

Revegetation of Fluvial Mine Tailing Deposits: The Use of Five Riparian Shrub Species

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ABSTRACT

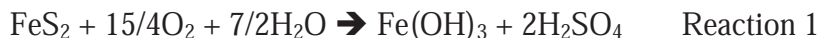
Fluvial deposition of mine tailings has caused extensive damage to riparian ecosystems throughout the West. Willows are often used for revegetation of fluvial mine tailing deposits but some species accumulate toxic concentrations of metals in leaves and stems. A greenhouse experiment was conducted to determine the value of thinleaf alder [*Alnus incana* (L.) Moench spp. *tinuifolia* (Nutt.) Breitung], water birch (*Betula occidentalis* Hook.), red osier dogwood (*Cornus sericea* L. spp. *sericea*), and shrubby cinquefoil [*Dasiphora fruticosa* (L.) Rybd.] compared to Geyer willow (*Salix geyeriana* Andersson) for revegetation of fluvial tailing deposits along the Upper Arkansas River. Bare root shrubs were grown in tailings amended with lime and composted biosolids. Tailings were collected from three acidic and metal contaminated deposits along the Arkansas River south of Leadville, Colorado. All shrubs survived the two month experiment. Averaged across source deposits, total biomass during the experiment increased for alder, birch, dogwood, cinquefoil, and willow by 831, 689, 579, 525, and 683%, respectively. All species concentrated Pb and Zn belowground. Dogwood assimilated little Zn (44.0 mg kg⁻¹) into its leaves and stems, but showed signs of nutrient deficiency which could have been induced by metal stress. Alder and cinquefoil partitioned Pb aboveground, 30.3 and 26.1 mg kg⁻¹, respectively, which is unusual, but concentrations were below toxicity thresholds for humans and animals. All species evaluated did not exhibit greater growth when compared to Geyer willow, but the other four riparian species had metal partitioning characteristics valuable for managers planning for *in situ* restoration of mine tailing deposits.

INTRODUCTION

Ecological problems associated with abandoned mine waste affect terrestrial and aquatic ecosystems and a wide variety of organisms, including humans. Prior to federal regulations for mine waste disposal, many companies stockpiled tailings near their operations or discarded mine wastes into nearby rivers. Large flood events have eroded sediment from stockpiles, depositing highly acidic, metal-laden soils in downstream flood plains (Toevs et al. 2006, Wielinga et al. 1999, Merrington and Alloway 1994). Heavy metals from fluvially deposited acid mine waste can leach for decades to centuries after mining has stopped (Modis et al. 1998, Marcus et al. 2001). About 40%, or approximately 19,000 km, of all the waterways in the Western United States are contaminated by metals from acid mine waste (Da Rosa et al. 1997).

Extraction of metaliferous ores from the mined rock is a process that can result in acidic waste products. Mined rock often contains pyritic minerals that oxidize when exposed to oxygen in the

atmosphere. Pyrite (FeS_2) loses electrons to oxygen which can then create sulfuric acid when precipitation or river water penetrates the oxidized pyrite (Reaction 1).



When the pH is below 4, heavy metals cannot chemically bind to soil substrates, thus increasing metal solubility. Protons are so numerous in acidic soils that they bind to most or all of the available sites on soil substrates, such as organic matter, primary and secondary minerals, as well as clays. Metals and other ions present in the bulk soil enter soil solution where they are available for plant uptake or susceptible to leaching. Arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) are some of the metals in tailings that cause water quality problems or can be toxic to plants, animals, or humans.

Acid mine waste is considered one of the major water pollution concerns associated with mining (Nelsen et al. 1991, Sheoran and Sheoran 2006). Tailing deposits remain acidic and metal precipitates can wash back into stream channels from reductive dissolution during seasonal flooding (Toevs et al. 2006). Once mobile, metals can be ingested by fish (Schmitt et al. 2006) and beaver (Nolet et al. 1994), and bioaccumulate in aquatic invertebrates (Cain et al. 2004).

Many strategies exist to alleviate the environmental impacts of mine tailing deposits. Contaminated soil excavation and storage is very expensive and use of this method may be limited by site accessibility. Phytoremediation may also be expensive because contaminated plants must be incinerated and new ones replanted. One much less expensive method is *in situ*, or onsite, restoration of fluvial mine tailing deposits. Woody shrubs with fibrous root systems, such as willows (*Salix* spp.), can reduce erosion by stabilizing the tailing deposits (Morgan and Rickson 1995).

Improving soil chemistry is necessary prior to revegetation. Low pH and organic matter content, and high concentrations of heavy metals make it difficult or impossible for plants and soil microbes to survive. Incorporation of a base (e.g. CaCO_3) raises pH and causes heavy metals to bind to the surface of clay minerals and organic matter, precipitate into solid metals, and/or reduce into less harmful metal species. The addition of organic matter increases the surface area for binding, adds nutrients (e.g. nitrogen, phosphorus, and potassium), and can inoculate the deposit with a new microbial community. Lime and organic matter are common soil amendments used in riparian areas affected by mine tailing deposits (Brown et al. 2005, Boyter 2006, Kramer et al. 2000).

