GROWTH RESPONSE AND METALS UPTAKE OF NATIVE BUNCHGRASSES DURING ORGANIC AMENDMENT-ASSISTED PHYTOSTABILIZATION OF ALKALINE MINE TAILINGS

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ABSTRACT

Tailings management is one of several challenges faced by mines operating in dry environments. If tailings are exposed, with no vegetative cover, dust from mine sites can spread over long distances through eolian dispersion and water erosion, posing a risk to human and environmental health. Phytostabilization is a remediation technique which involves promoting vegetation growth on mine sites in order to prevent erosion and stabilize metals belowground. Here we report the results of a greenhouse study in which two native bunchgrasses (bluebunch wheatgrass and rough fescue) were evaluated for their ability to grow on alkaline mine tailings from the historic Afton mine when amended with a range of concentrations of locally available soil amendments (compost and wood ash). The addition of compost lowered tailings pH and increased organic matter content. A positive correlation between compost concentration and total biomass was observed, likely due to improved soil conditions. Shoot and root biomass of bluebunch wheatgrass was significantly greater than that of rough fescue on all treatments. Both candidates minimized shoot accumulation of metals with the exception of Mo which exceeded the domestic animal toxicity limit. The results revealed that compost is a promising amendment for promoting bunchgrass growth on these tailings.

Key Words: mine reclamation, compost, wood ash, native species, grassland restoration

INTRODUCTION

As of 2015, there were 36 major metal and coal mines operating in British Columbia (BC) (Mining Association of British Columbia 2015). Several of these projects are located within the interior semi-arid grasslands, which are a unique ecoregion characteristic of hot, dry summers and minimal precipitation. Controlling dispersion of metal contaminants from tailings and other mine waste sites is one of several challenges faced by mines operating within these grasslands and other dry environments. If left barren, dust from mine waste sites can spread over long distances through eolian dispersion and water erosion (Munshower 1994), posing a risk to human and environmental health. For example, fine particulate waste materials (e.g. tailings) stored on mine sites are often high in toxic metals and other contaminants which can cause adverse health effects including respiratory disease, heart failure, and lung cancer, and also impact the surrounding environment by altering water chemistry and causing soil contamination (EPA 2016). Conventional remediation methods for controlling tailings dust include chemical (e.g. industrial tackifiers) and physical (e.g. waste rock, gravel, or clay capping) stabilization, however, these methods

pose economic challenges and do not provide a long term solution (Mendez and Maier 2007), especially during the post-closure phase of the mine cycle. Phytostabilization is an emerging technology which involves promoting vegetation growth on barren mine lands to control erosion and stabilize metals belowground, and may be a more sustainable alternative compared to conventional remediation techniques.

The goal of phytostabilization is to create a long-term vegetative cap in order to limit the movement of harmful metal contaminants from mine sites. Once established, the aboveground portion of the vegetation (canopy) acts to reduce wind erosion, whereas the belowground portion (roots) limits water erosion and immobilizes metals in the rhizosphere. The belowground processes involved in phytostabilization include precipitation of metals by bacterial and root surfaces, precipitation of metals by bacterial and root secretions, bacterial uptake of metals, and root uptake of metals (Mendez and Maier 2007). Phytostabilization differs from phytoextraction (another phytoremediation technology) in that the aim is to contain metals on site by minimizing shoot uptake and limiting metal bioavailability and subsequent entry into the food chain via livestock, wildlife, and human consumption. Contrarily, phytoextraction involves remediating contaminated materials by promoting hyperaccumulation of metals in shoots and requires removal of the toxic plant biomass from site which can be laborious and costly (Bolan et al., 2014). As mandated by federal and provincial regulations, reclamation of land disturbed from mining is the responsibility of the mining company (Government of British Columbia, Ministry of Energy 2008); if successfully implemented, phytostabilization in BC's semiarid grasslands can meet reclamation targets, while initiating ecological restoration and providing long term environmental and socioeconomic benefits (Wilson 2009).

The starting point for any revegetation project is the soil, or the degraded material left over from disturbance that has potential to develop into a soil over time (Bradshaw 1987). Mine tailings are the by-product of ore processing and consist of fine particulate matter which often lacks the physical, chemical, and biological properties of a productive soil (Gardner et al. 2010; Gardner et al. 2012), and therefore, are not a suitable growth medium for most terrestrial plants. In general, tailings are high in toxic metals such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), and zinc (Zn), which, contrary to organic contaminants, can persist in soils for long periods of time (Bolan et al., 2014). The mobility and bioavailability of most metals (e.g., Zn, Cu) increases with soil acidity, with a few exceptions such as molybdenum (Mo) and aluminum (Al) which can become available in alkaline conditions (Delhaize and Ryan 1995; EPA 2007). In addition to metal toxicity, abnormal pH levels, altered soil microbial communities, limited pore space, low amounts of plant nutrients, and poor water retention make up the factors limiting vegetation establishment success on mine tailings (Sheoran et al. 2010). Soil amendments are commonly used in revegetation projects to mitigate these ecological shortcomings and have been proven successful in several scenarios (Brown et al. 2007; Shrestha et al. 2009; Gardner et al. 2010; Drozdowski et al. 2012; Gardner et al. 2012).

