PLANTS GROWING IN CLOSED MINES OF NORTHWESTERN ONTARIO ARE USEFUL FOR PHYTOSTABILIZATION POTENTIAL OF ARSENIC, ANTIMONY AND MOLYBDENUM IN MINE RECLAMATION

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Introduction

Northwestern Ontario is a boreal forest region on the Canadian Shield rich in mineral resources with active, closed and abandoned mines. These areas could be reclaimed with phytoremediation species to prevent wind and water erosion to reduce metals from entering the food chain and waterways (Salt *et al*., 1995; McIntyre, 2003; Pilon-Smits, 2005; Peer *et al*., 2005). Whereas organic contaminants can be degraded and reduced to less toxic components, metals are non-biodegradable, persist in waterways near the contaminated area, can endure within the soil for the unforeseeable future and be taken up by plants including agricultural crops and natural revegetation of the region (Ashraf *et al*., 2011; Nouri *et al*., 2008).

Naturally occurring plants on closed mine sites are best adapted for growth on soils with elevated metal composition (Salt, 1995). They are often highly tolerant of metals that remain directly from the mining processes that occurred in the area, via direct contribution from mining or indirectly from air, water and soil erosion (Freitas *et al*., 2004). Metallophytic plants have evolved to grow on elevated metal soils. They are useful for reclamation purposes of other industrial sites or to indicate potential ore bodies (Whiting *et al*., 2004). Ideal candidate species for rehabilitation are thriving in the elevated metal contaminated soils, produce high amounts of biomass and are well adapted to the local climate (Khan *et al*., 2000; Dickinson *et al*., 2009; Chen *et al*., 2012; Majumder and Jha, 2012).

Phytoremediation with native plants is a multifaceted approach using metallophytic species more suited to the local environment as compared to agricultural or introduced plantings (Oppelt, 2000; Pilon-Smits, 2005). Various phytoremediation strategies exist including phytoextraction and phytostabilization. Phytoextraction removes the contaminant from the soil profile and known as accumulators. If the concentration of the contaminant in the plant is extremely high in comparison to the concentration in the soil, these are known as hyperaccumulators (Kumar *et al*., 1995; Peer *et al*., 2006). Phytostabilizers do not uptake metals and other contaminants into the above ground tissues of the plant and called excluders (Wong, 2003). Phtyostabilization prevents the elevated metal contents of the soil from spreading into the food chain via plant uptake and helps contain the contamination to the affected area (Mendez and Maier, 2008). Metal pollution on these mine sites is directly influenced by the type of deposit mined, so phytostabilizers need to cater to each type of circumstance. Absence and/or excess of various metals and nutrients can affect the success of each type of plant as well as other characteristics such as lifespan, size, root systems, and predation. Identification of specific plant species can cater to these contaminated areas (Tordoff *et al*., 2000; Mendez and Maier, 2008).

This paper explores the phytostabilization potential of plants naturally regenerating on closed mines in Northwestern Ontario. Data will be presented on their associated soils including pH and the various metal concentrations. The sites chosen for this study are mines that operated in the 20th century in Northwestern Ontario, located on the Canadian Shield and are in the Lake Superior and Seine River Watersheds. Minerals mined varied but two mines had elevated levels of As, Mo and Sb. Remediation of these sites would help to meet government environmental requirements as well as reduce the impact on the local populations of humans and wildlife. Surface soil contaminants from these sites have the potential to runoff into surface waters, leach into ground waters, and negatively impact the local food chains. A combination of native boreal species can be planted on closed mines in order to restrain the movement of these potentially harmful metals. Chlorophyll content of the leaves are measured as a sign of plant health and can aid in identification of healthy metallophytes.

The objectives of the study were i) to identify plants growing on these closed mines, and ii) to examine the variation in the soil conditions natural regenerating species experience on these closed mines.. Plant communities will be found that are tolerant to metal stress.

