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The Relationship between Site Contamination and Native Plant Success in Butte, MT: Implications for Future Restoration

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The Relationship between Site Contamination and Native Plant Success in Butte, MT: *Implications for Future Restoration*

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Abstract
Interest in ecological restoration of mining impacted areas, as opposed to traditional reclamation practices, has been increasing in recent years. Holistic ecological restoration is preferable to traditional reclamation practices as it provides better ecological function, biodiversity, and human enjoyment. However, legacy effects of mining, such as metals contamination and poor growing substrates, has hampered restoration efforts and further, there is little research on primary succession of these novel ecosystems. There is also scant information on many native plant species responses to contamination.

This study focuses on developing plant-contaminant relationships to guide restoration planting decisions. We look at Butte, MT, an area severely impacted by mining operations for over 100 years. Many plant species have spontaneously colonized this contaminated environment, and we show that low pH and elevated levels of Zn are limiting factors to plant growth and we present new information showing Cu as a driver for exotic species invasion. Many native plant species such as *Agrostis scabra*, *Oryzopsis hymenoides*, and *Mentzelia laevicaulis*, are highly tolerant to a range of contaminants. This new information can be used to guide future ecological restoration projects to produce more desirable outcomes while also serving to protect human health. More research, however, should be done to investigate contaminant relationships with additional soil parameters and how they affect native plant colonization.

Introduction
For over 100 years the area around Butte, MT was mined first for gold and silver and then for copper which provided much of the nation's copper needs through the 20th century. Tailings and waste rock were piled up in and around the town and left there until the town was

declared a US Environmental Protection Agency (EPA) Superfund site in the 1980s and remediation finally began (U S Environmental Protection Agency 2006). Traditional remediation practices were used, including removing waste and using a “waste in place” policy where the waste was just covered up with topsoil and a layer of lime to isolate it. A limited number of non-native reclamation species were then seeded in, including *Agropyron cristatum, Medicago sativa, Bromus inermis*, and *Festuca ovina*, producing a highly novel ecosystem. Novel ecosystems arise when species occur in combinations and relative abundances that have not occurred previously within a given biome (Chapin and Starfield 1997; Hobbs et al. 2006). Some contaminated areas, however, have been left alone and plant communities have colonized these areas through natural immigration, also forming novel ecosystems.

Restoration of native plant communities provides the most robust stabilization, ecological function, and recreational opportunities following mining disturbance. However, reclamation using mostly non-native grasses and forbs has been the preferred tool used post-mining. Consequently, most phytotoxicity research has focused on agricultural and reclamation species (non-natives and cultivars) and little is known about the tolerance of the native plants to contaminants produced by mining and smelting (Fletcher et al. 1988; Tordoff et al. 2000).

The areas in and around Butte, MT have significantly elevated levels of metals and metalloids (MM) namely As, Cu, Pb and Zn and areas of low pH as a result of mining activities. Although natural primary succession of plant communities has occurred on these impacted sites, they face significant physicochemical barriers to soil formation and plant growth (Wong 2003). Further environmental barriers such as the absence of topsoil, erosion, compaction, shortage of essential nutrients, limitations to immigration, extreme temperature fluctuations, and so on, further retard plant colonization (Bradshaw 1983; Ash et al. 1994; Wong 2003). Most research on natural succession of mine wastes has focused on detecting hyperaccumulator species for use in phytoremediation and phytostabilization of mine waste (Mendez and Maier 2008; Nouri et al. 2011; Favas et al. 2014; Bacchetta et al. 2015; Sánchez-López et al. 2015; Lauder et al. 2017; Fernández et al. 2017).

These metals and metalloids have been shown to be toxic to plants in the following concentrations: 60-125 ppm Cu, 70-400 ppm Zn, 20-50 ppm As, and 100-400 ppm Pb (Alloway 1995; Nagajyoti et al. 2010). On the other hand, both Cu and Zn are essential plant micronutrients at low levels but cause adverse effects at higher concentrations. At normal levels, Cu assists in CO_2 assimilation, enzyme activation, and ATP synthesis (Barbour et al. 1999; Marschner and Marschner 2012). However, concentrations within plants above 4-30 ppm, it causes reduced growth, leaf chlorosis, oxidative stress, and reactive oxygen species which in turn damages metabolic pathways (Alloway 1995; Nagajyoti et al. 2010; Marschner and Marschner 2012). Zn is also a micronutrient which activates some enzymes and is needed for protein synthesis and carbohydrate metabolism (Cakmak and Marschner 1993; Barbour et al. 1999; Marschner and Marschner 2012). But at concentrations over 15-100 ppm within a plant Zn causes reduced root and shoot growth, alters metabolism, and causes oxidative damage and may cause Cu, Mg, and Fe deficiency (Cakmak and Marschner 1993; Alloway 1995; Nagajyoti et al. 2010). Arsenic reduces root production and growth and when transported to the shoot interferes with metabolic processes and reproductive ability (Finnegan and Chen 2012). Finally, Pb has deleterious effects on plant morphology, growth and photosynthetic processes, and the uptake of nutrients (Seregin I.V. and Ivanov V.B. 2001; Nagajyoti et al. 2010).

Although metal and metalloid concentrations may be high in particular soils, their bioavailability is a complicated process that is influenced by many factors including, plant species, soil properties, microbial activity, metal speciation, and soil pH to name a few (Radanovic et al. 2002; Peralta-Videa et al. 2009). Soil pH has a significant effect on MM uptake of plants and is the only factor that will be investigated in this paper. In general, as soil pH increases the cationic metal solubility exponentially decreases for Cu, Pb, and Zn, however, this association is opposite and less pronounced with As (Alloway 1995).

The general ecological theory holds that harsh and unproductive environments are invaded less by exotic species than productive ones, such as agricultural fields because native species have developed mechanisms to tolerate stress and to maximize the use of limited resources (Alpert et al. 2000; Daehler 2003). However, in Butte, MT we have a novel semi-arid ecosystem of mine wastes to which native plant species have not evolved, and we would expect exotic species to perform better than natives because of their physiological plasticity and competitive ability. There is a limited body of research on exotic species invasion of contaminated mine sites, however, and Struckhoff et al. (2013) demonstrated that exotic species richness increased with increasing soil Pb and Zn levels from smelting operations in Missouri. On the other hand, Bes et al. (2010) showed that in a former timber treatment plant with high soil Cu concentrations exotic invasion did not increase with increasing Cu levels, but overall richness and diversity decreased with increasing contamination.

In this study, we aimed to investigate the unremeidiated areas to identify native species that show tolerance to contamination and their interactions with exotic species to understand which species should be used in future restoration and reclamation activities with an emphasis on ecological restoration. Further, we sought to identify local species that show tolerance to mine wastes and to investigate community composition in general for restoration purposes.