Areas of deposition occur on private and public lands, creating a myriad of concerned stakeholders. Groups across the state of Colorado, for example, have formed in response to the need for restoration. The Upper Arkansas River Restoration Project (UARRP) is a stakeholder group which formed in 1995 in Leadville, Colorado. The group identified a goal of “restoring the river and associated flood plain to a healthy and sustaining condition” (URS Operating Services 1997). An 18 km stretch of the Upper Arkansas River, starting at the confluence of California Gulch, has numerous fluvial mine tailing deposits that have affected water quality and ranching. Restoration studies by the Environmental Protection Agency (EPA), Bureau of Reclamation, US Geological Survey, Colorado State University, and University of Washington

have taken place along the 18 km stretch of the river following the creation of the UARRP (Walton-Day et al. 2000, Fisher 1999, Boyter 2006, Bourret 2004, Brown et al. 2005, 2007, Shanahan 2006).

Mountain (*S. monticola* Bebb.), Geyer (*S. geyeriana* Andersson), Drummond's (*S. drummondiana* Barratt ex Hooker), and planeleaf willow (*S. planifolia* Pursh var *planifolia*) have all been evaluated for their potential use in restoration efforts along the Upper Arkansas River (Fisher 1999, Boyter 2006, Bourret 2004, Shanahan 2006). Most research near Leadville by Colorado State University, concerning the restoration of contaminated sites, has focused on the use of willows. Expanding our knowledge of other riparian shrubs that can tolerate heavy metal contaminated soils will give restoration ecologists more choices to meet ecological goals.

OBJECTIVES/HYPOTHESES

Objective one was to compare survival and growth of thinleaf alder [*Alnus incana* (L.) Moench spp. *tinuifolia* (Nutt.) Breitung], water birch (*Betula occidentalis* Hook.), red osier dogwood (*Cornus sericea* L. spp. *sericea*), and shrubby cinquefoil [*Dasiphora fruticosa* (L.) Rybd.] to Geyer willow when grown in fluvial mine tailings amended with lime and composted biosolids. It was hypothesized that all species would have greater total growth as well as greater aboveground and belowground growth than Geyer willow.

Objective two was to characterize how thinleaf alder, water birch, red osier dogwood, shrubby cinquefoil, and Geyer willow partition Pb and Zn. Metal partitioning was hypothesized to vary by shrub species. It was further hypothesized that Pb would be excluded from aboveground plant parts and that thinleaf alder would exclude both metals from its aboveground parts.

LITERATURE REVIEW

The Western U.S. has a rich history of hard-rock, hydraulic, placer, and open pit mining, along with metal smelting. Before federal environmental regulations, most mining waste was uncontained in stockpiles near the mining operation or discarded in nearby streams and rivers. Regulation of pollutant discharge into U.S. water bodies and the establishment of water quality standards were initiated by The Clean Water Act of 1977. In 1980, the Comprehensive Environmental Response, Compensation, and Liability Act made mining operations liable for their release of chemical wastes. Federal control over mining activities created a focus on restoration of damaged areas, especially abandoned mining and smelting operations.

Tailings from abandoned mines can affect waterways for hundreds of years (Modis et al. 1998). Section 305(b) of the federal Clean Water Act mandates a biannual report from each U.S. state to the USEPA detailing water quality impairment information. In 2006, the Colorado Department of Minerals and Geology reported approximately 2092 km of rivers affected by inactive mining (Colorado Department of Minerals and Geology 2006). The Montana Department of Environmental Quality reported approximately 2896 km of rivers affected in their 2004 report (National Water Assessment Database 2004), while the Washington State Department of

Ecology reported nearly 161 km affected by surface mining and mine tailings in their 2000 305(b) report (Washington Department of Ecology 2000).

Colorado and Montana have extensive damage in riparian zones from fluvially deposited mine tailings. The Clark Fork River in western Montana has been affected by copper mining and smelting for over a century. High levels of Cu, Zn, and Pb are present in sediment and plant material adjacent to the river (Johns 1995). Further downstream, Soda Butte Creek in Yellowstone National Park has also been contaminated with heavy metals from the same mining and smelting. The Yellowstone site is also impacted by historic gold, silver, and copper mining wastes (Nimmo et al. 1998). Research has focused on characterizing site conditions and water quality issues.

Most of the damage near Leadville, Colorado is due to hydraulic placer and hard rock mining that occurred from the late 1800's to the mid 20th century. Mining wastes were generally not contained. Rather, they were either stockpiled or disposed of in the Arkansas River (URS Operating Services 1997). Currently, the largest ongoing impacts are to the surface water of the Arkansas River (MOUP CT 2002). Manganese, Cd, and Zn are the primary water quality concerns in the area (Walton-Day et al. 2000). Typically, fluvial mine tailing deposits vary in the depth of soil affected by acidity and heavy metals. Site characterization of the tailings along the Upper Arkansas River found that the deposits are generally less than one meter thick (MOUP CT 2002).

The use of a variety of riparian shrubs native to the Western U.S. in restoration research will expand our knowledge of species value for reclaiming heavy metal contaminated sites. Willow, birch, dogwood, and alder are common riparian shrubs that occur over a wide range of temperature and elevation gradients throughout the U.S. Cinquefoil is a woody shrub ubiquitous in the Western U.S., having ecological value in upland and riparian zones.

