through reductions in root growth and changes in branching pattern (Elobeid et al. 2012; Wang et al. 2014). In this study, the external roots exhibited different levels of blackening, and growth was stunted after exposure to heavy metals, regardless of heavy metal concentration. The root system exhibits a certain phenotypic plasticity: plants with plastic root systems can often cope with a wide range of soil factors (Keller et al. 2003). Avoidance of zones contaminated by heavy metals is an efficient survival strategy, especially for trees (Dickinson et al. 1991). For example, in this study, the root systems of *Q. phellos* and *Q. virginiana* only partially filled the containers and no toxicity symptoms were observed. This indicated that adaptation of the root system morphology was also a mechanism for plants to tolerate contaminated soils. Additionally, the ability of the root system to pump heavy metals and to fit and expand into the contaminated zone are key factors for phytoextraction efficiency (Keller et al. 2003). A similar phenomenon was observed in the present study, in which the root systems of *S. integra* filled the whole container and accumulated relatively high concentrations of Zn and Cd in the root and aboveground tissues.

Metal accumulation and translocation

Metal accumulation in roots and shoots of the five studied species was much lower compared with other phytoaccumulators (Lu et al. 2014; Leal-Alvarado et al. 2016). However, concentrations of heavy metals in plant tissues were similar (Seo et al. 2008) or higher than those of other species (Evangelou et al. 2012; de Souza et al. 2012). Heavy metal concentrations were higher in roots than in shoots of plants grown in different media (except for Zn and Cd in *S. integra*). This is the usual behavior of non-hyperaccumulator species (Evangelou et al. 2012). For example, the Pb TF values of the five species were similar to that of *Morus alba* (Zhou et al. 2015) and lower than those of *Erythrina speciosa* (TF = 0.37–0.56) and *Schizolobium parahyba* (TF = 0.27–0.47) (de Souza et al. 2012). However, plant capacity for heavy metal translocation and accumulation is highly variable, depending on genotypic and environmental traits (Pietrini et al. 2010; Baccio et al. 2014). In *Quercus* spp. and *S. matsudana*, the highest absolute concentrations of Pb, Zn, and Cd were in roots, in 100 rather than 50 % tailings (except for Cd; Fig. 4). Nevertheless, *S. integra* showed higher Zn and Cd concentrations in shoots, especially when grown on 50 % tailings. However, the indexes of absorption and translocation indicated that the different species, with the exception of *Q. virginiana*, limited metal absorption when the concentration in the substrate was too high, as shown by the higher BCF values of roots in 50 % tailings. Translocation of Pb to the shoot was particularly limited in *S. matsudana*. Cd was not detectable in shoots of *Quercus* spp. and, in contrast, Zn was well translocated from soil to shoot since it is an important micronutrient. Among the species, *S. integra* showed very good translocation of all three metals to the aboveground part, especially in 50 % tailings. In addition, the TF index confirmed this result, demonstrating that *S. integra* may be a candidate for phytoextraction of Zn and Cd (Vysloužilová et al. 2003; Vassilev et al. 2005; Yang et al. 2015).

Excessive amounts of Pb, Zn, and Cd in soil may retard plant growth. The threshold for heavy metal toxicity is highly variable even among within plant species (Shi et al. 2015). Kabata-Pendias and Pendias (2001) provided some general values for phytotoxicity. In the cases of Pb, Zn, and Cd, normal and phytotoxic concentrations were 0.5–10 and 30–300 mg kg^{-1}, 0.05–0.2 and 5–30 mg kg^{-1}, and 10–150 and >100 mg kg^{-1} dry weight in shoot tissues, respectively. In the present study, most Pb, Zn, and Cd were retained in roots (except for Zn and Cd in *S. integra*), with concentrations approaching or higher than the normal or phytotoxic levels in roots of seedlings grown in Pb/Zn mine tailings. These findings suggested that the roots may have functioned as a barrier to heavy metal transport to shoots, which contributes to heavy metal tolerance in woody plants (de Souza et al. 2012). Root tissues accumulated significantly higher concentrations of metals than did shoot tissues (Fig. 4), indicative of high plant availability of the metals as well as their limited mobility once inside the plant (de Souza et al. 2012; Wang et al. 2014). This is an important tolerance mechanism that can be exploited for phytostabilization processes (Rabeda et al. 2015).

