Research Article

Semiarid bunchgrasses accumulate molybdenum on alkaline copper mine tailings: assessing phytostabilization in the greenhouse

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Abstract
Phytostabilization is the use of plants and soil amendments to physically stabilize and remediate contaminated mine wastes and to control wind and water erosion in semiarid environments. The aim of this study was to evaluate two native bunchgrass species' (*Pseudoroegneria spicata* and *Festuca campestris*) biomass accumulation and metals uptake response to locally available soil amendments (compost, wood ash and wood chips) to determine their suitability for phytostabilization at an alkaline copper mine tailings site in British Columbia, Canada. In the greenhouse, bunchgrasses important as forage for livestock and wildlife were grown in tailings with various ash–compost–wood chip combinations and evaluated using a randomized complete block design with 13 treatments and 10 replicates. Plants were harvested after 90 d, and tissues were analyzed for root and shoot biomass. Tissue samples ($n = 3$) from three treatment subsets (ash, compost, blend) were selected for elemental analysis. Biomass increased with increasing compost applications, and the response was greatest for *P. spicata*. Shoot molybdenum exceeded the maximum tolerable level for cattle and was significantly higher when grasses were grown on the ash treatment (183–202 mg kg^{-1}) compared to the others (19.7–58.3 mg kg^{-1}). Translocation and root bioconcentration factors were highest on the ash treatment (2.53–12.5 and 1.75–7.96, respectively) compared to the other treatments (0.41–3.43 and 1.47–4.79, respectively) and indicate that both species are 'accumulators.' The findings suggest that these bunchgrasses were not ideal candidates for phytostabilization due to high shoot tissue molybdenum accumulation, but provide important considerations for mine restoration in semiarid grassland systems.

Article Highlights

- Compost increased *P. spicata* and *F. campestris* root and shoot biomass on alkaline copper mine tailings.
- Ash increased tailings pH and bunchgrass shoot uptake of molybdenum.
- *P. spicata* and *F. campestris* are 'accumulators' of molybdenum on alkaline copper mine tailings.

Keywords Phytoremediation · Soil amendments · Mine reclamation · Grassland restoration · *Pseudoroegneria spicata* · *Festuca campestris*

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1 Introduction

Sustainable management of mine tailings and other wastes presents several challenges during the mining life cycle [1, 2], particularly when sites are situated in arid or semiarid environments [3, 4]. If not effectively stabilized, the barren mine tailings can disperse to surrounding areas through airborne dust and water erosion, posing a risk to environmental and human health [5, 6]. Conventional methods for stabilizing tailings include use of chemical tackifiers and physical capping, but these options do not provide a long-term solution [7]. Phytostabilization is another approach and involves the use of vegetation to stabilize and remediate the contaminated mine waste [8, 9].

The goal of phytostabilization is to create long-term vegetative cover that minimizes wind and water erosion and immobilizes metals belowground [4, 10]. Once established, the canopy acts to reduce erosion, while the roots consolidate the loose material and facilitate plant and bacterial uptake and/or precipitation of metals in the subsurface [7]. Ideal species for phytostabilization have high biomass production and are 'excluder' plants which minimize shoot metal accumulation while tolerating elevated metals concentrations in the soil [4, 7, 8, 11]. Various plant species (e.g., *Brassica juncea*, *Medicago sativa*, *Agrostis tenuis*) have been assessed for phytostabilization across a wide range of environments [8, 12–14], but further research regarding site-specific and species-specific responses is required [4, 8]. If successfully implemented, phytostabilization can help mining companies mitigate pollution, restore native plant communities and return previous ecosystem services to the degraded land [8, 15, 16].

Mine tailings consist of fine particulate matter which lacks the physical, chemical and biological properties of a productive soil [17–19]. The material is often characterized as having unstable, extreme pH levels (<3 or >9), lack of plant nutrients, and poor water retention, which collectively act to hinder plant growth and establishment [20, 21]. The wastes are generally high in heavy metals [22, 23] that are toxic to plants [12, 24] and can persist in soils for long periods of time [25, 26]. Generally, the bioavailability of most heavy metals (e.g., copper, lead, nickel, zinc) increases with soil acidity [25, 27], with a few exceptions such as molybdenum and aluminum, which become available in alkaline conditions [28, 29]. Without a mechanism for stabilizing these contaminants, mine tailings can pollute surface- and groundwater (via run-off and leaching) and surrounding terrestrial environments (via wind erosion), or enter the food chain through plant uptake and herbivory [8, 10, 13].

Due to these physiochemical limitations, practitioners use soil amendments to ameliorate the tailings prior to revegetating [13, 30]. Soil amendments include organic amendments (e.g., municipal compost, biosolids, wood chips) and liming agents (e.g., wood ash, fly ash). Generally, organic amendments improve soil physiochemical conditions by providing key plant nutrients (e.g., N, P and K), enhancing microbial activity and nutrient cycling, and ameliorating unfavorable physical properties (e.g., high bulk density, low water holding capacity) [13, 17]; whereas high pH liming amendments can neutralize acidic soils, thereby immobilizing metals and mitigating phytotoxicity [31, 32]. Soil amendments are an important component of phytostabilization because they can improve plant growth and influence the availability of soil-borne metals for plant uptake [8, 25, 33].

The historic Afton tailings storage facility (HATSF), located at a decommissioned copper mine in Interior British Columbia (BC), Canada, is currently undergoing reclamation. The end land-use goals for the facility include: (1) restoring the native grassland community, (2) providing habitat and forage for wildlife and cattle and (3) stabilizing the tailings to mitigate potential adverse effects on the surrounding communities and ecosystems. The alkaline (pH > 8.5) mine tailings are high in copper (600 mg kg^{-1}) and molybdenum (10.5 mg kg^{-1}) and lie within a semiarid shrub–steppe ecosystem where predominant native forage plants include cool season bunchgrasses *Pseudoroegneria spicata* (Pursh) Á. Löve (bluebunch wheatgrass) and *Festuca campestris* Rydb. (rough fescue) [34]. As key components of the surrounding native grassland community, both species are potential candidates for phytostabilization at the site; however, few studies have assessed their tolerance to soil metal contaminants or their ability to grow on amended mine soils [12, 35–37]. Therefore, the HATSF provides us with a unique opportunity to assess locally available soil amendments and native bunchgrass performance on the alkaline copper mine tailings while contributing to the growing body of phytostabilization research.