Soil amendments include organic amendments (e.g. municipal compost, sewage sludge, wood chips) and liming agents (e.g. wood ash, fly ash). Generally, organic amendments are used to enhance plant growth by providing nutrients and improving soil physical properties, whereas liming amendments are used to reduce phytotoxicity by neutralizing acidic soils (EPA 2007), although, these effects can overlap. Manipulating soil properties such as pH and organic matter with soil amendments can influence the bioavailability of soil-borne metals and potentially mitigate any environmental or health risks caused by toxic metals (Bolan et al., 2014). In a greenhouse investigation Solís-Dominguez et al. (2012) used compost to increase the pH of acidic iron mine tailings, which reduced metal bioavailability and made the

tailings substrate more suitable for plant growth. In a review study Ussiri and Lal (2005) reported improved physical (bulk density, soil aggregation, and water-holding capacity) and chemical (pH and electrical conductivity) properties when coal mine soils were amended with fly ash (a by-product of coal combustion). Furthermore, in a greenhouse experiment, Piorkowski et al. (2015) found a positive synergistic effect on plant performance when a blend of biosolids and compost was utilized rather than a single amendment due to each amendment having its own unique properties. Although soil amendments can be the answer to poor productivity on mine sites, questions remain regarding the economics, particularly relating to the availability and the transport of large volumes. As such, investigation into sourcing economically and ecologically viable, locally available materials can be beneficial for mine operations conducting phytostabilization and other reclamation projects.

Once proper site preparation and soil amelioration is completed, revegetation can be conducted. Revegetation in arid and semiarid environments is exceptionally challenging due to a variety of environmental factors, such as reduced moisture availability and high temperatures, which can limit seed germination and establishment success (Padilla and Pugnaire 2006). Traditionally, non-native species have been utilized for mine reclamation because of their tendency to successfully germinate and establish in harsh environments, and also because of their low cost and ease of availability in large quantities (Burton and Burton 2002; Skousen and Venable 2008; Oliveira et al. 2012). Although, more recently, the disadvantages of using non-native species are becoming increasingly recognized. For example, evidence suggests that, due to their competitive nature, non-native species can alter the trajectory of ecological succession by preventing colonization and establishment of native species (Davis et al. 2005). In recent decades, attention has shifted towards restoration using native plant species for reclamation because of their potential to enhance ecosystem function and services, as well as socioeconomic benefits (Burton and Burton 2002; Skousen and Venable 2008; Kiehl et al. 2010). Researchers have explored the suitability of several plant species for phytostabilization across a wide range of environments, but we still remain in the information gathering stage regarding species-specific responses to mine tailings (Solís-Dominguez et al. 2012). It is known that candidate species must minimize accumulation of metals in their shoots and tolerate elevated metals, high salinity, and abnormal pH levels (Mendez and Maier 2007; Solís-Dominguez et al. 2012). Adaptation to the local climatic conditions is also ideal and thus native species are often preferred. Tremendous merit can be derived from investigating the suitability of native species for phytostabilization.

Bluebunch wheatgrass and rough fescue are both bunchgrass species with high forage value, and are native to BC's interior semiarid grasslands (Government of British Columbia 1991; USDA 2016). Bluebunch wheatgrass tends to occupy low elevation areas and can generally tolerate drier conditions compared to rough fescue which is predominant at higher elevations and is less suited to drought (Dobb and Burton 2013). Both of these grasses are potential candidates for phytostabilization of tailings sites located in interior BC, but little is known regarding their tolerance to soil metal contaminants and their ability to grow on amended mine spoils (e.g., Thorne et al., 1998). The tailings discharged from the historic Afton copper and gold mine (near Kamloops, BC) are currently undergoing reclamation, and dust mitigation is one of the primary objectives of the mining company that currently holds mineral title (KGHM International Ltd.). The historic Afton tailings are moderately alkaline (>8.5), high in copper (600 mg kg^{-1}) and molybdenum (10.5 mg kg^{-1}), and influenced by a semiarid climate, which provides us with a unique opportunity to conduct phytostabilization research using locally available soil amendments and native grassland species. This study summarizes the results of a greenhouse experiment which was designed to 1) evaluate two locally available organic amendments (municipal compost and wood ash) in terms of plant growth response on the historic Afton mine tailings; and 2) assess whether bluebunch

wheatgrass and rough fescue are suitable candidates for phytostabilization of these tailings in terms of growth response and metals uptake. Here we aim to couple phytostabilzation techniques with native grassland restoration practices in order to achieve both short and long term benefits from revegetation of the historic Afton tailings.

