Bioremediation of Contaminated Soils from Mine Sites Using Native Plants in Northwestern Ontario

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Abstract

Practical and scientific importance can be found in this research topic since the results directly apply to remediation of industrial and mined lands in the boreal forest region. Plants suitable for phytostabilization of As, Mo and Sb are identified as well as two hyperaccumulators of Zn. Using phytostabilization practices, metals are immobilized by the below ground components of the plants therefore restricting the flow into the ecosystem and lessening the impacts of metal pollution to the surrounding area. As long as there is little disturbance of the soil physically or chemically, the plants will continue to stabilize the metal in the organic portion of the deceased plants. The ease of replanting a site could incorporate successional ecosystem in the region by focusing on trees and shrubs that are earlier in the revegetation process after a disturbance. The addition of woodbark to the reestablishment of the top soil increases potential nutrients, organic matter, water holding potential as well as diluting potential harmful metal content of the soil and providing a mulching effect. Some concerns exist by using agronomic plant species as the sole part of revegetation as they have the potential to impact the wildlife in the region through excess Mo. Results from this thesis could be helpful for future mine closure plans and in the rehabilitation of other industrial sites.

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1. Introduction

1.1. Definition of the Problem

Mining has been a dynamic part of Ontario since before European settlement and has been constantly changing with discovery of new deposits, techniques and environmental practices (Ontario MNDM, 1986). The days of leaving a site as is after the ore is removed are in the past. Replanting a site with new green growth is a part of the closure and rehabilitation process in the life cycle of a mine. Even before the site has been opened for mineral production, plans are in the works for the life of the property after operations have finished. Soil cover, vegetation and surface water must return to as close as pre-development as possible (Ontario Mining Act RSO 1990, c. M.14-7). Revegetation of the soil is one part of this progression.

Revegetation is a complicated process even with no additional difficulties. When there are poor soil conditions, little to no seed sources, and contamination due to waste materials, the difficulty level increases (Renault, 2004). Soil conditions following mining show poor pH (either acidic or alkaline), low nutrients, minimal organic matter, low water potential and elevated metal contamination. Due to soil redistribution of stockpiled topsoil, water can also be poorly drained or the soil may not be able to hold any water. Many processes can occur to move localized metal contaminants into the surrounding systems via plant growth (Figure 1-1). Therefore phytoremediation practices should be included in closure plans for mines.

Phytoremediation techniques integrate the use of plants to rehabilitate soils, sludges, sediments, and water to remove, contain or degrade contaminants (UNEP, 2002). These procedures are a low cost method of remediation and are very useful on surface contamination or in circumstances with lower levels of contaminants. They can be used on their own or in conjunction with different methods of rehabilitation, including other mechanical, and biological practices.

