

Accumulation of metals in native wheatgrasses and wildryes when grown on metal-contaminated soil from three mine sites in Montana.

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Abstract

One of the biggest challenges to successfully phytoremediate contaminated mine land soils is the identification of native plants that possess a broad adaptation to ecological sites and either exclude or uptake heavy metals of interest. This study evaluated forage concentrations of aluminum (Al), arsenic (As), chromium (Cr), cadmium (Cd), copper (Cu), iron (Fe), magnesium (Mn), nickel (Ni), lead (Pb), strontium (Sr), and zinc (Zn) in native wheatgrasses (WG) and wildryes (WR) when grown in soil originating from mine tailings from Clark Fork, Cabbage Gulch, and Keating surface mines in western Montana. Despite having metal concentrations that exceeded the upper limits of normal plant tissue for As, Cd, Sr, and Zn, bioconcentration factors (BCF), an indicator of plants ability to extract metals from the soil, were ≥ 1 only for Cd and Mn and were soil specific. Charleston Peak slender WG appears to have some potential as a Cd accumulator when grown on soils with pH levels of 5.01 and 4.26 compared to a more basic soil found in the Clark Fork (pH = 7.64). Due to BCF values ≥ 1 for Mn uptake, all basin WR cultivars/germplasms studied, FirstStrike slender and Secar WG could be possible materials for Mn accumulators in a phytoextraction program. However, based on BCF values and soil types used, none of the WG and WR studied could be considered as hyperaccumulator species for Al, As, Cr, Cd, Cu, Fe, Mn, Ni, Pb, Sr, and Zn.

Keywords: Phytoremediation, Wheatgrasses, Wildryes, Metal uptake.

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Nomenclature:

USDA NRCS (2015)

Introduction

The excess of heavy metals released from mine tailings can cause severe damage to ecosystems including decreased biodiversity, soil microbial activity, animals, and human health by contaminating surrounding soil, water, and air [1-3]. Many of these mine tailings are associated with reduced annual precipitation, relatively high or elevated temperatures, and soil that exhibit low and high pH, salinity, and reduced essential nutrients which can reduce plant establishment, persistence, and biomass production [3-5].

Phytoremediation is the use of plants and soil microbes to reduce the concentrations or toxic effects of contaminants, such as metals, in the environment. It is a relatively recent technology and is perceived as cost-effective, efficient, novel, eco-friendly, and solar driven technology [6]. Phytoextraction is the main and most useful phytoremediation technique for removal of heavy metals from contaminated soils through the uptake of metals from soil or water by plant roots and their translocation to and accumulation in aboveground biomass [6,7]. Grasses are more preferred for phytoextraction than shrubs or trees because of their high growth rate, more adaptability to stress environments, and high biomass [8].

Bioconcentration factor (BCF) is defined as the ratio of metal concentration in the harvested tissue to the metal concentration in the soil [9]. Plant species with BCF values greater than one have the potential to be used for phytoextraction [6]. If all environmental factors are constant, the uptake of a metal by different species may be compared [6].

With increased emphasis on utilizing native grasses and the often harsh environments in mine land restoration, there is interest in native cool-season wheatgrasses (WG) and wildryes (WR) due to their ability to establish and persist in these environments [10]. These grasses are generally adapted to conditions ranging from sub-humid to arid climatic conditions in steppe or desert regions. In North America, the WG and WR are most prevalent in the northern Great Plains, as well as on the semiarid to arid rangelands of the Intermountain and Great Basin Regions of the western U.S. [11].

This study reports above ground metal concentrations (mg kg^{-1} DM) and BCF factors in native WG and WR for aluminum (Al), arsenic (As), chromium (Cr), cadmium (Cd), copper (Cu), iron (Fe), magnesium (Mn), nickel (Ni), lead (Pb), strontium (Sr), and zinc (Zn) when grown in the greenhouse on soils from three Montana mine sites designated as Clark Forks, Cabbage Gulch, and Keating.