MATERIALS AND METHODS

Mine tailings and amendment analysis
Bulk tailings samples were collected from the historic Afton mine, approximately 15 km west of Kamloops, British Columbia (GPS coordinates 50° 39' N, 120° 32' W; elevation 700 m). The compost amendment was produced from municipal yard waste at the Cinnamon Ridge compost facility (Kamloops, BC). The ash was sourced from the Domtar pulp mill (Kamloops, BC) and is a byproduct of waste wood (commonly referred to as 'hog fuel') incineration. Both amendments were available within a 15 km radius of the historic Afton tailings site. Samples were passed through a 2 mm sieve and analyzed for pH and electrical conductivity (EC) using a Hanna Combo electrode device in a 2:1 (soil: deionized water, by mass) solution reacted for 1 h (modified from Hayes et al. 2009). Soil texture was classified for tailings samples only, using the pipet sedimentation method (Hayes et al. 2009). Particle size distribution of the amendments was conducted using sieves with mesh sizes ranging from 0.1 to 16 mm. Organic content was determined by loss on ignition (550 °C for 6 h). Subsamples of the tailings and amendments were sent to the British Columbia Ministry of Environment Analytical Laboratory (BCMEAL) (Victoria, BC) for analysis of total carbon (C), total nitrogen (N) and metal concentration including the elements aluminum (Al), As, Cd, cobalt (Co), Cr, Cu, iron (Fe), Hg, molybdenum (Mo), nickel (Ni), Pb, and Zn.

Greenhouse experiment
The greenhouse experiment was conducted at the Thompson Rivers University Research Greenhouse (Fraser Lab) in Kamloops, BC. The experiment was designed to investigate the effects of compost and ash amendments on native bunchgrass growth, and to evaluate the suitability of the selected plant species for phytostabilization of the historic Afton tailings. Two representative forage bunchgrass species of the interior semiarid grasslands were selected using the 'species objective' filters in the British Columbia Rangeland Seeding Manual (Dobb and Burton 2013). Bluebunch wheatgrass was chosen primarily for its drought tolerance while rough fescue was selected for its tendency to occur naturally at similar elevations to the study site. A total of 13 ash-compost combinations ranging from 0-100% (w/w) of compost and wood ash, and 0-10% (w/w) of wood chips were evaluated using a randomized complete block design with 10 replicates. Three subcategories of treatments were selected for further analysis: 'ash' (100% ash), 'compost' (100% compost), and blend (40% ash, 50% compost, 10% wood chips). A separate germination trial was conducted to determine seed viability.

The growth experiment was conducted under controlled conditions (natural and artificial light day/night 18 h/6 h, temperature day/night 21 °C /15 °C, humidity 50-60%) in the research greenhouse. Half gallon nursery pots with drainage (15 cm top diameter X 14 cm height X 14 cm bottom diameter) were filled with 500 g of tailings and amended with 150 g (a field equivalent to 150 Mg ha^{-1}) of ash-compost-wood chip mixtures. Bluebunch wheatgrass and rough fescue seeds were sown at a density of 15 seeds per pot at a depth of approximately 0.5 cm. Pots were watered evenly on every second day using a garden hose fitted with a perforated spout. Plant root and shoot tissues were harvested 90 d after seeds were sown.

Plant productivity and metals uptake data collection
Bunchgrass shoots were clipped at the soil surface and roots were retrieved from the amended tailings substrate. Plant tissue samples were washed and dried (70 °C for 48 h), then weighed on an analytical scale to determine root and shoot biomass. Three composite biomass samples (roots and shoots) were

prepared from the amendment treatment subcategories ('ash', 'compost', and 'blend') for analysis of plant tissue elemental concentration by the BCMEAL.

Statistical analysis
Seedling germination rates, plant biomass, and tissue metal content data were analyzed in R version 3.2.3 "Wooden Christmas-Tree" (The R Foundation for Statistical Computing). Significances between species were determined using the Welch's two sample t-test. One-way and two-way analysis of variance (ANOVA) tests were employed to find significances between treatment means. Analysis of covariance (ANCOVA) was employed to control for seedling density when assessing plant productivity metrics. Treatments were grouped and ranked using Tukey's HSD test ($p < 0.05$).

RESULTS & DISCUSSION

Mine tailings and amendment characteristics
Soil texture analysis revealed that the historic Afton tailings had a sandy clay loam texture (52.9% sand, 26.5% silt, and 20.6% clay). Soil texture has a strong influence on water and nutrient retention, and generally, loamy soils are most preferred for agricultural production (Brady 1990). The volumetric water holding capacity (WHC) of the tailings was relatively high (Table 1), thus water retention was not considered a limitation. Although, high WHC can lead to poor drainage and anoxic conditions which can affect root productivity (Brady 1990). The tailings were also characterized by a moderately alkaline pH and low amounts of organic matter, total carbon, total nitrogen and phosphorus. Analysis of tailings for metals revealed high amounts of Cr, Cu, Mo, and Ni (Table 2). Of these metals, Cu, Cr, and Ni exceeded the CCME guidelines for industrial land use, while Mo exceeded the less rigid guideline for agricultural land use (Canadian Council of Ministers of the Environment 2014).

Table 1. Select chemical and physical parameters of mine tailings, organic amendments, and amendment mixtures used for this study.

