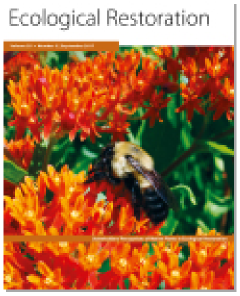
Cadmium Uptake and Growth of Three Native California Species Grown in Abandoned Mine Waste Rock

Jeffrey Lauder, Oriana Chafe, Jane Godfrey



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Cadmium Uptake and Growth of Three Native California Species Grown in Abandoned Mine Waste Rock

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Phytoremediation is the use of green plants to clean up environmental contamination from a variety of sources (Cunningham and Berti 1993). It has been suggested as a method for remediating metal contamination (Baker 1981, Chaney 1983), and numerous plants have since been identified as either tolerant to, or accumulators of various heavy metals, including lead (Huang and Cunningham 1996, Vassil et al. 1998, Chin 2007), cadmium (Dhankher et al. 2003, Podar et al. 2004), and arsenic (Zhaol et al. 2002, Meharg 2003, Rathinasabapathi et al. 2007).

Most phytoremediation studies to date have focused on the physiological mechanisms involved in the extraction, transport, and biomagnification or accumulation of metals in plants, primarily within a laboratory environment (Lasat 2002, Hansen et al. 2005, Maestri et al. 2010) and often focus on known accumulators of metals. While the mechanisms by which plants uptake and translocate metals are still being examined, identifying native species that thrive in contaminated soils as well as remove threats of metal exposure should be a primary objective. Approaching phytoremediation from both a theoretical perspective (i.e., which plants accumulate and how?) as well as an applied one (i.e., how effectively can phytoremediation be deployed at restoration sites?) leverages the potential two-fold benefit of both contamination removal and ecological restoration.

We took an exploratory approach to assess potential for three California native plant species to grow and accumulate heavy metals on gold-mine waste-rock-tailings piles within the Sierra Nevada Gold Country at an abandoned gold extraction site. We also looked at the potential heavy metal extraction capacity of these species as a phytoremediation-feasibility assessment for potential implementation as part of a broader clean-up effort.

We established open-air growth containers with plastic basins to hold all plants at the Providence Mine in Nevada City, California. The site was one of the richest mines during the California gold rush, and mining activities have left a legacy of heavy metal contamination. Preliminary soil sampling found elevated concentrations of arsenic (As), cadmium (Cd), and lead (Pb) with mean concentrations of 68.41 mg/kg As, 5.8 mg/kg Cd, and 240.77 mg/kg Pb. Soil screening levels (SSLs)—regulatory limits at time of site assessment—for each of these contaminants vary by location and sampling time, with recent California SSLs for each of the above metals being 0.07 mg/kg As, 1.7 mg/kg Cd, and 80.0 mg/kg Pb (CalEPA 2010). This restoration note focuses primarily on Cd due to significant uptake of Cd across sampled species as well as discovery of a previously unreported Cd accumulator.

Candidate species for this study were chosen following a previously conducted field-based-pilot feasibility analysis using a large selection of species (Lauder 2013). Species chosen for that pilot were selected based on their presence in the exhaustive PhytoRem phytoremediation literature database (McIntyre et al. 2003), as well as being described as locally native, and augmented with species yet untested in terms of metal tolerance. Pilot study results led to the selection of *Festuca rubra* (red fescue) and *Helianthus annuus* (sunflower) as candidates for controlled pot studies due to their nativity and demonstrated uptake capacity as well as biomass production (Lauder 2013). *Stipa pulchra* (= *Nassella pulchra* [purple needlegrass]) was added to the potted plant study due to its growth form, nativity, seed availability, and as an exploratory species that has not yet been tested for heavy metal uptake or tolerance. All three species were also selected due to their presence in commercially available hydro-seed mixtures which may be applied as part of larger, engineering-based clean-up efforts.

We planted candidates in equal amounts of contaminated homogenized soil taken directly from the mine tailings. All plants were direct-sown to simulate broadcast seeding similar to hydro-seed application. Each species was planted in triplicate with each replicate using the same amount of seed by weight (1.5 ounces [42 g] for both grasses and eight seeds per pot for *H. annuus*). Three one-gallon (5.7 l) pots of each plant contained no amendments, and three pots containing each species were amended with nitrate-rich compost (n = 3 per plant per treatment), due to previous findings of differences in biomass and shoot production in compost-amended plots (Lauder 2013). No plants were grown in control soil because the main question of this project was related to variation between species and potential germination for restoration, not whether growth is greater in contaminated or control soil. We watered plants daily with equal amounts of water until germination and every other day following germination. Plants were grown on-site to test growth under natural conditions. They were covered loosely with plastic during periods of rain to maintain a controlled watering regime and shade cloth during hot weather to prevent sun-scald. Potted plants were harvested approximately ten weeks after planting. We extracted all biomass, with roots and shoots intact, by gently removing plants from the potting soil, rinsing in tap water and de-ionized water, and then laying them on paper towels to air dry. Pre-planting soil metal concentrations and post-planting plant shoot metal uptake were measured using inductively coupled plasma-atomic emission spectrometry (ICP-AES) at Excel-Chem laboratories in Rocklin, CA.