This study summarizes the results of a greenhouse study, which was designed to: (1) characterize the HATSF mine tailings and three locally available soil amendments (compost, wood ash and wood chips), (2) assess *P. spicata* and *F. campestris* biomass growth and metals uptake response on the amended tailings and (3) determine the suitability of these native bunchgrasses for phytostabilization of copper and molybdenum at the HATSF.

2 Materials and methods

2.1 Site description

The HATSF (owned by New Gold Inc.) is located approximately 15 km west of Kamloops, B.C., Canada (50°39′ N, 120°32′ W; elevation 700 m). The region experiences a semiarid climate (annual precipitation < 350 mm) and hot, dry summer months. The copper mine tailings are designated as unclassified unconsolidated material and originated from an alkaline suite porphyry copper deposit [38] mined from the Afton, Ajax, Pothook and Crescent deposits during previous operations, which spanned from 1977 to 1997 [39]. Reclamation was initiated at the ~ 75 hectare facility in 1978 to enhance wildlife forage and domestic rangeland, but activities were abandoned in 1992. At the time of this study, the relict plant community was sparse and consisted primarily of non-native, agronomic grasses (e.g., *Lolium cristatum*, *Lolium multiflorum*) and legumes (e.g., *M. sativa*).

2.2 Mine tailings and amendment analysis

Bulk tailings samples from the HATSF and local soil amendments were collected in 2014. The compost amendment was produced from municipal yard waste at the City of Kamloops Cinnamon Ridge composting facility; the ash was sourced from the Domtar pulp mill (Kamloops, BC, Canada) and is a by-product of waste wood (commonly referred to as 'hog fuel' and derived from softwood) incineration; the wood chips were waste produced from a local veneer/plywood factory. The amendments were available within a 30-km radius of the HATSF, and therefore, they may be a more economically viable option for reclamation.

Samples of tailings and amendments (three of each) were passed through a 2-mm sieve and analyzed for pH and electrical conductivity (EC) (Hanna Combo HI-98130 handheld electrode device, Hanna Instruments Inc.; Woonsocket, RI, USA) in a 2:1 (soil: deionized water) solution reacted for 1 h (modified from Hayes et al. [3]). Soil texture was classified (for tailings samples only) using the pipet sedimentation method [3]. Particle size distribution of the amendments was determined using sieves with mesh sizes ranging from 0.1 to 16 mm. Organic matter (OM) content was determined for all samples by loss on ignition (550 °C for 6 h) (Thermo Scientific™ Thermolyne F62735, Waltham, MA, USA), and the values were used to estimate organic C (for amended tailings only) using the methods outlined by Nelson and Sommers [40]. Gravimetric water-holding capacity (WHC) was determined using the methods described by Haney and Haney [41]. Subsamples of the tailings and amendments (ash and compost only) were tested for total carbon (C) and total nitrogen (N) (Thermo Scientific™ Flash 2000 combustion elemental analyzer, Waltham, MA, USA). Mineralogical analysis was conducted on tailings subsamples by a third-party commercial laboratory via X-ray diffraction (XRD) using the Rietveld method [42]. Total elemental concentrations of phosphorus (P), potassium (K), aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), molybdenum (Mo), nickel (Ni), lead (Pb) and zinc (Zn) were determined with acid digestion and inductively coupled plasma mass spectrometry analysis (ICP-MS) (EPA Method 200.2/6020B) (Teledyne Leeman Labs Prodigy 7 dual-view ICP-MS, Mason, OH, USA). The acid digestion was conducted using nitric acid in a microwave digestion system (Milestone ultraWAVE, Shelton, CT, USA). Available metals were determined using weak acid digestion with a Mehlich-3 solution [43] and ICP-MS analysis as described above. The methods employed for these elements were intended to target metals that were environmentally available in the soil. The elemental analysis was performed by the British Columbia Ministry of Environment and Climate Change Strategy Analytical Chemistry Services Laboratory (NRL) in Victoria, BC. Metal concentrations were compared to the Canadian Council of Ministers of the Environment (CCME) soil quality guidelines for industrial and agricultural land uses [44].

2.3 Plant species selection

Given the intended use of the site for native grassland, two representative forage bunchgrass species of the interior semiarid grasslands were selected using the 'species objective' filters in the British Columbia Rangeland Seeding Manual [45]. *P. spicata* was chosen primarily for its drought tolerance, while *F. campestris* was selected for its tendency to occur naturally at similar elevations to the study site. With respect to their natural habitats, *P. spicata* typically occurs in low elevation, semiarid shrub–steppe systems, whereas *F. campestris* occupies higher elevation grasslands with more moisture [45, 46]. In the field, both species grow well on well-drained, medium-to-coarse textured soils [45] and have been demonstrated to tolerate metal-contaminated soil [12, 35].

2.4 Greenhouse experiment

The greenhouse experiment was conducted from January to March 2016 at the Thompson Rivers University Research Greenhouse in Kamloops, BC The study was designed to investigate the effects of soil amendments on native bunchgrass growth and to evaluate tissue metals uptake to determine the suitability of the selected plant species for phytostabilization of the HATSF mine tailings. A total of 13 ash–compost combinations ranging from 0 to 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 (Table 1). Three treatment subsets were selected for further analysis: 'ash' (100% ash), 'compost' (100% compost), and 'blend' (40% ash, 50% compost, 10% wood chips) ($n = 3$).

The growth experiment was conducted under controlled conditions (natural and artificial light: day/night 18/6 h; temperature: day/night 21/15 °C; humidity 50–60%) in the research greenhouse. Two-liter nursery pots with drainage (15 cm top diameter × 14 cm height × 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 homogenized ash–compost–wood chip mixtures. Seeds of *P. spicata* and *F. campestris* (obtained from Pickseed Canada Inc., Abbottsford, BC) were sown at a depth of approximately 0.5 cm with a density of 15 seeds per pot. 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. Prior to harvesting, seedling emergence was counted and shoot heights were measured in natural repose.