Materials and Methods

Study soils (Figure 1)

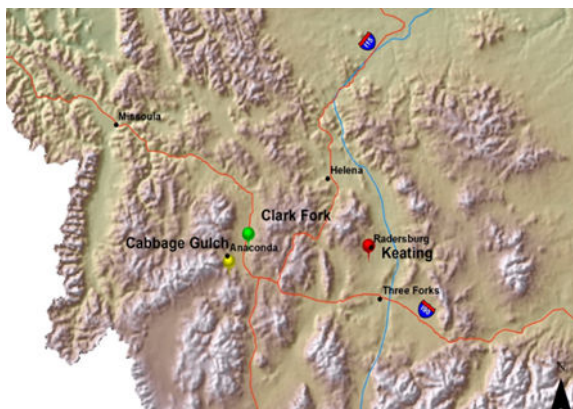


Figure 1. Study of soils.

Mine tailing soils from three western MT mines designated as 1) Clark Fork; 2) Cabbage Gulch; and 3) Keating were used. Clark Fork [Lat. 46° 11' 41" N, 112° 46' 07" W; elevation 1470 m asl; pH 7.64; EC (dSm⁻¹) 18.3] tailings were contaminated by sediments originating from an open-pit copper mine. Cabbage Gulch [Lat. 46° 04' 24" N, 112° 55' 18" W; elevation 1718 m asl; pH 5.01; EC 0.6] tailings were contaminated by aerial emissions from the Anaconda smelter that were deposited over approximately 100 square miles. Keating [Lat. 46° 10' 55" N, 111° 39' 21" W; elevation 1386 m asl; pH 4.26; EC 4.4] tailings were produced by early between 1870 and 1948 from gold and copper mining operations. Soil samples from multiple locations within the site were collected from the top 30 cm of soil from each site and mixed to uniform appearance, and partitioned into racked plant growing containers with a cell diameter and length of 3.8 x 21 cm.

Plant materials

The following species and cultivars/germplasm were used in the study basin WR [*Leymus cinereus* (Scribn. & Merr.) Á. Löve; cv. Continental, Trailhead, Magnar, Washoe, and Acc: 636], bluebunch WG [*Pseudoroegneria spicata* (Pursh) Á. Löve; cv. Anatone, Goldar, and P-7], creeping WR [*L. triticoides* (Buckley) Pilg.; cv. Shoshone], slender WG [*Elymus trachycaulus* (Link) Gould ex Shinners; cv. Copperhead, FirstStrike, and Pryor], Snake River WG [*E. wawawaiensis* J. Carlson & Barkworth; cv. Discovery and Secar], and thickspike WG [*E. lanceolatus* (Scribn. & J.G. Sm.) Gould; cv. Critana and Bannock II].

Experimental design

On 30 March 2009, six seeds from the above species and cultivars were hand planted at a depth of 1 cm directly into each container filled with soil from one of the three sites. At the bottom of each container a 10 x 10 cm felt liner was placed over the opening allowing for water movement, but restricting the soil from washing away. Plots consisted of five 3.8 x 21 cm

plant containers arranged in a randomized complete block design with four replications for a total of 240 plots.

Plants were watered every other day (~20 ml) with water and fertilized once each week for 180 days with a water-soluble fertilizer comprised of 20% nitrogen (4.1% ammonical nitrogen, 5.5% nitrate nitrogen, and 10.4% urea nitrogen), 20% phosphate (P₂O₅), and 20% potash (K₂O), 0.05% Mg, 0.0125% B, 0.0125% Cu, 0.050% chelated Fe, 0.025% chelated Mn, 0.005% Mn, 0.005% Mo, and 0.025% chelated Zn. Greenhouse temperatures ranged from 12.0 to 41.8°C, and supplemental lighting was measured as the photosynthetically active radiation (PAR) that occurs between 400 to 700 nm. Photosynthetically active radiation at noon on a cloudless day in the greenhouse averaged 383 ± 108 μmol m⁻²s⁻¹. Flats, replications kept together, were rotated randomly in the greenhouse each week to reduce the effect of light or temperature variability on plant growth. After 180 days, above ground plant parts (forage) was taken from each tube and bulked by plot.

