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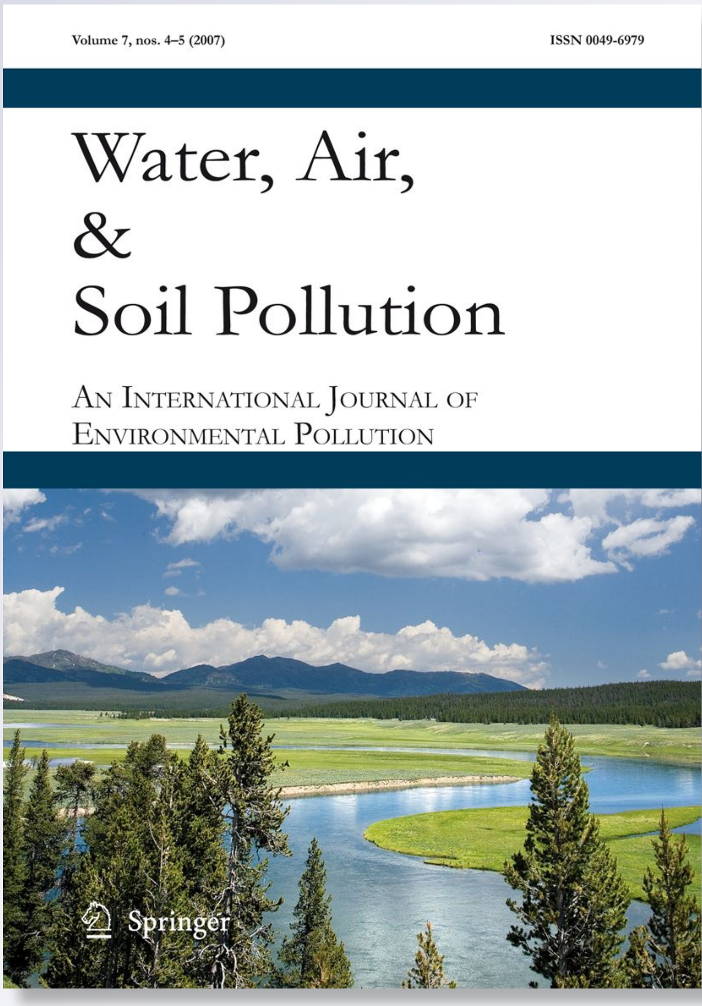
Water, Air, & Soil Pollution
An International Journal of Environmental Pollution

ISSN 0049-6979
Volume 223
Number 4

Water Air Soil Pollut (2012)
223:1815-1828
DOI 10.1007/s11270-011-0986-3



Springer

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Received: 10 June 2011 / Accepted: 28 September 2011 / Published online: 19 October 2011

Abstract Fluvial mine tailing deposition has caused extensive riparian damage throughout the western USA. Willows are often used for fluvial mine tailing revegetation, but some accumulate excessive metal concentrations potentially detrimental to browsers. This greenhouse experiment evaluated growth and metal accumulation of Geyer willow (*Salix geyeriana* Andersson), Drummond's willow (*Salix drummondiana* Barratt ex Hook.), diamondleaf willow (*Salix planifolia* Pursh), Bebb willow (*Salix bebbiana* Sarg.), thinleaf alder [*Alnus incana* (L.) Moench spp. *tenuifolia* (Nutt.) Breitung], water birch (*Betula occidentalis* Hook.), red-osier dogwood (*Cornus sericea* L. spp. *sericea*), and shrubby cinquefoil [(*Dasiphora fruticosa* (L.) Rydb. ssp. *floribunda* (Pursh) Kartesz)]. Bare-root shrubs were grown in tailings collected from three acidic, metal-contaminated (i.e., Cd, Cu, Pb, and Zn) fluvial deposits near Leadville, Colorado, USA. Tailings were amended with only lime to raise the soil pH to 7 s.u., or with lime and composted biosolids (224 Mg ha^{-1}). All shrubs survived in the amended tailings; composted biosolids had little effect on plant biomass. Aboveground and belowground biomass increased during the 2-month greenhouse study by 3–9 and 1.5–5 times initial values, respectively. Most shrubs accumulated Pb and Cu in roots, and belowground Pb concentrations in all shrubs were significantly reduced by the addition of composted biosolids. Compared to other species, alder and cinquefoil accumulated Pb in aboveground growth, and concentrations exceeded animal toxicity thresholds, but these shrubs normally comprise a small proportion of animal diets. Dogwood, alder, and cinquefoil contained low Cd concentrations in aboveground new growth, whereas Bebb and Geyer willow contained zootoxic concentrations. Dogwood, alder,

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and cinquefoil are three good candidates for mine tailing revegetation, especially in fluvial deposits with elevated Cd concentrations.

Keywords Riparian restoration · Heavy metal toxicity · Aboveground metal concentrations · Belowground metal concentrations · *Salix* · *Alnus* · *Betula* · *Dasiphora* · *Cornus*

1 Introduction

Prior to environmental regulations, mining waste was often uncontained in stockpiles near mining operations or discarded in nearby waterways (Da Rosa et al. 1997). Over time, large flood events have eroded stockpiles, depositing acidic and metal-laden tailings in downstream floodplains (Merrington and Alloway 1994; Wielinga et al. 1999). Metals from these fluvially deposited mine wastes can subsequently leach into rivers and streams for decades or centuries after mining activities have ceased (Marcus et al. 2001; Modis et al. 1998).

The Upper Arkansas River near Leadville, Colorado, USA is typical of areas affected by abandoned mining activity and fluvial deposition of mine tailings. Current water quality concerns resulting from fluvial mine tailing deposition along the Upper Arkansas River are related to elevated concentrations of Mn, Cd, and Zn (Walton-Day et al. 2000), and metal leaching due to acid generation. Without considering acid generation and appropriate amendments to counteract it, deposit restoration and stabilization using plants could be limited.