2.5 Plant tissue analysis

After 90 d, bunchgrass shoots were clipped at the soil surface and roots were retrieved from the substrate. Plant tissue samples were washed and dried (70 °C for 48 h) and then weighed on an analytical scale (Fisher Scientific™ accu225D, Waltham, MA, USA) to determine root and shoot biomass. Three composite biomass samples (roots and shoots) were selected from the amendment treatment subcategories ('ash', 'compost', and 'blend') and submitted to the NRL for analysis of plant tissue elemental concentration of standard metals (Al, Cu, Fe, Mn, Mo, Zn) based on the tailings being the product of a copper mine. Samples were dried at 70 °C for 24 h and ground (Foss™ Tecator™ Cyclotec Cyclone Mill, Hillerod, Denmark), and elemental analysis was performed by NRL using VHP closed vessel microwave acid digestion using ICP-MS (Teledyne Leeman Labs Prodigy 7 dual-view ICP-MS, Mason, OH, USA). The tissue metal concentrations were compared to the maximum tolerable levels for domestic cattle [47]. Metal concentrations were used to calculate the ratio of elemental concentration of shoots versus roots, or translocation factor (TF), using the equation:

$$\mathrm{TF} = \frac{\mathrm{Metal\ (shoot)}}{\mathrm{Metal\ (root)}}$$

and the ratio of elemental accumulation in roots compared to the substrate, or bioconcentration factor (BCF), using the equation:

$$\mathrm{BCF} = \frac{\mathrm{Metal\ (root)}}{\mathrm{Metal\ (substrate)}}$$

which are modified from Mendez and Maier [10]. The indices were used to evaluate phytostabilization potential of the candidate species.

Table 1 Amendment composition for all treatments ($n = 10$) and treatment subsets ($n = 3$) used for growth experiment

Treatment (subset)	Ash (%)	Compost (%)	Wood chips (%)	Field application rate (Mg/ha)
1 (ash)	100	0	0	150
2	90	0	10	150
3	80	10	10	150
4	70	20	10	150
5	60	30	10	150
6	50	40	10	150
7 (blend)	40	50	10	150
8	30	60	10	150
9	20	70	10	150
10	10	80	10	150
11	0	90	10	150
12 (compost)	0	100	0	150
13 (control)	0	0	0	0

2.6 Statistical analysis

Seedling emergence rates, plant biomass and tissue metal content data were analyzed in R version 3.2.3 (codename 'Wooden Christmas Tree') (The R Foundation). All data were checked for normality using boxplots and residual plots. Homogeneity of variance was assessed using the Fligner–Killeen test, and when necessary, data were transformed using a natural logarithm or a square-root function. One-way and two-way analysis of variance (ANOVA) tests were employed to find significant differences between treatment means. Analysis of covariance (ANCOVA) was used to control for seedling density when assessing plant productivity metrics. Treatments were grouped and ranked using Tukey's HSD test ($P < 0.05$). Significant differences between species metal accumulation and soil properties relative to the control were determined using the Welch's two-sample t-test.

3 Results

3.1 Tailings and amendment characteristics

The tailings were comprised of 26% plagioclase feldspar, 16% chlorite, 11% K-feldspar, 11% muscovite/illite, with lesser amounts of other phyllosilicates (kaolinite and smectite) and carbonates (calcite, dolomite and siderite). Soil texture analysis indicated that tailings were a sandy clay loam (USDA soil texture classification [48]), with 52.9% sand, 26.5% silt and 20.6% clay. The substrate was alkaline with low concentrations of OM, total C, N, P and K (Table 2). The gravimetric WHC was relatively high compared to the other materials, and levels of dissolved salts were adequate (not limiting plant growth) based on EC values below 4 dS m^{-1} [49]. With respect to metals concentration, the tailings contained elevated levels of total Cr, Cu, Mo, and Ni above the CCME agricultural land-use guideline, along with high levels of Al (Table 3). Available metals' content of the tailings ranged from 0.3 to 50% of total concentrations.

The municipal compost contained organic material and sands ranging from 0.1 to 4 mm with some large woody debris and coarse (≤ 16 mm diameter) rocks (data not shown). The amendment was slightly alkaline with a C:N ratio of 21:1 and the highest total N of the substrates studied (Table 2). Concentrations of metals in the compost were below the CCME guidelines with the exception of Cu, which exceeded the agricultural guideline (Table 3).

The wood ash was composed primarily of fine-to-medium particles ranging from 2 to 4 mm (data not shown). The material was very strongly alkaline and was high in P, K and Mn compared to the other amendments (Tables 2, 3). Despite having comparable OM and C to the compost, the amendment was low in N, which led to a high C:N ratio. All metals analyzed for in the ash were below the CCME guideline except for Cu, which exceeded the agricultural criteria [44].

The wood chips ranged from 1 to 16 mm in size (data not shown) and were primarily composed of OM with relatively low EC and WHC compared to the other materials (Table 2).

The addition of ash and compost treatments significantly ($P < 0.05$) affected tailings pH levels, while the blended amendment had no effect (Table 2). Tailings pH increased from 8.7 to 9.3 when ash was added and decreased from 8.7 to 8.1 when compost was added. All three treatments had a significant effect on tailings OM content with the compost treatment having the highest levels, and the ash and blend amendments being statistically similar to one another. Electrical conductivity increased with compost and decreased with ash, while remaining relatively unchanged with the blend treatment. Gravimetric WHC decreased when both compost and blended amendments were added.

3.2 Growth response to soil amendments

Seedling germination occurred within four to ten days depending on the species and treatment applied. Productivity on the unamended tailings was sparse and many plants that germinated did not survive through the 90 d. Plants grown in the ash-amended tailings were stunted and showed signs of nutrient deficiency (e.g., discoloration of shoots). The addition of soil amendments had a significant ($P < 0.05$) effect on seedling emergence after 90 d growth, with the exception of *F. campestris* on the compost-amended tailings, which was statistically similar to the control (Fig. 1a). Seedling emergence ranged from 42 to 79% on the amended tailings compared to 18 to

Table 2 Select physiochemical parameters of mine tailings, organic amendments, and amendment mixtures

Parameter	Tailings and soil amendments				Treatments (amendment mixtures)		
	Tailings	Ash	Compost	Wood chips	100% ash	100% compost	Blend
pH	8.7 ± 0.07	10.3 ± 0.02	7.8 ± 0.05	7.5 ± 0.10	9.3 ± 0.04^{a,*}	8.1 ± 0.09^{c,*}	8.7 ± 0.04^{b}
OM[a] (%)	0.22 ± 0.02	24.3 ± 1.23	23.9 ± 2.40	97.7 ± 0.90	3.9 ± 0.20^{b,*}	4.6 ± 0.28^{a,*}	4.3 ± 0.32^{b,*}
C[b] (%)	1.12	22.5	24.3	56.7 ± 0.52	2.3 ± 0.11	2.7 ± 0.17	2.5 ± 0.19
N[c] (%)	0.01	0.05	1.18	–	–	–	–
C:N ratio	112:1	450:1	21:1	–	–	–	–
P (%)	0.11	0.465	0.304	–	–	–	–
K (%)	0.281	1.769	0.793	–	–	–	–
EC[d] (dS m^{-1})	2.1 ± 0.02	2.0 ± 0.02	3.5 ± 0.23	0.5 ± 0.04	2.4 ± 0.01^{b,*}	3.0 ± 0.17^{a,*}	2.0 ± 0.05^{c}
WHC[e] (%)	69.5 ± 0.66	31.0 ± 0.72	50.2 ± 3.44	22.9 ± 1.36	63.6 ± 1.01^{b,*}	69.0 ± 0.54^{a}	65.4 ± 0.26^{b,*}

Values are means ± standard error ($n = 3$). Values without standard errors represent a single homogenized sample. 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.

