# PLUMBUM/ZINC ACCUMULATION IN SEEDLINGS OF SIX AFFORESTATION SPECIES CULTIVATED IN MINE SPOIL SUBSTRATE

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WANG Y, BAI S, WU J, CHEN J, YANG Y, ZHU X & ZHU T. 2015. Plumbum/zinc accumulation in seedlings of six afforestation species cultivated in mine spoil substrate. Mining activities generate spoils and effluents that are harmful to ecosystems and human health due to their extremely high toxic metal concentrations. Plants have been planted on these spoils to uptake heavy metal and to revegetate for phytoremediation. Soil obtained from Pb/Zn mine tailing areas was used to conduct a greenhouse pot experiment to compare the growth and toxic metal accumulation of six tree species for afforestation in southern China. One year growth indicated that the six species tolerated toxic metals and their accumulation differed with plant species, their parts and kinds of metals. Height and biomass of *Betula alnoides* were significantly greater than the rest of the species. *Alnus nepalensis* had the highest translocation (0.86 in root and 0.69 in shoot) and bioconcentration (0.62 in root and 0.51 in shoot) factor values for Pb, while *Pinus yunnanensis* had higher translocation and bioconcentration factor values (0.78, 0.45 in root and 0.84, 0.48 in shoot respectively) for Zn. *Alnus nepalensis* was the best candidate species for phytoremediation of Pb/Zn mine tailing areas due to its good nitrogen fixation, greater translocation and bioconcentration factor values for Pb, while and larger biomass, accumulating an average of 4.70 mg Pb and 6.92 mg Zn.

Keywords: Toxic metal accumulation, soil remediation, Pb/Zn mine tailings, phytoremediation

# **INTRODUCTION**

Industrial mining activities produce tailings that are rich in heavy metals such as plumbum (Pb) and zinc (Zn) which can contaminate air, water, soils and plants. Most mine tailing disposal areas are bare lands without vegetation and are considered environmentally harmful to ecosystems and human health (Smuc et al. 2012). To reduce the environmental impacts of mining waste, a variety of measures have been taken for many years. Many methods such as dig-and-dump, encapsulation, soil dressing or soil washing have been used to remediate contaminated soil. These civil engineering techniques are often appropriate only for small areas where rapid, complete decontamination is required (Martin & Bardos 1996, BIO-WISE 2000). These approaches are expensive and may even damage soil structure and weaken biological activity. Phytoremediation, the use of plants to remove pollutants from the environment or to render them harmless, has been considered as ecofriendly and low cost potential strategy for rehabilitating contaminated soil (Baker et al. 1994, Kyung et al. 2008).

Revegetation of contaminated areas is possible because of the effective mechanism of plants to accumulate heavy metals from the environment. Metal concentrations in plants vary with plant species (Secu et al. 2008). However, it is difficult to revegetate some mine tailing areas because of toxicity of heavy metals and low nutrient availability. Selecting plant species that can tolerate barren condition and accumulate heavy metal remains a worldwide restoration challenge (Kyung et al. 2008). Under natural growing conditions, plants can accumulate more metal ions than their surrounding medium by passive mass flow of water into the roots or active

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transport of metal ions into root epidermal cells (Yoon et al. 2006). Even though contaminants can be considered as quite insoluble and poorly leachable, their vertical transfer to aboveground vegetation is feasible because of their interactions with exudates released by roots.

Pb is extremely toxic to human and ecosystems and Zn is an essential element for plants that is toxic in high doses. These two metals are released from Pb/Zn mine activities and deposited in high levels in mine tailing areas. Some herbaceous species such as Aeluropus littoralis (Mohammad & Faezeh 2011), Medicago sativa (Shi et al. 2012), Bidens humilis (Bech et al. 2002) and Cyperus microiria and Hippochaete hiemale (Miao et al. 2011) can grow normally in Pb/Zn mine tailing areas. This suggests that these herbaceous species can be potentially used as suitable vegetation cover for lands contaminated with heavy metal or soil polluted by mining. Most research has focused on the potential use of grasses in revegetation, though a few studies have evaluated the utilisation of fast-growing tree species for remediation and revegetation of mine tailing areas, especially in southern China (Shi et al. 2011a).