Materials and Methods

Study area

The Butte, MT area is home to some of the most prolific mining operations in North America. For almost 100 years starting in the 1860's, gold, silver, and later copper mining fueled the world's mineral demands. Unlike many mining operations located in remote areas, the town of Butte is located within the operations. Waste rock was piled up in yards, tailings and smoke spilled from the smelters, and the area became virtually devoid of life. In 1984 the area became a U.S. EPA Superfund site, and *in-situ* remediation began by covering the waste with imported soils and revegetating with Eurasian grasses. However, many smaller areas that were not deemed a priority to protect human health have not been remediated; this study focuses on remediation of such areas (Figure 1).

Site and plot selection

Five sites were selected in the Butte Priority Soils Operable Unit (BPSOU) and another five in the area West of Montana Tech of the University of Montana for a total of 10 sites (Figure 1). Sites were selected if they were clearly impacted by mine waste but had some vegetative cover. If the area did not contain any vegetative cover, it was not considered. Sites also had to be over approximately 50 m^2 to qualify. In most of the degraded areas, vegetation was not universally distributed but rather formed islands of vegetation usually surrounded by unvegetated, barren land that we presumed to be contaminated (Figure 2).

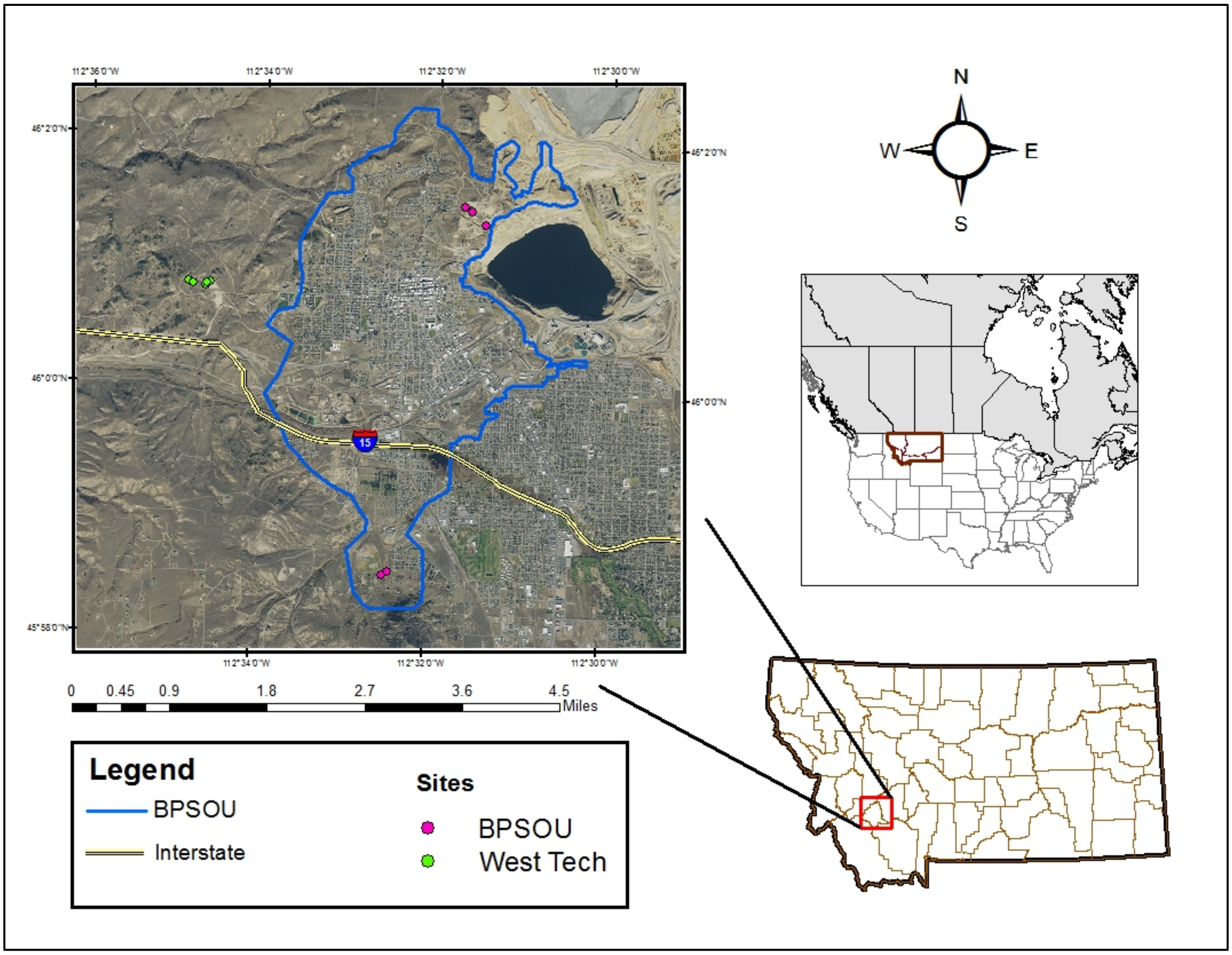

Figure 1. Locations of study areas. Butte, MT

To further investigate what causes these vegetation islands we employed both transect and random sampling methods at each site. Transects were used to investigate how the supposed contamination gradient affected the presence and absence of vegetation, while randomly selected sites were used to describe the conditions in which vegetation exists, more thoroughly. At each site, slope, aspect, and GPS coordinates were recorded.

Figure 2. Example of a typical study area where an island of vegetation exits surrounded by barren land.

Transects consisted of 10 sequentially placed 1x1m quadrats along a field tape measure starting from barren land outside the vegetated areas and working into the vegetation (Figure 3). We tried to have the fifth quadrat land at approximately the edge of the vegetation. Random sampling with 10 1x1m quadrats was employed within the vegetation islands. Location of quadrats was determined by selecting a direction, in degrees, and a distance in paces for each quadrat. Both direction and distance were selected using a random number generator. For direction, 0-360 which corresponds to a degree on a compass, and 1-25, which determines the distance in paces. For the first plot, the surveyor would start at the approximate middle of the site and select a degree and distance. If the distance exceeded the boundary of the site, the surveyor would “bounce” off the edge of the site and continue walking the same direction until the selected distance was reached.

Figure 3. Vegetation sampling using the transect method.

Plant community sampling

Within each 1x1m quadrat, each plant species was identified, and the cover was recorded to the nearest one-tenth of one percent (Daubenmire 1959). If a plant could not be keyed out in the field, a specimen was taken and *"Manual of Montana Vascular Plants"* (Lesica 2012) was used to identify it in the lab. Ground cover in the form of rocks, litter, cryptogamic, and vegetative ground cover were also recorded to the nearest one-tenth of one percent.

Soil sampling and analysis

Soil Field Sampling

Soil samples were collected at a depth of 15 cm from the middle of all quadrats in both transects and random sampling regimes for a total of 200 samples. We followed standard operating procedures for soil sampling recommended by US EPA (U.S. Environmental

Protection Agency 2000). Litter and other surface debris were first removed and then using a transplanting spade a 15cm hole was excavated. Then again using the spade, the soil was uniformly removed vertically and extracted from the hole on the spade. A horie knife was then used to cut the sample lengthwise down the spade on both sides to make a uniform sample (Figure 4). The sample was then labeled and placed in a plastic bag and stored in a refrigerator.