Materials and Methods

Site Description

This study examines three closed mines areas in Northwestern Ontario with no replanting of plants or trees following closure. Located on the Canadian Shield, these mines sites sampled have been closed or not replanted for a minimum of 25 years and had restricted road access.

Steep Rock Iron Mines, encompassing just over 100 square kilometers, are located near Atikokan, ON and operated as a source of high grade hematite from goethite-hematite deposits for 30 years from the 1950s to the early 1980s (Ontario Geological Survey *et al*., 1972). The Winston Lake mine near

Schreiber, ON which produced primarily zinc, silver and copper with secondary amounts of gold began in 1988 and ended in the late 1990s (LaFrance *et al*., 2004). The third area consists of several properties managed by Premier Gold located in the Beardmore/Geraldton greenstone Belt: Northern Empire and Leitch mines. Northern Empire was and underground operation producing gold from 1934 to 1941 with other exploration occurring since that time period. Leitch was mined for gold from 1936 to 1968 (GEDC, 2005).

Field and Sample Preparation

Thirty metre long transects were placed on the mined rock piles, tailings areas and former building locations to determine naturally regenerating plant communities on the closed mines. On these transects samples were collected as follows: 15 1 m^2 quadrats of herbaceous plants, 6 5 m^2 quadrats of shrubs and 3 10 m^2 quadrats of trees (Bagatto and Shorthouse 1999). Plants used for metal analysis in this investigation were identified and sampled based on abundance, amount of biomass in root and shoot tissue, healthy leaf colour and active growth on the sites. Chlorophyll content was used as a method to determine health status in their mine environment (Walters 2005). Chlorophyll content of species were obtained using the CCM-300 Chlorophyll content meter using an average of 3 readings per measurement (Gitelson, 1999).

Plant and soil samples were taken along these transects: 3 soil samples per transect and three plant samples per species. Plant samples were identified following local plant identification guides and verified at the Lakehead University Herbarium. The foliar samples were rinsed with distilled water, air-dried at room temperature for several weeks, and the samples were ground to a homogeneous powder. Analysis of metals in the plants encompassed all aboveground plant material in late August including twigs, leaves or needles, and flowers. Soil samples were collected in the rhizosphere of the plant, not always at the same depth due to plant type and variations in soil depth. Plants were dug out of the ground and shaken over a bag for the soil. Soils were air-dried and sieved with a 2mm mesh to remove plant matter and rocks.

Laboratory analysis

Analyses were done at the Lakehead University Environmental Laboratory (LUEL) according to the LUEL (2012) Quality Assurance/Quality Control (QA/QC) protocols. Soils and plants were analyzed for moisture,pH, conductivity, organic matter and total metal concentrations of Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mo, Na, Ni, P, Pb, Sb, Sr, Ti, Va, and Zn.

Statistical Analysis

Statistical analysis were performed using SPSS 23 package for Windows as follows:

i. For the transect data from the plant populations, herb, shrub and tree data were summarized using species richness (mean number of species and identity per mine) and the density (mean number of stems per mine)

(Magurran 2013). Differences in species richness and density at all stand levels among the three mines were examined using ANOVA.

ii. Plant species data were analyzed using ordination and classification (non-metric multi-dimensional scaling (NMS) and cluster analysis), to identify species with similar habitats. Vegetation data were screened for outliers, normality, and heteroscedasticity (McCune, 1996). The PROXSCAL algorithm with a Torgerson start and Chi-square measure for count data was applied because it allows similarity matrices to be used. Cluster analysis using Ward's linkage and Squared Euclidean distance was performed to confirm separation of the species.

iii. For the mine soils, mean and standard deviation of metals were determined for each mine. Data was log transformed to curtail skewness. Discriminant function analysis on the soil characteristics was performed. Statistical significance was defined as $P < 0.05$.

