

Uptake of As, Cd, Cu, Fe, Mn, Pb, and Zn in pasture grasses on three metal contaminated soils from Montana.

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Abstract

Successful phytoremediation contaminated mine land soils require the identification of plant species that with wide-spread adaptation to multiple ecological sites that exclude or uptake heavy metals of interest. This study observed metal uptake of arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), magnesium (Mn), lead (Pb) and zinc (Zn) in forage (mg kg⁻¹ DM) of alfalfa and nine other dryland and irrigated grass species when grown on soil from Clarks Fork, Cabbage Gulch, and Keating mine sites in Montana. On the Clarks Fork soil, increased tissue concentrations of As, Cd, Cu, and Zn; were greater than observed in normal plant tissue. However, bio-concentration factors (BCF) were < 1 in all plant materials in the study, suggesting that under these soil conditions they may not be good options for phytoextraction. BCF >1 in crested wheatgrass, intermediate wheatgrass, meadow and smooth brome grasses, Russian wildrye, tall wheatgrass, orchardgrass, and tall fescue suggest that these species have the ability to uptake Cd particularly on soils with pH levels of 5.01 and 4.26 found Cabbage Gulch and Keating soils compared to more basic Clarks Fork soil. Observed BCF >1 in Hycrest II crested wheatgrass, AI intermediate wheatgrass, Regar and Cache meadow bromegrass, Manchar smooth bromegrass, NewHy RS-hybrid wheatgrass, Bozoisky II Russian wildrye Alkar tall wheatgrass, Paiute and UTDG-101 orchardgrass, and Fawn tall fescue for Mn accumulation warrants further investigation as another option Mn phytoextraction. Only RS-H RS-hybrid wheatgrass had a Zn BCF >1 and may be a candidate for Zn accumulation.

Keywords: Phytoremediation, Metal uptake, Agropyron, Bromus, Fescue, Dactylis, Thinopyrum.

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Introduction

Heavy metal accumulation in plant species in the western U.S. are often associated with mine tailings or overburden piles left after mining activities [1]. Many of the chemical and physical treatments used in mining areas can irreversibly affect soil properties, destroy biodiversity, and often render the soil devoid of vegetation along with stressing the heterotrophic microbial community [2,3]. Characteristic of these soils is wide pH ranges and reduce plant nutrients. For instance, a vegetative shift from an evergreen ecosystem to bare ground around smelters in southwest Montana, which increased bare ground, wind and water erosion, and density of weedy species [4].

Due to the increasing costs of mine tailing remediation, phytoremediation has been used as a viable option for reclamation [2]. Phytoremediation has been defined as the use of plant based communities to mitigate contaminated environments [5]. On arid and semiarid rangelands where annual precipitation ranges from 15 to 40 cm, phytostabilization and phytoextraction are perhaps better options for phytoremediation. Phytostabilization tries to establish a vegetative cap where metals are immobilized in the plant rhizosphere [2]. Some have questioned the sustainability of phytostabilization because metal contaminants are not removed, but rather “stabilized” [1]. The biggest challenge to phytostabilization is the identification of region and climate-

specific plants that do not accumulate metals in their above ground parts [2]. To achieve self-sustainable vegetation on metal contaminated soils, it is essential to choose plant materials tolerant of specific metals and then rotate grasses with legumes to restore soil fertility and accelerate ecological succession to more optimal states [6].

Alternately, phytoextraction involves the translocation from roots to shoots and the hyperaccumulation of metals in concentrations of 100-fold or greater than non-accumulating plant species in above-ground plant parts [7]. Metals are then mechanically removed from the site by subsequent plant harvest. Again critical to the success of phytoextraction are the identification and/or development of plants and plant communities that have a wide range of ecological adaptation, are able to establish and persist, exhibit high biomass, fast growth, and have profuse root systems capable of transporting metals to their shoots [2,3,8]. However, plant materials that are broadly adapted, easy to establish and persist, with high biomass are frequently used as a forage base for livestock and wildlife; thus, increasing the risk of poisoning if not managed properly.