Willows (*Salix* spp.) are often used for riverbank stabilization (Morgan and Rickson 1995; Pezeshki et al. 2007; Schultz et al. 1995), and some willows tolerate elevated heavy metal concentrations (Cosio et al. 2006; Vandecasteele et al. 2005). Lead is generally not found in willow leaves and stems, but Zn, Cd, and Cu are often found in above- and belowground tissue (Bourret et al. 2009; Fischerova et al. 2006; Pahlsson 1989; Vandecasteele et al. 2005; Vervaeke et al. 2003). Bourret et al. (2009) reported aboveground Cd and Zn concentrations in mountain willow (*Salix monticola* Bebb) that were potentially toxic to livestock and wildlife (McDowell 2003). Thus, screening of shrubs from other genera for their potential use in revegetation of fluvial mine tailing deposits may be important.

Birch (*Betula* spp.) might be appropriate for revegetation of fluvial tailing deposits because shrubs and trees in this genus have been used in North America (Lautenbach et al. 1995; Winterhalder 1995) and Russia (Kozlov 2005; Kozlov and Haukioja 1999) for restoration of unamended smelter-damaged sites. Silver birch (*Betula pendula* Roth) and downy birch (*Betula pubescens* Ehrh.) are some of the first shrubs to colonize metal-contaminated areas, suggesting an evolved tolerance to Cd, Pb, and Zn (Pahlsson 1989; Utriainen et al. 1997). Silver and water birch (*Betula occidentalis* Hook.) trees have shown tolerance to a wide range of Cd, Zn, and Pb concentrations (Klassen et al. 2000; Margui et al. 2007).

Alders (*Alnus* spp.) are also useful for restoration of areas with heavy metal contamination (Mertens et al. 2004; Rosselli et al. 2003) as aboveground metal concentrations of most species tend to be low when grown in heavy metal-contaminated soils (Gaulke et al. 2006; Mertens et al. 2004; Rosselli et al. 2003). An additional benefit of alders is their ability to fix N_2 and increase N availability in soils. Symbiotic nitrogen fixing bacteria in the genus *Frankia* are responsible for this reaction, and the *Alnus–Frankia* relationship does not appear to be affected by high metal concentrations in soils (Gaulke et al. 2006).

Dogwood (*Cornus* spp.), a common riparian shrub, can tolerate elevated Ni and Cu concentrations (Heale and Ormrod 1982). Shrubby cinquefoil [*Dasiphora fruticosa* (L.) Rydb. ssp. *floribunda* (Pursh) Kartesz] is native to most of the USA, and both herbaceous (Pearce et al. 1998) and woody (Marosz 2004) cinquefoil species can be useful in revegetation efforts.

The objectives of this study were to: (1) compare growth of thinleaf alder [*Alnus incana* (L.) Moench spp. *tenuifolia* (Nutt.) Breitung], water birch, red-osier dogwood (*Cornus sericea* L. spp. *sericea*), shrubby cinquefoil, Geyer willow (*Salix geyeriana* Andersson), Drummond's willow (*Salix drummondiana* Barrat ex Hook.), diamondleaf willow (*Salix planifolia* Pursh), and Bebb willow (*Salix bebbiana* Sarg.) when grown in mine tailings from three separate fluvial deposits amended with only lime or lime and composted biosolids and (2) characterize plant accumulation of Cd, Cu, Pb, and Zn in aboveground new growth and roots when grown in the amended tailings.

2 Materials and Methods

2.1 Tailings Collection and Amendment

Tailings used in this study were collected from three sites located approximately 8 km south of Leadville, Colorado at an elevation of 2,800 m. Deposit 1 (39° 11′53.27″N 106°21′03.16″W), deposit 2 (39°07′ 50.14″N 106°18′47.54″W), and deposit 3 (39°07′ 48.93″N 106°18′44.35″W) contained elevated metal concentrations and had low pH (Table 1). Approximately 230 L of tailings was collected at each deposit from three or four 60-cm deep holes excavated in November 2006. Because the top 20 cm of tailings was frozen at this time of year, it was not collected. Tailings were placed in barrels for transport to Colorado State University where they were air-dried and homogenized.

Following homogenization, tailings were analyzed for total (methods 200.2 and 200.7; U.S. Environmental Protection Agency 1994) and bioavailable Cd, Cu, Pb, and Zn (Mehlich 1984), and the other properties presented in Table 1. Tailings from each deposit were amended either with lime only or with lime and composted biosolids in early February 2007. The quantity of lime ($CaCO_3$ powder ground to pass a 200-mesh screen) needed to neutralize tailings from each deposit was calculated by adding the lime requirement indicated by the Shoemaker–McLean–Pratt (SMP) single buffer method to achieve a target pH of 7 s.u. (Sims 1996), with the lime requirement indicated by the acid–base potential based on pyritic sulfur (multiply −31.25 by percent pyritic sulfur; Sobek et al. 1978). An additional 25% lime was added to account for heterogeneity in the tailings. Based on these calculations, 8.0, 21.1, and 39.1 g $CaCO_3$ kg^{-1} tailings was required to neutralize tailings from deposit 1, 2, and 3, respectively. Municipal biosolids, composted with wood chips, were added to improve soil structure, inoculate the soil with a microbial community, and supply N, P, and K. Composted biosolids were obtained from Gunnison County, Colorado and added at the rate of 42.3 g kg^{-1} (dry weight) based on a previous study in the Leadville area (Brown et al. 2005). A cement mixer was used to ensure thorough mixing of amended tailings, which were then allowed to chemically react for 14 days to stabilize pH prior to planting shrubs. After stabilization, tailings from each deposit were again analyzed for bioavailable Cd, Cu, Pb, and Zn (Table 1).