Results

Plant Characterization

Sampling at the mines resulted in a collection of 36 plant species, from 31 genera and 14 families, with richness and density data shown in

Table 1. Winston Lake and Premier had more plant species and a larger cover but less trees than Steeprock, which had an even richness of herbs, shrubs and trees. None of the species were found at all of the transect sites but willow (*Salix* spp. L), white birch (*Betula papyrifea* Marshall*),* goldenrod *(Solidago canadensis* L*),* hawkweed (*Hieracium canadense* Michx*), and* trembling aspen (*Populus tremuloides* Michx) were found at all three mining areas. Pearly everlasting (*Anapahlis margaritacea* L*)* was found solely at Winston Lake mine. At Steep Rock Mine, there are either older trees and very little understory with very few herbaceous species or areas with no trees, some shrubs and sparse herbs. Soils and surrounding water have vivid multicoloured hues with very low populations of unhealthy herbs. Many of these trees are seen with fungal mycorrhizae to aid in their growth. Sites investigated at Premier's properties showed stunted shrub-like trees and some herbaceous species but no large overstory. Winston Lake had no trees with some areas with shrubs and intermittent herbs.

Table 1 Mean number of herbaceous, shrub, and tree species (richness) and mean values of stand structure characteristics in three mines in northwestern Ontario*

	Herbs		Shrubs		Trees	
	Richness	Density (stems/m^2)	Richness	Density (stems/100 m^2)	Richness	Density (stems/ha)
Premier	4.7 $_a$	47.6 $_a$	2.4 $_a$	207.5 $_a$	0.0 $_a$	0.0 $_a$
Steep Rock	1.5 $_b$	12.3 $_b$	1.1 $_b$	183.9 $_a$	1.4 $_b$	10888.9 $_b$
Winston Lake	3.6 $_c$	42.1 $_a$	2.3 $_a$	281.1 $_a$	0.0 $_a$	0.0 $_a$

*Values within the rows with the same letters (a, b, and c) are not significantly different at the P <0.05 level.

The proximity values for the plant species at the three mines are represented by a two-dimensional NMS map based on the resulting raw stress of 0.05007 and a Stress-I value of 0.22377 (**Error! Reference source not found.**). The stress values reflect how well the solution summarizes the distances between the data so a low stress value shows a good fit ordination. Cluster analysis was run for 39 cycles to also determine the groups of plant species and compare to the NMS results (Figure 2). With a line drawn at the 6 distance in the cluster analysis, the majority of the plant species are separated in two clusters as well as outliers of the grass species of false melic grass (*Schizachne purpurascens* Torr.), and horsetail (*Equisetum* spp.) The next group of species features birdsfoot trefoil (*Lotus corniculatus* L), hawkweed (*Hieracium canadense* Michx), as well as raspberry (*Rubus idaeus* L), pearly everlasting and the shrub height of white birch. The last cluster is the remaining plant species, which can also be seen within the circle of the NMS diagram as seen in **Error! Reference source not found.**: white pine (*Pinus strobus* L), white spruce (*Picea glauca* Moench), willow, white birch, trembling aspen, red pine (*Pinus resinosa* Aiton), balsam fir (*Abies balsamea* (L) Mill), heart leaved aster (*Symphyotrichum cordifolium* L), fireweed (*Epilobium angustifolium* L), jack pine (*Pinus banksiana* Lamb.), blueberry (*Vaccinium angustifolium* Aiton), tamarak (*Larix laricinia* Michx), dandelion (*Taraxacum officinale* FH Wigg), yarrow (*Achillea millefolium* L), sedge (*Carex brunnescens* Pers.), daisy (*Leucanthemum vulgare* Lam), balsam poplar (*Populus balsamifera* L) , strawberry (*Fragaria vesca* L), cedar (*Thuja occidentalis* L), red clover (*Trifolium pratense* L), bladder campion (*Silene vulgaris* Poir.), goldenrod (*Solidago canadensis* L), and primrose (*Oenothera biennis* L).