Implications for practice

According to Mendez and Maier (2008b), plants that show TF and BCF values of <1.0 and shoot concentrations of Pb, Zn, and Cd of <100, <500, and <10 mg kg^{-1}, respectively, are appropriate for phytostabilization programs. For growth in tailings in temperate regions, plants are required to adapt to slightly anaerobic and wetland conditions (Craw et al. 2007). The five species of the present study are adaptable to wetland conditions. In arid and semiarid regions, drought-, metal-, and salt-tolerant plants are required (Mendez and Maier 2008a). *Salix* spp., and especially *Q. virginiana*, are salt-tolerant plants. Comparison of the TI values of the species in the present study and concentrations of heavy metals in shoots indicated that *Q. virginiana* was the most suitable for phytostabilization programs. From a toxicology perspective, this would be desirable to preclude metals from entering the food chain and so avoid potential environmental risks (Deng et al. 2004). According to Swiss regulations (FOEN 2005), the maximum allowed trace element concentrations in fodder (dry weight) are 150, 40, and 1 mg kg^{-1} for Zn, Pb, and Cd, respectively. The corresponding concentrations in shoots of *Quercus* spp. never exceeded these values (except for Zn of *Q. phellos*) and, because of high tannin concentrations in leaves, they are not eaten by deer and other animals (Evangelou et al. 2012), and thus pose a low risk for herbivores. *S. integra* accumulated relatively high concentrations of Zn and Cd in the aboveground tissues in our pot experiment. Through short rotations, *S. integra* could fulfill the purpose of phytoextraction from tailings in temperate regions driven by its high biomass production. However, the Zn and Cd concentrations in willow shoots surpassed the guideline limits, and the high Cd concentration represents a high risk for herbivores. *S. matsudana* and *Q. shumardii* displayed toxicity symptoms of leaf wilting and chlorosis when grown in 100 % tailings, and their sensitivity to heavy metal stress means they are unsuitable for phytoremediation under the experimental conditions.

Conclusions

The five tested tree species exhibited distinctly different responses of biomass yield when grown in tailings. However, they had specific heavy metal uptakes independent of the tailings treatment. Under the experimental conditions, *Q. virginiana* was highly tolerant to heavy metals. Variability in the root system development might explain why the *Quercus* spp. were more tolerant of tailings than were *Salix* spp. in this study. All species showed low BCF and TF values, except for the TF value for Zn and Cd of *S. integra*. Using short rotations, *S. integra* may be suitable for phytoextraction of tailings in temperate regions. Based on the present findings, *Q. virginiana* was the most metal-tolerant species at the seedling stage and is a potential candidate for phytostabilization of mine tailings in southern China.

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References

Acosta JA, Faz A, Martinez-Martinez S, Zornoza R, Carmona DM, Kabas S (2011) Multivariate statistical and GIS-based approach to evaluate heavy metals behavior in mine sites for future reclamation. Jc Geochem Explor 109:8–17

Aldrich PR, Cavender-Bares J (2011) Quercus. In: Kole C (ed) Wealth of wild species: genetic, genomic and breeding resources. Springer-Verlag, New York, pp. 89–130

Algreen M, Trapp S, Rein A (2014) Phytoscreening and phytoextraction of heavy metals at Danish polluted sites using willow and poplar trees. Environ Sci Pollut Res 21:8992–9001

Baccio DD, Castagna A, Roberto T, Ranieri A, Sebastiani L (2014) Early responses to cadmium of two poplar clones that differ in stress tolerance. J Plant Physiol 171:1693–1705

Bhargava A, Carmona FF, Bhargava M, Srivastava S (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manag 105:103–120

Brunner I, Luster J, Günthardt-Goerg MS, Frey B (2008) Heavy metal accumulation and phytostabilisation potential of tree fine roots in a contaminated soil. Environ Pollut 152:559–568

Cano-Reséndiz O, de la Rosa G, Cruz-Jiménez G, Gardea-Torresdey JL, Robinson BH (2011) Evaluating the role of vegetation on the transport of contaminants associated with a mine tailing using the Phyto-DSS. J Hazard Mater 189:472–478