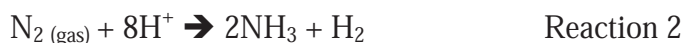
Willows have been used for riverbank stabilization (Morgan and Rickson 1995, Schultz et al. 1995, Pezeshki et al. 2007), improvement of soil structure (Stott 1992), and some species are known to tolerate elevated heavy metal concentrations (Cosio et al. 2006, Vandecasteele et al. 2005). While metal uptake varies by species, Pb is generally not concentrated in aboveground leaves and stems whereas Zn is often taken up by both plant roots and shoots (Vandecasteele et al. 2005, Pahlsson 1989). In a Belgium field study, *S. viminalis* L. 'Orm' accumulated an average of 18 and 3 mg kg⁻¹ Pb, and 243 and 363 mg kg⁻¹ Zn in roots and leaves, respectively, when grown in soil contaminated with 143 mg kg⁻¹ Pb and 437 mg kg⁻¹ Zn (Vervaeke et al. 2003). *Salix smithiana* Willd. c.f. *dasyclados*, *S. dasyclados* Vimm., and *S. caprea* L. concentrated 432, 591, and 471 mg kg⁻¹ Zn in aboveground biomass when grown in a soil containing 279 mg kg⁻¹ Zn (Fischerova et al. 2006). Geyer willow had a leaf tissue concentration of 641 mg kg⁻¹ Zn in a greenhouse study using mine tailings amended with lime and composted biosolids with an initial Zn concentration of 1935 mg kg⁻¹ (Bourret 2004). Willows are largely used for phytoremediation purposes because of their ability to hyperaccumulate one or more heavy metals.

Both shrub and tree life forms of birch have been used in North America (Lautenbach et al. 1995, Winterhalder 1995) as well as Russia (Kozlov 2005, Kozlov and Haukioja 1999) for restoration of smelter-damaged sites because they have a general tendency to naturally survive in

unamended, heavy metal contaminated soils. *Betula pendula* Roth. and *B. pubescens* Ehrh. are pioneer species in metal contaminated areas, showing an evolved tolerance to Pb and Zn (Utriainen et al. 1997, Pahlsson 1989). European white birch (*B. pendula*) trees have shown tolerance to Zn and Pb metal contaminated soil, accumulating a maximum of 3,100 and 530 mg kg⁻¹ Zn and Pb, respectively, in their leaves (Margui et al. 2007). River birch (*B. occidentalis* Hook.) grown in a greenhouse study in unamended soil that had a Pb content of 12,914 mg kg⁻¹ translocated 202 mg kg⁻¹ Pb aboveground (Klassen et al. 2000).

Dogwood shrubs, a common species in riparian zones, can tolerate saline soil conditions. Revegetation after strip mining for bitumen from oil sands in Alberta, Canada has included the use of red-osier dogwood (*C. sericea* L. subsp. *sericea* and *C. stolonifera* Michx.) because of its tolerance to elevated salt concentrations (Redfield et al. 2003, Renault et al. 2001).

Alders are beneficial restoration species for areas with heavy metal issues (Mertens et al. 2004, Rosselli et al. 2003). *Alnus glutinosa* L. Gaertn. grown on dredged sediment polluted with heavy metals accumulated Pb (5 mg kg⁻¹) and Zn (65 mg kg⁻¹), but not enough to be toxic (Mertens et al. 2004). *Alnus incana* used for revegetation of sediment contaminated by sewage sludge borne heavy metals also had very low leaf and stem Zn concentrations (Rosselli et al. 2003). *Alnus rubra* Bong. had similar Zn uptake regardless of whether it was planted in biosolids with low (279 mg kg⁻¹) or high (1760 mg kg⁻¹) Zn content (Gaulke et al. 2006). Alders are also beneficial because they are actinorhizal N₂-fixing shrubs that increase the availability of ammonia (NH₄⁺) in soils. Symbiotic nitrogen fixing bacteria in the genus *Frankia* are responsible for this reaction (Reaction 2). *Frankia* are filamentous actinomycetes that form a symbiotic relationship with actinorhizal plants, living within globular structures attached to primary and secondary roots. The *Alnus-Frankia* relationship does not appear to be affected by high metal concentrations in soils (Gaulke et al. 2006).



Shrubby cinquefoil is native to most of the U.S., occurring in all states west of the 100th meridian. Erosion was reduced when heavy rainfall was simulated in riparian zones where *Potentilla gracilis* Dougl ex. Hook was grown in conjunction with grasses (Pearce et al. 1998). *Potentilla fruticosa* 'Longacre' was found to be useful for landscaping roadsides where de-icing resulted in highly saline soils (Marosz 2004). Cinquefoil shrubs not only occur in the flood plains of streams and rivers in the Western U.S., but are also commonly found throughout the world.

MATERIALS AND METHODS

Growth Media Collection and Amendment

Tailings from three USEPA characterized sites approximately 8 km south of Leadville, Colorado, were used: FF (39°11'53.27"N 106°21'03.16"W), QO (39°07'48.93"N 106°18'44.35"W), and QN (39°07'50.14"N 106°18'47.54"W) (Figure 1). Two of the sites occurred at 2,799 m and were located on Colorado State Public land; the third site was located at 2,882 m in elevation and located on private ranch land owned by Dr. Bernard Smith. The three tailings deposits contained elevated Zn concentrations and low pH. A total of 227 L of tailings

was collected from each site in November 2006. Three or four 60 cm deep holes were excavated at each site and tailings were placed in barrels for transport to Colorado State University. The top 20 cm of soil was frozen and was not collected. The tailings from each site were air dried and homogenized during December and January 2007.