Substrate/ Treatment	pH	Organic Matter (%)	C (%)	N (%)	C:N	P (%)	K (%)	EC* (dS/m)	WHC** (%)
Tailings	8.7	0.1	1.12	0.01	112:1	0.11	1.09	2.1	69.5
Compost	7.8	26.8	24.3	1.18	21:1	0.30	1.32	3.5	50.2
Ash	10.3	23.9	22.5	0.05	450:1	0.47	2.49	2.0	31.0
Wood chips	7.5	97.7	56.7	0.12	473:1			0.5	22.9
100% ash	9.3	3.9	2.3					2.4	63.6
100% compost	8.1	4.6	2.7					3.0	69.0
Blend	8.7	4.3	2.5					2.0	65.4

*EC, electrical conductivity
**WHC, volumetric water holding capacity

The municipal yard waste compost was mostly made up of organic material and sands ranging from 0.1 to 4 mm, but also contained some large woody debris and coarse rocks ($\leq$ 16 mm diameter). The compost was characterized by a slightly alkaline pH, adequate total nitrogen, and a well-balanced C: N ratio (Table 1). Of the substrates studied, the compost had the highest electrical conductivity (EC), though, remained below the threshold of 4 dS m^{-1} at which plant growth becomes inhibited (Drozdowski et al. 2012). Metal contents of the investigated compost met the CCME guidelines for both agricultural and industrial land uses (Table 2).

The wood ash amendment was primarily composed of fine particles ranging from 2 to 4 mm. This amendment had a considerably high pH and C: N ratio (Table 1), as well as elevated levels of Al and Zn (Table 2), which raised concern regarding its suitability for this study. In spite of these limitations we assessed the ash in this study because of its potential to enhance plant performance due to other potentially favourable characteristics such as high levels of phosphorus (P) and potassium (K), which are also important plant nutrients.

The wood chips (used for the "blend" treatment) ranged from 1 to 16 mm in size and were primarily composed of organic matter and carbon. Because of these properties, wood chips can be a useful tool for adjusting the C:N ratio of reclamation materials and also for preventing leeching of N from the rooting zone (Piorkowski et al. 2015).

Table 2. Select metal and metalloid concentrations (mg/kg) of mine tailings and amendments used for this study compared to CCME guidelines for agricultural and industrial uses.

Element	Ash	Compost	Tailings	CCME* (agricultural)	CCME (industrial)
Al	1967	828	74.6	-	-
As	< 3.0	< 3.0	< 3.0	12	12
Cd	< 1.0	< 1.0	< 1.0	1.4	22
Co	25.7	16.9	30.8	40.0	300
Cr	51.8	52.2	138	64	87
Cu	70.7	77.9	600	63	91
Fe	486	545	525	-	-
Hg	< 3	< 3	< 3	6.6	60
Mo	3.15	3.81	10.5	5	40
Ni	30.9	26.1	90.7	45.0	89
Pb	< 1.0	6.6	< 1.0	70	600
Zn	216	106	19.6	200	360

*CCME, Canadian Council of Ministers of the Environment soil quality guidelines (2014).

Effects of amendments on tailings characteristics

We compared soil chemical and physical parameters of the tailings before and after amendments were added. As expected, the addition of soil amendments, regardless of treatment, increased the amount of organic matter and total C in the tailings (Table 1). The addition of organic matter is beneficial for soil reclamation because doing so leads to improved nutrient availability and enhanced soil physical and chemical conditions (Shrestha et al. 2009; Drozdowski et al. 2012). For example, in a tailings reclamation study, Gardner et al. (2010) attributed increases in cation exchange capacity and water holding capacity to organic matter inputs from biosolids. Further, organic matter increases biochemical activity by enhancing microbial decomposition (Brady 1990). Gardner et al. (2010) also found increased numbers of aerobic heterotrophic bacteria in the amended tailings compared to the unamended tailings. Although we did not assess microbial populations in this study, it is likely that the addition of compost and ash had a positive effect on their numbers and biological activity.

The compost amendment lowered tailings pH, but the effect was suboptimal as the amended tailings remained moderately alkaline (pH > 8). In a recent review, Sheoran et al. (2010) reported that mine soil

pH range of 6 to 7.5 is adequate for agronomic or horticultural uses, however in arid environments it is common for pH to be slightly to moderately alkaline (pH between 7 and 9) (Brady 1990). The ash amendment increased the tailings pH level from moderately alkaline to strongly alkaline (> 9), which is above what is deemed normal in most soils (normal range is 5.5 to 8.5). Abnormally high soil pH can lead to mobility of As, Mo, and Se as well as reduced availability of P and certain micronutrients (e.g., B, Mn, Fe) (EPA 2007). When a blend of the amendments was used, the pH remained moderately alkaline and organic matter and total C content were intermediate. In general, the addition of organic matter increased the EC of the tailings with the exception of the blend which had little or no effect. In all treatments, EC remained below the critical level of 4 dS m^{-1}. Based on these parameters, the compost treatment appeared to provide the most suitable soil conditions for revegetation of the historic Afton tailings.