2.2 Shrub Selection and Purchase

Eight shrub species were chosen based on several criteria. First, we wanted to test four species of *Salix* L. and four species from other genera for their ability to grow in amended fluvial mine tailings. The USDA PLANTS Database was used to find facultative or facultative wetland shrubs native to Chaffee, Eagle, Gunnison, Lake, Park, and Summit counties in Colorado, USA and expected to tolerate a pH of 4–5 s.u. (USDA PLANTS Database 2009). Shrubs came from three different suppliers and were of different ages and sizes (Table 2). Thinleaf alder, red-osier dogwood, Bebb willow, and Geyer willow were purchased from Plants of the Wild (Tekoa, WA USA). Drummond's and diamondleaf willow were purchased from the Montana Conservation Seedling Nursery (Missoula, MT USA). Water birch was purchased from Lawyer Nursery (Plains, MT USA), and shrubby cinquefoil from Rocky Mountain Native Plants (Rifle, CO USA). Plant nomenclature follows the USDA PLANTS Database (USDA PLANTS Database 2009).

2.3 Plant Growth, Harvesting, and Plant Metal Concentrations

Shrubs were dormant when purchased in January 2007, except for shrubby cinquefoil which had leaves. Roots of all plants were washed free of potting soil at the beginning of the study to ensure they were in direct contact with amended tailings. Each bare-root individual was planted in a conical pot (6.4 cm diameter × 17.8 cm deep) filled with amended tailings. Eighteen individuals of each shrub species were planted in tailings from each of the three deposits amended with lime only or lime and composted biosolids (864 plants total). Ten randomly chosen pots were arranged in 20-cell support trays leaving every other position empty. The location of potted plants in support trays and the arrangement of support trays in the greenhouse were completely random.

Table 1 Characterization of composted municipal biosolids and mine tailings used in a greenhouse study to determine growth and elemental uptake by eight riparian shrub species grown in the media

Parameter	Units	Composted biosolids	Deposit 1			Deposit 2			Deposit 3		
			Prior to amendment	No biosolids	With biosolids	Prior to amendment	No biosolids	With biosolids	Prior to amendment	No biosolids	With biosolids
Texture	N/A	N/A	Sand	–	–	Sandy loam	–	–	Sandy loam	–	–
Paste pH	Standard	7.10	4.00	6.40	6.40	3.30	6.20	6.70	2.00	6.20	6.30
SMP buffer pH[a]	Standard	–	6.00	–	–	4.50	–	–	2.00	–	–
Pyritic S[b]	%	–	0.215	–	–	0.078	–	–	0.736	–	–
Total S[c]	%	0.161	0.411	–	–	0.473	–	–	1.70	–	–
OM[d]	%	72.2[e]	0.8	–	–	0.4	–	–	1.1	–	–
Cd–Mehlich 3[f]	mg kg^{-1}	<0.010	16.0	6.70	5.00	2.90	1.00	1.30	2.60	2.20	2.60
(Total) [g]	mg kg^{-1}	(2.10)	(17.7)	–	–	(8.90)	–	–	(18.5)	–	–
Cu–Mehlich 3	mg kg^{-1}	67.3	82.3	20.0	15.1	67.3	13.2	16.3	65.9	4.90	6.50
(Total)	mg kg^{-1}	(268)	(197)	–	–	(280)	–	–	(151)	–	–
Pb–Mehlich 3	mg kg^{-1}	15.3	270	78.8	63.2	210	99.4	133	190	62.0	118
(Total)	mg kg^{-1}	(26.3)	(1,540)	–	–	(3,100)	–	–	(5,250)	–	–
Zn–Mehlich 3	mg kg^{-1}	20.4	87.8	24.5	24.0	81.2	7.50	9.30	92.0	11.5	13.5
(Total)	mg kg^{-1}	(39.4)	(3,380)	–	–	(1,670)	–	–	(2,640)	–	–

Tailings were collected from three fluvial mine tailings deposits along the Arkansas River near Leadville, Colorado. All tailings received a lime ($CaCO_3$) addition; some tailings were also amended with composted municipal biosolids

[a] Shoemaker et al. (1961)

[b] Williams and Schuman (1987)

[c] Tabatabi (1996)

[d] Organic matter (Nelson and Sommers 1996)

[e] Expressed as weight loss on ignition

[f] Values for elemental analysis are expressed as Mehlich 3 extractable amounts to indicate bioavailable concentration of shown element (Mehlich 1984)

[g] Values for elemental analysis are expressed in parenthesis to indicate ICPOE total recoverable metals of shown element (US EPA 1994)

Table 2 Characteristics of thinleaf alder [*Alnus incana* (L.) Moench spp. *tenuifolia* (Nutt.) Breitung], water birch (*Betula occidentalis* Hook.), red-osier dogwood (*Cornus sericea* L. spp. *sericea*), shrubby cinquefoil [(*Dasiphora fruticosa* (L.) Rydb. ssp. *floribunda* (Pursh) Kartesz)], Geyer willow (*Salix geyeriana* Andersson), Drummond's willow (*Salix drummondiana* Barratt ex Hook.), diamondleaf willow (*Salix planifolia* Pursh), and Bebb willow (*Salix bebbiana* Sarg.) used in a 2-month greenhouse experiment

Shrub	Age (years)	Pot size or plant height	Source of plant material
Thinleaf alder	1	25 cm^3 pots	Northern Idaho
Water birch	2	15–30 cm tall	Montana
Red-osier dogwood	2	25 cm^3 pots	Northern Idaho and central Washington
Shrubby cinquefoil	1	25 cm^3 pots	Rocky Mountains, Colorado
Geyer willow	2	25 cm^3 pots	Northern Idaho
Bebb willow	1	25 cm^3 pots	Northern Idaho
Drummond's willow	2	76 cm^3 pots	Northern Montana
Diamondleaf willow	2	76 cm^3 pots	Northern Montana

Support trays were randomly rearranged in the greenhouse twice during the study maintaining 15 cm of open space between trays at all times. The greenhouse was kept at ~25°C, and the shrubs were watered with municipal water two or three times daily to maintain turgor pressure.