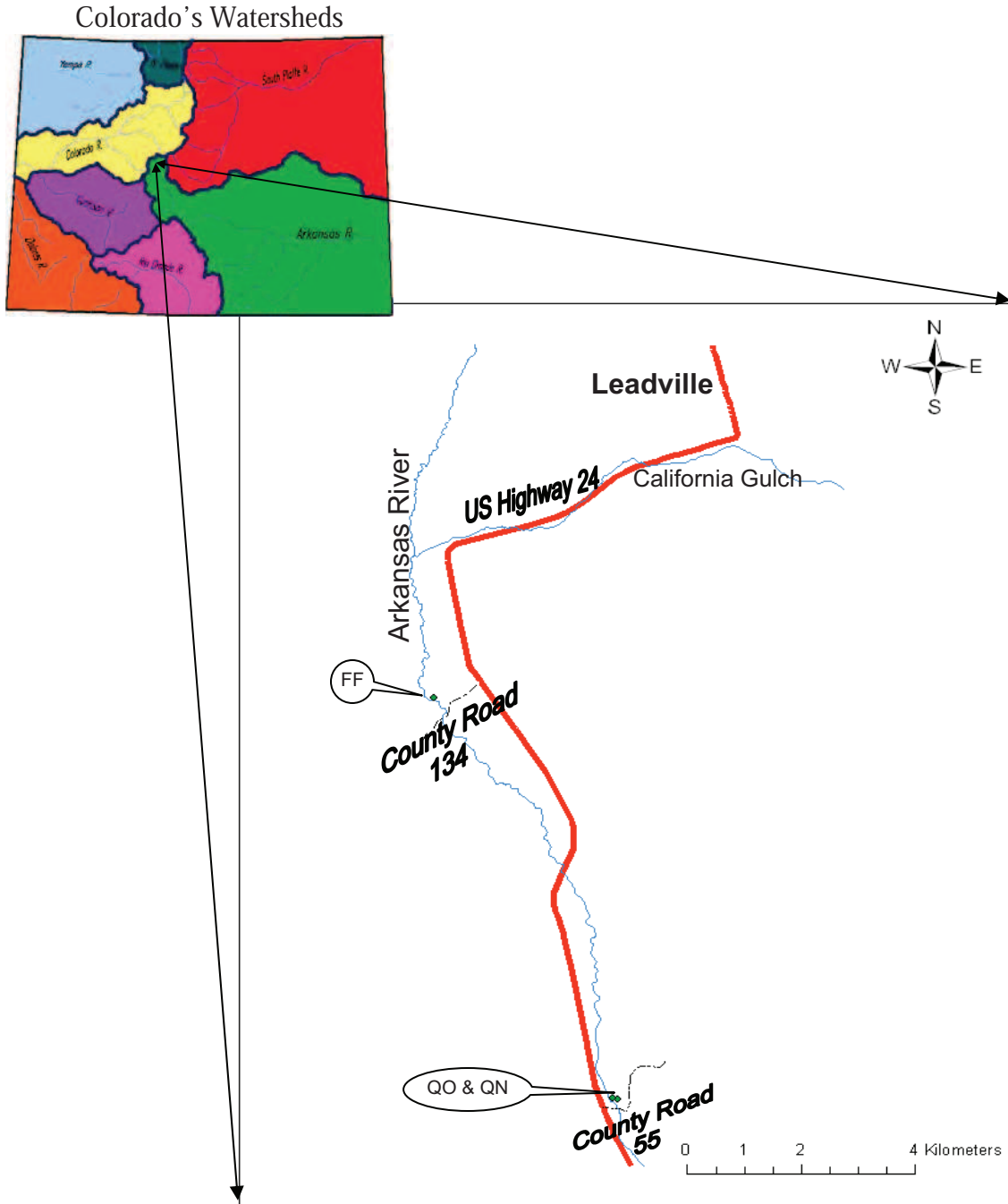


Figure 1: Map showing the location of study sites FF, QO, and QN near Leadville, Colorado.

Once tailings were homogenized within a source deposit, each was analyzed for total Cd, Cu, Pb and Zn (U.S. Environmental Protection Agency 1994) as well as bioavailable Pb and Zn (Mehlich 1984). Unamended tailings contained 1,540 to 5,250 mg kg⁻¹ total Pb and 1,670 to 3,380 mg kg⁻¹ total Zn. Bioavailable Pb ranged from 190 to 210 mg kg⁻¹, and bioavailable Zn ranged from 81 to 92 mg kg⁻¹.

Tailings were then amended with lime and composted biosolids in early February 2007. Lime (CaCO₃ powder ground to pass a 200-mesh screen) was added to raise the soil pH to 7, based on the Shoemaker-McLean Pratt (SMP) Single Buffer Method (Sims 1996; Table 1). Municipal biosolids, composted with wood chips, were added to improve soil structure, inoculate the soil with a microbial community, and supply the tailings with N, P, and K. Composted biosolids were obtained from Gunnison County, Colorado and added at the rate of 224 Mg ha⁻¹ 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, and then the amended tailings were allowed to chemically react for an additional 14 days.

Table 1: The sites, initial tailing soil pH, and amount of lime required to raise the tailing soil pH to 7.