*Indicates values are statistically significant compared to the tailings (control)

[a] *OM* organic matter

[b] Total carbon (except values for wood chips and treatments are organic C estimated from OM content)

[c] Total nitrogen

[d] *EC* electrical conductivity

[e] *WHC* gravimetric water holding capacity

Table 3 Select available and total elemental concentrations (mg kg^{-1}) of mine tailings and amendments compared to the Canadian federal soil quality guidelines for agricultural and industrial land use

	Available			Total				
Element	Tailings	Ash	Compost	Tailings	Ash	Compost	CCME[a] (AL)	CCME (IL)
Al	74.6	1,967	828	22,658	17,742	13,670	–	–
As	< 3.0	< 3.0	< 3.0	< 8.0	< 8.0	< 8.0	12	12
Cd	< 1.0	< 1.0	< 1.0	< 2.0	< 2.0	< 2.0	1.4	22
Co	1.1	< 1.0	< 1.0	30.8	25.7	16.9	40	300
Cr	1.2	< 1.0	< 1.0	**115.2**	48.0	57.8	64	87
Cu	73.0	11.3	11.9	**600**	**70.7**	**77.9**	63	91
Fe	525	486	545	21,285	16,062	16,544	–	–
Hg	–	–	–	< 3	< 3	< 3	6.6	50
Mo	< 1.0	< 1.0	< 1.0	**10.5**	3.15	3.81	5	40
Mn	23.7	680	83.8	303.6	3551	339.6	–	–
Ni	1.6	2.8	1.4	**76.4**	32.5	38.3	45	89
Pb	< 1.0	< 1.0	6.6	< 2.0	< 2.0	14.9	70	600
Zn	< 3.0	82.9	29.3	19.6	216	106	250	410

Values represent concentrations of single homogenized samples

Bolded values for total metals are in exceedance of at least one of the referenced guidelines

[a]*CCME* Canadian Council of Ministers of the Environment Soil Quality Guidelines for the Protection of Environmental and Human Health [44], *AL* agricultural, *IL* industrial

20% for the control (Fig. 1a). When comparing between species, *P. spicata* emergence was significantly ($P < 0.05$) higher than *F. campestris* on the ash-amended tailings and similar on all other treatments.

Shoot heights of bunchgrasses were significantly ($P < 0.05$) affected by the addition of soil amendments with compost (Fig. 1b). The compost and blend treatments increased shoot heights by 165 and 74% (*P. spicata*) and 185 to 153% (*F. campestris*), respectively.

The shoot heights on the ash-amended tailings did not significantly differ from the control. Shoots of *P. spicata* (10–26 cm) were generally taller than *F. campestris* (4.5–13 cm), but significant differences were detected on the ash- and compost-amended tailings only. There was evidence of a positive correlation between amendment relative compost concentration and seedling heights, with *P. spicata* ($R^2 = 0.40$, $P < 0.0001$) having a slightly greater growth response compared to *F. campestris* ($R^2 = 0.30$, $P < 0.0001$) (see supplementary data).

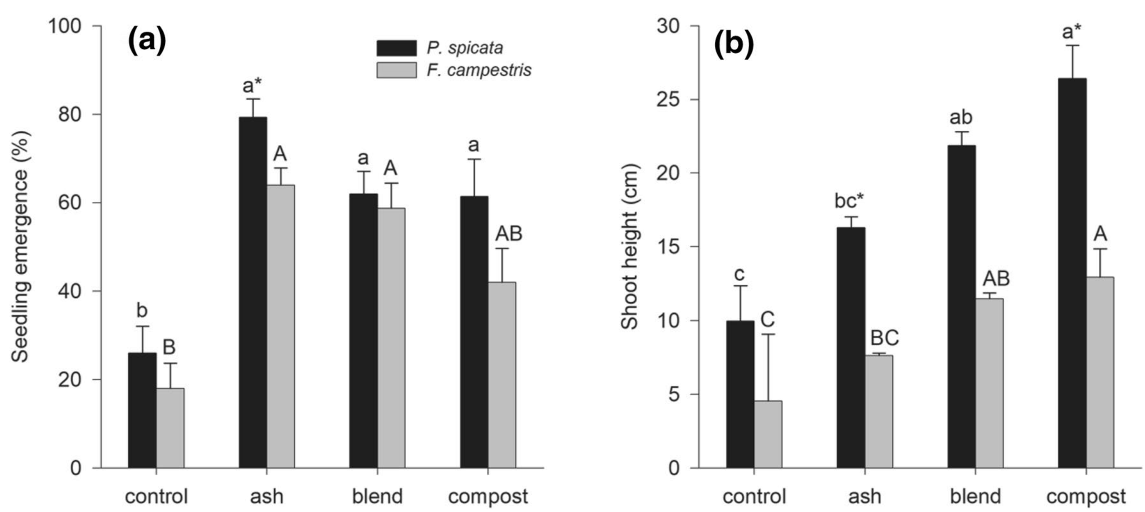

Fig. 1 Mean *Pseudoroegneria spicata* and *Festuca campestris* **a** seedling emergence and **b** shoot heights by treatment after 90-d growth in amended mine tailings. Error bars are standard errors of the mean ($n = 10$). Treatments with different letters are statistically significant within species (lower case = *P. spicata*; upper case = *F. campestris*) at $P < 0.05$ (one-way ANOVA, Tukey's HSD). *Represents a statistical significance between species (determined by Welch's *t*-test) for that treatment

Shoot and root biomass of bunchgrasses were significantly ($P < 0.05$) affected by soil amendments with the compost yielding the highest results (Fig. 2a). Plants grown on the compost-amended tailings produced 14–21 times more total biomass than the control and 1.5–3.4 times more biomass than the ash treatment. Amendment compost concentration had a positive linear effect on total biomass for both species, while ash had a negative effect (Fig. 2b). When controlling for seedling density (using ANCOVA), the relationship between compost concentration and plant biomass was strengthened ($R^2 = 0.48$ and 0.45 for *P. spicata* and *F. campestris*, respectively). Total biomass of *P. spicata* (0.05–0.75 g per pot) was significantly higher than *F. campestris* (0.01–0.24 g per pot) on all treatments. Root-to-shoot ratios were similar for both species and ranged from < 1:1 on the unamended tailings to up to 3:1 with ash treatment.