All plants were harvested after 61 days of growth in the greenhouse. Each plant was cut at the soil surface to separate aboveground from belowground parts. New growth included leaves and twigs produced during the study, and this material was bagged separately from the plant material that existed at the beginning of the study (stem). All aboveground biomass was rinsed with deionized water to remove tailings. Roots were collected from all species. Because diamondleaf and Drummond's willow were propagated from cuttings, roots were separated from the original stem used for propagation. Tailings were thoroughly washed from roots using a 0.5-mm sieve and a gentle stream of tap water. Roots were then soaked for 1 min in 0.01 M Na_2H_2-EDTA to remove cations adsorbed to the root surface (Kalis et al. 2006; Slaveykova and Wilkinson 2002). All plant biomass was weighed after oven drying at 55°C for 72 h.

Cadmium, Cu, Pb, and Zn concentrations were determined in aboveground growth and root biomass collected from six randomly selected individuals of each species in each deposit/amendment combination (a total of 288 aboveground growth samples and 288 root biomass samples analyzed). Biomass was ground to pass through a 2-mm screen. Then, samples were weighed (~1.00 g) and digested with concentrated nitric acid and 30% hydrogen peroxide (Huang and Schulte 1985). Final dilution factors were taken into account after determining Cd, Cu, Pb, and Zn concentrations using inductively coupled plasma–atomic emission spectroscopy. Metal concentrations were expressed as milligrams per kilogram of dry weight plant material.

2.4 Experimental Design and Data Analysis

A true control was not incorporated into the study because unamended acidic mine tailings with high metal content can rarely support plant growth, and so this is not a realistic option for restoration. A pilot greenhouse study confirmed that shrub survival in unamended tailings was very low.

Initial plant size of the shrubs used in this study varied by species, so plant proportional growth was used in this study to facilitate comparisons among species. Proportional growth was calculated by standardizing ending biomass by initial biomass for each species. Initial biomass was determined for each species at the beginning of the experiment. Eighteen individual plants of each species were selected at random from the purchased plant material. These plants were separated at the root crown into aboveground and belowground components. Aboveground biomass was washed in a deionized water bath. Belowground biomass was washed free of potting soil, and all plant material was oven dried and weighed as previously described.

At the end of the experiment, aboveground growth was defined as the weight of new growth for an individual plant plus the stem weight divided by the average initial stem weight for each species. Belowground growth was defined as ending root weight divided by the average initial root weight for that species.

Individual plants were treated as subsamples, and deposit averages for growth and metal concentrations were used as observations resulting in three replicates per treatment. Proportional growth and metal concentration datasets did not meet assumptions of normality and were log transformed prior to analysis. Analysis of variance was used to determine the effects of amendment, shrub species, and their interaction on growth and metal concentrations. Proc GLM in SAS 9.2 (SAS Institute Inc. 2008) was used for all analyses. Separate analyses were conducted for aboveground growth, belowground growth, total growth, and above- and belowground concentrations of Cd, Cu, Pb, and Zn. Tukey's method with an alpha of 0.05 was used to separate means in the transformed scale where F tests were significant. Treatment means in figures and tables are presented in the original, untransformed scale.

3 Results

3.1 Growth

Aboveground, belowground, and total proportional growth of the eight shrubs grown in amended tailings were unaffected by amendment ($0.430<P<0.903$), and there were no interactions between amendment and species ($0.152<P<0.961$). All components of proportional growth varied by species ($P<0.001$ for all). Based on these results, mean proportional growth for the eight shrubs averaged over the two amendments was calculated (Fig. 1).

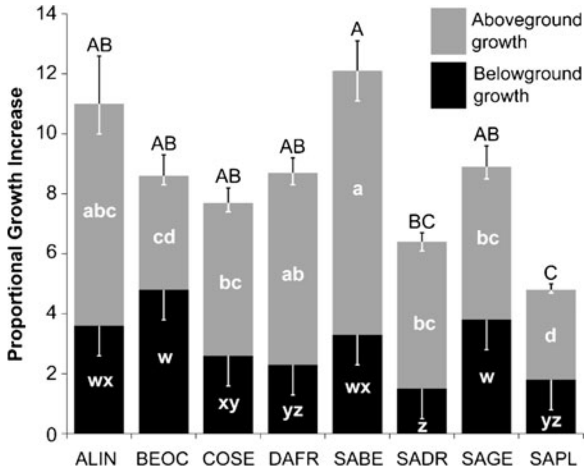

Fig. 1 Mean belowground (*black bars*), aboveground (*gray bars*), and total proportional growth (mean±SE, $n=6$) of eight shrubs grown in mine tailings from three deposits amended with lime ($CaCO_3$) to a pH of 7 s.u., or lime and composted biosolids (224 Mg ha^{-1}). Values presented are species main effect means (averaged across the three deposits and two amendments). Proportional growth represents increases in biomass during a 2-month greenhouse experiment. Standard errors of mean aboveground and belowground proportional growth are represented by *downward pointing*, *white error bars*. *Upward pointing*, *black error bars* depict standard errors of total proportional growth. Shrubs included thinleaf alder [*Alnus incana* (L.) Moench spp. *tenuifolia* (Nutt.) Breitung] (*ALIN*), water birch (*Betula occidentalis* Hook.) (*BEOC*), red-osier dogwood (*Cornus sericea* L. spp. *sericea*) (*COSE*), shrubby cinquefoil [(*Dasiphora fruticosa* (L.) Rydb. ssp. *floribunda* (Pursh) Kartesz)] (*DAFR*), Bebb willow (*Salix bebbiana* Sarg.) (*SABE*), Drummond's willow (*Salix drummondiana* Barratt ex Hook.) (*SADR*), Geyer willow (*Salix geyeriana* Andersson) (*SAGE*), and diamondleaf willow (*Salix planifolia* Pursh) (*SAPL*). *Means with the same letter* for belowground (*x–z*), aboveground (*a–d*), and total (*A–C*) proportional growth are not different (Tukey's HSD, $\alpha=0.05$)

Aboveground biomass of the eight shrubs increased by three to nine times the initial mean aboveground plant weights. Aboveground proportional growth of Bebb willow (nine times initial size) was similar to shrubby cinquefoil and thinleaf alder, but exceeded the other five shrubs. Diamondleaf willow had only a threefold increase in aboveground growth, similar to water birch, but less than the other six shrubs.