Soil samples were then prepared for metal and pH analysis. After all samples were collected they were air dried for one week and sifted through a No. 10 (2.00 mm) sieve, the material that passed through was then pulverized using a laboratory disk pulverizer until it could pass through a No. 120 (0.125 mm) sieve. Pulverizing the sample also acted to homogenize the sample. The sample was then placed in a new bag and relabeled. Between each sample, the disk pulverizer was cleaned by spraying it with compressed air and wiping it down with acetone and paper towels.

Figure 4. A soil sample is taken with a transplanting spade.

Soil Metals Analysis

Pulverized soil samples were placed into specialized soil sample cups designed for pXRF analysis, covered with a Mylar film and labeled. Soil metals analysis was carried out with a solid-state Cd source Thermo Scientific Nitron XLp 300 series analyzer in "bulk sample" mode. Before sample testing, the pXRF was tested for accuracy against nationally certified prepared samples from the manufacturer. The prepared sample cups were placed into the pXRF test stand and tested by the remotely operated pXRF for 100 seconds each. To ensure the accuracy of pXRF results, 20 soil samples were selected for inductively coupled plasma mass spectrometry (ICP-MS) analysis by Marcom Labs in Butte, MT. We selected the 20 samples by taking the sum of all metals found by the pXRF for each sample and sorting them from highest to lowest concentrations and selecting every tenth sample for laboratory analysis. In this way, we were able to test the pXRF along a linear contamination gradient to ensure accuracy along the entire gradient.

Soil pH analysis

The same pulverized soil samples were used to determine the pH of all samples. We used an Ohaus Starter 300 series pH meter and calibrated it using a two-step standard pH 4.00 and 7.00 buffer procedure. We chose a 0.01 M $CaCl_2$ solution to measure soil pH because it is a widely used procedure, it is not affected within a range of the soil to solution ratios used, and prolonged storage (up to one year) does not affect the reading (Peech 1965; Davey & Conyers 1988; Conyers & Davey 1988). To make the $CaCl_2$ (.01M) solution we dissolved 2.940 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in distilled deionized water in a 2 L volumetric flask which was repeated five times to make a total of 10 L. The electrical conductivity of the solution was 2.3 mS cm^{-1} at 25° C. The electrical conductivity was checked each time the solution was used to ensure it stayed within the recommended 2.24 and 2.40 mS cm^{-1} range. We then weighed out 15 mg of soil sample and placed it into a 50 mL graduated centrifuge tube and added 30 mL $CaCl_2$ solution to the centrifuge tubes. The tubes were then placed into test tube racks and shaken until all of the sample was dissolved into the solution. For one hour the solution was shaken every 10 minutes, and after the last shaking, the tubes were left to sit for an additional 10 minutes before testing. The electrode was lowered into the solution, and the reading was recorded once the pH meter stabilized.

Data Analysis

Portable X-ray Fluorescence Performance

ICP-MS laboratory results were compared to results to test the accuracy of pXRF. Deming regression was performed in R software (R Core Team 2017) using the *Deming* package (Therneau 2014) to compare the results because it accounts for errors on both the *x*- and the *y*-axis, rather than parallel to the *y*- axis as a traditional least squares method measures. PXRF results were then referenced against US EPA standards (Table 1) and elements not meeting the “definitive” or “quantitative” quality level were thrown out.

*Table 1. US EPA criteria for establishing data qua*lity (US EPA 1998)

Data quality level	**Statistical requirement**
Definitive	r^2= 0.85-1.0. Relative standard deviation (RSD) ≤10%. Inferential statistics must indicate the two datasets are statistically similar (at the 5% level), i.e., relationship $y = x$ accepted.
Quantitative screening	r^2= 0.70-1.0. Relative standard deviation (RSD) < 20%. Inferential statistics indicate the two data sets are statistically different i.e. relationship $y = mx$ *or* $y = mx + c$ accepted.
Qualitative Screening	r^2< 0.70. Relative standard deviation (RSD) > 20%. Inferential statistics indicate two data sets are statistically different.

Nonmetric Multidimensional Scaling

Using the *Vegan* package (Oksanen et al., 2018) for R software Nonmetric Multidimensional Scaling (NMDS) was used to understand numerically, and graphically, the differences in plant community composition between plots and to identify significant environmental variables for both random and transect sites. Species names were abbreviated by combining the first three letters of the genus and species names separated by a period, so for example*, Festuca idahoensis* was transformed to "Fes.ida." Only species which occurred in ≥5% of the plots for each sampling type were included in this analysis to dampen the effects of rare species on the ordination. The NMDS was then performed using Bray-Curtis dissimilarities based on the square root, and Wisconsin-double-standardization (species are first standardized by maxima and then site by site totals) of species cover percentages. The combination of these two standardizations improves the quality of the ordinations. The ordination was performed with 999 permutations.

In addition to environmental values observed in the field, the richness of native and exotic species, (nR, and eR, respectively), Shannon-Wiener diversity of native and exotic species (nH and eH), and the richness of exotic species divided by the richness of native species (R) were calculated and fitted onto the plot to understand how observed environmental factors affected native and exotic species composition. Aspect in degrees was converted to linear form by creating variables "Eastness" (1= East or 90°, 0 = no aspect to East or West; -1 = West) and "Northness" (1 = North; 0 = no aspect; -1 South). The function *envfit* in the vegan package was used to calculate correlation coefficients and *P* values for each of the environmental variables about the species scores. The environmental values were then plotted as vectors in the NMDS.

Linear Modeling

In addition to NMDS, we used linear modeling to investigate more thoroughly how plant coverage responds to environmental variables. We used the *lm* function in the *stats* package in R Software to analyze the response variables total cover, exotic cover, and native cover with environmental variables as predictor terms for both transects and randomly sampled sites. To reduce collinearity among covariates, we followed the advice of Zurr et al. (2010) and used their function *corvif* in R software to sequentially remove the environmental factor with the highest Variance Inflation Factor (VIF) until all VIF's were below at or below 3. We also repeated these models with additional for interactions between pH and each metal as additional terms.

Interaction Plots

We also wanted to identify species that show promise for restoration of contaminated sites, and to show how exotic species are influenced by contamination. Similarly, we wanted to examine if multiple species occupy the same environmental niches. To visualize these response/interactions we created interaction plots with post-hoc Tukey HSD tests (95% family-wise comparison) was performed on each species to determine which species differed significantly *(p*-value < 0.05) with respect to each environmental variable. We selected species that occurred in ≥5% of all plots, sorted them into exotic and native species, and made interactions plots for each examined metal within each group using Least Squares Means (LS Means) at a 95% confidence level.

Results

A total of 52 species were found within all the sites (Tables 2 and 3). Of the 52 species, 17 were exotic, and 35 were native. MM and pH levels consistently exceed normal background soil levels (Table 4.)

Table 2. All observed exotic plant species.