To reduce potential wildlife and livestock poisoning, there is a need to identify plant materials that do not accumulate critical levels of arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), magnesium (Mn), lead (Pb), and zinc (Zn) in dryland and irrigated pasture grasses. Conversely, if the objective is to

remove the metals from the soil, plant materials with BCF >1 need to be identified. Herein, we report the forage tissue concentrations and bioconcentration factor (BCF) values of (As), cadmium (Cd), copper (Cu), iron (Fe), magnesium (Mn), lead (Pb) and zinc (Zn) of 10 dryland and irrigated pasture grasses and alfalfa when grown on soils from three Montana mine sites designated as Clarks Fork, Cabbage Gulch, and Keating.

Materials and Methods

Study soils

Details regarding the location elevation, soil characteristics, and tailing information was previously reported in [9] and can

Table 1. Heavy metal soil analysis from three mining sites in Montana Clark Fork, Cabbage Gultch, and Keating.

[This table was reproduced from Jensen et al. 2018; †Ranges taken from Kabata-Pendias (2001); ‡Ranges taken from Haque et al.]

Soil	mg kg ⁻¹												
	pH	EC (dSm ⁻¹)	Al	As	Cr	Cd	Cu	Fe	Mn	Ni	Pb	Sr	Zn
Normal soil range†			10,000-300,000	0.1-40	5-1,500	0.1-1	2-250	100->100,000	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	7.64	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	5.01	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	4.26	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	7.46	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

Plant materials

Species used in the study included alfalfa (cv. Ladak), crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] (cv. Douglas and Hycrest II), intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) (Exp. breeding line 'AI'), meadow brome grass (*Bromus riparius* Rehmman) (cv. Cache and Regar), smooth brome grass (*Bromus inermis* Leyss.) (cv. Manchar), RS-Hybrid (*Elymus hoffmannii* K.B. Jensen & K.H. Asay) (cv. NewHy and RS-H), Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski] (cv. Bozoisky II), tall wheatgrass [*Th. ponticum* (Podp.) Z.-W. Liu & R.-C. Wang] (cv. Alkar), orchardgrass (*Dactylis glomerata* L.) (cv. Paiute and UTDG-101 germplasm), and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] (cv. Fawn).

Experimental design

The experimental design was described in a previous paper [9] that observed metal uptake in native wheatgrasses and wildryes. Briefly, on 30 March 2009 seeds from the above

species were planted in containers containing soil from the different mine sites at a depth of 1 cm. Plots consisted of five containers arranged in a randomized complete block design with four replications for a total of 240 plots. The study was conducted for 180 days. Irrigation and fertilizer rates, greenhouse minimum and maximum temperatures, and photo synthetically active radiation at noon were previously reported in [9]. After 180 days, above ground plant parts (forage) was taken from each tube and bulked by plot.

be found in Table 1. A brief summary follows: Clarks Fork tailings were contaminated by sediments originating from an open-pit copper mine. Cabbage Gulch tailings were contaminated by aerial emissions from the Anaconda smelter that were deposited over approximately 100 square miles. Keating tailings were produced by early (1870-1948) from gold and copper mining operations. Soil samples consisted of four to five subsamples within each mine tailing site collected from the top 30 cm of soil. Soil samples were homogenized in a cement mixer to uniform appearance, and placed into containers with a cell diameter and length of 3.8 x 21 cm.

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Soil	mg kg ⁻¹												
	pH	EC (dSm ⁻¹)	Al	As	Cr	Cd	Cu	Fe	Mn	Ni	Pb	Sr	Zn
Normal soil range†			10,000-300,000	0.1-40	5-1,500	0.1-1	2-250	100->100,000	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	7.64	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	5.01	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
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Soil mineral analysis

Procedures pertaining to soil mineral analysis for Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr, and Zn were described previously [9]. Briefly, three replicate soil samples were taken before the greenhouse growth experiment. Mineral concentrations in the extracts were measured using ICP spectrometer (iCAP 6300, Thermo Fisher Scientific USA). The pH value was determined using a saturated paste, with direct measurement of the pH in the paste [10].

Plant mineral analysis

Above ground biomass samples for heavy metal uptake were dried at 60°C in a forced-air oven to a constant weight and ground using a coffee bean grinder. The digestibility procedures to prepare the samples for analysis with the inductively coupled plasma (ICP) mass spectrometer (MS) were reported previously [9]. Above ground plant tissue and soil metal concentrations were used to calculate the BCF values. BCF is 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]. Plant materials with BCF values >1 have potential for used in phyto-extraction [11].