Belowground biomass increased from 1.5 to nearly five times initial belowground plant weight. Water birch and Geyer willow had the greatest mean proportional belowground growth, significantly greater than red-osier dogwood, shrubby cinquefoil, Drummond's willow, and diamondleaf willow. Shrubs with low belowground proportional growth included Drummond's willow, diamondleaf willow, and shrubby cinquefoil.

Total proportional growth ranged from five times initial weight for diamondleaf willow to 12 times initial weight for Bebb willow. Five other shrubs had values of total proportional growth similar to Bebb willow. Total proportional growth of diamondleaf willow was similar to that of Drummond's willow, but lower than the other six shrubs.

3.2 Plant Metal Concentrations

There were no interactions between species and amendment for any of the metal concentrations in aboveground or belowground plant material ($0.872 > P > 0.999$). There were also no effects of amendment detected for aboveground Pb concentration or aboveground and belowground Cd, Cu, and Zn concentrations ($0.150 < P < 0.883$). Only belowground Pb was affected by amendment ($P = 0.0172$), and the concentration was greater for plants grown in tailings amended with lime only (515 ± 69.3 mg kg^{-1}, mean± SE, $n = 24$) compared to those grown in tailings amended with lime and composted biosolids (292 ± 38.3 mg kg^{-1}). Metal concentrations in aboveground and belowground plant tissue did vary by species with the exception of belowground Zn ($P = 0.726$). Aboveground and belowground metal concentrations averaged over the amendment treatment, along with SE and P values for the species main effect, are presented in Table 3.

Aboveground Cd concentrations were greatest for Bebb and Geyer willow; intermediate for Drummond's willow, diamondleaf willow, water birch, and shrubby cinquefoil; and lowest for thinleaf alder and red-osier dogwood. Aboveground Cu concentration of thinleaf alder was greater than water birch, red-osier dogwood, Geyer, and diamondleaf willow. Aboveground Pb concentrations of thinleaf alder and shrubby cinquefoil were greater than all other shrubs. The greatest aboveground concentrations of Zn occurred in water birch and the four willows, and the lowest concentration was in red-osier dogwood. Belowground Cd, Cu, Pb, and Zn were relatively consistent across all species.

4 Discussion

4.1 Soil Chemistry and Plant Growth

Typical soil background total metal concentrations range from: Cd, 0.01 to 2.0 mg kg^{-1}; Cu, 2 to 250 mg kg^{-1}; Pb, 2 to 300 mg kg^{-1}; and Zn, 1 to 900 mg kg^{-1} (Sparks 2003). Tailings total Cd, Cu, Pb, and Zn contents were well above the average contents found in soils, ranging from 8.9 to 18.5, 151 to 280, 1,540 to 5,250, and 1,670 to 3,380 mg kg^{-1}, respectively (Table 1). Although tailings total metal concentrations will likely never be reduced by phytoextraction, the in situ technique of phytostabilization is cost-effective and a more practical remediation technique than other restoration options (Chaney et al. 1999; Li and Chaney 1998). However, due to the oxidation of pyrite, these tailings have a low pH, and the associated elevated heavy metal concentrations can make plant survival difficult or impossible. Incorporation of a liming agent can raise pH and immobilize heavy metals by causing them to bind to the surface of clay minerals and organic matter, or precipitate into solid phases. Thus, when utilizing phytostabilization on acidic mine tailings, pH adjustment is necessary to reduce metal bioavailability.

Tailings metal bioavailability appeared to be reduced by lime addition (Table 1). Neutralization of solution and reserve H^+, the concomitant rise in pH, coupled with composted biosolids addition theoretically should have increased the available exchange sites for metal attenuation. The retention of metals to organic phases is weaker at low pH, resulting in more available metals in the soil solution for root absorption (Prasad and Freitas 2003). Thus, increasing soil pH should have increased the pH-dependent exchange sites found on organic phases (i.e., composted biosolids). Illera et al. (2004) showed that dolomite residue reduced Cd, Cu, and Pb solution concentrations by 73%, 80%, and 97%, respectively, after performing metal speciation modeling with MINTEQA2 (v.3.11) (US Environmental Protection Agency 1997) that suggested Cd and Cu were controlled by the precipitation of $Cd(OH)_2$ and $Cu(OH)_2$, and Pb by the formation of laurionite or larnakite.

There were no growth differences between amendments, so the biosolids amendment did not improve the growth (above and belowground) of the eight shrubs. Usually, adding both biosolids and lime to mine tailings improves plant growth compared to amending only with lime (Svendson et al. 2007; DeVolder et al. 2003). Perhaps our greenhouse study was too short for the additional benefits of biosolids to become apparent. Longer-term studies might reveal benefits of biosolids for shrub growth when used for phytostabilization of fluvial mine tailing deposits by providing macronutrients and other constituents that bind metals.

Root biomass is often used as an indication of a plant's ability to stabilize soil (Morgan and Rickson 1995; Pezeshki et al. 2007; Schultz et al. 1995).