Site	pH	Lime Added (Mg/ha/20 cm)
FF	4.0	3.5
QO	2.0	17.2
QN	3.3	9.2

After amending, tailings from each site were analyzed for bioavailable Pb and Zn (Mehlich 1984). The lime and composted biosolids amendments reduced the soluble forms of Pb and Zn, making only 63 to 133 mg kg⁻¹ Pb bioavailable, and 9 to 24 mg kg⁻¹ Zn bioavailable.

Shrub Selection and Purchase

Five shrub species were chosen based on several criteria. The primary goal was to find species other than willow that would potentially grow in areas similar to those contaminated with heavy metals from fluvial deposition of mine tailings. The USDA Plants database was used to find facultative or wetland facultative shrubs native to Chaffee, Eagle, Gunnison, Lake, Park, and Summit counties in Colorado that could tolerate a pH of 4 - 5, for assurance that they would tolerate the study area growing conditions. Shrubs came from three different suppliers and were of different ages and sizes (Table 2). Thinleaf alder, red-osier dogwood, and Geyer willow were obtained from Plants of the Wild (Tekoa, WA). Water birch was obtained from Lawyer Nursery (Plains, MT). Shrubby cinquefoil was obtained from Rocky Mountain Native Plants (Rifle, CO).

The shrubs were dormant when purchased in January 2007, except shrubby cinquefoil which was leafed out. Roots of all plants were washed free of potting soil prior to being planted to ensure that the roots were in direct contact with amended tailings.

Table 2: Pre-greenhouse experiment characteristics of thinleaf alder, water birch, red osier dogwood, shrubby cinquefoil, and Geyer willow.

Species	Age (years)	Size	Seed Source
Alder	1	25 cm ³ pots	Northern Idaho
Birch	2	15-30 cm tall	Montana
Dogwood	1	25 cm ³ pots	Northern Idaho & Central Washington
Cinquefoil	1	25 cm ³ pots	Rocky Mountains
Willow	1	25 cm ³ pots	Northern Idaho

Experimental Design

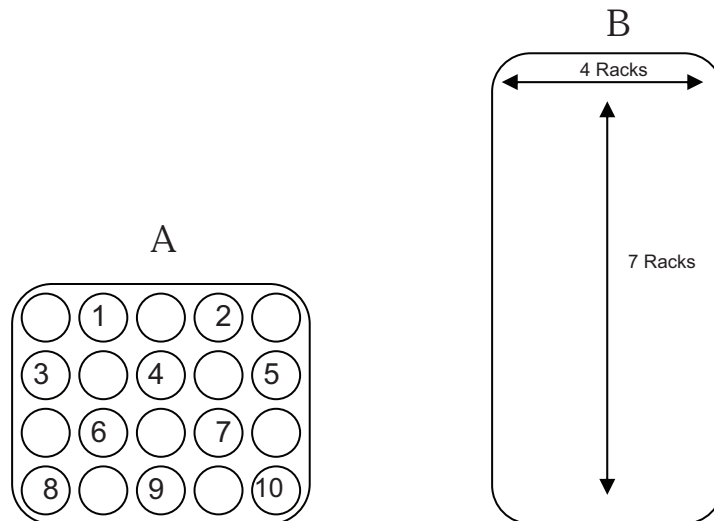


Figure 2: (A) Placement of individual plants into every other cell of a support tray. (B) Tray placement along the greenhouse bench.

Plants for this experiment were grown in the Colorado State University Plant Growth Facilities greenhouse from February 22, 2007 until April 24, 2007. The greenhouse was kept at ~25°C and the shrubs were watered with municipal water. The location of potted plants in support trays and the arrangement of support trays on the greenhouse bench were completely random. Eighteen replicates of each shrub species were planted in tailings from each of the 3 sites amended with lime and composted biosolids, for a total of 270 individual plants. Each bare root individual was planted in a 656 ml plastic pot containing amended tailings. Ten randomly chosen pots were placed in every other cell of a 20 cell support tray (Figure 2). Each support tray was randomly placed along a greenhouse bench, leaving 15 cm of open space between trays. The 27 support trays were re-randomized on the greenhouse bench twice during the study (March 15, 2007 and March 29, 2007).

Harvesting

All plants were harvested on April 24, 2007. Each plant was clipped at the soil surface to separate aboveground from belowground parts. Aboveground biomass was divided into two parts: new growth and stem (material that grew prior to the experiment). The new growth included any leaves and stems that grew during the study. This material was easily identified by the lighter stem color or pubescence. Any tailings particles present on the surface of leaves and stems were rinsed off with deionized water. Tailings were thoroughly washed away from roots using a 0.5 mm sieve and a gentle stream of water. Roots were then soaked for one minute in 0.01 M $\text{Na}_2\text{H}_2\text{-EDTA}$ to remove cations adsorbed to the surface of the root system (Kalis et al. 2006, Slaveykova and Wilkinson 2002). Plant biomass was oven dried at 55°C for 72 hours and weighed.

Plant Metal Concentrations

Lead and Zn concentrations were estimated for new above- and below-ground growth from six randomly selected individuals from every site/amendment combination. Aboveground and belowground plant biomass was ground to pass a 1 or 2 mm screen, depending on dried plant species size. Samples of one gram or less, depending on quantity available, were digested with concentrated nitric acid and 30% hydrogen peroxide (Huang and Schulte 1985). Samples were then analyzed for Pb and Zn using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) at the Plant Testing Laboratory.