Native bunchgrass response to soil amendments

Germination occurred on the amended tailings within four to ten days depending on species and growth medium. Soil amendments, regardless of composition, had a significant effect ($p < 0.05$) on bluebunch wheatgrass and rough fescue germination compared to the unamended tailings, with the exception of the fescue growing on the compost amended tailings (Figure 1a). Germination success was low on the unamended tailings for both grass species. When comparing germination rates between species, bluebunch wheatgrass outperformed rough fescue in all treatments, but statistical significances were only detected in the ash treatment. This could be attributed to the ability of bluebunch wheatgrass to germinate under a wider range of conditions, as shown by Young et al., (1981). For bluebunch wheatgrass, the germination rates obtained on the amended tailings were lower compared to those obtained during the germination trial, while rough fescue germination was comparable. Despite obtaining some statistical differences, no clear relationship was observed between amendment relative composition and seedling germination rates. In general, the soil amendments substantially improved seed germination of the grass species studied. This is likely due to enhanced availability of nitrates and other macronutrients resulting from organic matter addition (Rivard and Woodard 1989). Despite successful germination, neither one of the grass species developed seed heads in any of the treated soils during the 90 d of growth.

Bluebunch wheatgrass shoots were taller than rough fescue shoots on all treatments, but significances were only detected on the ash- and the compost-amended tailings (Figure 1b). The compost-amended tailings yielded the tallest shoots for both species, however, shoot heights were not statistically significant compared the blend treatment. The addition of compost significantly increased shoot heights for both species, likely due to improved nutrient availability (i.e. N) within the growth medium. There was evidence of a positive correlation (R^2=0.42, $p < 0.0001$) between compost concentration and bluebunch wheatgrass seedling height (data not shown). A similar pattern was observed for rough fescue, however, this association was less prominent (R^2=0.25, $p < 0.0001$).

Plant productivity was sparse on the unamended tailings; despite some of the seeds germinating, survival was low. Shoot and root biomass of bluebunch wheatgrass were significantly greater ($p < 0.05$) than that of rough fescue on all treatments (Figures 2a and 2b). When amendments were used, this difference was over twofold. In a field experiment conducted in Lac du Bois Grassland Provincial Park (near Kamloops, BC), (Carlyle 2012) reported greater relative growth rates and shoot and root biomass for bluebunch wheatgrass compared to rough fescue under natural and manipulated moisture conditions. The compost treatment yielded the highest shoot and root biomass (up to 1.4 g total dry biomass per pot) while the unamended tailings yielded the lowest; this was true for both species. In terms of total biomass, the ash was the least productive amendment while the blend was intermediate. Statistically, there were no differences in root biomass between the compost and the blend amendment. There were also no statistical

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differences in root biomass between the ash and the blend treatments. When comparing biomass productivity between species, bluebunch wheatgrass outperformed rough fescue in all treatments.
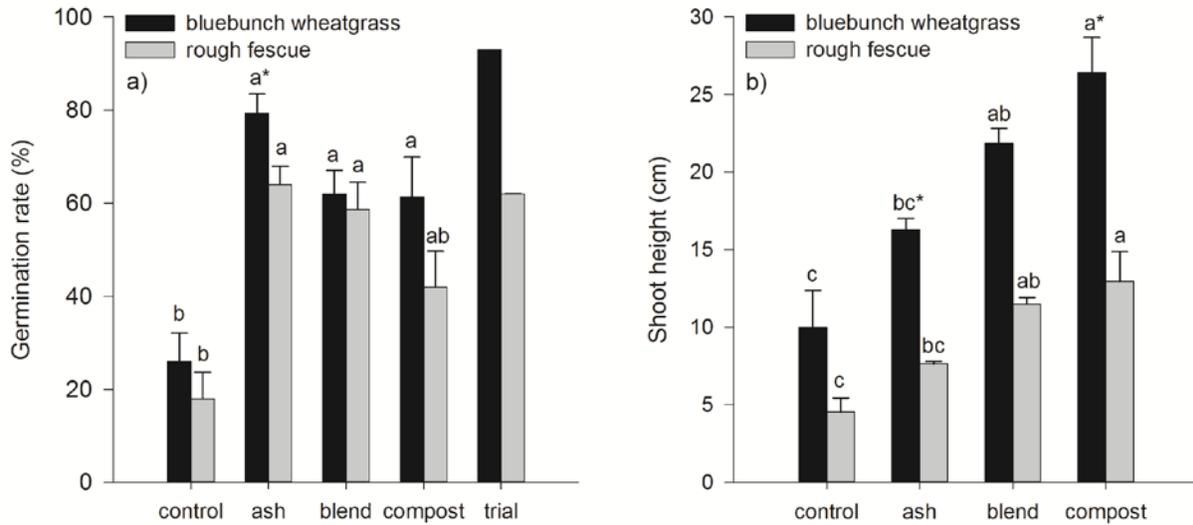