Table 3 Aboveground (AG) and belowground (BG) metal concentrations (milligrams per kilogram) in eight shrubs grown in amended mine tailings [means, n=6, and standard errors (SE)]

	Thinleaf alder	Water birch	Shrubby cinquefoil	Red-osier dogwood	Bebb willow	Drummond's willow	Geyer willow	Diamondleaf willow
AG Cd (SE) P<0.001	0.17 D (0.0558)	5.94 B (1.45)	1.88 C (0.328)	0.0472 D (0.0288)	21.5 A (2.39)	7.12 B (0.635)	24.3 A (4.14)	7.36 B (0.647)
BG Cd (SE) P=0.019	47.7 A (10.6)	22.1 AB (7.60)	18.3 AB (8.11)	42.7 AB (12.2)	13.9 AB (2.67)	13.0 AB (3.96)	21.5 AB (5.14)	9.69 B (1.80)
AG Cu (SE) P<0.001	16.6 A (2.19)	5.11 BC (1.59)	11.2 AB (2.29)	4.49 BC (0.307)	7.56 ABC (1.06)	4.94 ABC (0.656)	3.15 C (0.928)	3.39 BC (0.592)
BG Cu (SE) P<0.001	102 A (17.1)	74.6 A (16.1)	54.9 AB (5.95)	23.4 B (3.61)	81.6 A (14.1)	99.6 A (18.3)	88.6 A (22.3)	77.7 A (13.3)
AG Pb (SE) P<0.001	27.7 A (7.91)	1.40 B (0.780)	27.9 A (9.08)	1.68 B (1.02)	1.17 B (0.491)	0.0514 B (0.0513)	0.128 B (0.0790)	0.111 B (0.0872)
BG Pb (SE) P=0.004	588 A (113)	542 A (174)	380 AB (174)	122 B (37.3)	314 AB (71.4)	476 A (105)	424 A (101)	384 A (71.8)
AG Zn (SE) P<0.001	140 AB (41.2)	219 A (54.8)	113 AB (45.1)	48.1 B (18.9)	270 A (49.6)	160 A (33.9)	281 A (50.2)	184 A (37.0)
BG Zn (SE) P=0.726	430 (83.7)	368 (120)	295 (92.4)	267 (70.0)	242 (53.6)	260 (64.1)	243 (49.8)	236 (46.2)

Thinleaf alder [*Alnus incana* (L.) Moench spp. *tenuifolia* (Nutt.) Breitung], water birch (*Betula occidentalis* Hook.), red-osier dogwood (*Cornus sericea* L. spp. *sericea*), shrubby cinquefoil [*Dasiphora fruticosa* (L.) Rydb. ssp. *floribunda* (Pursh) Kartesz], Geyer willow (*Salix geyeriana* Andersson), Drummond's willow (*Salix drummondiana* Barratt ex Hook.), diamondleaf willow (*Salix planifolia* Pursh), and Bebb willow (*Salix bebbiana* Sarg.) were grown in tailings amended with lime ($CaCO_3$) to achieve a soil pH of 7 s.u., or lime and composted biosolids (224 Mg ha^{-1}). Values are averaged across the three deposits and two amendment treatments. P values associated with the species main effect from two-factor analyses of variance are presented in the left column. Means in a row with the same letter are not different (Tukey's HSD, α=0.05)

Based on that criterion and our results, water birch, thinleaf alder, and Bebb and Geyer willow would be likely candidates for stabilization of fluvial tailing deposits. Another important consideration for stabilization may be root structure. Shrubs with fibrous roots are often recognized as having the best phytostabilization potential (Mench et al. 2006), and willows are often recognized for their role in reducing erosion of riverbanks (Morgan and Rickson 1995; Pezeshki et al. 2007; Schultz et al. 1995). Two of the four willows we studied had relatively low root growth, but visual observation suggested that red-osier dogwood and all willows used in this study had thick, fibrous root systems. Considering root growth and root structure simultaneously, root systems of Geyer and Bebb willow may have the greatest potential for stabilizing fluvial tailings.

Previous studies suggested that Geyer willow did not grow well in metal-contaminated soils (Bourret et al. 2009; Boyter et al. 2009; Fisher 1999; Shanahan 2006). Geyer willow plants started from cuttings collected in the Leadville area had poor growth responses when lime and composted biosolids were used to amend tailings collected from the same general area as deposit 1 (Boyter et al. 2009). Boyter et al. (2009) found Geyer willow cuttings had 60% less total biomass when grown in tailings amended with lime and biosolids versus topsoil, and the clones that survived in the amended tailings had a large number of chlorotic leaves that tended to drop before the end of their 120-day study. Geyer willow plants used in our study were originally started from seed collected in Northern Idaho, were 1 year old when this study began, and had 100% survival in this greenhouse study. Although some plants did show mild chlorosis, they did not drop many leaves over the course of the experiment. Local willow stock may be the most economical choice for revegetation and is often preferred or required by land management agencies, but nonlocal stock should be considered as an alternative where applicable.

4.2 Plant Metal Concentrations

Cadmium Normally, Cd accumulates in roots, and small amounts are transported to shoots, but the distribution differs among plants (Chatterjee and Dube 2006). Silver birch (Gussarsson et al. 1995; Kelly et al. 1979) and thinleaf alder (Rosselli et al. 2003; Wickliff et al. 1980) accumulated more Cd in roots than shoots. Our results for thinleaf alder were similar in that this shrub had the lowest aboveground Cd concentrations and among the greatest belowground Cd concentrations (Table 3). Above- and belowground Cd concentrations in water birch, however, were similar to shrubs with the greatest or second greatest concentrations.