Chlorophyll concentrations in vegetation at the mines are shown by Table 2. Some of the plant species with leaves that were too chlorotic to give a chlorophyll content via the CCM-300 meter. Chlorophyll concentrations of the leaves ranged from 166 mg/m^2 to 718 mg/m^2. Higher concentrations of chlorophyll were found at Winston Lake where no chlorosis was evident. Many leaves on the plant species at Premier had chlorophyll levels below detection for the meter but the plants that did give readings were higher values than the plants found at Steep Rock, except for white birch and red clover.

Soil Characterization

A summary of the soil analysis of each of the studied mines is given in

Table 3. Metals with elevated concentrations in the soil samples were As, Mo and Sb at Steep Rock at levels of 320.65 mg As kg^{-1}, 4.46 mg Mo kg^{-1} and 674.91 mg Sb kg^{-1} respectively and Premier sites at levels of 2245.36 mg As kg^{-}, 7.74 mg Mo kg^{-1} and 1472.3 mg Sb kg^{-1} respectively. Winston Lake had elevated amounts of zinc at 787.61 mg Zn kg^{-1} due to ore mined at the site. The pH at the mines varied from slightly basic at Premier with a pH of 7.9 to Winston Lake with 6.8 and Steep Rock with the more acidic conditions at 5.86. Each location showed similar bulk density ranging from 0.87 at Premier to 0.91 at Winston Lake and 0.93 at Steep Rock.

All soils chemistry data was used in the discriminant function analysis that classified 100% of the samples collected correctly (Figure 3). Function 1 explained 72.8% of the and function 2 explained 27.2%. Function 1 could be interpreted as the ratio of Fe (negative coefficient) to P, As, V, Pb, and Co (positive coefficient). Function 2 has Fe, Ca, Mn and K as the positive coefficients and Mg, Cr and Co as the negative coefficients. Each of the mines is completely separated with different soil characteristics and so plants found at all three locations are possible universal candidates for rehabilitation.

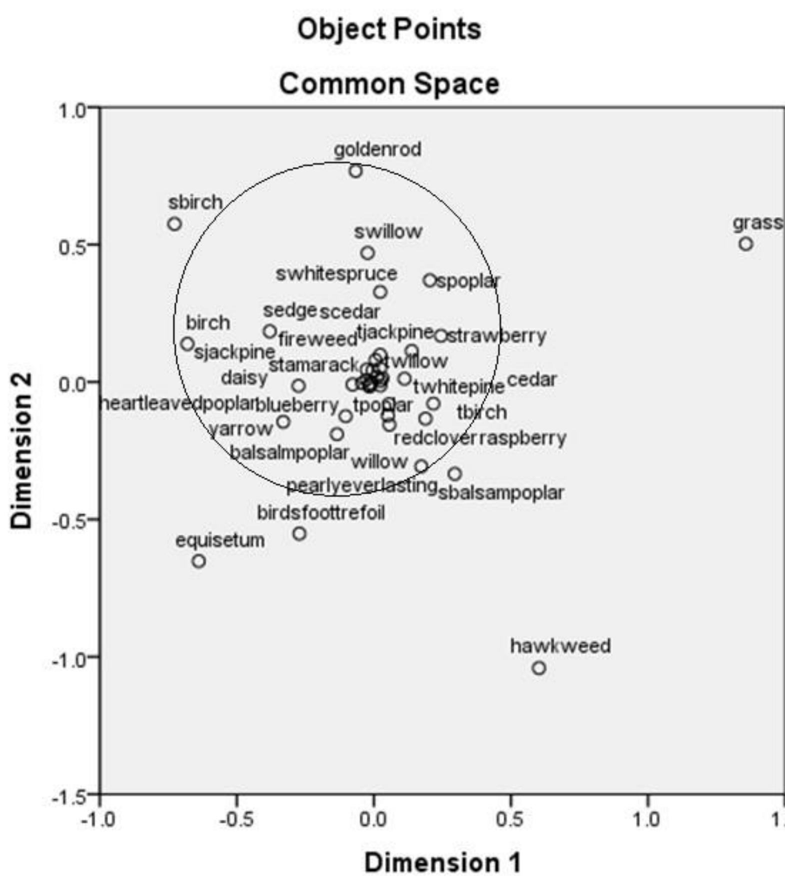

Figure 1 A two-dimensional ordination plot derived from non-metric multi-dimensional scaling (NMS) of 13 transects using herbaceous species composition and abundance data.