3.3 Plant metals uptake

Root and shoot concentrations of select metals Al, Cu, Fe, Mo and Zn were analyzed for both grass species after 90-d growth on three amendment mixtures (Fig. 3). Metals exceeding the maximum tolerable levels for cattle were Cu for *F. campestris* grown on the compost-amended tailings, Fe for *F. campestris* grown on the ash-amended tailings (although the exceedance was slight and data were highly variable), and Mo on all treatments. Shoot accumulation of Mn and Mo was significantly ($P < 0.05$) higher when grown on the ash-amended tailings compared to the other mixtures (Fig. 3d, e). Molybdenum concentrations in *P. spicata* and *F. campestris* shoot tissue were 5 to 10 times higher with ash compared to compost. The TF values were > 1 for Mn (ash only), Mo and Zn, and < 1 for the remaining metals (Table 4). The root BCF values were > 1 for Mo and Zn, and < 1 for the remaining metals (Table 5).

4 Discussion

4.1 Effect of soil amendments on tailings characteristics

Plant growth on mine tailings is facilitated by the addition of soil amendments, which are used to enhance physiochemical conditions for plant growth [4, 20]. Analysis of the HATSF mine tailings revealed high pH, WHC (or poor drainage) and metals, and low OM and nutrients. The combination of these poor physiochemical properties limited the establishment of plants on the unamended tailings.

Assessment of tailings characteristics before and after amendment addition revealed an increase in the OM content of the amended tailings (Table 2), which is consistent with other mine tailings studies [26, 50, 51]. The compost-treated tailings had the highest OM content, and the ash-treated tailings had the lowest. Organic matter is important for soil rehabilitation and reclamation because (1) the organic C provides an energy source

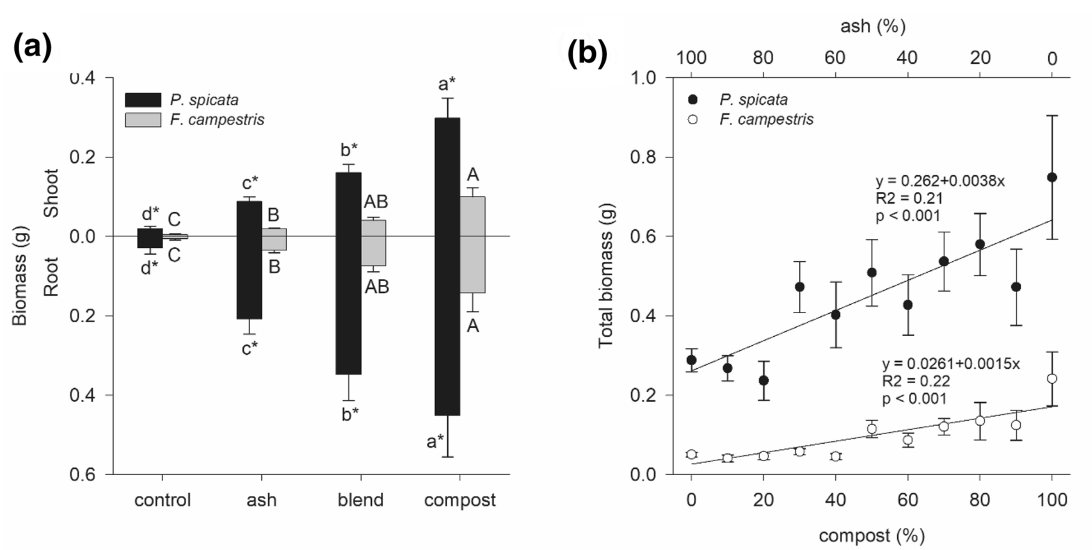

Fig. 2 Mean **a** *Pseudoroegneria spicata* and *Festuca campestris* shoot and root biomass per pot by treatment and **b** relationship between total biomass (roots and shoots) and relative concentrations of compost and ash in the soil amendment mixtures after 90-d growth in amended mine tailings. Error bars are standard errors of the mean ($n = 10$). Treatments with different letters are statistically significant within species (lower case = *P. spicata*; upper case = *F. campestris*) at $P < 0.05$ (one-way ANOVA, Tukey's HSD). *Represents a statistical significance between species (determined by Welch's *t*-test) for that treatment