Craw D, Rufaut C, Haffert L, Paterson L (2007) Plant colonization and arsenic uptake on high arsenic mine wastes, New Zealand. Water Air Soil Poll 179:351–364

de Souza SCR, de Andrade SAL, de Souza LA, Schiavinato MA (2012) Lead tolerance and phytoremediation potential of Brazilian *leguminous* tree species at the seedling stage. J Environ Manag 110:299–307

Deng H, Ye ZH, Wong MH (2004) Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal–contaminated sites in China. Environ Pollut 132:29–40

Dickinson CD, Altabella T, Chrispeels MJ (1991) Slow-growth phenotype of transgenic tomato expressing apoplastic invertase. Plant Physiol 95:420–425

Dimitriou I, Aronsson P, Weih M (2006) Stress tolerance of five willow clones after irrigation with different amounts of landfill leachate. Bioresource Technol 97:150–157

Domínguez MT, Marañón T, Murillo JM, Schulin R, Robinson BH (2008) Trace element accumulation in woody plants of the Guadiamar Valley SW Spain: A large–scale phytomanagement case study. Environ Pollut 152:50–59

Domínguez MT, Madrid F, Marañón T, Murillo JM (2009) Cadmium availability in soil and retention in oak roots: potential for phytostabilization. Chemosphere 76:480–486

Elobeid M, Göbel C, Feussner I, Polle A (2012) Cadmium interferes with auxin physiology and lignification in poplar. J Exp Bot 63:1413–1421

Evangelou MWH, Robinson BH, Gunthardt-Goerg MS, Schulin R (2012) Metal uptake and allocation in trees grown on contaminated land: implications for biomass production. Int J Phytorem 15:77–90

Evlard A, Sergeant K, Printz B, Guignard C, Renaut J, Campanella B, Paul R, Hausman JF (2014) A multiple-level study of metal tolerance in *Salix fragilis* and *Salix aurita* clones. J Proteome 101:113–129

FOEN (2005) Manual on risk assessments and measures for polluted soils. Federal Office for the Environment, Bern

Gogorcena Y, Larbi A, Andaluz S, Carpena RO, Abadía A, Abadía J (2011) Effects of cadmium on cork oak (*Quercus suber* L.) plants grown in hydroponics. Tree Physiol 31:1401–1412

Haliru M, Ajibola VO, Agbaji EB (2009) Evaluation of the uptake and accumulation of metals by some commonly irrigated vegetables in soils treated with different concentrations of these metals. J Appl Sci 9:1573–1577

He JL, Qin JJ, Long LY, Ma YL, Li H, Li K, Jiang XN, Liu TX, Polled A, Liang ZH, Luo ZB (2011) Net cadmium flux and accumulation reveal tissue-specific oxidative stress and detoxification in *Populus* × *canescens*. Physiol Plantarum 143:50–63

He JL, Li H, Ma CF, Zhang YL, Polle A, Rennenberg H, Cheng XQ, Luo ZB (2015) Overexpression of bacterial c-glutamylcysteine synthetase mediates changes in cadmium influx, allocation and detoxification in poplar. New Phytol 205:240–254

Kabata-Pendias A, Pendias H (2001) Trace element in soils and plants, 3rd ed. CRC Press, Florida

Keller C, Hammer D, Kayser A, Richner W, Brodbeck M, Sennhauser M (2003) Root development and heavy metal phytoextraction efficiency: comparison of different plant species in the field. Plant Soil 249: 67–81

Kumar A, Prasad MNV (2015) Lead-induced toxicity and interference in chlorophyll fluorescence in *Talinum triangulare* grown hydroponically. Photosynthetica 53:66–71

Leal-Alvarado DA, Espadas-Gil F, Sáenz-Carbonell L, Talavera-May C, Santamaría JM (2016) Lead accumulation reduces photosynthesis in the lead hyper-accumulator *Salvinia minima* baker by affecting the cell membrane and inducing stomatal closure. Aquat Toxicol 171: 37–47