Table 2 Chlorophyll Concentrations of various plant species found at mine locations in Northwestern Ontario

			Mine Location					
			Premier		Steep Rock		Winston Lake	
			Concentration (mg/ m^2)					
			Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Species	Balsam poplar	*Populus balsamifera*	535.7	116.3				
	White birch	*Betula papyrifera*	330.0	134.4	453.5	79.3	569.3	226.3
	Birdsfoot trefoil	*Lotus corniculatus*			479.7	76.6	349.0	215.0
	Blue spruce	*Picea pungens*					386.0	0.0
	Cedar	*Thuja occidentalis*	289.0		250.0			
	Goldenrod	*Solidago canadensis*	471.0		316.0	114.6	440.0	79.7
	Horsetail	*Equisetum* spp	166.0					
	Pearly everlasting	*Anaphalis margaritacea*					339.3	119.5
	Trembling aspen	*Populus tremuloides*			648.3	218.7	667.0	173.4
	Evening primrose	*Oenothera biennis*					244.0	
	Red clover	*Trifolium pratense*	278.0		538.5	47.4		
	Red pine	*Pinus resinosa*			300.5	68.6		
	Sedge	*Carex gynocrates*					347.0	0.0
	White pine	*Pinus strobus*			432.5	57.3		
	White spruce	*Picea glauca*	318.0		265.0	65.0	389.0	92.2
	Wild strawberry	*Fragaria vesca*			560.0			
	Willow	*Salix* spp.	540.8	149.8	405.3	130.8	718.0	174.4
	Yarrow	*Achillea millefolium*					351.5	111.0