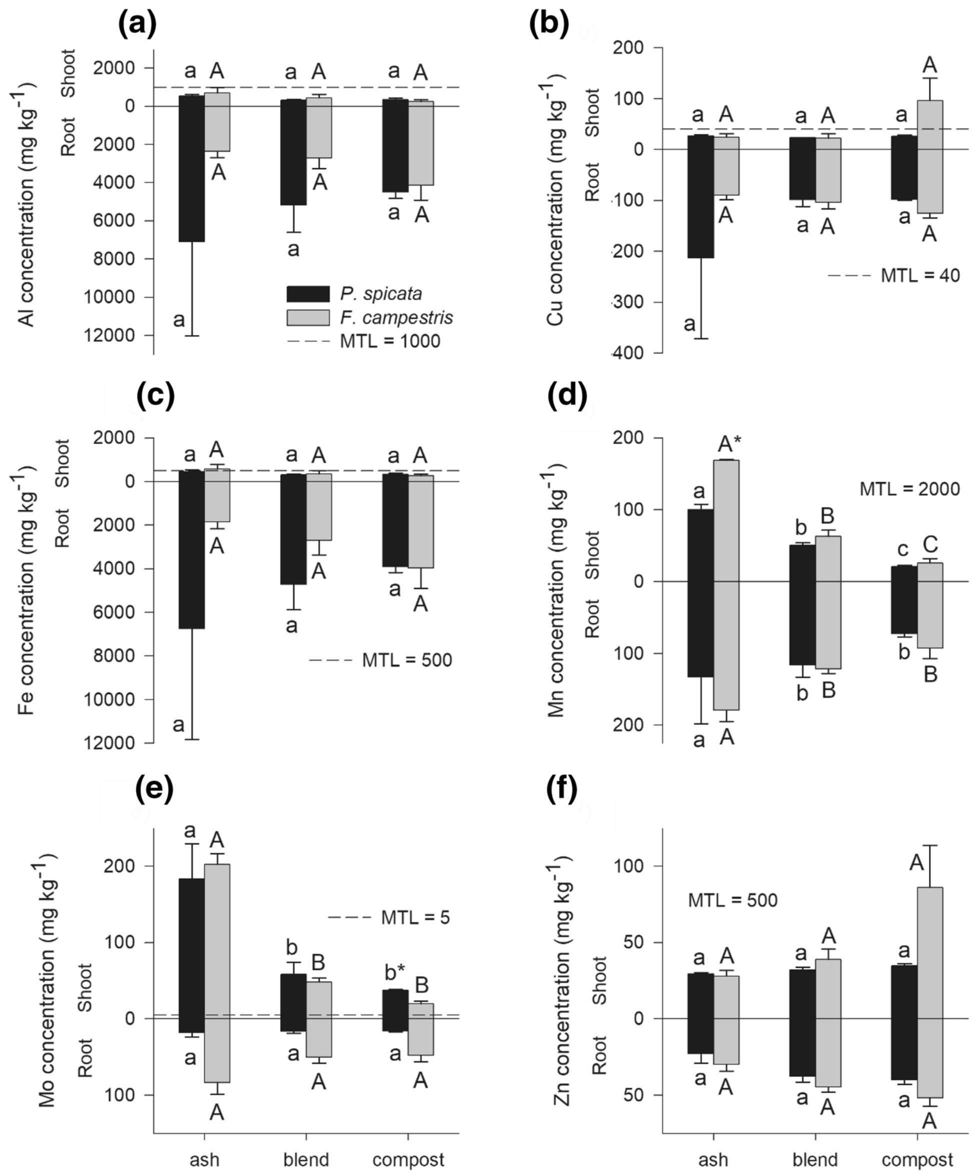

Fig. 3 Mean *Pseudoroegneria spicata* and *Festuca campestris* shoot and root concentration of select metals and metalloids by treatment after 90-d growth in amended mine tailings. *MTL* maximum tolerable levels for cattle [47] (indicated by the dotted line). Error bars are standard errors of the mean ($n=3$). Treatments with different letters are statistically significant within species (lower case = *P. spicata*; upper case = *F. campestris*) at $P<0.05$ (one-way ANOVA, Tukey's HSD). *Represents a statistical significance between species (determined by Welch's *t*-test) for that treatment

Table 4 Mean *Pseudoroegneria spicata* and *Festuca campestris* translocation factors (the shoot: root ratio of the concentration of the corresponding element) for select metals and metalloids after 90-d growth on amended mine tailings

Element	*P. spicata*			*F. campestris*		
	Ash	Blend	Compost	Ash	Blend	Compost
Al	0.15 ± 0.06	0.07 ± 0.02	0.08 ± 0.02	0.30 ± 0.11	0.15 ± 0.04	0.07 ± 0.04
Cu	0.33 ± 0.14	0.24 ± 0.03	0.50 ± 0.04	0.28 ± 0.09	0.21 ± 0.07	0.12 ± 0.02
Fe	0.18 ± 0.08	0.08 ± 0.02	0.08 ± 0.02	0.33 ± 0.11	0.12 ± 0.03	0.08 ± 0.03
Mn	1.22 ± 0.54	0.47 ± 0.11	0.29 ± 0.05	0.96 ± 0.08	0.53 ± 0.10	0.29 ± 0.08
Mo	12.5 ± 4.62	3.43 ± 0.47	2.44 ± 0.16	2.53 ± 0.30	1.01 ± 0.18	0.41 ± 0.02
Zn	1.46 ± 0.34	0.87 ± 0.08	0.89 ± 0.04	1.02 ± 0.28	0.90 ± 0.22	1.60 ± 0.47

Values are means ± standard error of the mean ($n=3$)

for soil microorganisms, which accelerates decomposition and nutrient cycling [4, 7], (2) long-term plant nutrient availability is enhanced because N is in an organic form and is slowly released over time [50], (3) the OM improves soil physical conditions such as water retention and bulk density [17, 49, 51, 52], and (4) some organic residues (e.g., waste from agri-food industry, seaweed) have high concentrations of base cations, which increase

Table 5 Mean *Pseudoroegneria spicata* and *Festuca campestris* bioaccumulation factors for roots (the root: substrate ratio of the concentration of the corresponding element) for select metals and metalloids after 90-d growth on amended mine tailings

	P. spicata			*F. campestris*		
Element	Ash	Blend	Compost	Ash	Blend	Compost
Al	0.31 ± 0.22	0.23 ± 0.06	0.20 ± 0.01	0.10 ± 0.02	0.12 ± 0.02	0.18 ± 0.03
Cu	0.36 ± 0.26	0.16 ± 0.02	0.16 ± 0.003	0.15 ± 0.02	0.17 ± 0.02	0.21 ± 0.01
Fe	0.32 ± 0.24	0.22 ± 0.06	0.18 ± 0.01	0.09 ± 0.02	0.13 ± 0.03	0.19 ± 0.04
Mn	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.004	0.28 ± 0.05	0.17 ± 0.03	0.16 ± 0.03
Mo	1.75 ± 0.51	1.57 ± 0.20	1.47 ± 0.12	7.96 ± 1.48	4.79 ± 0.73	4.58 ± 0.78
Zn	1.16 ± 0.31	1.91 ± 0.20	2.03 ± 0.16	1.51 ± 0.24	2.28 ± 0.17	2.65 ± 0.27

Values are means ± standard error of the mean ($n = 3$)

pH and cation exchange capacity [13]. Because of these properties, the longevity of positive restorative effects is often greater when using organic amendments compared to traditional reclamation methods such as inorganic fertilizers [18, 53].

The addition of amendments had a significant effect on tailings pH levels (Table 2). The compost amendment lowered tailings pH, but the effect was suboptimal as the amended tailings remained moderately alkaline (pH > 8). The ash amendment increased the tailings pH level from moderately alkaline to strongly alkaline (pH > 9), which is above the preferred range for most plants (pH 5.5 to 8.5) [48]. In a recent review, Sheoran et al. [54] reported that mine soil pH range of 6 to 7.5 is adequate for agronomic or horticultural uses of mine sites. Although, in arid environments, it is normal for pH to be slightly to moderately alkaline (pH between 7 and 9) [48]. Abnormally high soil pH can increase mobility of metals such as As, Mo, and Se and limit the availability of P and certain micronutrients (e.g., B, Mn, Fe) [25, 55].

