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Phytoremediation of cadmium and lead-polluted watersheds

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ABSTRACT

Abandoned hard rock mines and the resulting acid mine drainage (AMD) are a source of vast, environmental degradation that are toxic threats to plants, animals, and humans. Cadmium (Cd) and lead (Pb) are metal contaminants often found in AMD. In our mine outwash water samples, Cd and Pb concentrations were 300 and 40 times greater than EPA Aquatic Life Use water quality standards, respectively. We tested the phytoremediation characteristics, accumulation and tolerance of Cd and Pb contamination, for annual aboveground biomass harvest of three montane willows native to the Rocky Mountains: *Salix drummondiana, S. monticola,* and *S. planifolia*. We found *S. monticola* best suited for Pb remediation based on greater growth and tolerance in response to the low Pb treatment compared to the high Pb treatment. *Salix monticola* stems also contained higher Pb concentrations in control treatment compared to *S. planifolia*. We found *S. planifolia* and *S. drummondiana* best suited for Cd remediation. *Salix drummondiana* accumulated higher concentrations of Cd in stems than both *S. monticola* and *S. planifolia. Salix planifolia* accumulated nearly 2.5 times greater concentrations of Cd in stems in control treatment than did *S. drummondiana. Salix planifolia* also contained more total Cd in stems than did *S. monticola* in Cd treatments. Based on our results, *S. drummondiana* and *S. planifolia* could aid in reduction of Cd in watersheds, and *S. monticola* is better suited than is *S. planifolia* for aboveground accumulation and tolerance of Pb pollution.

1. Introduction

Anthropogenic disturbances, including hard rock mining and the resulting outwash, are significant sources of metal pollution in the global environment over the last century (Jung, 2001; Wahsha et al., 2012; Fig. A.1; US EPA, 2014b; US EPA, 2016). There are over 500,000 abandoned, hard rock mine sites in the U.S., of which 38,500 are on National Forest System lands, polluting watersheds and ecosystems for decades or more (Carr, 2005). Acid mine drainage (AMD) from the abandoned mines contains toxic concentrations of metals that cause significant environmental damage because the metals cannot be chemically degraded (Salt et al., 1995). Remediation of abandoned mine lands and watersheds is important, especially in Colorado where mining activities have been active since the mid-1800s. One-third of EPA's Region 8 (6 states: MT, WY, ND, SD, CO, and UT) superfund sites are in Colorado as of 2014 (US EPA, 2014a). 2,100 km of streams in Colorado are significantly polluted with AMD and many metals. 89% of total maximum daily load of Colorado water impairments are due to many of the 23,000 abandoned mine features (CDPHE, 2012).

Metal contaminants not only accelerate environmental degradation but also are detrimental to humans as well (Feleafel and Mirdad, 2013; Jarup, 2003; Leaño and Pang, 2010; Thompson and Bannigan, 2008; Méndez-Armenta and Ríos, 2007; Rosas et al., 1984). Dust from dry and loose tailings causes and exacerbates respiratory diseases in human populations near abandoned mine disposal sites (Mendez and Maier, 2008). More specifically, Cd and Pb are common, biologically non-essential metal pollutants in mine tailings and outwash (Das et al., 1997, Flora et al., 2012; Fowler, 2009; Nagajyoti et al., 2010). According to the most recent USEPA watershed assessment for Colorado (US EPA, 2010), nearly 1,300 and 300 km of streams are impaired by Cd and lead, respectively. As of 2011, 938 million gallons of water per year are treated near abandoned mining sites in the Colorado, which is effective but expensive (CDPHE, 2012).

The US Government Accountability Office found that the US EPA spent $2.2 billion on abandoned hard rock mine land remediation between 1997 and 2008 (Nazzaro, 2008). The most common approach to mine tailings and waste is piling and containing the waste tailings without chemical or metal removal treatments (Mendez and Maier, 2008). Isolating tailings with embankments does prevent the escape and spread of pollutants but does not decrease or remove them (Cunningham and Berti, 1993).

Phytoremediation is a cheap alternative to complement common, conventional methods. Phytoremediation is a broad term for using plants for cleanup of environmental metal pollution through phytoex-

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traction, rhizofiltration, or phytostabilization (Salt et al., 1995). Here we focused on phytoextraction and permanent removal of metal contaminants using native montane willows (*Salix* spp.) of the Central Rockies in Colorado, near abandoned mine lands at elevations above 2,400 m. Phytoextraction is the ability of plants to accumulate metal contaminants in their aboveground biomass, for harvesting and contaminant removal (Salt et al., 1998). Best practices of phytoremediation, such as phytoextraction, use regionally appropriate plants including Brassicaceae (mustards), Poaceae (grasses), and Salicaceae (poplars and willows) (Salt et al., 1995). Willows are the dominant riparian vegetation at these elevations and found along EPA impaired and BLM high priority watersheds near abandoned mine lands above elevations of 2,400 m in Colorado. Willows are also easy to propagate and establish in the field with very deep and extensive root systems (Vangronsveld et al., 2009). Willows are known phytoremediation agents; they accumulate and concentrate metals, grow rapidly with a relatively high biomass (Pulford and Watson, 2003; Punshon and Dickinson, 1997), and translocate metals from roots to aboveground biomass (Wahsha et al., 2012).

Here we investigated the phytoremediation abilities of three common willows native to Colorado: *Salix drummondiana, S. monticola,* and *S. planifolia* for suitability in reducing Cd and Pb contamination along the 1,600 km of cadmium and lead-polluted streams across Colorado watersheds. These phytoremediation abilities would require annual harvests before leaf fall for optimal reduction of environmental Cd and lead. Our study is innovative as it is the first study to directly compare these three dominant willow species' phytoextraction and tolerance characteristics for Cd and Pb contamination. We tested the phytoremediation characteristics of these willows via hydroponic experiments, which are useful for screening willows' tolerance and accumulation characteristics (Huang and Cunningham, 1996; Watson et al., 2003; Dos Santos et al., 2007; Zhivotovsky et al., 2011a).