To provide highly efficient, sustainable and ecologically sound solution for reclamation of mine tailings, it is necessary that suitable tree species be selected as vegetation cover for phytoremediation of land contaminated by toxic metals. However, on highly contaminated soil, tree establishment may be inhibited by high concentrations of heavy metals (Pulford & Watson 2003). The aim of this study was to compare the growth and toxic metal accumulation of six species mainly used for afforestation in Yunnan Province, southern China. We also assessed the feasibility of utilising these trees for phytoremediation purpose by greenhouse pot experiment using soil obtained from Pb/Zn mine tailing areas.

# MATERIALS AND METHODS

### Site description and soil materials

This study compared seedling growth in spoils from an abandoned mine in Zhanyi county (25° 472' N, 103° 86' E), Yunnan province, China. The mine was exploited for Pb and Zn from 1923 till 2004. Toxicity levels of Pb and Zn in spoils of Zhanyi mine were 144 and 327% higher respectively than the allowable toxicity levels in China (Anonymous 1995).

Subsamples of mineral soil from the upper 30 cm of the Pb/Zn mine tailings were collected, mixed and analysed for physicochemical properties (Table 1). Total N was analysed colorimetrically with a continuous flow ion analyser following wet digestion in sulfuric acid (Bremner 1996). Total P was analysed by molybdenum blue method after digestion with  $1.0 \text{ g of } \text{K}_{\circ}\text{SO}_{4} \text{ and } 5 \text{ mL of concentrated } \text{H}_{\circ}\text{SO}_{4}$ (Deng et al. 2004) and pH was determined in a 1:5 soil to distilled water slurry after 1 hour of stirring. The concentrations of Pb and Zn were determined for 10-g soil subsamples extracted in 50 mL of 0.1 mol/L HCl after 1 hour of stirring. Soil extracts were filtered and the concentrations were measured using a spectrometer. The Pb/Zn spoils contained Pb and Zn in concentrations of 1220 and 2134 mg kg<sup>-1</sup> respectively. Average pH value was 7.38 and the total N and P contents were 0.08 g kg<sup>-1</sup> and 0.46 mg kg<sup>-1</sup> respectively.

## **Plant material**

Six species selected for this study are afforestation tree species in southern China. *Pinus yunnanensis* is native to Yunnan province where it is considered one of the most economically and culturally important species. *Cupressus duclouxiana* is endemic to China. Both species are commonly used to reclaim degraded lands in southern China. *Alnus nepalensis* grows

 Table 1
 Physical and chemical characteristics of soil from Pb/Zn mine tailing area

Total N	Total P	K	рН	Pb	Zn
(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )		(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )
$0.08 \pm 0.01$	$0.46\pm0.03$	$1.35\pm0.06$	$7.38 \pm 0.13$	$1219.60 \pm 116.24$	2134.18 ± 226. 42

Values are means with standard errors (n = 5)

rapidly, with symbiotic association with nitrogenfixing actinomycetes. *Betula alnoides* is a pioneer species which grows rapidly in warm climates. *Alnus nepalensis* and *B. alnoides* are fast-growing and high biomass-accumulation species. *Eucalyptus globulus* and nitrogen-fixing *Acacia dealbata* are native to Australia, and both were widely introduced decades ago and are now becoming naturalised across southern China. For this experiment, we collected about 1 kg of seeds for each species from trees growing in Zhanyi County.

#### **Greenhouse experiment**

The experiments were performed in a greenhouse. Seedlings of six tree species were lifted from bare root nursery beds and transplanted into large plastic pots (0.021 m<sup>3</sup>) in March 2010. Seedling shoots and root systems were well developed and structurally sound. There were five replicates and 10 pots were used for each replicate, totalling 300 pots in a randomised block design. Height and root collar diameter of each seedling were measured at the beginning of experiment (Table 2). Pots were filled with 12 kg of Pb/Zn mine spoil substrates. Pots were watered by controlled irrigation which was scheduled according to soil moisture measured by tensiometer to maintain moisture content around field capacity.