Species	Species
Alyssum murale	*Poa pratensis*
Bromus tectorum	*Rumex hydrolapathum*
Centaurea stoebe ssp. micranthos	*Salsola tragus*
Elymus elymoides	*Silene vulgaris*
Euphorbia esula	*Taraxacum officinale*
Gypsophila paniculata	*Tragopogon dubius*
Linaria dalmatica	*Trifolium pratense*
Linaria vulgaris	*Verbascum thapsus*
Phleum pratense	*Poa compressa*

Table 3. All observed native plant species

Species	Species
Achillea millefolium	*Leymus cinereus*
Agropyron trachycaulum	*Lithospermum ruderale*
Agrostis scabra	*Lupinus sericeus*
Allium cernuum	*Machaeranthera canescens*
Artemisia ludoviciana	*Mentzelia laevicaulis*
Artemisia tridentata	*Oenothera biennis*
Astragalus atropubescens	*Oryzopsis hymenoides*
Astragalus laxmannii	*Penstemon eriantherus*
Calamagrostis purpurascens	*Phacelia hastata*
Carex douglasii	*Phlox hoodii*
Chaenactis douglasii	*Pseudoroegneria spicata*
Deschampsia cespitosa	*Pseudotsuga menziesii*
Elymus canadensis	*Solidago missouriensis*
Elymus cinereus	*Stipa comata*
Ericameria nauseosa	*Stipa viridula*
Erigeron compositus	*Symphyotrichum spathulatum*
Festuca idahoensis	*Thlaspi arvense*
Heterotheca villosa	

Table 4. Summary of MM levels (mg/kg) and pH

Factor	Low	High	Average
Copper	0	884	177
Zinc	56	11,538	1,667
Lead	24	7,560	1,025
Arsenic	0	782	70
pH	3.54	7.05	5.27

Portable X-ray Fluorescence Performance

The pXRF performed well against ICP-MS metals analysis for the MM we were interested in. Zn, Cu, and Pb had r^2 values of 0.998, 0.970, and 0.968 respectively (Figure 5) and therefore meet US EPA standards of definitive data quality level (Table 1). Arsenic, meanwhile, had an r^2 value of 0.805 which meets US EPA quantitative screening level. The pXRF tended to over-report As, Cu, and Pb levels at low concentrations and underreported at high concentrations compared to ICP-MS. For Zn, the pXRF was extremely accurate at low concentrations and tended to report slightly higher levels than ICP-MS at higher concentrations.

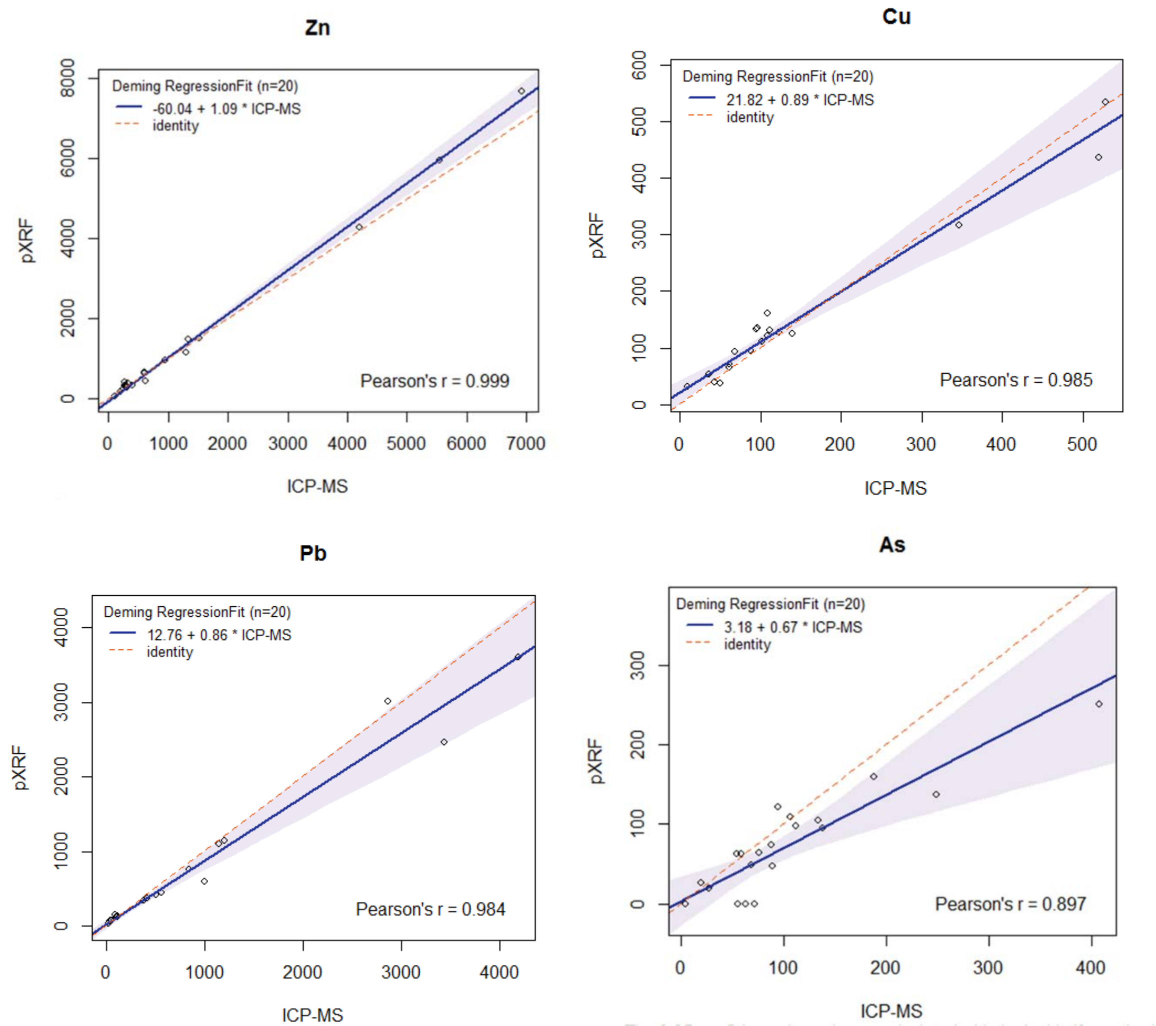

Figure 5. Regression analysis of pXRF measurements against ICP-MS analysis. Deming relationship (solid red line) and 100% recovery (dashed red) are shown in each plot all measurements are in mg kg^{-1}.

Nonmetric Multidimensional Scaling

Transects

The NMDS model produced a two-dimensional solution with final stress of 0.15. All environmental variables were statistically significant at *P* <.01 or less (Table 5). Most of the metals (Pb, Zn, As) are highly associated with the NMDS1 axis, while Cu is nearly evenly split between the two axes (Figure 5). Exotic diversity, exotic richness, and pH were highly associated with the NMDS2 axis while slope, northness, native richness, native diversity, and eastness were nearly split between the two axes. pH and Cu had the highest correlation values of r^2 = 0.27 and 0.28 respectively (Table 5). pH had a strong positive influence on exotic species richness and diversity and the ratio of exotic richness to native richness while increasing pH levels had a less significant effect on native richness and diversity. *Agrostis scabra* showed a strong affinity for acidic environments, while A*chillea millefolium* also showed a preference for acidic environments. Increasing Cu levels also had a substantial effect on the ratio of exotic richness to native richness and a moderate positive correlation with exotic richness and diversity. Cu had little effect on native richness and diversity while Pb and Zn had a substantial negative effect. The grasses *Elymus trachycaulum, Stipa comata,* and *Stipa viridula* all were negatively associated with Pb and Zn and positively correlated with increasing As. Native richness and diversity were also negatively associated with slope and north facing aspects, while the east-west aspect had no effect.