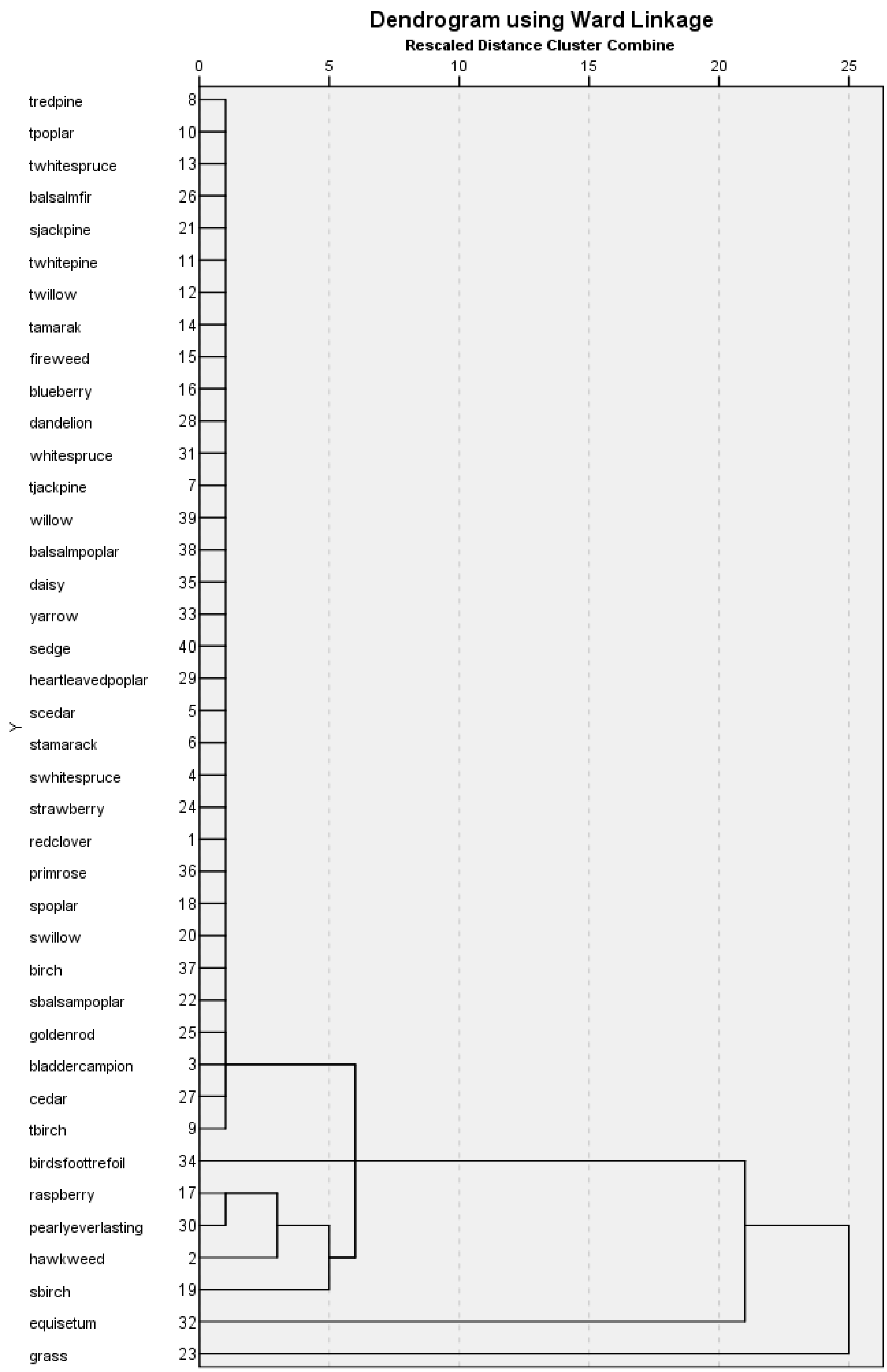

Figure 2 Hierarchical cluster analysis of 13 transects using herbaceous species composition and abundance data.

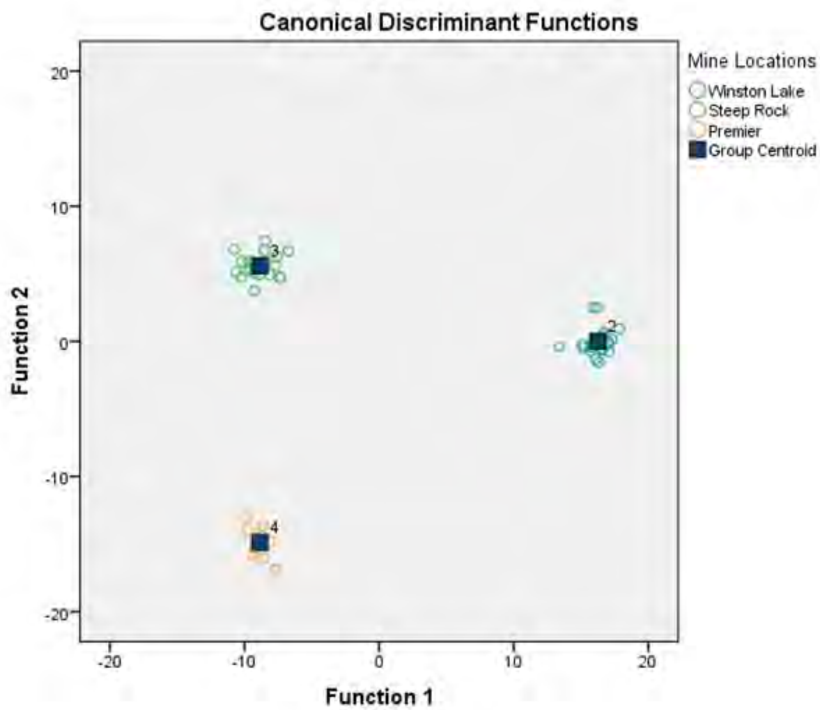

Figure 3 Discriminant Function Analysis Plot with all the soil variables at the mine locations. Standardized discriminating function 1 Fe -4.08, Ni - 1.70, K -1.38, Cr -1.29, Co and Pb +1.85, V +2.27, As, +2.28, P +2.34. Function 2 Mg -0.94, Cr -0.92, Co -0.84