# Seedling survival, biomass and heavy metal accumulation

The stress experiment was conducted from March 2010 till March 2011. Seedling survival, height and root collar diameter were assessed on all seedlings at the end of experiment period and all plants were harvested to determine their biomass and Pb/Zn concentrations. When the plants were harvested, their shoots and roots were washed with deionised water to remove tailing particles and dissected into leaf, stem and root tissues. Each subsamples were dried at 75 °C to constant mass and weighed. The subsamples were ground to pass 850-µm mesh sieve and were wet digested (0.2 g) in 4 mL of HNO<sub>3</sub> and 1 mL of HClO<sub>4</sub> mixture. Metal concentrations (Pb and Zn) were determined using flame atomic absorption spectroscopy. Heavy metal accumulation was calculated by multiplying the biomass and elemental concentrations of each seedling component (root, shoot and leaf).

#### Data analysis

Translocation factors were used to estimate the ability of plants to translocate metals from roots to aboveground components (Yoon et al. 2006, MacFarlane et al. 2007). The translocation factor of each species was calculated as:

$$TF = C_{\text{shoot (leaf)}} / C_{\text{root}}$$
(1)

where TF = translocation factor and  $C_{shoot (leaf)}$ and  $C_{root}$  = total heavy metal concentration in the shoot (leaf) (mg kg<sup>-1</sup>) and root of plant (mg kg<sup>-1</sup>) respectively. Greater translocation factor shows more effective translocation of metals occurred from root to shoot (Zhang et al. 2002, Fayiga & Ma 2006).

Bioconcentration factors was used to estimate the ability of plants to accumulate metals from soil (Yoon et al. 2006). Bioconcentration factors of Pb and Zn was calculated as:

$$BCF = C_{shoot (leaf)} / C_{soil}$$
(2)

Species	Height (cm)	Root collar diameter (mm)		
Alnus nepalensis	$12.6 \pm 1.3$	$2.2 \pm 0.2$		
Betula alnoides	$15.4 \pm 1.2$	$2.7 \pm 0.4$		
Cupressus duclouxiana	$7.2 \pm 1.4$	$1.0 \pm 0.3$		
Pinus yunnanensis	$10.3 \pm 1.5$	$1.4 \pm 0.2$		
Acacia dealbata	$14.7\pm1.2$	$2.1 \pm 0.4$		
Eucalyptus globules	$14.3 \pm 1.3$	$2.4 \pm 0.3$		

 Table 2
 Seedling sizes for six afforestation species at the beginning of the experiment

Values are means with standard errors (n = 50)

where BCF = bioconcentration factor and  $C_{shoot\ (leaf)}$  and  $C_{soil}$  = total heavy metal concentrations in the plant shoot (leaf) (mg kg<sup>-1</sup>) and soil (mg kg<sup>-1</sup>) respectively. Plant with bioconcentration factors were categorised further as hyperaccumulators (> 10), accumulators (between 1 and 10) and excluders (< 1) (Ma et al. 2001, Cluis 2004).

All indices were analysed by one-way ANOVA and LSD using SPSS 18. Differences at p < 0.05 were considered significant.

# RESULTS

#### Seedling growth

Survival of the six species ranged from 70 to 96% after 12 months in contaminated soil (Figure 1). About 96% *A. nepalensis* and 90% *A. dealbata* survived in mine tailing, significantly higher than the other four species (p < 0.05). Highest mortality occurred in *P. yunnanensis* and *C. duclouxiana*, reaching 30 and 24% respectively.