Data Analysis

Initial plant size varied among the shrub species used in the study. In order to make comparisons of growth over the course of the experiment, a starting baseline biomass was determined for each of the five species at the beginning of the experiment. Eighteen individual plants of each species were selected at random from the purchased plant material for starting biomass determinations. These plants were separated at the root crown into root and stem samples. Aboveground leaves and stems with any soil particles visible on the epidermis were washed in a deionized water bath. Roots were washed free of potting soil and all plant material was oven dried and weighed as described above. Proportional aboveground growth was defined as the weight of new growth for an individual plant divided by the average baseline stem weight for that species. Proportional belowground growth was defined as ending root weight divided by the average baseline root weight for that species. Metal concentrations were expressed as mg kg^{-1} of dry weight plant material.

Raw proportional growth data and metal concentrations did not meet assumptions of normality and were log transformed for analysis. Growth and metals data were averaged across all three tailings materials in order to focus on species response rather than individual site characteristics. Differences among species for proportional aboveground and belowground growth, as well as metal concentrations, were analyzed using the Proc GLM model in SAS version 9.1 (SAS institute, 2002). Differences were examined using analysis of variance (ANOVA) at a significance level (α) of 0.05 for the experimentwise error rate, with Bonferroni's inequality used for pairwise comparisons of the *a priori* comparisons among the 5 shrub species.

RESULTS

Growth

Species proportional growth, as compared to Geyer willow, is presented in Table 3. Aboveground growth of water birch was less than all other species. Water birch and Geyer willow exhibited the greatest belowground growth, which was significantly greater than thinleaf alder, red-osier dogwood, and shrubby cinquefoil. There was no difference among species in total growth during this experiment ($p=0.23$). The hypothesis that aboveground, belowground, and total proportional growth of Geyer willow would be significantly less than each of the other four species was rejected.

Table 3: Aboveground, belowground, and total growth for thinleaf alder, water birch, red osier dogwood, shrubby cinquefoil, and Geyer willow averaged across the three tailings soils. Mean \pm SE Aboveground n=54, Belowground n=27, Total n=27.

Species	Proportional Growth (%)		
	Above	Below	Total
Alder	536.2 \pm 65.3 a [§]	293.9 \pm 33.0 b	831.1 \pm 114.9 a
Birch	196.1 \pm 14.2 b	466.7 \pm 46.0 a	688.9 \pm 66.5 a
Dogwood	306.9 \pm 16.7 a	258.6 \pm 18.4 bc	578.6 \pm 39.5 a
Cinquefoil	361.0 \pm 27.4 a	194.6 \pm 17.0 c	525.5 \pm 43.8 a
Geyer	309.1 \pm 9.7 a	381.3 \pm 18.1 a	682.7 \pm 26.8 a

§ Within columns, lower case letters show significant differences ($p \leq 0.05$) among species for proportional growth using Bonferroni's inequality method of analysis.

Plant Metal Concentrations

Lead

Plant tissue Pb concentration results are summarized in Table 4A. Thinleaf alder and shrubby cinquefoil concentrated the most Pb in new aboveground growth over the course of the experiment. The hypothesis that Pb would be excluded from aboveground parts was rejected. Red-osier dogwood excluded more Pb belowground than Geyer willow, whereas thinleaf alder accumulated more Pb belowground.

Zinc

Plant tissue Zn concentrations are summarized in Table 4B. Red-osier dogwood had the lowest Zn concentration in aboveground new growth, significantly less than Geyer willow. Shrubby cinquefoil and thinleaf alder aboveground Zn content was also significantly lower than Geyer willow. However, birch contained comparable aboveground Zn as Geyer willow (Table 4B). The hypothesis that thinleaf alder would exclude metals from aboveground biomass was rejected. Belowground biomass Zn concentrations were greatest for thinleaf alder and least for Geyer willow. Water birch, red-osier dogwood, and shrubby cinquefoil contained belowground Zn concentrations similar to Geyer willow.

Table 4: Aboveground, belowground, and total Pb (A) and Zn (B) concentrations for alder, birch, dogwood, cinquefoil, and willow grown in lime and composted biosolids amended mine tailings soil collected near Leadville, Colorado. Mean \pm SE, n=18.

A			
Pb Concentration (mg kg ⁻¹ dry weight)			
Species	Above	Below	Total
Alder	30.3 \pm 9.1 a [§]	544.1 \pm 88.7 a	574.4 \pm 97.7 a
Birch	2.5 \pm 1.3 ab	367.5 \pm 72.6 ab	370.0 \pm 73.8 ab
Dogwood	0.6 \pm 0.3 b	99.0 \pm 19.2 d	99.7 \pm 19.6 c
Cinquefoil	26.1 \pm 7.2 a	212.8 \pm 38.7 c	253.1 \pm 45.8 b
Geyer	0.1 \pm 0.1 b	295.5 \pm 39.3 bc	295.7 \pm 39.5 b
B			
Zn Concentration (mg kg ⁻¹ dry weight)			
Species	Above	Below	Total
Alder	125.7 \pm 21.8 b	471.6 \pm 90.4 a	597.4 \pm 112.1 a
Birch	210.9 \pm 32.5 a	364.7 \pm 81.5 b	575.6 \pm 113.9 a
Dogwood	44.0 \pm 8.3 d	250.8 \pm 35.4 b	294.7 \pm 43.7 b
Cinquefoil	100.8 \pm 20.8 c	262.8 \pm 60.0 b	363.6 \pm 80.8 b
Geyer	222.8 \pm 20.8 a	205.5 \pm 28.6 b	427.7 \pm 49.4 a

[§]Within columns, lower case letters indicate significant differences (P \leq 0.05) among species in metal concentration using Bonferroni's inequality method of analysis.