Figure 1. Mean bluebunch wheatgrass and rough fescue a) germination rates and b) shoot heights by treatment after 90 d growth in amended mine tailings. In a) "trial" represents results of 21 d seed viability study conducted in the greenhouse. Error bars are standard errors of the mean. Treatments with different letters are statistically significant at $p < 0.05$ (one-way ANOVA, Tukey's HSD). * represents statistical significance between species (determined by t-test) for that treatment.
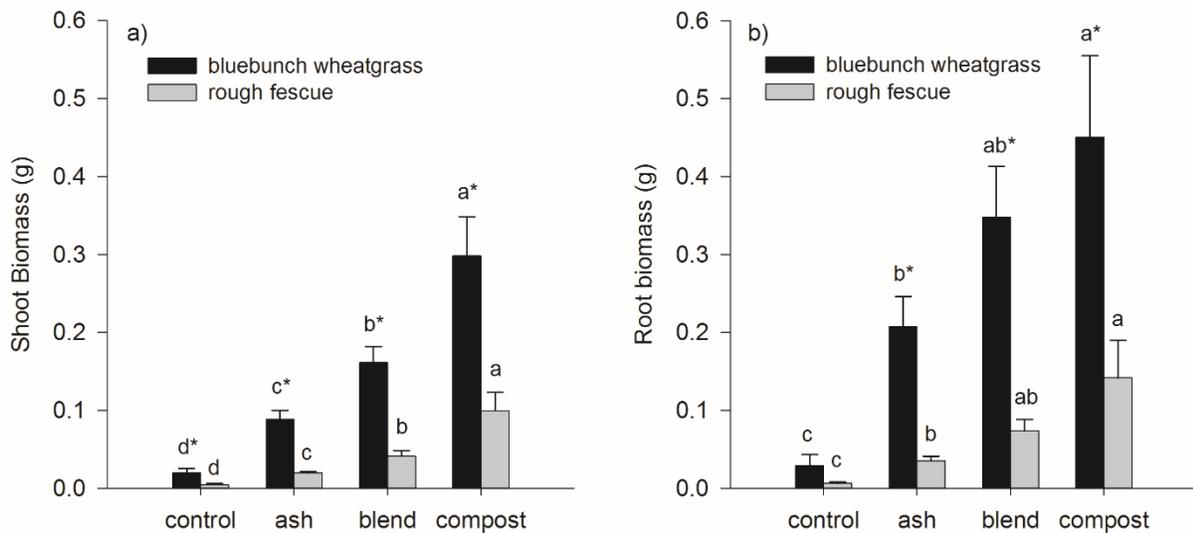

Figure 2. Mean bluebunch wheatgrass and rough fescue a) shoot biomass and b) root biomass per pot by treatment after 90 d growth in amended mine tailings. Error bars are standard errors of the mean.

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Treatments with different letters are statistically significant at $p < 0.05$ (one-way ANOVA, Tukey's HSD). * represents statistical significance between species (determined by t-test) for that treatment.

Plants growing in the ash amended tailings were stunted and showed signs of nutrient deficiency, probably a result of the poor soil chemical conditions such as high pH and lack of nitrogen. Under abnormal pH conditions (< 5.5 to > 8.5) certain nutrients (e.g. N, P, K) become limiting and microbial activity is stalled (EPA 2007). As such, incorporating the very strong alkaline ash material into the alkaline tailings was not an effective method for promoting plant growth.

A positive linear association between compost concentration and total biomass was detected (Figures 3a and 3b), although this relationship was weak. On the contrary, total biomass responded negatively to ash concentration. When we controlled for seedling density (using ANCOVA), this relationship was strengthened (R^2=0.48 and 0.45 for bluebunch wheatgrass and rough fescue, respectively). Although these data suggest that the compost is the most suitable amendment for plant growth on the historic Afton tailings at the rate studied (150 Mg ha^{-1}), it would be beneficial to further investigate plant biomass responses to higher rates of compost. We suspect such will result in a stronger association between biomass productivity and compost concentration.
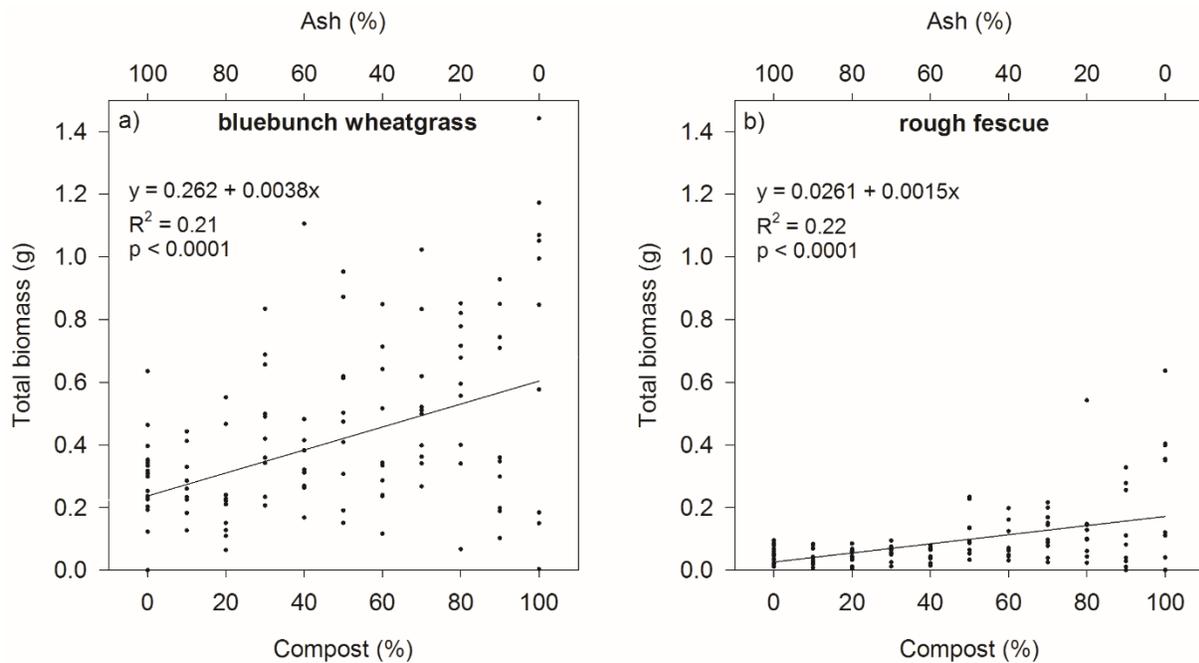