**Figure 1** Survival of seedlings of the six main afforestation species; vertical bars represent standard errors (n = 50)

Table 3Increment of height and root collar diameter and tissue biomass per seedling of six afforestation<br/>species grown in Pb/Zn tailings from March 2010 till March 2011

Tree species	Height (cm)	Root collar diameter (mm)	Root biomass (g)	Shoot biomass (g)	Leaf biomass (g)
Alnus nepalensis	55 ± 6 a	4.3 ± 0.3 a	$1.96 \pm 0.12$ a	2.24 ± 0.21 a	2.01 ± 0.14 a
Betula alnoides	$57 \pm 4$ a	$5.1 \pm 0.4$ a	$2.25\pm0.09~\mathrm{a}$	$2.84 \pm 0.11$ a	$2.41 \pm 0.08$ a
Cupressus duclouxiana	$16 \pm 1$ b	$2.1\pm0.3~b$	$0.19\pm0.02~{\rm c}$	$0.23\pm0.03~\mathrm{c}$	$0.20\pm0.01~{\rm c}$
Eucalyptus globulus	$52 \pm 4$ a	$3.6 \pm 0.6$ a	$0.73\pm0.04\;b$	$1.52\pm0.12\;b$	$1.50\pm0.10\;\mathrm{b}$
Acacia dealbata	$44 \pm 5$ a	$3.9 \pm 0.4$ a	$0.31\pm0.03~{\rm c}$	$0.48\pm0.06~c$	$0.37\pm0.02~{\rm c}$
Pinus yunnanensis	$22 \pm 3$ b	$3.1 \pm 0.2$ b	$0.25 \pm 0.01 \text{ c}$	$0.29 \pm 0.02$ c	$0.54 \pm 0.03$ c

Values are means with standard errors, means with similar alphabets in a column are not significantly different

Death of seedlings was observed mainly in the first three months. During the last six months of study, survival of *A. nepalensis* and *A. dealbata* was constant, and that of the other four species declined a little.

Growth of A. nepalensis, B. alnoides, A. dealbata and E. globulus was significantly greater (p < 0.05) compared with P. yunnanensis and C. duclouxiana (Table 3). Mean stem height increment was 57 cm for B. alnoides and 16 cm for C. duclouxiana. Root diameter growth per seedling for species reflected similar trends as height. Mean absolute stem root collar diameter increased 5.1 mm in B. alnoides and 2.1 mm in C. duclouxiana. Total biomass of B. alnoides seedlings was the highest (7.50 g seedling<sup>-1</sup>), followed by *A. nepalensis* (6.21 g seedling<sup>-1</sup>). *Cupressus duclouxiana* had the lowest biomass (0.62 g seedling<sup>-1</sup>).

#### Heavy metal concentrations

The six afforestation species had higher capability of accumulating Zn, consistent with heavy metal concentrations in tailing (Figures 2 and 3). Pb/Zn concentrations in the six species were generally in the order of *A. nepalensis* > *P. yunnanensis* > *B. alnoides* > *E. globulus* > *A. dealbata* > *C. duclouxiana.* Heavy metal concentrations were significantly higher in root tissue than in aboveground components (leaf and stem).



**Figure 2** Pb concentrations in six afforestation species seedlings; vertical bars represent standard errors



Figure 3 Zn concentrations in six afforestation species seedlings; vertical bars represent standard errors

The six species demonstrated different ability to take up heavy metals. Concentrations of Pb and Zn were highest in the root of A. nepalensis seedlings and lowest in C. duclouxiana. Pb and Zn concentrations in root were 13 and 4.5 fold higher in A. nepalensis seedlings compared with C. duclouxiana respectively. Concentrations of Pb and Zn were highest in stem and leaf of A. nepalensis seedlings. Pb and Zn concentrations in stem and leaf showed similar trends, i.e. 22.3 to 23.8 fold higher in A. nepalensis stem and 5.4 to 22.3 fold higher in A. nepalensis leaf. Except for *P. yunnanensis*, Pb concentrations in the seedlings were generally in the order of root > shoot > leaf. Except for B. alnoides, Zn concentrations in the seedlings were generally in the order of root > leaf > shoot.