Statistical analysis

Data were analyzed within mine soils using the GLM procedure of SAS with a random statement [12]. The main

effects species, cultivars (species), and soil type were treated as fixed effects and replication as a random effect. 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

Accumulation of heavy metals in plants on Clarks Fork soil

Soil metal concentrations of As, Cd, Cu, and Zn exceeded the normal soil range reported [7] at 453 ± 8 , 6.0 ± 0.2 , 2101 ± 41 , and 1295 ± 23 , respectively (Table 2).

Table 2. Metal concentrations and BCF (F) values in alfalfa and nine grass species on contaminated soil from the Clarks Fork area in Montana, USA.

[F Bioconcentration factor (BCF) defined as $BCF = [C_{\text{harvested}}]/C_{\text{soil}}$, where $C_{\text{harvested}}$ is the tissue concentration of the target metal in harvested tissue and C_{soil} is the concentration of the same metal in the soil (substrate) values].

	mg kg ⁻¹ dry-matter							BCF values						
	As	Cd	Mn	Zn	Cu	Fe	Pb	As	Cd	Mn	Zn	Cu	Fe	Pb
Alfalfa														
Ladak	6.3	1.5	32	71.6	17.6	49.5	0.0	0.014	0.251	0.060	0.055	0.008	0.003	0.000
Crested WG														
Douglas	6.4	0.8	24	152.0	33.7	87.4	1.4	0.015	0.134	0.044	0.117	0.016	0.005	0.000
Hycrest II	4.6	0.7	23	164.5	28.9	75.5	0.5	0.011	0.115	0.043	0.127	0.014	0.004	0.000
Intermediate WG														
AI	5.1	0.6	24	173.9	26.2	58.8	0.7	0.012	0.093	0.045	0.134	0.012	0.003	0.000
Meadow brome														
Regar	5.3	0.9	34	158.2	35.0	110.4	1.5	0.012	0.151	0.064	0.122	0.017	0.006	0.000
Cache	4.4	0.8	36	130.4	30.5	65.4	1.0	0.011	0.140	0.067	0.101	0.015	0.004	0.000
Smooth brome														
Manchar	2.8	0.5	33	183.0	37.5	73.2	0.4	0.006	0.078	0.061	0.141	0.018	0.004	0.000
RS-Hybrid														
NewHy	4.9	0.9	26	187.5	28.9	70.8	1.2	0.011	0.154	0.048	0.145	0.014	0.004	0.000
RS_H	4.4	0.6	29	131.5	21.3	54.5	0.0	0.010	0.093	0.054	0.101	0.010	0.003	0.000
Russian WR														
Bozoisky II	2.3	0.4	11	154.5	18.7	30.5	0.0	0.005	0.072	0.021	0.119	0.009	0.002	0.000
Tall WG														
Alkar	4.0	1.4	29	187.8	19.2	44.8	0.0	0.009	0.236	0.054	0.145	0.009	0.003	0.000
Orchard grass														
Paiute	4.6	0.7	52	191.7	17.6	54.5	0.0	0.010	0.109	0.098	0.148	0.008	0.003	0.000

UTDG-101	4.2	0.4	30	120.4	20.9	44.9	0.0	0.010	0.065	0.056	0.093	0.010	0.003	0.000
Tall fescue														
Fawn	2.8	1.3	32	200.0	21.6	45.3	0.5	0.006	0.212	0.060	0.154	0.010	0.003	0.000
LSD _(0.05)	2.1	0.5	17	ns	8.9	ns	ns	0.005	0.081	0.031	ns	0.004	ns	ns

Concentrations of As in ‘Ladak’ alfalfa (6.3 mg kg⁻¹ DM), ‘Douglas’ (6.4) crested wheatgrass, AI (5.1) intermediate wheatgrass, and ‘Regar’ (5.3) meadow bromegrass exceeded the upper normal concentration limit of 5.0 mg kg⁻¹ DM for normal plant tissue [7]. In general, background As concentrations in grasses range between 0.28 and 0.33 mg kg⁻¹ DM while tissue concentrations between 5 and 20 mg As kg⁻¹ DM can be toxic to plants [7]. Arsenic concentrations above 50 mg kg⁻¹ DM are considered toxic to livestock and wildlife [7].