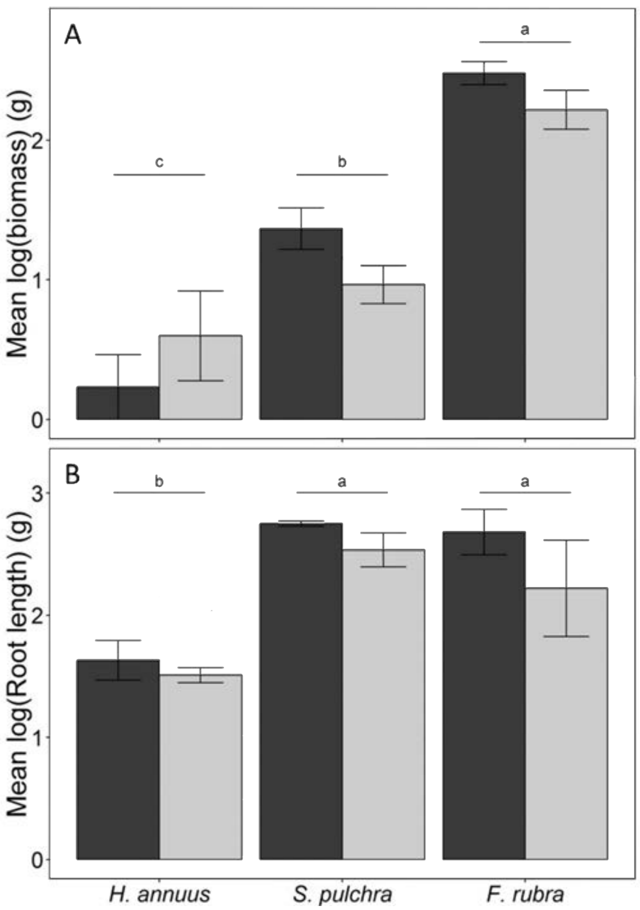

Figure 1. Mean log-transformed biomass production (A) and root growth (B) by species and amendment in *Helianthus annuus, Stipa pulchra,* and *Festuca rubra* planted in metal-contaminated soils. Light grey bars represent compost-amended soils, and dark grey bars represent non-amended soils. Different lower-case letters represent significant differences between species (ANOVA $p < 0.05$). Error bars represent standard error about the mean.

We used mixed effects analysis of variance (ANOVA) to evaluate between-species variation in biomass production, root growth, shoot growth, and total Cd uptake. All variables were examined for normality, and biomass and root growth were log (x + 1)-transformed for final analysis. All models included pre-planting Cd, As, and Pb soil concentrations as covariates due to lack of any collinearity. Pre-planting soil pH was removed from further analysis due to a lack of significant variation between pots (ANOVA $F_{3,6} = 0.18$, $p = 0.90$). Step-wise model reduction was performed using Akaike's Information Criteria (AIC) for variable reduction (pH, soil metal concentrations, amendment type, and plant species) and final model selection.

Biomass production differed by species when accounting for pre-planting metal concentrations ($F_{5,12} = 21.54$, $p < 0.001$), with *S. pulchra* ($\overline{x} = 2.33$ g) producing significantly

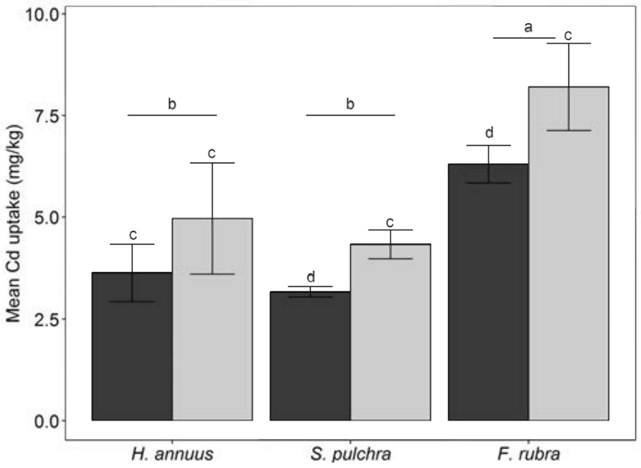

Figure 2. Mean cadmium (Cd) uptake by species and amendment in *Helianthus annuus*, *Stipa pulchra*, and *Festuca rubra* planted in metal-contaminated soils. Light grey bars represent compost-amended soils, and dark grey bars represent non-amended soils. Error bars represent standard error about the mean. Different letters above error bars represent significant within-species, between-amendment differences (ANOVA $p < 0.05$). Letters above horizontal bars represent between-species differences.

more biomass than *H. annuus* ($\bar{x} = 0.67$ g), and *F. rubra* ($\bar{x} = 9.67$ g) producing significantly more biomass than *S. pulchra* (Figure 1A). The species × amendment interaction was not significant at $\alpha = 0.05$, but compost did appear to slightly decrease biomass production in the two greatest biomass-producing species (*F. rubra* and *S. pulchra*).