In general, the addition of organic amendments increased the EC of the tailings, with the exception of the blend which had little or no effect (Table 2). In all treatments, EC remained below the critical level of 4 dS m^{-1} at which plant growth is negatively affected [49].

The high gravimetric WHC (Table 2) in the tailings indicates water retention was not a limitation; however, substrates with high WHC (> 80%) can have poor drainage leading to anoxic conditions, which can affect root productivity and reduce overall revegetation success [48]. The addition of both amendments reduced the WHC of the tailings, likely because the addition of larger organic particles reduced bulk density and improved drainage of the substrate.

Overall, the compost treatment appeared to provide the most suitable soil conditions for revegetation of the HATSF mine tailings; however, lowered pH from the addition of OM could result in the release of heavy metal cations, such as Ni and Cr, from the substrate, causing potential negative impacts to the surrounding environment and groundwater through leaching and mass flow, especially if the site is irrigated [8, 13]. Under such conditions, environmental and human health risk may be elevated as elements become phytoavailable and enter the food chain through consumption by primary consumers [8, 10].

Changes in soil physiochemical properties following the 90-d growth period were not assessed in this study; however, based on the previous research, we expect plant establishment to aid in further improving the growing conditions of the amended tailings. It is well known that plant establishment improves tailings properties over time by initiating soil development processes such as nutrient cycling, soil aggregation and accumulation of organic C [50, 56, 57]. For instance, Cele and Maboeta [26] reported significant improvements in soil fertility parameters such as OM, WHC, CEC, and plant nutrients after 12 weeks of *Cynodon dactylon* (perennial, rhizomatous grass) growth on Fe mine tailings. Furthermore, Antonelli et al. [50] noted improvements in soil C, N, P content and stable pH levels (around the neutral range) 13 years after revegetating Cu and Mo tailings with agronomic pasture grasses. The latter study noted the role of plants and OM in stabilizing tailings pH levels over time by slowing the rate of carbonate activity and other geochemical weathering processes.

4.2 Growth response to soil amendments

The results of the experiment indicate that the addition of amendments, regardless of treatment, improved seedling germination and growth of native bunchgrass species *P. spicata* and *F. campestris* on the HATSF mine tailings. Of the treatments investigated, the compost was the most effective at promoting above- and belowground growth of both species. The positive influence of compost on plant productivity was likely a result of improved tailings biological and physiochemical conditions [51, 58]. In two greenhouse studies, Solís-Dominguez et al. [4] and Gil-Loaiza et al. [59] reported improvements in tailings pH, EC, organic C, total N and neutrophilic heterotrophic bacteria numbers when compost was applied to acidic mine tailings. The neutrophilic heterotrophic community is thought to play an important role in promoting C and N nutrient cycling by offsetting chemoautotrophic microbial activity (e.g., iron and sulfur oxidation) and is an important indicator of tailings conditions for plant growth [4, 59].

Plants growing in the ash treatment did not appear healthy; this can be attributed to the lack of N coupled with increased pH levels, which created less favorable conditions for plant growth. In high pH environments, bacterial and fungal activity is restricted, which has a negative effect on nutrient cycling [55, 60, 61]. Because of these properties, it is possible that the nutrients (namely P and K) contained in the ash were not available for plant uptake. The data suggest that incorporating the very strongly alkaline ash material into the alkaline tailings was not an effective method for optimizing plant growth. In a recent review assessing remediation technologies on high pH bauxite residue, Santini et al. [61] suggested inoculating the substrate with alkaline-resistant microorganisms (alkaliphiles) to enhance nutrient cycling. A combination of the ash amendment with an inoculation treatment should be investigated as an option for enhancing the tailings conditions. Assessment of post-treatment nutrient concentrations and neutrophilic heterotrophic bacteria numbers would have revealed more insight as to the limitations of the ash material as a soil amendment.

Root-to-shoot ratios were around 1 for the compost treatment, which indicates balanced biomass allocation and adequate nutrient availability in the amended substrate [62]. Generally, when nutrients are limiting, plants will allocate more resources to their roots, which increases the root-to-shoot ratio [63]. The high root-to-shoot ratios of plants growing in the ash-amended tailings can be explained by the lack of N in the growing medium, which may have forced plants to allocate more effort into root production at the cost of shoot production. The positive response to compost addition (Fig. 2) suggests that N was a limiting factor for plant growth on these tailings. Several studies have underscored the importance of soil N in mine reclamation because it is an essential plant nutrient, yet it is often limiting in mine soil ecosystems [51, 56, 57].

With respect to plant growth and overall productivity, *P. spicata* outperformed *F. campestris* on all treatments including the control (Figs. 1a, 2). This can be partially attributed to the ability of *P. spicata* to germinate under a wider range of conditions compared to other grassland species [64]. The results were consistent with a recent field study where Carlyle [65] reported higher relative growth rates and shoot and root biomass for *P. spicata* compared to *F. campestris*.

4.3 Effect of soil amendments on plant metals uptake

Shoot and root concentrations of select metals were assessed for both species after 90 d of growing in the amended tailings. The results indicated high shoot concentrations of Mo, which exceeded maximum tolerable level for cattle [47] in all treatments, most notably when the ash amendment was used (Fig. 3e). The uptake in Mo on the amended tailings is attributed to the alkaline pH (8.3 to 9.3) of the mixtures, which led to increased mobility of Mo in the soil. According to Goldberg et al. [66], Mo availability increases with increasing pH due to weaker adsorption of Mo to clay minerals under alkaline conditions. The enhanced Mo uptake in plants grown on the ash treatment can be attributed to a combination of the high pH and P content relative to the other treatments. Neunhäuserer et al. [29] mention that P additions to soil can reduce the sorption of Mo to clay minerals, thereby increasing the metal's availability to plants. In the same study, sewage sludge applications of pH 12.1 increased extractable Mo on contaminated pasturelands but decreased plant uptake; it was suspected that the effect was due to other cations such as Ca^{2+} interacting with Mo to compete for plant absorption. Doran and Martens [31] found similar effects of soil pH on Mo uptake when growing *M. sativa* in a fly-ash amendment. In the Neunhäuserer et al. [29] study, Mo uptake increased in the field due to increased moisture availability. In the current study, Mo uptake may decrease in the field due to lack of moisture, high soil and air temperatures, and other abiotic constraints expected in a semiarid environment [7].