Tissue Cd concentrations in Ladak (1.5 mg kg⁻¹ DM), ‘Alkar’ (1.4) tall wheatgrass, and ‘Fawn’ (1.3) tall fescue were equal to or exceeded the upper limit of 1.3 mg Cd kg⁻¹ DM [7]. Tissue Cu concentrations exceeding 25 mg kg⁻¹ DM were observed in Douglas (33.7) and ‘Hycrest II’ (28.9) crested wheatgrass, AI (26.2) intermediate wheatgrass, Regar (35.0) and ‘Cache’ (30.5) meadow bromegrass, ‘Manchar’ (37.5) smooth bromegrass and ‘NewHy’ (28.9) RS-Hybrid. With the exception of Ladak alfalfa, Zn tissue concentration exceeded the upper normal limit of 125 mg kg⁻¹ DM [7]. Based on the maximum tolerable dietary level for cattle and horses of 500 mg Zn kg⁻¹ DM [11], plant materials in this study did not accumulate forage Zn concentrations above 200.0 mg kg⁻¹ DM (Fawn tall fescue). On this soil, all plant metal concentrations had BCF factors < 1 for As, Cd, Cu, and Zn and may not be good options for use a phytoextraction. However, they may

warrant further investigation into possible phytostabilization species.

Accumulation of heavy metals in plants on Cabbage Gulch soil

Cabbage Gulch soil concentrations of As, Cd, and Pb were greater than the upper soil range [7]. Plant tissue As concentrations were greater than 5.0 mg As kg⁻¹ DM in Ladak (6.1) alfalfa, Douglas (7.8) and Hycrest II (7.2) crested wheatgrass, AI (6.1) intermediate wheatgrass, and Regar (5.5) and Cache (6.2) meadow bromegrass (Table 3). With the exception of Manchar smooth bromegrass and ‘Bozoisky II’ Russian wildrye all other species and cultivars within species exceeded 1.3 mg Cd kg⁻¹ DM (Table 3). Tissue Zn concentrations exceeded 125 mg kg⁻¹ DM in the crested wheatgrass and RS-H Hybrid wheatgrass (Table 3). Douglas crested wheatgrass and ‘Alkar’ tall wheatgrass had BCF values > 1 for Cd (Table 3), while RS-H RS-hybrid germplasm, a natural cross between *Elytriga repens* L. and old world bluebunch wheatgrasses, had a BCF value of 1.350 for Zn uptake. These drought tolerant species should be considered as possible options when the objective is to accumulate Cd or Zn from the soil. Due to their high palatability, caution is needed if grazing either by wildlife or livestock is going to occur on these mine sites.

Table 3. Metal concentrations and BCF (F) values in alfalfa and nine grass species on contaminated soil from the Cabbage Gulch area in Montana, USA.

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

	mg kg ⁻¹ dry-matter							BCF values						
	As	Cd	Mn	Zn	Cu	Fe	Pb	As	Cd	Mn	Zn	Cu	Fe	Pb
Alfalfa														
Ladak	6.1	2.2	26	47.2	12.7	152.6	0.4	0.133	0.471	0.091	0.257	0.780	0.013	0.001
Crested WG														
Douglas	7.8	6.6	30	152.8	23.3	215.5	2.3	0.169	1.426	0.105	0.831	0.144	0.018	0.006
Hycrest II	7.2	3.3	37	138.5	22.9	135.1	1.6	0.157	0.713	0.130	0.752	0.141	0.011	0.004
Intermediate WG														
AI	6.1	2.0	25	85.3	17.4	149.7	1.3	0.134	0.435	0.085	0.464	0.107	0.013	0.003
Meadow brome														
Regar	5.5	2.9	39	65.8	18.7	91.4	0.9	0.119	0.620	0.134	0.357	0.115	0.008	0.002
Cache	6.2	2.8	36	58.7	18.8	97.5	1.5	0.135	0.600	0.124	0.319	0.116	0.008	0.004
Smooth brome														