Root length differed by species when accounting for pre-planting metal concentrations ($F_{6,11} = 6.65$, $p < 0.01$), with both *S. pulchra* and *F. rubra* producing significantly longer roots ($\bar{x} = 13.2$ cm and 11.79 cm, respectively) than *H. annuus* ($\bar{x} = 3.88$ cm, Figure 1B). There was no significant species × amendment interaction observed in root length. Shoot length did not significantly differ by species but did differ by amendment ($F_{12,5} = 17.269$, $p < 0.01$). Composted *H. annuus* and *F. rubra* grew shoots more than 10 cm longer than non-amended plants.

Cadmium uptake was significantly greater in *F. rubra* ($\bar{x} = 7.25$ mg/kg) than either *S. pulchra* ($\bar{x} = 4.3$ mg/kg) or *H. annuus* ($\bar{x} = 3.75$ mg/kg; Figure 2) while controlling for pre-planting metal concentrations ($F_{10,7} = 6.543$, $p < 0.05$), and composted pots showed significantly greater Cd uptake than non-amended pots in both *F. rubra* ($\bar{x} =$ 8.2 mg/kg versus 6.3 mg/kg) and *S. pulchra* ($\bar{x} = 4.97$ mg/kg versus 3.63 mg/kg).

The observed decreased biomass production in both grass species in amended pots was unexpected. It was consistent, however, with observations made by Leavitt et al. (2000), who reported significantly reduced grass cover on mine waste amended with fertilizer when compared to unamended mine waste. It is unknown if the likely cause of the observed decrease in biomass is due to application rates causing a spike in soil acidity. Increased uptake is consistent, however, with recent work that shows increased Cd mobility when N-rich fertilizer is applied and pH is reduced (Zhou et al. 2015). These results show that soils do not need to be amended to produce significant growth and slope stabilization, but should be considered if phytoextraction is a goal.

Strong root growth is a key component in angle of repose mine waste revegetation (Leavitt et al. 2000), as roots support erosion control. *Festuca rubra* produced significantly higher biomass than the other tested species, and is a clear choice for early establishment of native plants in the area. The growth form of *S. pulchra* (a bunchgrass), combined with its nativity should be considered in any waste restoration efforts focusing on ecological restoration objectives, such as restoring plant diversity. *Helianthus annuus* has been shown to exhibit allelopathy toward weedy broad-leaved invasive species such as *Brassica* sp., and in general has been shown to inhibit invasive weed cover following multiple growing seasons (Leather 1983). Thus, a combination of all three species used in this study, as well as other native species such as nitrogen-fixing legumes, would provide the most comprehensive re-vegetation plan for mine waste restoration. *Festuca rubra* and *S. pulchra* also have the benefit of being readily available in hydro-seed mixtures, allowing for cost-effective application during reclamation efforts.

Hyperaccumulation (extreme accumulation of metals relative to that of surrounding species) or accumulation of Pb and Cd have been previously reported in both *H. annuus* (Adesodun et al. 2010) and *F. rubra* (Vangronsveld et al. 2009), but no previous known work has demonstrated significant uptake of Cd in *S. pulchra* under ambient field conditions. Results from this experiment show that all three species, *F. rubra*, *S. pulchra*, and *H. annuus* are viable revegetation options for derelict mine sites, particularly when erosion potential and habitat restoration are primary goals. The added benefit of uptake can also be considered if long-term harvest plans are considered. However, the lack of hyperaccumulation in these plants makes simple non-harvest, vegetative succession plans viable, as small amounts of uptake can still limit dust exposure and erosion into waterways, while not providing a significant threat of bioaccumulation.

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Evidence of Increased Soil Organic Matter Accumulation in a Tropical Alpine Wetland After Cattle Removal

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Tropical alpine ecosystems, regionally known as "páramos", are key to healthy socio-environments in the Andean region because of the environmental services they provide. Rivers and streams originating in these ecosystems supply water to most Andean cities, agricultural activities, and hydropower generation. Ranging from the border of the tree line to the permanent snow line, these ecosystems develop in valleys and plains of glacial origin and include small lakes, peat bogs, wet grasslands, shrublands, and dispersed short-height forest patches (Buytaert et al. 2006). At sites with low disturbances, soil organic matter (SOM) content is high, commonly above 40% (Buytaert et al. 2005). In addition, they are important biodiversity hotspots due to the high level of endemism of species adapted to this unique environment.

The ecological functions of tropical alpine ecosystems are threatened by increasing pressures due to climate change, agriculture, mining, and forestry. Furthermore, scientific knowledge of these ecosystems is low relative to other tropical ecosystems. Therefore, conservation and restoration of degraded portions of these ecosystems is critical. A common management practice to enhance restoration in these ecosystems is the removal of stressors through land purchase and posterior designation as conservation sites. However, how the removal of disturbances in tropical alpine wetlands improve ecosystem functions and services is poorly reported in scientific literature, preventing the overall evaluation of this practice and the

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