Figure 3. Relationship between a) bluebunch wheatgrass total biomass (roots + shoots) and b) rough fescue total biomass per pot and relative concentrations of compost and ash in the soil amendment mixtures. Data points are untransformed raw data.

Native bunchgrass root to shoot ratios ranged from $< 1:1$ on the unamended tailings up to 3:1 in the ash treatment (data not shown). Root: to shoot ratios were around 1 for the compost treatment which indicates

balanced biomass allocation and adequate nutrient availability in the amended substrate. Generally, when nutrients are limiting, plants will allocate more resources to their roots (Ågren and Franklin 2003). In this study, the high root: shoot ratios of plants growing in the ash-amended tailings can be explained by the lack of nitrogen in the growing medium which may have forced plants to allocate more effort into root production at the cost of shoot production. The low root: shoot ratio of grasses growing on the unamended tailings was attributed to limited seedling survival.

Metals accumulation in plant tissues

Shoot concentrations of select metals were determined for both plant species growing on the three amendment mixtures (Table 3). Analysis indicated that Cu, Fe, and Mo concentrations of plant tissues were greater than the domestic animal toxicity limit in several of the treatments. Although, the only significant treatment effect was for Mo; both grass species accumulated a substantially greater amount of Mo when growing in the ash-amended tailings compared to the other treatments. We also looked for differences in shoot metal accumulation between species and found that Mo concentration was significantly greater (nearly twofold) in bluebunch tissue compared to rough fescue when grown on compost-amended tailings.

Table 3. Plant shoot accumulation (mg kg^{-1}) of select metals and metalloids after 90 d growth in amended mine tailings

Element	Total[a]	MTL[b]	Amendment treatment	shoot tissue metal accumulation			TF[e] (BB)	TF (RF)
				Bluebunch wheatgrass	Rough fescue	BB vs. RF (t-test)[d]		
Al	75	1000	100% compost	361 ± 93.0 a	245 ± 108 a	NS	0.08 ± 0.02	0.07 ± 0.04
			100% ash	526 ± 99.4 a	697 ± 291 a	NS	0.15 ± 0.06	0.3 ± 0.11
			blend	323 ± 46.6 a	442 ± 202 a	NS	0.07 ± 0.02	0.15 ± 0.04
Cu	600	40	100% compost	26.4 ± 1.69 a	96.5 ± 43.9 a	NS	0.27 ± 0.02	0.76 ± 0.36
			100% ash	27.1 ± 2.25 a	24.0 ± 7.53 a	NS	0.33 ± 0.14	0.28 ± 0.09
			blend	22.7 ± 0.68 a	22.3 ± 8.54 a	NS	0.24 ± 0.03	0.21 ± 0.07
Fe	525	500	100% compost	331 ± 65.1 a	273 ± 71.4 a	NS	0.08 ± 0.02	0.08 ± 0.03
			100% ash	457 ± 72.8 a	576 ± 216 a	NS	0.18 ± 0.08	0.33 ± 0.11
			blend	308 ± 32.1 a	343 ± 147 a	NS	0.08 ± 0.02	0.12 ± 0.03
Mo	21.9	5	100% compost	37.4 ± 1.32 b	19.7 ± 3.28 b	*	2.44 ± 0.16	0.41 ± 0.02
			100% ash	183 ± 46.5 a	202 ± 14.4 a	NS	12.5 ± 4.62	2.53 ± 0.30
			blend	58.3 ± 15.7 b	48.4 ± 5.41 b	NS	3.43 ± 0.47	1.01 ± 0.18
Zn	< 3.0	500	100% compost	34.9 ± 1.18 a	85.9 ± 27.7 a	NS	0.89 ± 0.04	1.60 ± 0.47
			100% ash	29.3 ± 1.0 a	28.1 ± 3.78 a	NS	1.46 ± 0.34	1.02 ± 0.28
			blend	32.1 ± 1.95 a	38.8 ± 6.96 a	NS	0.87 ± 0.08	0.90 ± 0.22

[a]Total elemental concentration of mine tailings prior to planting. Values are means ± standard error of the mean. [b]MTL = maximum tolerable levels of metals in the shoots; values are for cattle (National Research Council 2005). [c]Treatment means with different letters are statistically significant at $p < 0.05$ (one-way ANOVA, Tukey's HSD) for each species corresponding to each element. [d]Welch's two sample t-tests were performed for each row (NS = non-significant; * = significant difference); BB = bluebunch

wheatgrass, RF = rough fescue. [e]TF = translocation factor; the shoot:root ratio of the concentration of the corresponding element.