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Manchar	4.6	0.1	42	62.4	19.0	126.6	1.1	0.099	0.180	0.147	0.339	0.117	0.011	0.003
RS-Hybrid														
NewHy	4.8	2.2	28	79.2	21.2	104.9	2.1	0.103	0.469	0.099	0.430	0.131	0.009	0.005
RS_H	6.0	1.6	27	249.1	19.9	130.7	1.3	0.130	0.339	0.092	1.350	0.123	0.011	0.003
Russian WR														
Bozoisky II	4.3	1.2	27	86.4	17.2	283.7	3.5	0.094	0.259	0.093	0.470	0.106	0.024	0.008
Tall WG														
Alkar	2.3	4.9	31	101.9	16.9	137.5	0.5	0.051	1.056	0.108	0.554	0.104	0.012	0.001
Orchardgrass														
Paiute	3.4	1.6	93	110.8	12.8	114.5	2.2	0.074	0.439	0.325	0.602	0.079	0.010	0.005
UTDG-101	4.9	1.6	112	96.9	13.4	154.7	3.2	0.106	0.350	0.392	0.527	0.083	0.013	0.008
Tall fescue														
Fawn	2.6	3.9	38	115.8	16.4	131.9	1.5	0.055	0.853	0.133	0.629	0.102	0.011	0.003
LSD _(0.05)	2.1	1.0	23	122	6.1	ns	2.1	0.046	0.216	0.812	0.663	0.038	ns	0.005

Accumulation of heavy metals in plants on Keating soil

Tissue As concentrations and BCF values when grown in the Keating soil were low ranging from 0.0 mg kg⁻¹ DM to 1.0, yet the soil As concentrations of 128 mg kg⁻¹ DM were high (Table 1). A possible explanation for this observation is that soluble Fe [Fe (II)] in an alkaline pH soil controls speciation and solubility of As [10], thus affecting ability to move from the soil to plant. Since the Keating soil contained 38,900 mg Fe kg⁻¹ with a pH of 4.26 this likely contributed to the reduced As uptake.

Background Cd concentrations in plant tissue range from 0.0 to 1.3 mg kg⁻¹ DM [7]. Cadmium tissue concentrations were greater than 1.3 mg Cd kg⁻¹ DM in all plant materials, except UTDG-101 orchardgrass [7]. Species rankings for Cd levels were tall fescue (5.0 mg Cd kg⁻¹ DM), tall (4.8), crested (3.7), and intermediate (3.6) wheatgrasses, Russian wildrye (3.4), meadow bromegrass (3.0), alfalfa (2.0), RS hybrid wheatgrass (1.7), smooth bromegrass (1.5), and orchardgrass (1.4). Tissue Cd levels ranged from 1.3 mg Cd kg⁻¹ DM to 5.0 in UTDG-101 orchardgrass and Fawn tall fescue, respectively (Table 4).

Table 4. Metal concentrations and BCF (F) values in alfalfa and nine grass species on contaminated soil from the Keating area in Montana, USA. [F Bioconcentration factor (BCF) defined as $BCF = [C_{\text{harvested}}]/C_{\text{soil}}$ where $C_{\text{harvested}}$ is the tissue concentration of the target metal in harvested tissue and C_{soil} is the concentration of the same metal in the soil (substrate) values].

	mg kg dry-matter							BCF values						
	As	Cd	Mn	Zn	Cu	Fe	Pb	As	Cd	Mn	Zn	Cu	Fe	Pb
Alfalfa														
Ladak	0.0	2.0	279	181.2	12.5	120.6	0.0	0.000	0.817	0.654	0.372	0.060	0.003	0.000
Crested WG														
Douglas
Hycrest II	0.0	3.7	477	130.3	15.1	47.6	0.0	0.000	1.521	1.120	0.268	0.072	0.001	0.000
Intermediate WG														
Al	0.3	3.6	529	100.1	17.8	188.2	0.7	0.003	1.479	1.242	0.206	0.086	0.005	0.001
Meadow brome														
Regar	0.6	2.9	484	59.2	16.2	130.5	0.0	0.004	1.191	1.136	0.122	0.078	0.003	0.000
Cache	0.3	3.2	598	68.8	18.7	91.0	0.4	0.003	1.329	1.405	0.141	0.090	0.002	0.001
Smooth brome														