In the current study, Cd concentrations in aboveground growth were higher for willows and water birch compared to the other shrubs (Table 3). Bebb and Geyer willow had Cd concentrations in aboveground growth that were greater than values reported by some (Rosselli et al. 2003; Stoltz and Greger 2002; Vervaeke et al. 2003) and above toxicity thresholds for animals (National Research Council 2005). Domestic animals, including horses, cattle, and sheep, can tolerate a Cd concentration of 10 mg kg^{-1} in their diet (National Research Council 2005). Willows are known to accumulate greater than the 10 mg Cd kg^{-1} threshold (Pulford and Watson 2003; Punshon and Dickinson 1997; Rosselli et al. 2003; Stoltz and Greger 2002; Vervaeke et al. 2003). However, we found that Cd concentrations in Drummond's and diamondleaf willow were below the threshold and similar to those reported by others (Rosselli et al. 2003; Stoltz and Greger 2002; Vervaeke et al. 2003). It is important to note that if an herbivore's diet contains small amounts of shrubs with elevated Cd concentrations, there would be little chance of toxicity.

Willows have been studied extensively and used for phytoextraction of Cd because they translocate the metal to aboveground tissues (Pulford and Watson 2003; Punshon and Dickinson 1997; Rosselli et al. 2003; Stoltz and Greger 2002; Vervaeke et al. 2003). Our results for Bebb and Geyer willow support this contention, with aboveground Cd concentrations the greatest among all species studied (Table 3).

Previous studies document relatively consistent, low aboveground Cd concentrations in various species of alder despite a wide range in total Cd in the growth media. Red alder (*Alnus rubra* Bong.) had less than 0.6 mg kg^{-1} foliar Cd in a field experiment when grown in soil with 1.2–32.5 mg kg^{-1} Cd (Gaulke et al. 2006). Black alder [*Alnus glutinosa* (L.) Gaertn.] leaves had less than 0.23 mg kg^{-1} Cd 2 years after being planted in a field setting on dredged sediment containing 5.7–5.9 mg kg^{-1} total Cd (Mertens et al.

2004). Rosselli et al. (2003) found no detectable Cd in aboveground tissues of thinleaf alder grown in sewage sludge containing 1.71 mg kg^{-1} Cd. Averaged across amendment treatments, the Cd concentration we found in thinleaf alder aboveground growth (Table 3) was toward the low end of the range reported in the studies cited above, even though our plants were grown in tailings containing a total Cd content of 8.9–18.5 mg kg^{-1}.

The consistently low aboveground Cd concentrations observed in alders have led to the suggestion that members of this genus either exclude heavy metals at the root or retain them in belowground plant tissue (Rosselli et al. 2003). The high Cd concentration we observed in thinleaf alder roots (Table 3) provides evidence that this shrub does not exclude Cd at the root, but retains Cd in belowground tissue. Based on our results for belowground growth and belowground Cd concentrations in thinleaf alder, it is reasonable to expect this shrub to provide an effective means of immobilizing Cd and preventing it from being leached. Root accumulation of Cd may be considered biochemical immobilization and compliments the physical stabilization resulting from roots stabilizing fluvial tailing deposits.

Unlike alder, there is inconsistency in the literature regarding the amount of Cd translocated to aboveground tissues of various birch species. Silver birch, grown in biosolids containing less Cd than found in the mine tailings we used, contained a greater aboveground Cd concentration (46.36 mg kg^{-1}; Rosselli et al. 2003) than we found in water birch (Table 3). Yellow birch (*Betula alleghaniensis* Britton) grown in media containing approximately the same Cd content as the tailings we used had a much greater aboveground Cd concentration (39.7 mg kg^{-1}; Kelly et al. 1979) than we detected in water birch.

Copper Copper mobility in plants is typically low, and thus, Cu tends to concentrate in roots. Copper accumulation in roots has been observed for birch (Gussarsson et al. 1995; Kelly et al. 1979; Utriainen et al. 1997), alder (Kramer et al. 2000), and willow (Punshon and Dickinson 1997; Rosselli et al. 2003; Stoltz and Greger 2002; Vervaeke et al. 2003). Our belowground Cu concentration results for thinleaf alder, water birch, and all four willows (Table 3) were consistent with previously reported plant Cu immobility.

All shrub aboveground Cu concentrations (Table 3) were below known tolerance levels for most domestic animals (National Research Council 2005). Cattle and sheep have the lowest tolerance thresholds at 40 and 15 mg kg^{-1}, respectively. Of the species studied, only thinleaf alder exceeded the sheep threshold.

Aboveground copper concentrations have been shown to be inconsistent across alder species. Black alder grown in dredged sediment, containing approximately the same total Cu concentration as the tailings used in our study, accumulated ~10 mg kg^{-1} less Cu in aboveground tissue (Mertens et al. 2004) than we observed in thinleaf alder (Table 3). Gray alder [(*A. incana* (L.) Moench)] grown in biosolids containing three times the Cu concentrations found in the amended tailings we used had approximately the same Cu concentration in leaves and twigs (Rosselli et al. 2003) as we report for thinleaf alder (Table 3).

Lead Lead is considered a nonessential nutrient, but can be passively absorbed by roots whereby it is incorporated into cell walls, thus preventing translocation to shoots (Kabata-Pendias 2001). All shrubs accumulated Pb in roots to a greater extent than the aboveground tissue, and biosolids consistently reduced root Pb concentrations in all shrubs. The addition of biosolids, therefore, did not promote Pb stabilization in plant roots, but likely reduced its bioavailability. The addition of P to soils, via composted biosolids, could have caused complexation and thus a reduction in bioavailable metal phases. Although biosolids P content was not determined in the current study, total P content of biosolids typically range from ~1.0% to 4.0%. Phosphorus addition causes Pb in soils to shift from highly available to more strongly bound and non-bioavailable forms such as pyromorphite (Miretzky and Fernandez-Cirelli 2008) and has been used to limit Pb bioavailability (Brown et al. 2004).