Soil mineral analysis

Minerals were analyzed including Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr, and Zn in soil samples, except soil Si, which was not measured. Trace mineral grade nitric acid was used for all digestions, which included standardized reference material (SRM) from the United State Department, National Institute of Standards and Technology (NIST). Three replicate soil samples were taken before the greenhouse growth experiment. Soil samples were digested using a standardized protocol [12] with a blank and standardized reference (NIST SRM 2711 Montana Soil II). Mineral concentrations in the extracts were measured using ICP spectrometer (iCAP 6300, Thermo Fisher Scientific USA) with analytical standards prepared from stock mixtures and customized mixtures prepared from individual element stock solutions (Ultra Scientific USA). The pH value was determined using a saturated paste, with direct measurement of the pH in the paste [13].

Plant mineral analysis

Plot forage samples were dried at 60°C (to a constant weight) in a forced-air oven and milled using a coffee and spice grinder, which was modified to reduce size of the original 75 g grinding chamber. A quantity of 0.50 was weighed in 50 ml disposable digestion tubes with ultra-low leachable metal content (Digi tubes, SCP Science, USA), except that blue caps were replaced with colorless caps having the same appropriate specifications. Sets of 48 samples were predigested with 9 ml of concentrated nitric acid (68-70%) overnight and then incubated in a heat block (90°C) for a minimum of 1.5 continuous hours. Digested samples were diluted with ultra-pure water to final nitric acid content of 5%, which matches the analytical standards. Each set of forage extractions included one blank sample and one standardized reference (NIST SRM 1547 Peach Leaves). Mineral concentrations in the plant extracts were measured using an inductively coupled plasma (ICP) mass spectrometer (MS). Standard curves and quality

control samples were analyzed every five samples. All tissue concentrations are reported on a dry matter basis.

To determine the potential of the WR and WG for use in phytoextraction projects, forage and soil mineral concentrations (g kg^{-1}) were used to calculate the BCF defined as $[C_{\text{harvested tissue}}]/C_{\text{soil}}$ where $C_{\text{harvested tissue}}$ is the concentration of the target metal in harvested tissue and C_{soil} is the concentration of the same metal in the soil (substrate) [6].

Statistical analysis

Data were analyzed within and across mine soils using the GLM procedure of SAS with a random statement [14]. The main effects species, cultivars (species), and soil type were

Table 1. Heavy metal soil analysis from three mining sites in Montana Clark Fork, Cabbage Gulch, and Keating. [[†]Ranges taken from Kabata-Pendias (2001), [‡]Ranges taken from Haque, et al.]

Soil	pH	EC(d Sm ⁻¹)	mg kg ⁻¹										
			Al	As	Cr	Cd	Cu	Fe	Mn	Ni	Pb	Sr	Zn
Normal soil range [†]			10,000-300,00	0.1-40	5-1,500	0.1-1	2-250	100->100,00	2-7,000	2-750	2-300	<5-3,000	1-900
Normal plant range			2.6-14,500 [†]	0.01-5.0 [‡]	0.2-5.0 [‡]	0.03-1.30 [†]	5-25 [‡]	18-320 [†]	80-1,840 [†]	1-10 [‡]	0.1-5.0 [‡]	6-37 [†]	17-125 [†]
Clark Fork	7.6	18.3	6,300±58	436±8	7.7±0.2	6.0±0.2	2101±41	17,880±58	534±35	4.7±0.1	2331±97	74±1	1295±23
Cabbage Gulch	5.0	0.6	18,567±769	46±3	34.2±2.4	4.6±0.2	162±11	11,900±839	287±21	19.4±1.3	430±10	90±2	184±10
Keating	4.3	4.4	9,400 ± 153	127±1	10.8±0.1	2.4±0.0	208±1	39,833±426	426±11	4.7±0.1	541±4	145±3	487±4
Control Soil	7.5	0.9	3633±176	0.0±0.2	1.3±0.1	0.0±0.0	3.7±0.2	1,900±173	97±3	2.4±0.1	12±1	10±1	12±1