Li MS, Luo YP, Su ZY (2007) Heavy metal concentrations in soils and plant accumulation in a restored manganese mineland in Guangxi, South China. Environ Pollut 147:168–175

Li ZY, Ma ZW, van der Kuijp TJ, Yuan ZW, Huang L (2014) A review of soil heavy metal pollution from mines in China: pollution and health risk assessment. Sci Total Environ 468–469:843–853

Lu LL, Liao XC, Labavitch J, Yang XE, Nelson E, Du YH, Brown PH, Tian SK (2014) Speciation and localization of Zn in the hyperaccumulator *Sedum alfredii* by extended X-ray absorption fine structure and micro-X-ray fluorescence. Plant Physiol Bioch 84: 224–232

Ma YL, He JL, Ma CF, Luo J, Li H, Liu TX, Polle A, Peng CH, Luo ZB (2014) Ectomycorrhizas with *Paxillus* involutus enhance cadmium uptake and tolerance in *Populus* × *canescens*. Plant Cell Environ 37: 627–642

Mallick N, Mohn FH (2003) Use of chlorophyll fluorescence in metal-stress research: a case study with the green microalga *Scenedesmus*. Ecotox Environ Safe 55:64–69

Marques APGC, Moreira H, Rangel AOSS, Castro PML (2009) Arsenic, lead and nickel accumulation in *Rubus ulmifolius* growing in contaminated soil in Portugal. J Hazard Mater 165:174–179

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence-a practical guide. J Exp Bot 51:659–668

Mendez MO, Maier RM (2008a) Phytostabilization of mine tailings in arid and semiarid environments–an emerging remediation technology. Environ Health Persp 116:278–283

Mendez MO, Maier RM (2008b) Phytoremediation of mine tailings in temperate and arid environments. Rev Environ Sci Biotechnol 7:47–59

Mertens J, Vervaeke P, Schrijver AD, Luyssaert S (2004) Metal uptake by young trees from dredged brackish sediment: limitations and possibilities for phytoextraction and phytostabilisation. Sci Total Environ 326:209–215

Metwally A, Safronova VI, Belimov AA, Dietz KJ (2005) Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J Exp Bot 56:167–178

Monterroso C, Rodríguez F, Chaves R, Diez J, Becerra-Castro C, Kidd PS, Macías F (2014) Heavy metal distribution in mine-soils and plants growing in a Pb/Zn-mining area in NW Spain. Appl Geochem 44:3–11

Paniz JNG, Flores EMM, Dressler VL, Martins AF (2001) Flow injection turbidimetric determination of total organic carbon with a gas-liquid transfer microreactor. Anal Chim Acta 445:139–144

Parra A, Zornoza R, Conesa E, Gómez-López MD, Faz A (2014) Seedling emergence, growth and trace elements tolerance and accumulation by *Lamiaceae* species in a mine soil. Chemosphere 113: 132–140

Pérez-Esteban J, Escolástico C, Ruiz-Fernández J, Masaguer A, Moliner A (2013) Bioavailability and extraction of heavy metals from contaminated soil by *Atriplex halimus*. Environ Exp Bot 88:53–59

Pietrini F, Zacchini M, Iori V, Pietrosanti L, Ferretti M, Massacci A (2010) Spatial distribution of cadmium in leaves and its impact on photosynthesis: examples of different strategies in willow and poplar clones. Plant Biol 12:355–363

Pottier M, de la Torre VSG, Victor C, David LC, Chalot M, Thomine S (2015) Genotypic variations in the dynamics of metal concentrations in poplar leaves: a field study with a perspective on phytoremediation. Environ Pollut 199:72–83

Prasad MNV, Freitas H (2000) Removal of toxic metals from solution by leaf, stem and root phytomass of *Quercus ilex* L. (holly oak). Environ Pollut 110:277–283

Rabeda I, Bilski H, Mellerowicz EJ, Napieralska A, Suski S, Woźny A, Krzesłowska M (2015) Colocalization of low-methylesterified pectins and Pb deposits in the apoplast of aspen roots exposed to lead. Environ Pollut 205:315–326

Seo KW, Son Y, Rhoades CC, Noh NJ, Koo JW, Kim JG (2008) Seedling growth and heavy metal accumulation of candidate woody species for revegetating Korean mine spoils. Restor Ecol 16:702–712