# Translocation and accumulation of metals in plants

The ability to translocate metals differed between tree species. Translocation factor values of the six species subjected to growth in tailings are listed in Table 4. In general, Pb and Zn mainly accumulated in the root of plants compared with stem and leaf. All translocation factors of the six species were lower than 1. Alnus nepalensis shoot had the highest translocation factor values for Pb (0.86). Its leaf had the highest translocation factor value for Zn (0.73). Except for *P. yunnanensis*, the other five species had higher ability of translocating Pb in shoot than in leaf. Except for *B. alnoides*, the other five species had higher ability of translocating Zn in leaf than in shoot. Translocation factor values of Zn in leaves of the six species were higher than that of Pb. However, shoot translocation factors had no specific trend.

For all species, shoot bioconcentration factors ranged from 0.03–0.62 for Pb and 0.05–0.46 for Zn, while leaf bioconcentration factors ranged from 0.02–0.51 and 0.09–0.48 respectively (Table 4). *Alnus nepalensis* shoot had the highest bioconcentration factor value for Pb (0.62) and its leaf had the highest for Zn (0.47). *Cupressus duclouxiana* leaf had the lowest bioconcentration factor value for Pb (0.02) and the shoot had the lowest value for Zn (0.05). Except for *P. yunnanensis*, the other five species had higher ability of accumulating Pb in shoot than in leaf. Except for *B. alnoides*, the rest of the species accumulated higher Zn in leaf than in shoot. Except for *A. nepalensis*, bioconcentration

Table 4Translocation factors and bioconcentration factors for Pb and Zn in seedlings of six<br/>afforestation species

Species	Translocation factor				Bioconcentration factor			
	Pb Shoot	Pb Leaf	Zn Shoot	Zn Leaf	Pb Shoot	Pb Leaf	Zn Shoot	Zn Leaf
Alnus nepalensis	$0.86 \pm 0.08$ a	$0.69 \pm 0.07$ a	$0.71 \pm 0.05$ a	$0.73 \pm 0.06$ a	$0.62 \pm 0.04$ a	$0.51 \pm 0.04$ a	$0.46 \pm 0.03$ a	$0.47 \pm 0.04$ a
Betula alnoides	$0.31 \pm 0.03$ c	$0.20\pm0.04~c$	$0.56\pm0.08\;b$	$0.55\pm0.07\;b$	$0.13 \pm 0.02$ c	$0.08\pm0.01~b$	$0.19\pm0.03~b$	$0.19 \pm 0.02 \text{ b}$
Cupressus duclouxiana	$0.46\pm0.04~b$	$0.40\pm0.05\;b$	$0.36 \pm 0.03$ c	$0.61\pm0.06\;\mathrm{b}$	$0.03 \pm 0.00 \text{ c}$	$0.02\pm0.00~b$	$0.05\pm0.00~\mathrm{c}$	$0.09 \pm 0.01$ a
Eucalyptus globulus	$0.29\pm0.03~\mathrm{c}$	$0.27\pm0.02~\mathrm{c}$	$0.42\pm0.06~\mathrm{c}$	$0.55\pm0.05~b$	$0.06 \pm 0.01 \text{ c}$	$0.05\pm0.00~b$	$0.11 \pm 0.01 \text{ c}$	$0.14\pm0.01~b$
Acacia dealbata	$0.44\pm0.06\;b$	$0.32\pm0.04~b$	$0.31 \pm 0.05 \text{ c}$	$0.62\pm0.05\;b$	$0.07\pm0.01~\mathrm{c}$	$0.05\pm0.00~b$	$0.06\pm0.00~\mathrm{c}$	$0.11\pm0.01~b$
Pinus yunnanensis	$0.38\pm0.03~b$	$0.66 \pm 0.05$ a	$0.78 \pm 0.07$ a	$0.84 \pm 0.06$ a	$0.24\pm0.01~b$	$0.43 \pm 0.02$ a	$0.45 \pm 0.03$ a	$0.48 \pm 0.04$ a

Values are means with standard errors; different letters in the same column indicate significant differences between species (p < 0.05)

factors of Zn in the leaves of the rest of the species were higher than Pb.