Here, we propose four hypotheses. First, three common native willow species will differ in stem and leaf accumulation concentrations and total amounts (concentration x final mass) after growing in Cd and Pb treatments. Second, three common willow species will differ in Cd and Pb stem concentrations after growing in low and high metal concentration levels of Cd and lead. Third, three common native willow species differ in their relative tolerance (growth) to environmental representative Cd and Pb treatments. Lastly, three common native willow species differ in total metal amounts and concentrations of Cd and Pb without any additional access to metals in the control treatment.

2. Materials and methods

2.1. Collections

We sampled 160 individual willow genets (unique individual willow shrubs, not connected by roots) in November 2013, and from April to June 2014, at elevations above 2,400 m throughout five counties in Colorado: San Juan, Ouray, Lake, Clear Creek, and Summit. These areas of interest were near abandoned mine lands and designated as high priority (BLM, 2013) or impaired (US EPA, 2010) watersheds (Fig. 1). We collected all unique willow species in an area within 5 m of waterway edge. If there were different willow species, we collected from no more than three unique genets (not connected by roots) from each of the different species. If there was only one species in an area, we collected no more than three unique genets of that species.

We collected for diversity in the areas of interest in April – June 2014. Of the 160 individual willow collections, we identified eight unique willow species. We found the three most common willow species to be *Salix monticola, S. planifolia,* and *S. drummondiana*. Once we identified the three most common willow species, we collected 12 cuttings (20–40 cm in length) from 69 genets of the three most common willow species in August and October of 2014 for the greenhouse experiment. We collected cuttings from 32 genets of *S. monticola,* 19 genets of *S. drummondiana,* and 18 genets of *S. planifolia.* Individual genets (shrub masses) of same species were at least 20 m apart, to ensure genetic diversity. Genets were tagged in the field with sequential numbers on aluminum tags. Furthermore, previous studies of willow phytoremediation focused on plants from a limited geographic or genetic range. We selected plants from 10 sites from both eastern and western slopes of the Rocky Mountains with at least 18 unique genets of each species, with species often intermixed or at the same site. These sites offered various redox states and metal concentrations.

2.2. Hydroponic greenhouse experiment

We conducted a four week accumulation and tolerance greenhouse experiment with the three willow species of interest: *S. drummondiana, S. planifolia,* and *S. monticola.* We brought cuttings to the greenhouse at the University of Denver immediately after field collections, and placed in bunches of 12 cuttings in each deepot cone (6.4 cm by 36 cm) in every other slot in 20 slot support trays (Stuewe & Sons, Corvallis, OR). We used cupcake papers to prevent cuttings from slipping out of the cones. We submerged each cone in each slot tray in 17 cm of deionized (DI) water supplemented with 132 mL FloraGro (2-1-6 NPK ratio) nutrient solution per 100 L of water. University of Denver's Olin Hall greenhouse has natural, south facing light. We completely replaced the water every other week and supplemented with FloraGro fertilizer once a week.

We grew cuttings for 6 weeks for root development. We discarded cuttings that did not show signs of root or leaf development. For living cuttings, we assigned a root score $(0-4)$ at the beginning and end of experiment (Fig. A.2). For each genet, we chose five living cuttings giving them a letter (a, b, c, d, and e) and then assigned them randomly to each treatment. We successfully rooted 405 cuttings of 828 collected from the 69 willow genets.

We conducted the experiment with five hydroponic treatments: control (DI water; FloraGro), low Cd (11 ppb or μg/L; 0.10 μM), high Cd (300 ppb; 2.56 μM), low Pb (15 ppb; 0.07 μM), and high Pb (145 ppb; 0.70 μM). Hydroponic experiments that use unrealistically high metal concentrations can lead to 'forced' metal accumulation and their results have no biological relevance (van der Ent et al., 2013). Here, we used relatively lower (~1 μM) concentrations of metals, representing more environmentally and biologically relevant levels (Table A.1; US EPA, 2014a, 2014b; US EPA, 2016). We added metals via a stock solution of 7.6 mM of cadmium chloride ($CdCl_2$) and 2.2 mM of lead chloride ($PbCl_2$) weekly to obtain the treatment concentrations. We placed one cutting from three unique genets of each of the three species in a 26.5 L Sterilite storage bin ("block"), for a total of 9 cuttings in each block. We established a randomized block factorial design, randomizing the location of each cutting. Out of the 69 willow genets, there were eight genets that had 10 or more successfully growing cuttings that were included as replicates for genets in treatments and were nested for the genet. We filled remaining blocks with these replicate cuttings. We replicated each treatment block nine times for a total of 45 blocks and a total of 405 cuttings.

We conducted the experiment for four weeks from December 2014 to January of 2015, with diurnal temperatures ranging from 16 °C to 23 °C. Growing lights, in addition to natural light, were on for 12 h per day, from 6 am to 6 pm. We randomly organized blocks on the greenhouse benches, and moved all blocks each week to randomize lighting conditions in the greenhouse and minor irregularities of depth profiles in the blocks.

We counted leaves weekly, survival at week 3 and 4, and the biomass of each cutting before and after the experiment for the percent change. Survival was the presence of healthy roots or leaves (Figs. A.2, A.3). We only counted fully developed, healthy leaves. Leaves that were shriveling or showing chlorosis were considered dead and were not counted. At the beginning and end of the experiment, we recorded biomasses of the entire cutting to the nearest 0.1 g.