Table 3 Soil chemistry characteristics of the studied areas on the three mines (mg kg^{-1}) includes total metal concentrations in mg kg^{-1}, % moisture, conductivity, bulk density, % organic matter and pH

	Mine Location					
	Premier		Steeprock		Winston Lake	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
% Moisture	19.54	7.87	9.23	3.52	15.54	6.23
Conductivity (us/cm)	347.35	157.86	139.79	101.31	235.71	229.09
Bulk Density (g/cm^3)	.87	.22	.93	.21	.91	.20
Organic Matter	2.72	1.13	5.80	1.16	4.57	7.23
pH	7.90	.17	5.86	1.94	6.36	.90
Aluminum	1.2%	0.3%	0.6%	0.2%	0.8%	0.2%
Arsenic	2245.36	3106.81	320.65	104.37	2.00	0.00
Barium	41.16	32.73	77.41	225.74	21.70	9.15
Calcium	2.2%	1.2%	1.7%	4.5%	0.19%	0.18%
Cobalt	17.83	8.57	25.12	11.74	2.47	1.09
Chromium	34.46	25.06	313.81	214.59	35.91	14.11
Copper	51.04	21.82	41.93	13.99	54.39	63.29
Iron	4.4%	1.5%	4.6%	17.6%	1.4%	0.36%
Potassium	2265.84	1275.54	573.20	340.97	304.39	151.20
Magnesium	1.0%	0.34%	0.81%	1.65%	0.39%	0.19%
Manganese	762.66	422.32	2624.90	2213.43	133.02	70.77
Molybdenum	7.74	24.37	4.46	12.06	2.00	0.00
Sodium	422.42	291.58	76.53	43.05	99.72	22.66
Nickel	53.66	43.90	124.94	39.50	20.10	8.84
Phosphorus	355.64	93.11	227.90	51.74	304.81	156.11
Lead	28.97	33.98	61.60	19.81	4.51	1.30
Antimony	1472.30	805.71	674.91	1001.75	2.00	0.00
Strontium	86.04	26.38	34.40	20.21	4.48	1.62
Titanium	298.14	152.31	306.89	162.44	410.64	75.70
Vanadium	32.17	20.57	125.90	65.92	23.83	4.50
Zinc	78.37	24.43	112.88	45.87	787.61	953.26

Discussion

Plant Characterization

Species richness and density was much lower than typical southern boreal forests in Canada. Very few species were tabulated compared to Haeussler *et al*. (2002). They found that species richness was higher in clear cut forests compared to old growth forests. Heavy mechanical soil disturbance and removed soil organic layers could drastically decrease the residual and resprouting species so as to shift to pioneering species growing from seeds and spores, providing an opening for non-native species invasion. The results of the NMS data and cluster analysis provide evidence of several factors: invasive species ability to adapt to the site conditions, type of soil conditions following mining operation and differences in the age of the stands due to time since closure. None of the mines had a completely unique set of plant species. The first group of plants in the NMS/Cluster analysis included the outlier species that have been classified as monocultural, invasive or exotic. With the alteration of the landscape, monocultures of these species occur due to their quick adaptation to the soil conditions, open sunlight, little competition and their ease of reproduction through seed or rhizomes (Bosdorff *et al*., 2005). They also tend to have hermaphroditic sex habits, extended flowering, small seeds, and a short lifespan (Cadotte and Lovett-Doust, 2001). Plants like *Equisetum* spp. can improve the soil compaction and lower the conductivity of the soil as well as improve soil nutrition (Young *et al.,* 2013). The next group of plants in the analysis were found at the Winston Lake location which had different soil conditions compared to the other sites, so these plants can be found on disturbed soils but not necessarily elevated metal contaminated soils. While all three sites were disturbed from mining operations, Winston Lake had levels of As, Sb and Mo in the soil considered normal to plants so plant species growing at this site are living on generally disturbed soils (Kabatas-Pendias, 2010). All of the other plants investigated in this study are in the last group of the analysis: white pine, white spruce, willow, white birch, trembling aspen, red pine, balsam fir, jack pine, blueberry, tamarack, dandelion, yarrow, sedge, daisy, balsam poplar, strawberry, cedar, red clover, bladder campion, and primrose . This group contains all of the older trees and are found on the majority of the transects. All of these plants can be considered potential candidates for rehabilitation purposes as they are found on a variety of disturbed soils and have a wide range of habitat for wildlife, and growth habits.