Although Mo is an essential trace element for plants and animals, elevated levels in forage can lead to molybdenosis (induced Cu deficiency) when ingested by cattle or other ruminants [17, 66]. The condition is influenced by relative concentrations of Cu, Mo and sulfur [28] and can be detrimental to ruminant health. In general, the risk of molybdenosis increases when the Cu: Mo ratio is < 2:1 [67]. In the current study, Cu: Mo ratios ranged from 0.1 to 0.7 (calculated from Fig. 3b, e), with the lowest value being for *F. campestris* growing on the ash-amended tailings. As the ratios were all well below the tolerance level, the grasses would not be ideal for ruminant forage under the conditions studied.

The metals Cr and Ni exceeded the soil quality guidelines in tailings but were not analyzed for in plant tissues. Available metal concentrations (Table 3) and pH of the substrates analyzed indicate these metals were not readily available for plant uptake, as noted in other studies with similar pH values [13, 25]. Rodríguez-Vila et al. [33], the addition of compost (pH ~ 9) and biochar (pH ~ 10) to copper mine tailings, reduced the 'phytoavailable' amounts of Ni in the soil as determined by $CaCl_2$ extraction techniques. They determined that a mixture of 19% compost and 1% biochar was most effective at reducing the Ni concentrations in foliage of *B. juncea*. At the same site, Forján et al. [13] reported reduced Ni uptake when biochar was used as an amendment. Aluminum concentration of the tailings was notably high (Table 2), and when treated with ash the material had ideal conditions for the formation of soluble Al in the form of aluminate ($Al(OH)_4^{-1}$), which can cause soil toxicity [68]. This may be a reason for the stunted growth and poor plant health observed on the ash treatment. According to Hodson [24], some plants are able to tolerate excessive levels of Al and other metals by avoiding shoot uptake and concentrating them in their roots. Both plant species used in this study accumulated substantially more Al in their roots (up to seven times) compared to their shoots (TF values 0.07–0.3), which provides some indication of their tolerance to Al (Table 4). Our results suggest that these species may be useful for remediating tailings and other mine wastes that are high in Al.

While changes in available metals or chemical speciation in the tailings following amendment addition and plant establishment were not assessed in the current study, we would expect the compost treatment to have had a positive effect on metals immobilization due to the increase in root biomass and OM present in the rhizosphere. (The roots provide a surface for metal sorption and organic C offers binding sites for complexation with metals.) For example, the key metals in this study, Cu and Mo, have a strong tendency to sorb to clay minerals and bind to organic products on amended soils [29, 69]. Bolan et al. [69] observed an increase in Cu adsorption and formation of Cu-organic complexes on sandy mineral soils amended with organic manure. In Neunhäuserer et al. [29], Mo availability decreased with OM, clay minerals, and Fe-oxide content, and increased with addition of P-rich fertilizer amendments.

4.4 Evaluation of bunchgrasses for phytostabilization

Vegetation plays a key role in the physical stabilization of barren tailings [4, 7]. Aboveground, the plant shoots, stems and leaves provide cover which protects against wind and water erosion [8, 10]. Belowground, the plant roots act to amalgamate the loose material and reduce the vertical transport of soil particles and contaminants carried by water through the soil profile [8]. We suspect that the observed improvement in seedling emergence and biomass productivity (Figs. 1, 2) with the addition of soil amendments would translate to enhanced physical stabilization in the field. In theory, the plants with higher shoot and root biomass would be more capable of protecting the ground surface from wind erosion and stabilizing the surficial soils [7].

TF and BCF indices are used for measuring metal accumulation in plant tissues and characterizing plant species strategies for growing in metal-contaminated substrate [7, 13, 33]. Generally, plants with TF values < 1 and root BCF values > 1 are considered excluder plants, whereas those with TF and BCF values > 1 are accumulator or hyperaccumulator plants, depending on the total concentration of the metal sequestered in shoots (generally above or below 1000 mg/kg) [8, 33]. Suitable candidate plant species for phytostabilization have high biomass and are excluders: those which minimize shoot accumulation without limiting root uptake [7, 10, 35], allowing for metals to be stabilized in the rhizosphere and preventing potential translocation to shoots or leaching to groundwater [8]. In the current study, TF values generally remained below 1 for most of the metals investigated, except for Mn, Mo and Zn which exceeded (or were close to) the threshold for all treatments (Table 4). Root BCF values also remained below 1 except for Mo and Zn. These results suggest that both species are accumulators of Mo and Zn; however, with no significant difference in shoot Zn concentration or exceedance of maximum tolerable level for cattle, the effect of amendments on Zn is negligible. Similar TF values were obtained by Forján et al. [13] for Zn uptake on copper mine tailings amended with a mixture of compost and biochar.

In addition to promoting physical stabilization and translocation of metals from the soil into root and shoot tissues, plants encourage phytostabilization by immobilizing metals in the rhizosphere through a variety of biogeochemical processes [60]. On metal-contaminated soils, OM and plant roots act to reduce available metals via precipitation, root sorption, or formation of metal–organic complexes [7]. These products are typically less soluble compared to their previous form, allowing the metals to be stabilized in place [7]. Root exudes also play an important role in modifying metal availability in the rhizosphere by influencing redox conditions and facilitating microbial transformation of cations [60].

Despite adequate biomass production, the high shoot concentration of Mo coupled with the TF and root BCF values indicates that both *P. spicata* and *F. campestris* are accumulators of Mo and therefore are not suitable for phytostabilization of the HATSF tailings under these conditions. A better use of the grasses would be for phytoextraction where shoot uptake is encouraged (i.e., high TF values) and vegetation is subsequently harvested [36, 70].

5 Conclusions

Of the locally available soil amendments investigated in this study, compost was the most effective at ameliorating the alkaline copper mine tailings and promoting native bunchgrass (*P. spicata* and *F. campestris*) growth; however, practitioners must caution the use of this amendment in large quantities, as release of heavy metals from lowered pH can cause adverse effects to surrounding ecosystems or groundwater. The Mo concentration in plant shoots exceeded the maximum tolerable level for cattle, which is not favorable when considering the end land-use objectives of wildlife use or cattle grazing. While biomass response was positive, the shoot Mo concentrations and observed values for TF (> 1) and BCF (> 1) suggest that *P. spicata* and *F. campestris* are accumulators of Mo and therefore are not suitable candidates for phytostabilization of the HATSF tailings under the conditions examined.

In summary, this study provides practical information regarding the suitability of soil amendments and native grassland species for restoration of alkaline copper mine tailings. While the research was intended to broadly address various land management issues relating to mine restoration in semiarid environments, it also contributes valuable pieces of knowledge to the field of phytostabilization research. In addition to this information being directly applicable the HATSF, it may also be useful for land-use planning and implementation at other degraded sites located in similar environments.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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