### Metal accumulation in biomass

The six studied species accumulated large quantities of Pb/Zn during the 1-year growth in tailings (Figure 4). Pb/Zn accumulation by A. nepalensis was significantly greater than other five species, averaging 4.70 mg seedlings<sup>-1</sup> for Pb and 6.92 mg seedlings<sup>-1</sup> for Zn. Except for A. nepalensis, the Pb/Zn accumulated by *B. alnoides* was significantly greater than the other four species (average 1.85 mg Pb seedling<sup>-1</sup> and 3.74 mg Zn seedling<sup>-1</sup>). The accumulation of Pb and Zn ranged from 0.03-4.70 and 0.12–6.92 mg seedling<sup>-1</sup> respectively. For all plant species studied, both the accumulation of Pb/Zn in the biomass per seedling was generally in the order of A. nepalensis > B. alnoides > P. yunnanensis > E. globulus > A. dealbata > C. duclouxiana.

# DISCUSSION

#### Seedling growth performance

Plant tolerance of heavy metals is a prerequisite for phytoremediation of land contaminated by heavy metals (Shi et al. 2011a). After 12 months growing in Pb/Zn tailings, all six species survived but with different rates (Figure 1). Pb and Zn concentrations in the soil of this study are greatly higher than 100–400 and 70–400 mg kg<sup>-1</sup> respectively, which are considered toxic to plants (Kabata-Pendias & Pendias 1984). With such high contamination, the soil used in this study inhibited growth of all six afforestation species. Normal tissue concentrations of heavy metals in plants range from 0.5 to 10 mg kg<sup>-1</sup> and phytotoxic concentration, from 30 to 300 mg kg<sup>-1</sup> for Pb, while those for Zn are 10 to 150 mg kg<sup>-1</sup> and 500 to 1500 mg kg<sup>-1</sup> respectively (Levy et al. 1999). However, Pb/Zn concentrations of most seedlings in this study were higher than the phytotoxic range. Excess Pb and Zn contents in soil may retard plant growth (Kamal et al. 2004). Our findings agree with many previous studies that trees can generally survive in metal-contaminated soil, albeit usually with reduced growth rates. These results indicated that the six tree species growing on the site contaminated with heavy metals were tolerant of these metals.

In addition, the deficiency of nutrients such as N, P and K in Pb/Zn tailings may be another major factor in the reduced plant growth on tailings (Ye et al. 2002). Inherent low nutrients of N, P and K in tailing soil are important limitations to plant growth (Levy et al. 1999). In our study, although some species seedlings grew slowly, *A. nepalensis* and *B. alnoides* showed superior growth performance most probably as a result of symbiotic nitrogen fixation. Many studies have documented positive plant responses to fertilisation in tailings. For example, fertilisation improved survival, growth and biomass of



Figure 4 Accumulation of Pb and Zn in biomass per seedling of six afforestation species; vertical bars represent standard errors

trees planted on reclaimed mined lands in the Appalachians (Casselman et al. 2006) and increased the biomass of native species grown on neutral mine tailings (Conesa et al. 2007). Our study showed that *A. nepalensis* and *B. alnoides* could adapt to high concentrations of Pb and Zn in nutritionally poor soil better than the rest of the tested seedlings.

# Accumulation and translocation of metals in tree species

Metal concentrations in plants vary with plant species (Alloway et al. 1990, Secu et al. 2008). In this study, Pb concentrations in the six tree species ranged from 27.6–893.2 mg kg<sup>-1</sup>, while Zn, 108.9–1378.2 mg kg<sup>-1</sup> in the order of *A. nepalensis* > *P. yunnanensis* > *B. alnoides* > *E. globulus* > *A. dealbata* > *C. duclouxiana*. Since hyperaccumulators are defined as plants that accumulate >1000 mg kg<sup>-1</sup> of Pb or >10,000 mg kg<sup>-1</sup> of Zn into their aboveground biomass (Baker & Brooks 1989), none of the six tree species were hyperaccumulators. However, they showed acceptable capacity of accumulating heavy metals.