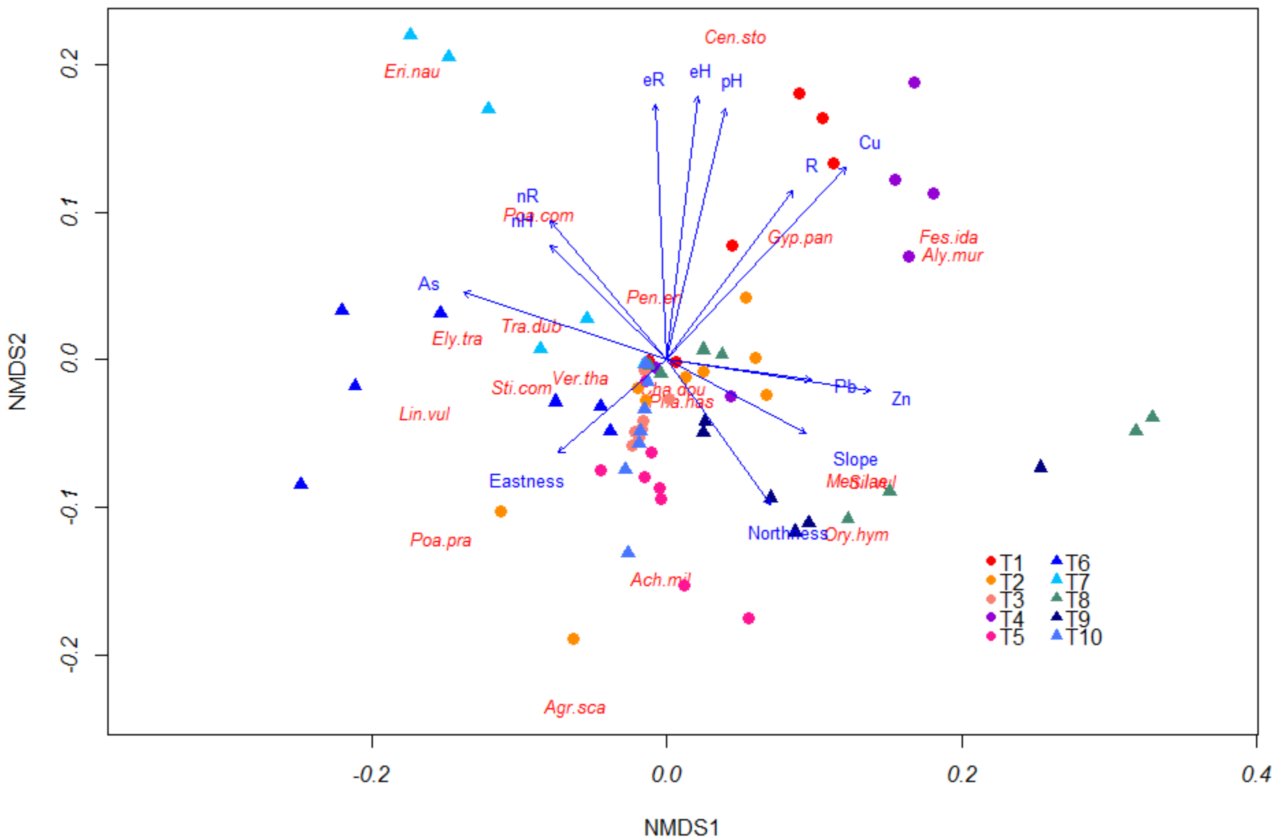

Figure 5. Transect NMDS ordination of Bray-Curtis dissimilarities on species abundance data with environmental vectors (p <.05) overlaid. Sites labeled with a circle are those within the BIPSOU while sites labeled with a triangle are those west of Montana Tech. Different colors indicate sites within each group and where nH = native diversity, nR = native richness, eR = exotic richness, eH = exotic diversity, and R = exotic richness divided by native richness.

Table 5. Significance codes: 0 '***' 0.001 '**' 0.01 '*' where nH = native diversity, nR = native richness, eR = exotic richness, eH = exotic diversity, and R = exotic richness divided by native richness.

Factor	**NMDS1**	**NMDS2**	**r^2**	***P***	**Sig**
pH	0.22622	0.97408	0.2712	0.001	***
Pb	0.99063	-0.13657	0.0861	0.022	*
As	-0.94977	0.31295	0.1868	0.001	***
Zn	0.98801	-0.15442	0.1721	0.004	**
Cu	0.68018	0.73305	0.2807	0.001	***
Northness	0.5797	-0.81483	0.1289	0.003	**
Eastness	-0.75711	-0.65329	0.0832	0.016	*
Slope	0.88374	-0.46798	0.1008	0.01	**
eH	0.11442	0.99343	0.2859	0.001	***
eR	-0.0464	0.99892	0.2655	0.001	***
nH	-0.71473	0.6994	0.1084	0.004	**
nR	-0.64233	0.76642	0.134	0.002	**
R	0.59777	0.80167	0.1808	0.001	***

Random Samples

The NMDS model produced a two-dimensional solution with the final stress of 0.15. All environmental variables were significant at $P < 0.01$ or less besides northness and native diversity, which are not included in the model (Table 6). In this model, Zn, Pb, and slope were mostly associated with the NMDS1 axis, while, eastness, native richness, exotic richness, exotic diversity, the ratio of exotic richness to native richness, and Cu trended along NMDS2 (Figure 6). As and pH were roughly split between the two axes. Slope and pH again had some of the highest r^2 values at 0.47 and 0.46 respectively, but Zn also showed high values with 0.41 (Table 6). Native richness was strongly associated with east aspects and negatively associated with Cu, exotic diversity and richness, and a high ratio of exotic to native richness. Native richness was also associated with As and higher pH values. *Elymus trachycaulum, Stipa comata, Ericameria nauseosa* and *Stipa viridula* are negatively associated with Cu and positively correlated with As. Exotic richness and diversity, and the ratio of exotic to native richness was strongly associated with Cu and had a negative correlation with As, and pH. Lead, and Zn had almost no association with any of the diversity or richness measures but had a strong correlation with *Mentzelia laevicaulis,* and *Silene vulgaris* and a negative relationship with *Agrostis scabra, Calamagrostis purpurascens, Deschampsia cespitosa,* and *Leymus cinereus*.