Shi WG, Li H, Liu TX, Polle A, Peng CH, Luo ZB (2015) Exogenous abscisic acid alleviates zinc uptake and accumulation in *Populus* × *canescens* exposed to excess zinc. Plant Cell Environ 38:207–223

Shu WS, Ye ZH, Lan CY, Zhang ZQ (2001) Acidification of lead/zinc mine tailings and its effect on heavy metal mobility. Environ Int 26: 389–394

Solís-Dominguez FA, White SA, Hutter TB, Amistadi MK, Root RA, Chorover J, Maier RM (2012) Response of key soil parameters during compost-assisted phytostabilization in extremely acidic tailings: effect of plant species. Environ Sci Technol 46:1019–1027

Suchkova N, Tsiripidis I, Alifragkis D, Ganoulis J, Darakas E, Sawidis T (2014) Assessment of phytoremediation potential of native plants during the reclamation of an area affected by sewage sludge. Ecol Eng 69:160–169

Ucisik AS, Trapp S (2008) Uptake, removal, accumulation, and phytotoxicity of 4-chlorophenol in willow trees. Arch Environ Contam Toxicol 54:619–627

Vassilev A, Manolov P (1999) Chlorophyll fluorescence of barley (*Hordeum vulgare* L.) seedlings grown in excess of Cd. Bulg J Plant Physiol 25:67–76

Vassilev A, Perez-Sanz A, Semane B, Carleer R, Vangronsveld J (2005) Cadmium accumulation and tolerance of two Salix genotypes hydroponically grown in the presence of cadmium. J Plant Nutr 28: 2159–2177

Vernay P, Gauthier-Moussard C, Jean L, Bordas F, Faure O, Ledoigt G, Hitmi A (2008) Effect of chromium species on phytochemical and physiological parameters in *Datura innoxia*. Chemosphere 72:763–771

Vysloužilová M, Tlustoš P, Száková J (2003) Cadmium and zinc phytoextraction potential of seven clones of *Salix* spp. planted on heavy metal contaminated soils. Plant Soil Environ 49:542–547

Wang SF, Shi X, Sun HJ, Chen YT, Pan HW, Yang XE, Rafiq T (2014) Variations in metal tolerance and accumulation in three hydroponically cultivated varieties of *Salix integra* treated with lead. PLoS One 9:e108568

Yang WD, Zhao FL, Zhang XC, Ding ZL, Wang YY, Zhu ZQ, Yang XE (2015) Variations of cadmium tolerance and accumulation among 39 *Salix* clones: implications for phytoextraction. Environ Earth Sci 73:3263–3274

Zacchini M, Pietrini F, Mugnozza GS, Iori V, Pietrosanti L, Massacci A (2009) Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. Water Air Soil Pollut 197:23–34

Zacchini M, Pietrini F, Mugnozza GS, Iori V, Pietrosanti L, Massacci A (2011) Cadmium accumulation and tolerance in *Populus nigra* and *Salix alba*. Biol Plantarum 55:383–386

Zhang X, Zhu YG, Zhang YB, Liu YX, Liu SC, Guo JW, Li RD, Wu SL, Chen BD (2014) Growth and metal uptake of energy sugarcane (*Saccharum* spp.) in different metal mine tailings with soil amendments. J Environ Sci-China 26:1080–1089

Zhao XL, Zheng LY, Xia XL, Yin WL, Lei JP, Shi SQ, Shi X, Li HQ, Li QH, Wei Y, Chang EM, Jiang ZP, Liu JF (2015) Responses and acclimation of Chinese cork oak (*Quercus variabilis* Bl.) to metal stress: the inducible antimony tolerance in oak trees. Environ Sci Pollut Res 22:11456–11466

Zhou LY, Zhao Y, Wang SF, Han SS, Liu J (2015) Lead in the soil–mulberry (*Morus alba* L.)–silkworm (*Bombyx mori*) food chain: translocation and detoxification. Chemosphere 128:171–177

Zhuang P, McBride MB, Xia HP, Li NY, Li ZA (2009) Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. Sci Total Environ 407:1551–1561