Cabbage Gulch soil exceeded normal metal soil concentrations for As, Cd, and Pb at 46, 4.6, and 430 mg kg^{-1} , respectively. Keating soil exhibited above normal soil concentrations for As, Cd, and Pb at 127, 2.4, and 541 mg kg^{-1} , respectively (Table 1). All metal concentrations in the control were within the normal soil ranges reported in [15]. Soil pH was 4.26, 5.01, 7.64, and 7.46 in the Keating, Cabbage Gulch, Clark Fork, and control soils, respectively (Table 1). Soil electrical conductivity was 0.6, 0.9, 4.4, and 18.3 dS m^{-1} in Cabbage Gulch, control, Keating, and Clarks Fork soils, respectively (Table 1).

treated as fixed effects and replication as a random effect. Main effects and interactions were tested with their first-order interactions with replications as the error terms. Due to a non-significant soil x species interaction in minerals Al, Fe, and Pd these will be described across species while all other minerals will be presented within sites. Mean separations by species were based on species averages in accordance with Fisher's protected least significant difference (LSD) at the $P < 0.05$ level of probability.

Results and Discussion

Metal concentrations of each soil type, Clarks Fork, Cabbage Gulch, Keating, and control are listed in Table 1.

Accumulation of heavy metals in plants on Clark Fork soil (Figure 2)



Figure 2. Clarks fork soil.

Tissue As, Cd, Cu, Pb, and Zn concentrations were above the normal soil concentration range reported [15] at 436, 6.0, 2101, 534, 2331, and 1295 mg kg^{-1} , respectively (Table 1). Corresponding species and cultivars within species tissue concentrations for Cd, Pb, and Zn were within the normal range [2,15]. Tissue As concentrations in FirstStike slender WG (7.1 mg kg^{-1}), and Secar (6.0) Snake River WG exceeded

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the upper normal concentration of 5.0 mg kg⁻¹ for normal plant tissue [15]. With the exception of Continental basin WR, P-7 bluebunch WG, Copperhead slender WG, and Critana thickspike WG all other plant materials exceeded the upper

limit of 125 mg Zn kg⁻¹ as the upper limit [15]. However, regardless tissue metal concentration, all species or cultivars within species had BCF values < 1; hence, not good choices for phytoextraction at this site (Table 2).

Table 2. Mean metal tissue concentrations and bioaccumulation factors (BCF) of As, Cd, Mn, and Zn in five native grasses when grow on Clarks Fork, Gabbage Gulch, and Keating soils.