When water birch was grown in the greenhouse over a 4-month period in tailings from the Pacific Mine in Utah, 90% of plant Pb accumulated in the roots (Klassen et al. 2000). Total Pb levels in the Pacific Mine tailings (30,000 to 130,000 mg kg^{-1}) were much greater than the Leadville tailings (1,540 to 5,250 mg kg^{-1}), yet water birch performed similarly in the two studies. Silver birch and downy birch have been shown to accumulate greater aboveground Pb concentrations (Margui et al. 2007;

Pahlsson 1989; Utriainen et al. 1997) and would be better suited for phytoextraction.

Cattle and sheep can tolerate 100 mg kg^{-1}, but consuming vegetation containing greater than 10 mg kg^{-1} Pb can be toxic to most other domestic animals (National Research Council 2005); thinleaf alder and shrubby cinquefoil exceeded 10 mg kg^{-1} Pb (Table 3). Thinleaf alder is highly palatable to browsing animals (USDA Plants Database 2009) and cinquefoil has a moderate palatability (USDA Plants Database 2009), but they do not generally comprise a large proportion of browsing animal diets. Thus, concerns regarding Pb consumption are minimal.

Zinc Plant roots can absorb Zn both actively and passively from the soil (Kabata-Pendias 2001). Once absorbed, Zn has intermediate to high mobility in plants depending on the concentration of Zn available to a plant. Greater soil Zn concentration leads to greater plant Zn mobility (Kabata-Pendias 2001).

Alder tends to concentrate Zn in its roots, and the amount of Zn translocated aboveground varies by species and location. Our results (Table 3) were similar to those of Mertens et al. (2004) who reported black alder concentrated 90% of Zn in roots. Sitka alder [*Alnus viridis* (Chaix) DC. ssp. *sinuata* (Regel) A. Löve & D. Löve] had greater Zn concentrations in aboveground tissue than roots when grown in unamended copper mine tailings or tailings capped with biosolids and gravel (Kramer et al. 2000). Gaulke et al. (2006) grew red alder in biosolids with a Zn concentration similar to our tailings and reported aboveground Zn concentrations similar to those we detected in thinleaf alder. Rosselli et al. (2003) reported thinleaf alder had greater aboveground Zn concentrations when grown in a field setting with total Zn concentrations similar to the tailings we used.

Above- and belowground Zn concentrations in various birch species appear fairly uniform when roots are exposed to relatively low Zn concentrations, but exposure to excessive Zn concentrations apparently causes root accumulation. In a hydroponic experiment, silver birch had similar Zn concentrations in roots and shoots when grown in solution with 3.3 mg L^{-1} Zn, but accumulated Zn in belowground tissues when grown in solutions with 52.3 and 261 mg L^{-1} Zn (Utriainen et al. 1997). In a field experiment, Rosselli et al. (2003) found that silver birch growing in metal-contaminated biosolids (with less total Zn concentration than the tailings used in our study) concentrated more Zn in aboveground tissues than we measured in water birch (Table 3). A fairly uniform distribution of Zn in above- and belowground plant parts was reported for yellow birch grown in soils without elevated Zn content (Kelly et al. 1979). In our study, Zn concentrations in aboveground growth and roots of water birch appeared to be similar (Table 3).

Willows often concentrate Zn in aboveground tissues whether they are grown in high Zn media in a greenhouse (Boyter et al. 2009; Vysloužilová et al. 2003), in the field (Vervaeke et al. 2003), or using hydroponic solutions (Punshon and Dickinson 1997; Stoltz and Greger 2002). The four willows in our study appeared to have an even aboveground and belowground Zn distribution (Table 3), suggesting that there may be a limit to the amount of Zn readily translocated to aboveground tissues.

Most domestic animals can tolerate high Zn concentrations in their diet. The toxicity threshold for cattle is 500 mg kg^{-1} (National Research Council 2005); mean aboveground tissue Zn concentrations were below this threshold so Zn toxicity from the eight shrubs we studied is unlikely. Willows are much more palatable to browsing animals than the non-willow species and may comprise a larger portion of an animal's diet.

5 Summary and Conclusions

Previous research suggests that amending acidity and adding organic matter to fluvial mine tailing deposits is a first step in facilitating the return of characteristic sedges, grasses, and forbs. Restoring shrubs at the time of amendment is a method to hasten recovery of the woody component of denuded riparian systems. All eight shrubs we studied survived and grew well in amended fluvial tailing deposits so should be considered reasonable candidates for revegetation and restoration of fluvial tailing deposits. Composted biosolids offered no detectable short-term benefits in terms of shrub growth or metal uptake, but did result in rapid and consistent reductions in belowground Pb concentrations for all shrubs. Most shrubs appeared to accumulate Cd, Cu, Pb, and Zn to a greater degree in roots compared to shoots, resulting in biochemical stabilization and likely reduced leaching of metals from tailing deposits.

Variation observed in aboveground vegetation metal concentrations showed strong species dependence and underscores the importance of species selection in mine site revegetation. When choosing shrubs for revegetation of fluvial mine tailing deposits similar to those used in our study, and considering the potential for herbivore consumption, the following points should be considered. Bebb and Geyer willow accumulated potentially zootoxic aboveground Cd concentrations. The only potential threat for Cu toxicity appeared to be for sheep consuming thinleaf alder. Thinleaf alder and shrubby cinquefoil contained Pb concentrations in aboveground tissues considered toxic to domestic animals. None of the shrubs we studied are likely to pose a threat of Zn toxicity.

We report an additional insight regarding Cd uptake and movement in thinleaf alder. Our results disprove previous suggestions that alders exclude Cd at the root (Rosselli et al. 2003), but support the theory that this metal is retained in belowground plant parts (Rosselli et al. 2003).

Acknowledgment This research was funded by the Colorado State University Agricultural Experiment Station, with Dr. Lee Sommers as the Director.

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