Manchar	0.4	1.5	958	102.1	21.8	125.4	0.5	0.003	0.640	2.250	0.210	0.105	0.003	0.001
RS-Hybrid														
NewHy	0.0	2.0	443	71.6	20.3	76.5	0.0	0.000	0.823	1.040	0.147	0.098	0.002	0.000
RS_H	0.4	1.4	312	57.3	22.8	119.3	0.5	0.003	0.580	0.733	0.118	0.109	0.003	0.001
Russian WR														
Bozoisky II	0.0	3.4	529	159.3	14.7	79.3	0.0	0.000	1.406	1.241	0.327	0.070	0.002	0.000
Tall WG														
Alkar	0.6	4.8	860	87.0	17.1	187.0	0.0	0.005	1.988	2.014	0.179	0.082	0.005	0.000
Orchard grass														
Paiute	0.0	1.6	2143	143.8	13.6	123.6	0.0	0.000	0.651	5.030	0.295	0.065	0.003	0.000
UTDG-101	0.8	1.3	1440	79.1	16.7	204.3	0.4	0.006	0.528	3.380	0.162	0.080	0.005	0.001
Tall fescue														
Fawn	1.0	5.0	859	128.6	19.6	221.8	1.4	0.008	2.006	2.017	0.264	0.094	0.006	0.003
LSD _(0.05)	ns	1.6	384	42.3	8.6	ns	ns	ns	0.645	0.901	0.087	0.041	ns	ns

BCF values for Cd were > 1 were ranked as: Fawn tall fescue, Alkar tall wheatgrass, Hycrest II crested wheatgrass, AI germplasm intermediate wheatgrass, Bozoisky II Russian wildrye, and Cache and Regar meadow bromegrass (Table 4). A decrease in soil pH was associated with an increase in tissue Cd levels. Whereas, a decrease in tissue Cd concentrations were observed when grown in soils with increased levels of Zn and Cu (Table 4) [10].

Tissue Mn concentrations ranged from 279 to 2,143 mg Mn kg^{-1} DM with 'Paiute' orchardgrass exceeding the 1,840 mg Cd kg^{-1} DW as the upper limit in plant tissue (Table 4) [7].

All plant materials except Ladak alfalfa and RS-H hybrid wheatgrass had Mn BCF values > 1 (Table 4), suggesting that they may be candidates for phytoextraction. Kabata-Pendias [7] suggested that an excess in phytoavailable Mn is associated with strongly acid soils (pH 5.5 or below), anaerobic condition, and heavily limed soils. Acidic soil (pH 4.26) and poor soil aeration in the Keating soil likely contributed to the observed increase in forage Mn levels. Increased concentrations of soil Zn have, in fact, been reported to depress Mn uptake, which may have contributed to the lack of forage Mn observed in the Clark Fork soil [7]. All orchardgrass Mn concentrations exceeded the minimum tolerable threshold of 1,000 mg kg^{-1} DM determined for cattle [13,14].

Tissue Zn concentrations in alfalfa, crested wheatgrass, Russian wildrye, orchardgrass, and tall fescue were greater than 125 mg Zn kg^{-1} DM reported in normal plant tissue [7]. However, all BCF values were < 0.4 suggesting limited value in phytoextraction of Zn from this soil (Table 4).

Conclusion

The need to identify plants that are adapted to environmental stresses present on most mine land reclamation sites is critical to the success of phyto remediation projects. In this study, metal

tissue concentrations of As, Cd, Mn, and Zn exceeded the upper limits of normal plant tissue (Tables 2-4). In addition, BCF values, an indicator of plants ability to extract metals from the soil, were > 1 for Cd, Mn, and Zn. Crested wheatgrass, intermediate wheatgrass, meadow and smooth brome grasses, Russian wildrye, tall wheatgrass, orchardgrass, and tall fescue appear to have some potential as a Cd accumulator when grown on soils with pH levels of 5.01 and 4.26 (Table 1). Due to BCF values > 1 for Mn uptake in this study, Hycrest II crested wheatgrass, AI intermediate wheatgrass, Regar and Cache meadow bromegrass, Manchar smooth bromegrass, NewHy RS-hybrid wheatgrass, Bozoisky II Russian wildrye Alkar tall wheatgrass, Paiute and UTDG-101 orchardgrass, and Fawn tall fescue could be possible materials for Mn accumulators in a phytoextraction program. Only RS-H RS-hybrid wheatgrass had a Zn BCF value > 1 and may be a candidate for Zn accumulation.

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