DISCUSSION

Growth

All five species were able to survive in the amended tailings and grew five to eight times their original weight. The four shrubs that were transplanted when dormant were able to acquire the resources needed to break dormancy and produce new growth over the course of the study. Shrubby cinquefoil was not dormant when transplanted and did show some negative responses just after transplanting. The oldest leaves became chlorotic and many were shed within the first week, but all plants recovered. The composted biosolids used contained $326 \text{ mg kg}^{-1} \text{ NO}_3^-$, $2106 \text{ mg kg}^{-1} \text{ P}$, and $5013 \text{ mg kg}^{-1} \text{ K}$. Our results suggested that there was no difference in total proportional growth among the shrubs, but there were differences in where that growth occurred.

Geyer willow had not performed well in previous studies when grown in metal contaminated soils (Bourret 2004, Boyter 2006, Fisher 1999, Shanahan 2006). Previous greenhouse research showed Geyer willow plants started from cuttings collected in the Leadville area had poor growth response when using lime and composted biosolids amended tailings collected from the same area as one of our sites (Boyter 2006). Boyter (2006) found Geyer willow cuttings to have 60% less total biomass when grown in lime and biosolids amended tailings versus when grown in topsoil, with a large number of chlorotic leaves that tended to drop on the clones that survived in the amended tailings. Geyer willow plants used in our study were originally started from seed collected from Northern Idaho, were one-year old when this study began, and had 100% survival. Although some plants did show some chlorosis, they did not drop many leaves over the course of the experiment. Usually plants close to a metal contaminated site can evolve a tolerance to adverse soil conditions where plant biomass is not affected by soil toxicity (Chaney 1993, Pahlsson 1989). In the present experiment, Geyer willow seedlings had not been previously exposed to elevated heavy metal containing soils, but were resistant to biomass reduction over the course of the experiment. Thus, Geyer plants established from seed and/or the use of non-local stock can result in a greater growth response compared to the use of local cuttings.

Thinleaf alder did not show any signs of chlorosis. During the course of the study, all individuals of this species appeared healthy. Nodules were observed on roots of many thinleaf alder at the time of transplanting. Nitrogen production and nodule biomass have not been shown to be reduced by high Pb and Zn content in soil (Gaulke et al. 2006), and in the present study this symbiotic association perhaps benefited thinleaf alder. Zinc can suppress root nitrate reduction to ammonia in some plants (Pahlsson 1989), but because of *Frankia* bacteria, thinleaf alder was most likely able to acquire the ammonia it needed to synthesize plant biomass. Chlorosis, the result of a metal competing with Fe during chlorophyll synthesis, was not observed in thinleaf alder, possibly because nitrogen metabolism was not suppressed by Zn.

Aboveground growth of water birch was affected more than belowground growth. While water birch doubled in aboveground growth over the study, this growth was significantly less than the other four species. Instead, water birch concentrated its growth belowground, greater than all species except Geyer willow. Both water birch and Geyer willow belowground growth may help

secure rhizosphere soil from erosion, considering their belowground growth compared to the other species. This should be researched further.

Shrubby cinquefoil aboveground growth accounted for 69% of its total proportional growth. Just after transplanting, shrubby cinquefoil showed signs of shock. The oldest leaves became chlorotic and began wilting in the first week of the study. Yet, all plants were able to recover, survive, and produce as much aboveground biomass as Geyer willow and red-osier dogwood.

Red-osier dogwood grown in consolidated tailings produced greater belowground growth than aboveground over a one year greenhouse study (Redfield et al. 2003). In the present study, above and belowground biomass were nearly equal. Aboveground biomass was similar to Geyer willow, unlike root biomass which was lower than Geyer willow.

Lead

All species concentrated Pb belowground, ranging from 84 to 99% of the total Pb uptake. When water birch was grown in the greenhouse over a 4 month period in tailings from the Pacific Mine in Utah, 90% of the Pb concentrated in roots and no signs of nutrient deficiency or toxicity were observed (Klassen et al. 2000). In our 2-month greenhouse study, 99% of the Pb accumulated by water birch was concentrated in the roots. Lead 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 very similarly in both studies. Water birch exhibits a range of Pb tolerance implying that it is capable of some means of exclusion. By concentrating Pb belowground, water birch promotes Pb stabilization in the tailing deposits by reducing the amount of Pb within the tailings that would be susceptible to leaching. *Betula pubescens* and *B. pendula* are two species that can accumulate greater aboveground Pb concentrations (Pahlsson 1989, Utriainen et al. 1997, Margui et al. 2007) and would be better suited for phytoremediation. *Betula occidentalis* Hook. is better suited for *in situ* remediation of sites where aboveground Pb accumulation is not desired.