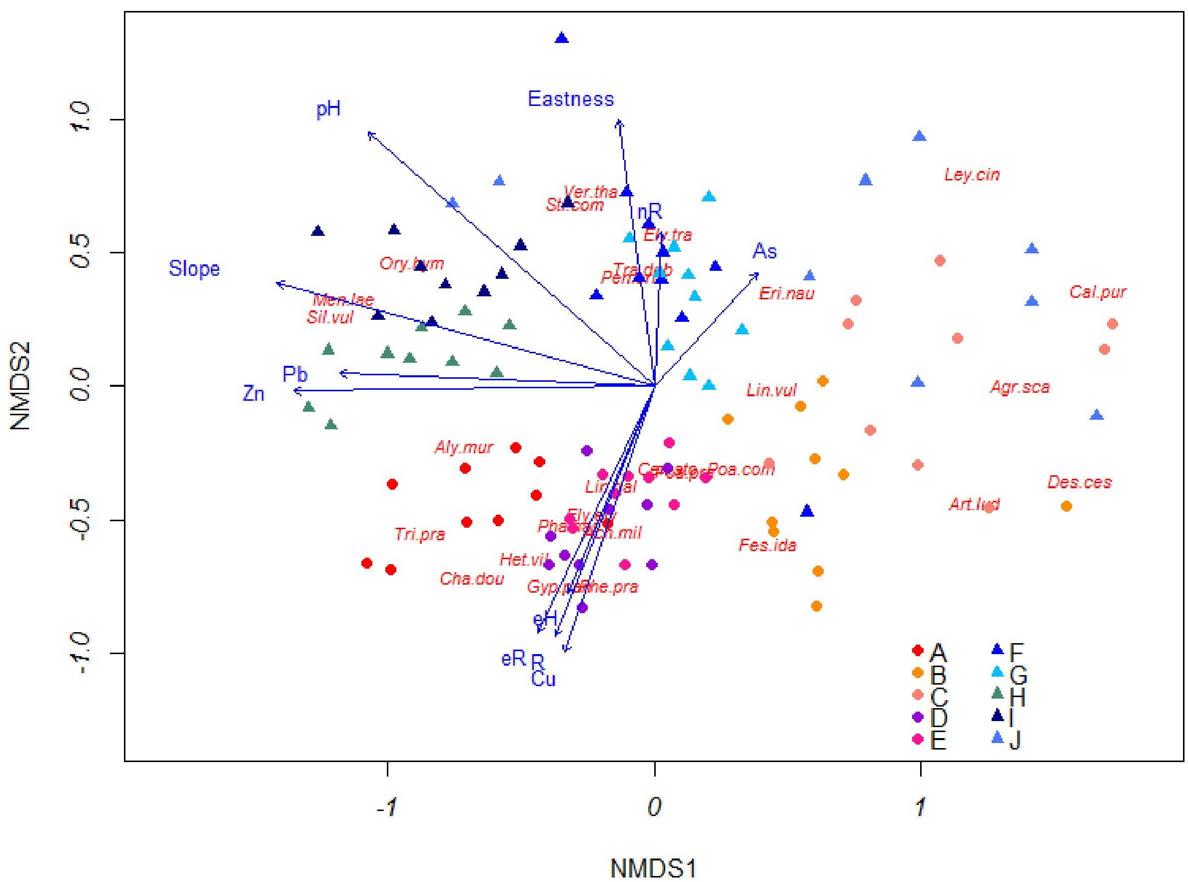

Figure 6. Randomly sampled sites NMDS ordination of Bray-Curtis dissimilarities on species abundance data with environmental vectors ($p < 0.05$) overlaid. Sites labeled with a circle are those within the BIPSOU while sites labeled with a triangle are those west of Montana Tech. Different colors indicate sites within each group and where nH = native diversity, nR = native richness, eR = exotic richness, eH = exotic diversity, and R = exotic richness divided by native richness.

Table 6. Significance codes: 0 '***' 0.001 '**' 0.01 '*' where, nH = native diversity, nR = native richness, eR = exotic richness, eH = exotic diversity, and R = exotic richness divided by native richness.

Factor	**NMDS1**	**NMDS2**	**r^2**	***P***	**Sig**
pH	-0.74937	0.66215	0.4563	0.001	***
Pb	-0.99918	0.04045	0.3093	0.001	***
As	0.67085	0.74159	0.0734	0.029	*
Zn	-0.99992	-0.01234	0.4053	0.001	***
Cu	-0.31967	-0.94753	0.2457	0.001	***
Eastness	-0.13632	0.99066	0.2251	0.001	***
Northness	0.15888	-0.9873	0.0035	0.857	
Slope	-0.96448	0.26416	0.4774	0.001	***
nH	0.24644	0.96916	0.0348	0.175	
nR	0.04371	0.99904	0.0739	0.021	*
eR	-0.42711	-0.9042	0.2306	0.001	***
eH	-0.38453	-0.92311	0.1564	0.001	***
R	-0.36724	-0.93013	0.2252	0.001	***

Linear Modeling

Transects

Linear modeling with the total cover as the dependent variable and all environmental terms as the independent variables showed pH and Zn to be the most significant predictors *p*- values of 0.001 and 0.01 respectively. Native cover also yielded pH and Zn as most significant with *p*-value of 0.05. With exotic cover as the response pH, eastness, and As were most significant with *p*- values of 0, 0.001, and 0.05 respectively. When we reran the models with pH interactions with all metals included in the models, there were no significant pH interactions with the metals.

Random Samples

Linear modeling with the total cover as the dependent variable and all environmental terms as the independent variables showed Cu, Pb, and eastness with *p* values of 0.01, 0.05, and 0.05 respectively. With native cover as the response, eastness was the only significant predictor with *p*-value of 0.01. For exotic cover as the response, Cu and Pb were significant at 0.001 and 0.05 respectively. With respect to pH metals interactions, when exotic cover was used as the response both Cu and As were significant at *P* 0.001 and 0.01 respectively. The native cover also showed pH interactions with As with *P* of 0.01.

Interaction Plots

Copper and lead

The exotic species *Alyssum murale, Centaurea stoebe, Gypsophila paniculata,* and *Linaria dalmatica* showed the highest tolerance to Cu while, *Verbascum thapsus, Tragopogon dubius, Silene vulgaris,* and *Linaria vulgaris* showed the lowest tolerance (Figure 7). Native species *Phacelia hastata, Festuca idahoensis, Ericameria nauseosa*, and *Achillea millefolium* showed the highest tolerance to Cu and *Stipa comata,* and *Elymus trachycaulum* showed the lowest tolerance.

For lead, the exotic species *Silene vulgaris* showed extreme tolerance and while *Alyssum murale, Verbascum thapsus,* and *Tragopogon dubius* were moderately tolerant, the rest of the exotic species significantly overlapped in lower concentration. The native species *Mentzelia laevicaulis*, and *Oryzopsis hymenoides* was extremely tolerant to Pb while *Agrostis scabra* was the least tolerant.