Previous studies showed that the metals accumulation by plants differed between tissues (Shi et al. 2011a). In most of the seedlings in this study, root Pb/Zn concentrations were much greater than those of shoot (leaf), indicating low mobility of Pb/Zn from the former to the latter and immobilisation of heavy metals in roots. Restriction of upward movement from roots into shoots can be considered as tolerance mechanism (Verkleij & Schat 1990). Bioconcentration and translocation factors can be used to estimate the potential of plant for phytoremediation purpose (Yoon et al. 2006). Most of the six afforestation species seedlings in this study had obvious translocation and bioconcentration factors, which indicated their ability to accumulated heavy metal and translocation (Figure 4). Tolerant plants tend to restrict soil-root and root-shoot (leaf) metal transfers and therefore has much less accumulation in their biomass, while hyperaccumulators actively take up and translocate metals into their aboveground biomass. In the present work, all tree species had bioconcentration and translocation factors of less than one. Translocation and bioconcentration factors of Pb/Zn for

A. *nepalensis* in this study was higher than that found by Shi et al. (2011b) in Vitex trifolia var. *simplicifolia* (translocation factors = 0.78 of Pb, 0.28 of Zn and bioconcentration factors = 0.22 of Pb, 0.02 of Zn). However, some studies suggest that plants exhibiting translocation factor and particularly bioconcentration factor values of less than one are unsuitable for phytoextraction (Fitz & Wenzel 2002); the possible reasons may be attributed to the differences between agricultural crops and tree species. Due to rapid growth, total mass of metals accumulated by A. nepalensis was 4.70 mg seedling<sup>-1</sup> for Pb and 6.92 mg seedling<sup>-1</sup> for Zn. Betula alnoides had 1.85 mg seedling<sup>-1</sup> of Pb and 3.74 mg seedling<sup>-1</sup> of Zn. These show that both species are suitable to be used for phytoextraction of Pb/Zn in mining areas.

# Potential uses of afforestation species in phytoremediation

Tree growth and establishment may be inhibited by high concentrations of heavy metals (Pulford & Watson 2003). It is very difficult to select the right tree species to cover and revegetate highly contaminated soils. Although no Pb/Zn hyperaccumulators were found in this study, some interesting observations were noted. Some of the tested species have good characteristics that make them suitable to be used in remediation of Pb/Zn contaminated land. These include the ability to grow on nutrient-poor soil, deep root system, fast growth rate, metal-resistance trait and economically viable secondary use (Punshon et al. 1996). Survival of all species was higher than 70%, confirming that they could all survive in experimental soil with very high Pb/Zn concentrations. This showed that the seedlings were metal-tolerant and would gradually grow up and establish to restore the vegetation in the area. Seedlings with fast growth accumulate higher biomass over time, so they possess specific capacity for heavy metal accumulation.

Alnus nepalensis is an N fixer that can adapt to growth on nutrient-poor soil to stimulate biomass production in Pb/Zn mine tailing area soil. Thus, it is suitable for use in phytoremediation. Alnus nepalensis and B. alnoides are two of the most widely used afforestation species in southern China (Liang et al. 2006, Li et al. 2008). These species are used to make plywood, high-grade furniture, wood flooring and interior decorative material. Heavy metals will not be released but instead will be safely stored in the end-products. Microenvironmental effects such as increasing soil moisture, additional organic matter and nutrient availability would be produced gradually after afforestation with these species. After that, some herbaceous and shrub hyperaccumulators will have the opportunity to establish under the tree canopy. Thus, formation of forest community dominated by these trees can not only translocate large amounts of heavy metals to trees biomass but also improve the ecosystem of the area.

# CONCLUSIONS

All six afforestation species in this study were able to grow in Pb/Zn tailings and had different tolerance levels to stress of heavy metals. All species were capable of accumulating heavy metals in their roots and some translocated metals into aboveground biomass. Although *A. nepalensis* and *B. alnoides* are not hyperaccumulators, they have a lot of excellent features such as a high survival rate in tailings, easy propagation, rapid growth, large biomass, good end-product uses, not associated with the food chain and ability to accumulate large amounts of heavy metals. *Alnus nepalensis* and *B. alnoides* could serve as appropriate species to be used in afforestation of mine tailing areas with high levels of Pb and Zn.

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