Thinleaf alder and shrubby cinquefoil concentrated an average of 30 and 26 mg kg⁻¹ Pb, respectively, into their new growth over the study. Lead can be taken up by plant roots, but is not commonly translocated to stems and leaves (Hettiarachchi and Pierzynski 2004, Pahlsson 1989, Klassen et al. 2000). Lead can be laterally transported and accumulated aboveground when applied to bark (Lepp and Dollard 1974). Perhaps the Pb in cinquefoil and alder was not translocated from the roots, but rather absorbed from tailings splashed onto aboveground woody stems and incorporated into plant tissues prior to harvest.

Consuming vegetation containing greater than 30 mg kg⁻¹ Pb is known to be toxic to cattle, sheep, pigs, horses, and rabbits (McDowell 2003). Lead is a toxic metal that can cause neurological damage to humans and pathological changes in kidneys, digestive tracts, and cardiac systems of animals (McDowell 2003). Most domestic animals can consume plants with 30 mg kg⁻¹ Pb or less (McDowell 2003), the approximate concentrations we observed in thinleaf alder and shrubby cinquefoil. Thinleaf alder is a highly palatable shrub to browsing animals and cinquefoil has a medium palatability (USDA Plants Database 2007), but they would not

generally comprise a significant proportion of the diet of any animal browsing along the Arkansas River.

Zinc

Soils often have total zinc concentrations between 10 and 300 mg kg⁻¹ while total Zn concentrations of plants typically range from 15 to 100 mg kg⁻¹ (Hagemeyer 1999). Domestic animals can tolerate a range of Zn concentrations, with the lowest tolerance at 300 mg kg⁻¹ for sheep (McDowell 2003). All shrubs in this study had less than 300 mg kg⁻¹ Zn in their aboveground growth. However, Zn uptake is often greater in greenhouse than field experiments due to greater greenhouse temperatures causing greater transpiration rates (Chaney 1993), and enough water supply to consistently maintain turgor pressure (Rosselli et al. 2003). All four species would likely concentrate less Zn aboveground in the field.

Thinleaf alder concentrated Zn in its new growth, but the genus *Alnus* is generally considered a heavy metal excluder. In our study, 79% of the Zn detected in alder was concentrated in the roots, which was greater than birch, cinquefoil and Geyer willow. Our results showed a similar relationship to a field study conducted in Switzerland at a high metal content sewage sludge capped landfill. In the Switzerland study, *A. incana* (L.) Moench had lower aboveground Zn concentrations than *B. pendula* and *S. viminalis* (Rosselli et al. 2003). *Alnus incana* in our experiment was consistent with other field studies where *A. glutinosa* concentrated 90% of Zn taken up in its roots (Mertens et al. 2004) and *A. rubra* concentrated 84% of Zn belowground (Gaulke et al. 2006).

General symptoms of Zn toxicity in plants include turgor loss and necrosis on older leaves (Hagemeyer 1999), water stress, wilting, and nutrient deficiency (Pahlsson 1989, Chaney 1993). Zinc can cause Fe and Mn deficiencies which then interferes with carbohydrate metabolism and translocation to growing plant parts, nitrogen metabolism, and photosynthesis (Pahlsson 1989). Only a few replicates of red-osier dogwood and water birch had necrosis on older leaves. Many of the red-osier dogwood replicates had purple spots on their leaves or a purple hue over the entire leaf, but never displayed multiple symptoms of metal stress on one individual plant. Regular spotting is indicative of cadmium and copper stress (Barcelo and Poschenrieder 1999), which was possible considering that the tailings had an average bioavailable content of 12.6 mg kg⁻¹ Cu and 3.0 mg kg⁻¹ Cd. The purple hue on the leaves resembled the fall coloration of this species, but the leaves were not senescing. The only leaves that died over the study were the oldest leaves due to necrosis. Red-osier dogwood showed visible signs that it suffered from some sort of metal induced nutrition deficiency.

CONCLUSION

No shrub species evaluated in this greenhouse experiment exhibited superior growth or metal uptake qualities when compared to Geyer willow. All five species could be used for *in situ* field restoration of fluvial mine tailing deposits because of their ability to exclude enough Pb and Zn as to not be toxic to wild and domestic animals. Overall metal uptake ought to be lower in the field than it was in the greenhouse study due to reduced transpiration. All five of these shrubs have shown their ability to grow when exposed to high Zn and Pb content in soils, but thinleaf

alder was the only species not to exhibit any visual signs of metal induced nutrient deficiencies. Where Zn and Pb are of concern for their effect on wildlife, red-osier dogwood would be an ideal species to use because of its ability to exclude both metals from aboveground growth.

Restoring vegetative cover to fluvial mine tailing deposits using thinleaf alder, water birch, red-osier dogwood, shrubby cinquefoil, or Geyer willow is a first step to reducing heavy metal contaminated sediment erosion. Establishing these shrubs could also significantly reduce the extent of waterways reported under section 305(b) of the Clean Water Act to be effected by inactive mining. Biodiversity should be considered when planning restoration projects by using a mixture of these facultative wetland species. Stabilization of fluvial tailing deposits with shrubs should also facilitate the return of characteristic sedges, grasses, and forbs. Nutrient cycling, another ecosystem function, would also be restored, especially by the addition of N, P, K, and microbial communities from composted biosolids application. Re-creating continuity between degraded areas with adjacent riparian zones as well as upland ecosystems would also reduce exposure to animals that typically have to cross contaminated zones to access river water.

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