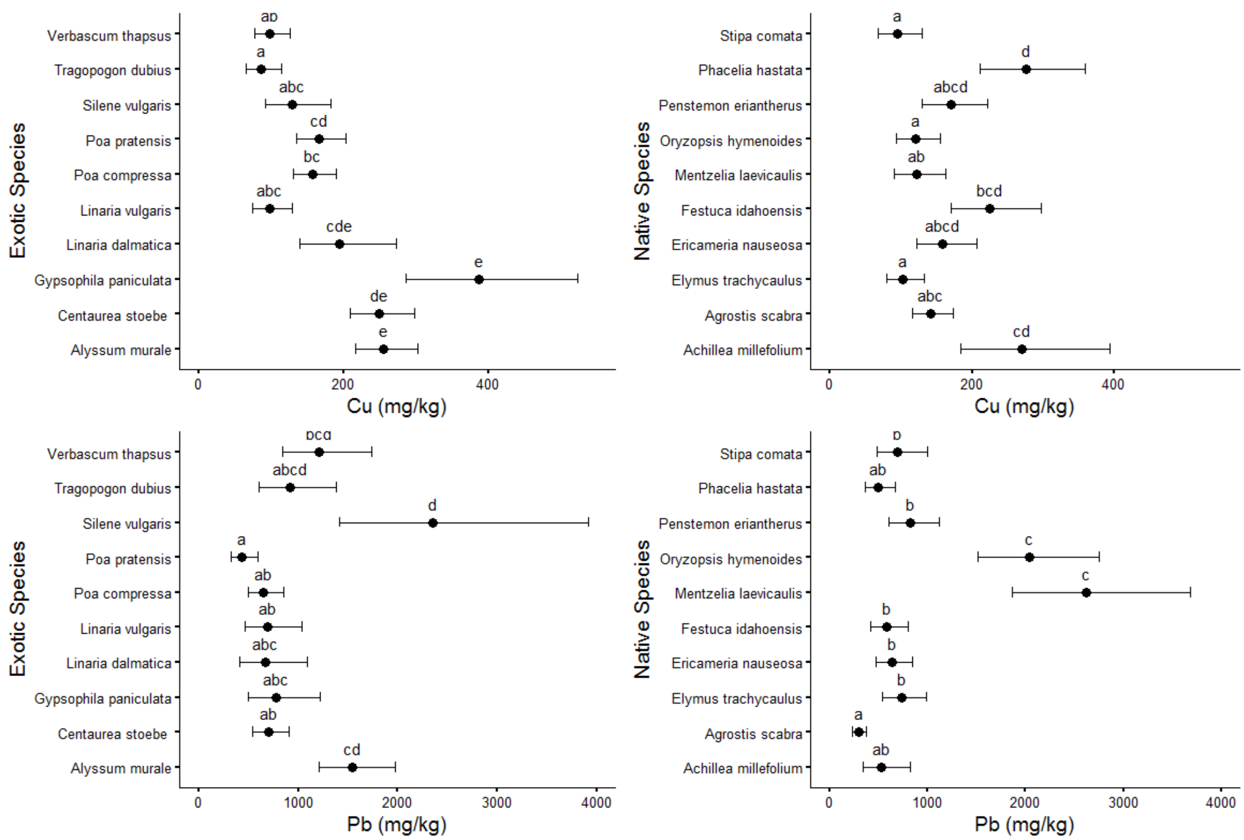

Figure 7. Exotic and native species tolerance to Cu and Pb. Circles indicate the LS mean. Error bars indicate the 95% confidence interval of the LS mean. Means sharing a letter are not significantly different (Tukey-adjusted comparisons).

Arsenic and zinc

The exotic species *V. thapsus, T. dubius,* and *L. vulgaris* were most tolerant of As, while *L. dalmatica* and *A. murale* were least tolerant (Figure 8). The native species *S. comata, P.*

eriantheus, Ericamerica nauseosa, and *E. trachycaulum* had the highest tolerance to As, while *A. millefolium* was the least tolerant. The exotic species *A. murale,* and *S. vulgaris* were most tolerant of Zn while the rest of the exotic species had similar lower tolerance. The native species *M. laevicaulis* and *O. hymenoides* had a high tolerance to Zn, while *A. millefolium* and *A. scabra* showed the lowest tolerance.

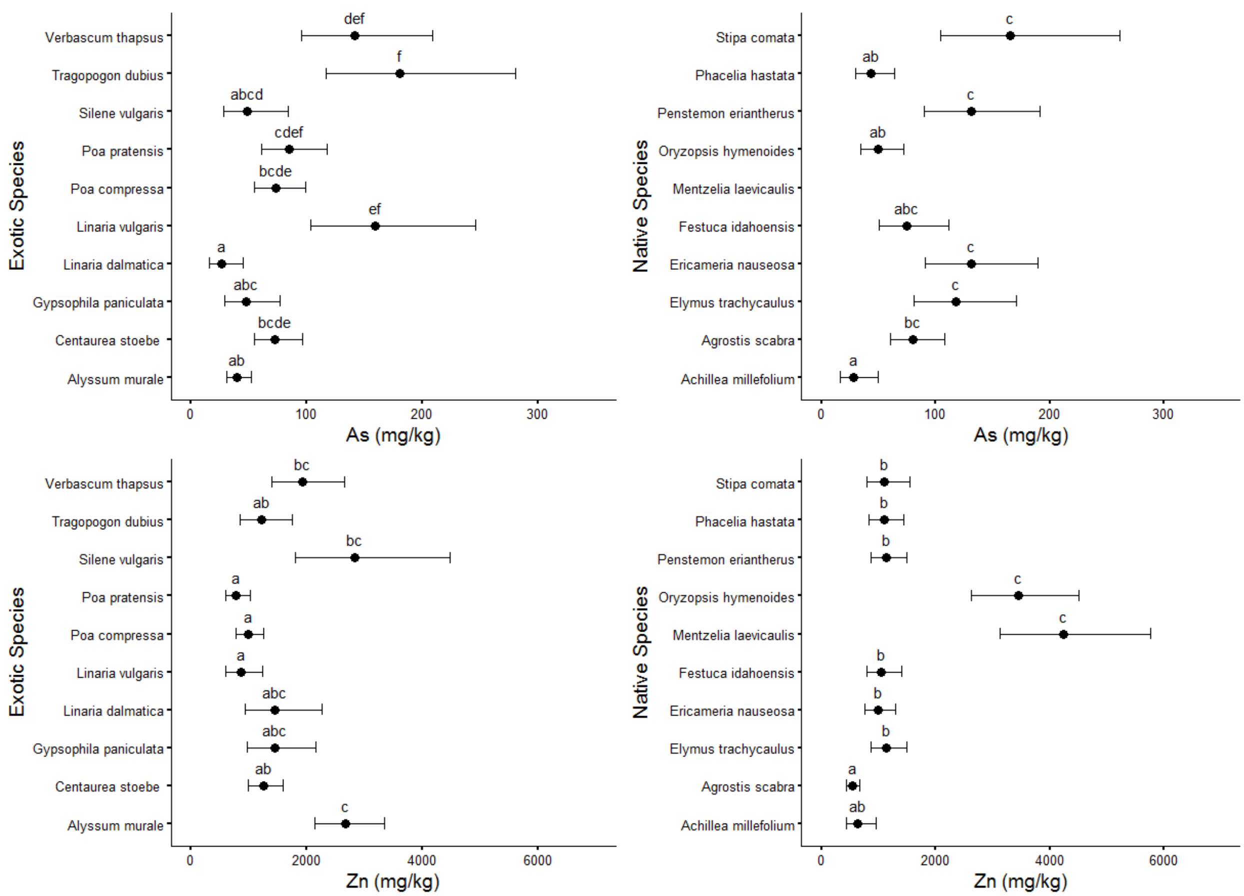

Figure 8. Exotic and native species tolerance to AS and Zn. Circles indicate the LS mean. Error bars indicate the 95% confidence interval of the LS mean. Means sharing a letter are not significantly different (Tukey-adjusted comparisons).

pH

The exotic species *V. thapsus, T. dubius,* and *S. vulgaris* had the lowest tolerance to acidic soil, while the rest of the species were slightly more tolerant (Figure 9). Native species *A.*

millefolium, A. scabra, F. idahoensis, and *P. hastata* were the most tolerant to low pH, while all other species trended towards neutral pH soils.