In examining the results more closely, Fe only exceeded the toxicity limit when fescue was grown on the ash-amended tailings and this exceedance was negligible when considering sampling error (i.e. high standard error). Copper concentration in fescue tissues also exceeded the toxicity threshold, but again the difference was minimal. It is worth noting that Cu and Fe toxicity is unlikely in alkaline soils because both metals are less available (Audebert and Sahrawat 2000; EPA 2007).

Of particular concern were the high concentrations of Mo for both plant species which exceeded toxicity limits in all treatments, most notably when the ash amendment was used. Elevated Mo levels can lead to molybdenosis (induced Cu deficiency) when ingested by cattle or other ruminants (Gardner et al. 2012). This condition is influenced by relative concentrations of copper, molybdenum, and sulfur. In general, the risk of molybdenosis increases when the Cu: Mo ratio is < 2:1 (Mason 1971; cited by Gardner et al., 2012). In this study, Cu: Mo ratios for all treatments were well below this threshold, with the highest ratio being for the wheatgrass growing in the compost treatment (0.7:1). The enhanced Mo uptake by grasses growing on the ash-amended tailings was likely the result of elevated soil pH levels. Doran and Martens, (1972) found similar effects using alfalfa and a fly-ash amendment. Under abnormally high pH conditions (pH > 8.5) molybdenum is soluble and readily available for plant uptake (EPA 2007). This information suggests that bluebunch wheatgrass and rough fescue may not be suitable for phytostabilization of these tailings due to their tendency to uptake molybdenum under alkaline conditions. However, further additions of compost and/or wood chips may be worth investigating because doing so may further neutralize tailings pH, thereby reducing the potential for Mo uptake and lowering the risk of molybdenosis.

Aluminum concentration of the ash amendment was notably high (Table 2). When coupled with its high pH (Table 1) this created ideal conditions for the formation of soluble Al in the form of aluminate ($Al(OH)_4^{-1}$) which can cause soil toxicity and inhibit plant growth (Fuller and Richardson 1986). Despite the high aluminum content of the wood ash, shoot tissue aluminum concentration remained below the domestic toxicity limit (Table 3). According to (Hodson 2012), some plants are able to tolerate excessive levels of aluminum and other metals by avoiding shoot uptake and concentrating them in their roots. Both plant species used in this study accumulated substantially more aluminum in their roots (up to seven times, data not shown) compared to their shoots which provides some indication of their tolerance to aluminum. These results suggest that these species may be useful for remediation of tailings and other mine wastes high in aluminum.

The translocation factor (TF) is a useful metric for measuring metal accumulation in plant tissues. Suitable candidate plant species for phytostabilization are those which minimize shoot accumulation without limiting root uptake, thus TF values of < 1 are preferred (Mendez and Maier 2007). While TF generally remained below this threshold in both species for most of the metals investigated, values for Mo and Zn exceeded (or were close to) this threshold in all treatments (Table 3).

CONCLUSION

Of the organic amendments investigated in this study, the City of Kamloops municipal compost was the most effective in promoting bluebunch wheatgrass and rough fescue growth on the amended tailings. Further investigation using higher rates of compost would be meritable because we suspect this will result

in enhanced plant performance and reduced Mo uptake. Due to its high pH and elevated aluminum content, the Domtar pulp mill wood ash was not suitable for amelioration of the alkaline mine tailings, as plants growing in the ash amended tailings were subjected to the ideal conditions for aluminate toxicity. However, there may be potential to use this amendment for remediation of acidic mine tailings such as those investigated by Solís-Dominguez et al. (2012).

Rough fescue growth on the amended tailings was sparse in comparison to bluebunch wheatgrass. Although the latter exhibited good germination and growth, it also accumulated elevated levels of Mo in its shoots which counted against its candidacy for phytostabilization and use as a forage species at the Historic Afton mine site. There may be potential to use this species in other technologies such as phytoremediation where shoot accumulation is encouraged and aboveground biomass is removed from site. Despite this verdict, further investigation of these grass species is required because it is likely that under optimal soil pH conditions (pH between 6 to 7.5), Mo uptake will decrease. Both grasses minimized shoot uptake of aluminum when present in high quantities by concentrating it in their roots, which prompts investigation of these species' performance on aluminum rich mine wastes.

In summary, our study provides practical information regarding the suitability of soil amendments available in the Kamloops region and the performance of native grassland species during restoration and phytostabilization of alkaline mine tailings. In addition to this information being directly applicable to reclamation at the historic Afton mine, it may also be useful for remediation planning and implementation at other sites located in similar environments. Further research is needed to investigate native bunchgrass performance on the compost amendment more closely, and to test the greenhouse results in the field.

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