Species	Mine soil											
	Clarks Fork				Gabbage Gulch				Keating			
	mg kg ⁻¹ †				mg kg ⁻¹ †				BCF‡			
	As	Cd	Mn	Zn	As	Cd	Zn	Cd	Cd	Mn	Cd	Mn
Basin wildrye												
Continental	3	0.9	14	113	3.9	2.1	89	0.43	4.2	583	1.75	1.37
Trialhead	4.3	0.7	19	153	3.7	3	136	0.66	3.3	464	1.38	1.09
Mangar	3.4	1	17	131	2.9	3.2	57	0.73	5.1	525	1.98	1.15
Washoe	3	0.8	24	187	2.3	2.3	86	0.5	4.5	549	1.86	1.29
Acc. 636	3.7	0.5	14	146	3	2	97	0.41				
Bluebunch wheatgrass												
Anatone					7.6	2.6	86	0.62				
Goldar					6.6	2.5	109	0.56	4.6	382	1.88	0.93
P-7	6.9	0.5	31	113	9.1	3	72	0.64	3.6	391	1.16	0.92
Slender wheatgrass												
Copperhead	3.3	1.2	30	63	3.1	2.7	38	0.58	3	396	1.27	0.93
FirstStrike	7.3	1.3	15	262	3.4	2.9	102	0.63	2.9	473	1.24	1.11
Pryor					4.3	2.1	97	0.46	3	361	1.19	0.85
Charleston Peak	3.9	0.8	24	197	4.4	6	128	1.31	6.3	417	2.64	0.98
Snake River wheatgrass												
Discovery	4.4	0.9	16	178	4.9	2.2	89	0.49	2.2	356	0.92	0.84
Secar	6	0.7	20	155	5	1.3	81	0.28	2	462	0.82	1.09
Thickspike wheatgrass												
Critana	2.1	0.2	66	97	3.6	1.5	61	0.34	2.3	349	0.98	0.82
Bannock II	4	0.3	18	133	4.1	1.2	51	0.27	2.1	243	0.86	0.57
LSD _{0.05}	2.4	0.4	36	77	1.7	1	26		1.6	177		

* Significant at the 0.05 probability level, ns = not significant.

† Based on a dry weight basis.

‡ Biocentration factor (BCF) is defined as $BCF = [C_{harvested\ tissue}] / [C_{soil}]$ where $C_{harvested\ tissue}$ is the concentration of the target metal in harvested tissue and C_{soil} is the concentration of the same metal in the soil.

Accumulation of heavy metals in plants on Cabbage Gulch soil (Figure 3)

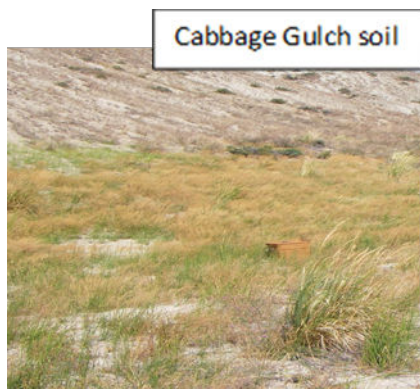


Figure 3. Cabbage gulch soil.

As and Cd levels exceeded the normal levels [15]. Observed As tissue concentrations in P-7 (9.1 mg kg^{-1}), Goldar (6.6), and Anatone (7.6) bluebunch WG exceeded the 5.0 mg kg^{-1} reported as the upper normal tissue limit [2] (Table 2). Observed tissue Cd concentrations ranged from 1.5 to 6.0 mg kg^{-1} in Critana thickspike WG and Charleston Peak slender WG, respectively, exceeding the upper normal tissue limit of $1.3 \text{ mg Cd kg}^{-1}$ [15] (Table 2). With the exception of Trailhead basin WR ($136 \text{ mg Zn kg}^{-1}$) and Charleston Peak slender WG ($128 \text{ mg Zn kg}^{-1}$), all other grasses ranged from 38 to $123 \text{ mg Zn kg}^{-1}$. Only Charleston Peak slender WG had a BCF value > 1 for Cd (Table 2). This rapidly establishing slender WG germplasm should be considered as a possible grass when the objective is to accumulate Cd from the soil.

Accumulation of heavy metals in plants on Keating soil (Figure 4)



Figure 4. Keating Soil.

Tissue Cd concentrations exceeded the normal level of 1.3 mg kg^{-1} [15] in all species and cultivars within species (Table 2) on this soil. Species ranking for Cd levels were Basin WR (4.7 mg kg^{-1}), bluebunch (4.1), slender (3.8), thickspike (2.2), and Snake River WG (2.1). Within each tissue Cd levels ranged from 2.0 to 6.3 mg kg^{-1} in Secar Snake River WG and Charleston Peak slender WG, respectively (Table 2). Basin WR, blue bunch WG and slender WG species and cultivars within species had BCF > 1 for Cd, suggesting that they may have potential as a phytoextraction species for soil Cd removal

in this type of soil (Table 2). Tissue Mn concentrations ranged from 65 to 180 mg kg^{-1} well within the normal 80 to $1,840 \text{ mg kg}^{-1}$ [15]. However, all basin WR plant materials, FirstStrike slender WG, and Secar Snake River WG had BCF values > 1 for Mn suggesting that they may have some potential as phytoextraction species for Mn.