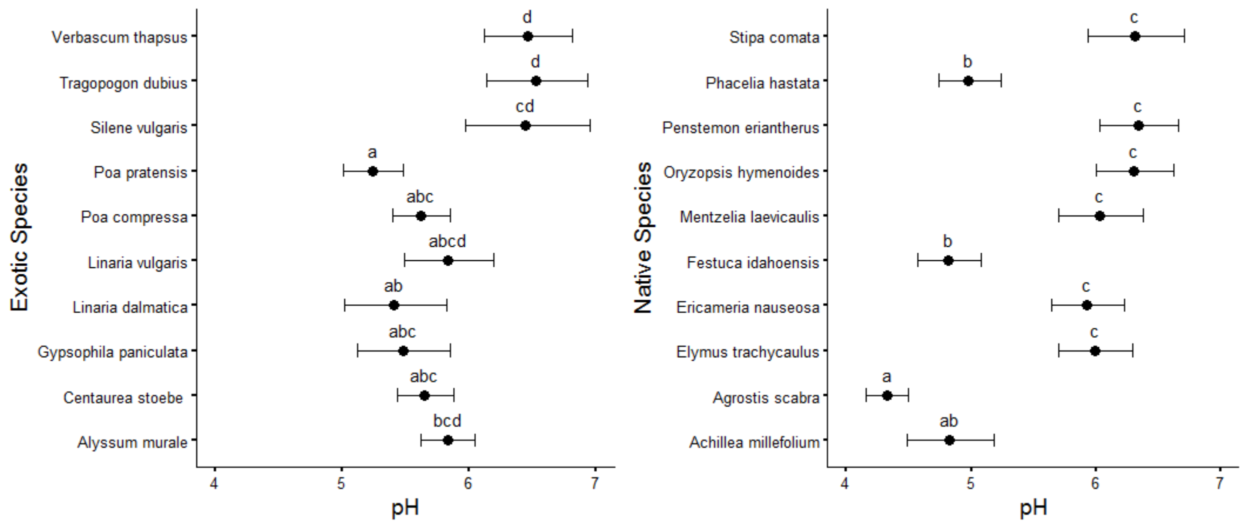

Figure 9. Exotic and native species tolerance to pH. Circles indicate the LS mean. Error bars indicate the 95% confidence interval of the LS mean. Means sharing a letter are not significantly different (Tukey-adjusted comparisons).

Discussion

Plant-contaminant relationships

The results of both the ordinations and linear modeling show that low soil pH, Cu, Zn, and Pb significantly influence plant colonization and vegetation on mining impacted sites. Transect sampling showed that pH had the most significant inhibitory effect followed by Zn on plant establishment while random sampling indicates Cu and Pb to be most influential in retarding plant coverages in plant islands. Our results showing pH as the primary driver for plant success are consistent with a large body of research (Wong et al. 1998; Gough et al. 2000; Pärtel 2002; Conesa et al. 2006; Eskelinen et al. 2009). Although not investigated here, low pH values decrease the availability of nutrients to plants, microbial activity, and can increase the bioavailability of most heavy metals, with the exception of As, (Alloway 1995; Peet et al. 2003;

Nordin et al. 2004; Eskelinen et al. 2009; Baker et al. 2010) which may contribute to the strong negative association of pH and plant establishment.

Within vegetation islands, heavy metals were negatively associated with plant community composition. We observed that increasing Cu, and to a lesser extent Pb, concentrations are strongly correlated with increasing exotic species cover, richness, and diversity and they are negatively correlated with native species richness. A literature review did not yield any studies which investigated the relationship between soil Cu contamination and quality of plant community diversity and richness. Thompson and Proctor (1983), however, found copper had little effect on determining vegetation abundance on waste from an abandoned copper and lead mine in Scotland but did not investigate richness or diversity. Nikolic et al. (2016) investigated plant communities impacted by Cu tailings in Serbia and found that Cu alone did not prevent affected plant communities from returning to the original vegetation but when Cu and low pH were present together a novel community was produced. Thus, the present study appears to be the first of its kind to investigate the quality of spontaneous vegetation establishment on Cu contaminated soils. When examining the effects of heavy metals together, similar results were found in nearby Anaconda, MT as a result of smelting emissions (Galbraith et al. 1995), Legeune et al. (1996) showed heavy metals reduced the compositional and structural heterogeneity on Silver Bow Creek and the upper Clark Fork River in southwest Montana but these studies did not examine the effects of specific metals on community composition.

Implications for Restoration

The use of native species in mine restoration is preferable to the use of exotic species. Species native to the degraded area are uniquely adapted to the local environment and are more resilient in the long term than exotic. Although holistic restoration of highly contaminated sites is not possible because of physio-chemical constraints, many native species have spontaneously colonized contaminated sites without traditional ameliorative activities such as liming, organic matter additions, isolation of mine waste, or irrigation. Direct planting or seeding of these species in MM contaminated soils could be used as a low-cost way of providing stabilization of contaminants where full restoration of native plant communities is not feasible. The native species referred to in Table 7 are the most abundant species observed on the sites investigated and are recommended for incorporation into future restoration seed mixes or planting. Seed mixes or plantings of these species can be tailored to meet the needs of sites with specific contaminants. For example, *Agrostis scabra* shows extreme tolerance to low pH and *Mentzalia laevicaulis* is very tolerant of Pb and Zn contaminated soils.

Table 7. Native species recommended for use in restoration

Species	**Species**
Stipa comata	*Penstemon eriantheus*
Phacelia hastata	*Ericamerica nauseosa*
Oryzopsis hymenoides	*Elymus trachycaulus*
Mentzalia laevicaulis	*Agrostis scabra*
Festuca idahoensis	*Achillea millefolium*

Conclusion

Unremediated mine waste areas in Butte, MT are extensively populated with native and exotic plant species. We have demonstrated that low pH and Zn are the main limiting factors to plant growth in these novel ecosystems, and our research is the first to show that soil Cu levels drive the increase in exotic plant richness and diversity. Future restoration projects in this area should consider the species identified in this paper for use in unremediated sites and to provide resiliency in areas that have been reclaimed with exotic species. Future research on this topic should examine other environmental properties such as soil nutrient levels, organic matter, and speciation of heavy metals.

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