Soil Characterization

The As and Sb levels at the Premier sites and at Steeprock are similar (Jana *et al*., 2012). These levels of As and Sb are quite elevated according to Canadian standards of soil quality of 12 mg As kg^{-1}, and (CCME, 2007) or worldwide values of 0.05 to 4 mg Sb kg^{-1} and 1.5 to 3.0 mg As kg^{-1} soils from igneous rocks and 1.7 to 400 mg As kg^{-1} from sedimentary rocks (Kataba-Pendias and Mukherjee,, 2007; Smith et al., 1998). Canadian soil quality standards have Mo at 5 mg Mo kg^{-1} so Premier is the only location with average amounts of 7.74 mg Mo kg^{-1} while Steep Rock shows borderline levels just under the limit. The high amounts of Ca, Mg, K, and Na at each site can negatively influence the plant metabolism especially in the higher pH conditions (Wong *et al*., 1998).

Conclusions

The mining areas of Steeprock, Premier and Winston Lake show a range of plants with varying tolerance to soil metal concentrations of As, Mo and Sb with a range of accumulations. The main findings are i) a variety of plant species can be found at all three locations with few species specific to each mine, ii) the soil characteristics were quite different at each of the closed mines , and iii) there were species with the potential to be metal excluders including white birch, willow*.,* trembling aspen, goldenrod, pearly everlasting and tamarack.

Since differences exist between disturbed forest soils and man-made unweathered mine soils, difficulty arises when planting directly on mine soils. For better success at replanting mine soils with phytostabilization species, soil improvements could improve plant survival and growth. The addition of some topsoil or organic amendments improves soil moisture and nutrient availability. These could include woodbark, composts or another local waste source. Insulating layers of subsoil including building rubble, refuse, or uncontaminated rock would help buffer the planted species from lower underground metal contamination and increase the success of the seedlings and cuttings (Zhang *et al*., 2001).

Rehabilitation of contaminated soils on closed mines will have to include a variety of species for the specific metal contamination so as to mimic the diversity of the surrounding boreal forest. Some metallophytic species have a natural drought tolerance so as to withstand the dry conditions of the mine soil. Perennial species, species with wide ranging root systems, and those adapted to cold winters, low nutrient, low organic matter and compacted soils can be included in closure replanting plans for mines in Northwestern Ontario. Focus should be placed on pioneer plant species, rather than the climax coniferous species such as white spruce due to their poor health after planting on these mine sites. Plantings should include a mix of grasses, herbs, shrubs and trees that will colonize the surrounding area, increase organic matter and improve fertility and soil characteristics like water retention, aeration and wildlife habitat. Hyperaccumulator plants should be avoided for planting, actively eliminated from areas through weeding or only planted on areas scheduled for regular harvesting for metal removal so as to reduce the hazard for the future land uses. Future research should include test plantings in various metal concentration and soil types for ease of use. as well as with various organic matter, fungal and bacterial amendments.

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