Across soil types, BCF values of ≤ 1 were observed for Al, Cr, Cu, Fe, Ni, and Pb, suggesting that these grasses are likely not candidates for use in phytoextraction. Tissue As concentrations reached 11 mg kg^{-1} in basin WR and 3 mg kg^{-1} in bluebunch WG near Anaconda, MT [16]. Of interest was the low as concentrations in forage grown on the Keating soil, which ranged from 0.0 to 1.4 mg kg^{-1} , yet it possessed a soil as concentration of 128 mg kg^{-1} . The edox potential with soluble Fe [Fe (II)] and alkaline pH were shown to control speciation and solubility of As [17], thus affecting its uptake. The Keating soil contained $38,900 \text{ mg Fe kg}^{-1}$ with a pH of 4.26 , which likely contributed to the reduced As uptake by grasses when grown on the Keating soil.

Although Cd is considered to be a nonessential element in plants, it is absorbed by both roots and leaves. In a world-wide review [15], background Cd concentrations in grass forage ranged between 0.03 to 1.3 mg kg^{-1} . In this study, forage Cd concentrations ranged from 0.2 to 6.3 mg kg^{-1} for Critana thickspike WG and Charleston Peak slender WG germplasm. Charleston Peak slender WG germplasm when grown on Cabbage Gulch and Keating soils had BCF values of 1.3 and 2.6 ; hence may be considered a possible plant option for use when Cd uptake is the goal [6]. Plant Cd concentrations generally increased when associated with a decrease in soil pH; and conversely, decreased in soils with increased levels of Zn and Cu possibly explaining the increased BCF values observed in Cd uptake when grown on the Keating soil (Table 2) [17]. The pH of Clark Fork soil is 7.64 , and possesses $1,394.7 \text{ mg Zn kg}^{-1}$, and $2,100.6 \text{ mg Cu kg}^{-1}$ compared to the Keating soil that has a pH of 4.26 , and a Zn and Cu concentration of 487 mg kg^{-1} , and 426 mg kg^{-1} , respectively (Table 2).

Forage Zn concentrations in bluebunch WG and basin WR were 42 mg Zn kg^{-1} and 26 , respectively, near Anaconda, MT [18]. Likewise, forage Zn concentrations ranging from 9 to 50 mg kg^{-1} in slender WG, 23 to 175 mg kg^{-1} in basin WR, 19 to 77 mg kg^{-1} (bluebunch WG), and 17 to 68 mg kg^{-1} (Snake River WG) were observed near Anaconda, MT [16]. All Zn BCF values were < 1 and did not appear to uptake Zn in quantities suitable for use in phytoextraction.

Conclusion

The need to identify native plants, particularly WG and WR because of their broad adaptation to ecological sites throughout the western US, that uptake heavy metals is critical for use in phytoextraction [8]. Despite having metal concentrations that exceeded the upper limits of normal plant tissue for As, Cd, and Zn (Table 2) BCF values, an indicator of plants ability to extract metals from the soil, were > 1 only for Cd and Mn and were soil specific. Charleston Peak slender WG appears to have some potential has a Cd accumulator when grown on soils

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with pH levels of 5.01 and 4.26 compared to a more basic soil observed in the Clark Fork soil (Table 1). Due to BCF values > 1 for Mn uptake, all basin WR plant materials studied, FirstStrike slender and Secar Snake River WG could be possible materials for Mn accumulators in a phytoextraction program.

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