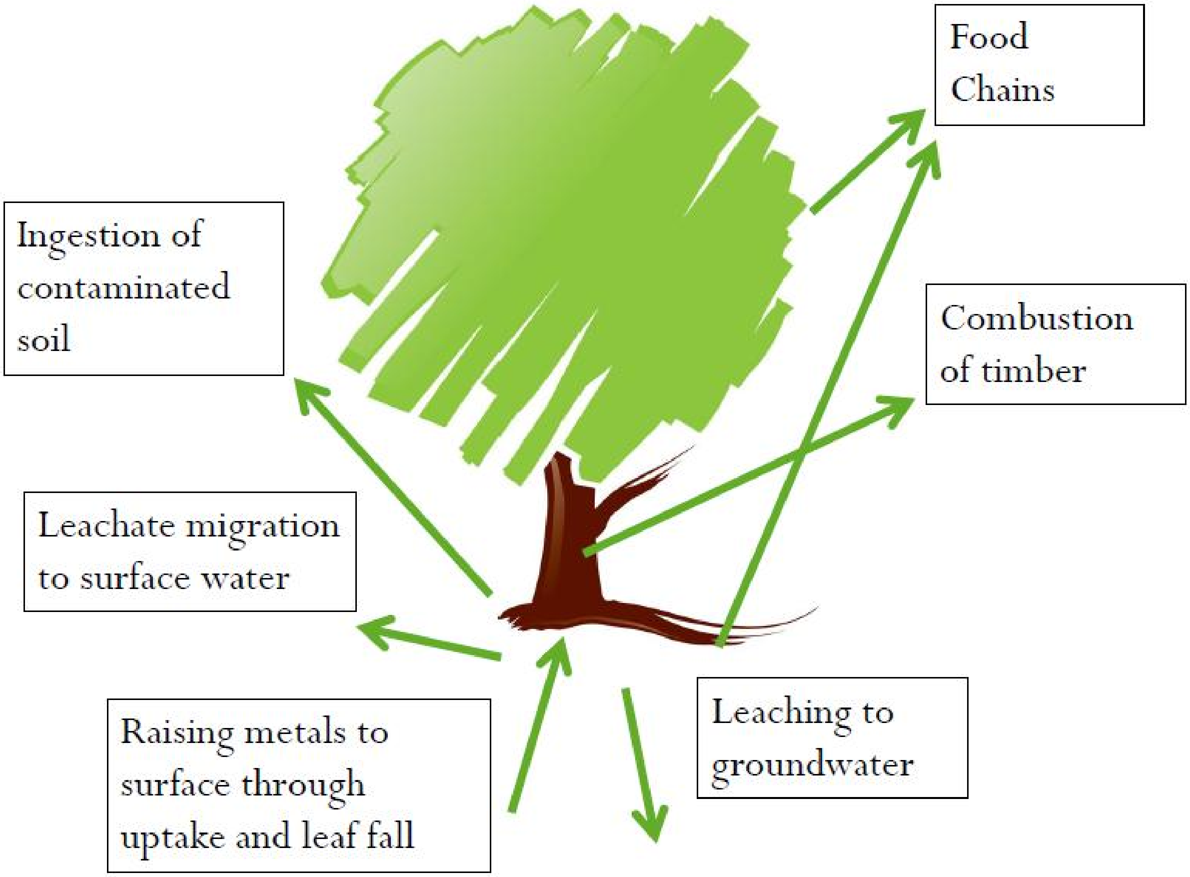


Figure 1-1 Hazard/pathway/target model of risks to be assessed through potential mobilization of heavy metals in soil by planting trees

Other remediation practices to control metal movement can be costly. Excavate and dispose is one method. The site has all the contaminated soil excavated, processed with biological or chemical agents and moved to a waste site (Smith and Underwood, 2000, Mulligan *et al*., 2001). This expensive practice tends to occur in the event of land sales or if the contamination is moving to neighbouring properties to remove any liabilities of soil contaminations. But this migration of contamination does not necessarily solve the problem of the soil once it has been moved and can completely change a landscape or require soil from a non-contaminated area to be backfilled into the site.

Another type of remediation involves treating the contaminated soil *in situ* with biological and chemical additives (Shilev *et al*., 2006; Gadd, 2010; Glick, 2010; Ma *et al*., 2011). Bioremediation with bacteria can help organic contaminants to degrade into less toxic counterparts. Pipes are distributed into the contaminated soil and injected with bacteria . Degradation would be monitored on a regular basis until the hydrocarbon contamination would be reduced to unregisterable levels. Other techniques involve engineered caps and terraces to contain the contamination behind a barrier and prevent soil and water movement (Buckley *et al*., 2012; Hosney and Rowe, 2014). These practices can be prone to leaks and require monitoring as well as a high expense involvement.

Past practices of revegetation involved planting commercially available seeds, herbs and trees. Strip coal mines in Appalachia tree planted the repositioned topsoil which was standard practice from the 1940s to the 1970s (Brenner, 1979; Wade and Thompson, 1990). Little concern was focused on regeneration of other species or the introduction on exotic species to the area.

In the early 70s, Peters (1984) combined his knowledge of agriculture with the reclamation process to re-green the mine impacted land in the Sudbury, ON region and his work became the standard practice around the world. Techniques included fertilizer, lime, and cover crops to build up the soil and ameliorate some of the contaminants from the mining processes (Peters, 1984) . Agricultural species were planted on mines, and his work yielded excellent results and changed the landscape for the re-greening of Sudbury (CLRA, 2015). Barley (*Hordeum vulgare* L.) is a hardy, annual crop species can prevent erosion, and improve leaching of ions in tailings (Renault *et al.,* 2003). However, some concerns exist over the long term results of these plantings. Few native species are able to compete with agronomic plant species and fertilizer and lime inputs are needed in some areas.

Not all plants or soils are created equal when it comes to phytoremediation. Soil metal availability depends on pH, organic matter content, particle size, total metal content, and mineral source material (Magua *et al*., 2007). Plants remove both essential and non-essential elements from the soil. Various species can withstand a variety of levels of different metals and variation can even occur within a species. Some of the metals are required by the plant for metabolic processes such as Fe, B, Zn, Cu, Mn and Mo (Mengel *et al*., 2001). Concentrations of these elements can be deficient, adequate, luxury consumption (in excess of what the plant actually needs but not impacting growth), or toxic. Some of these elements are easily transported to leaves including Zn, Cd, Co, B, and Mo. Others have limited mobility within plant via water movement in the xylem sap or more so translocated in the phloem saps (Hazama *et al.,* 2015). These include Cr, Pb, Hg, and Cu, which are . Other metals, still although not required for plant growth and metabolism, are taken up by plants, such as Pb, Ni, Cd, Ti, As, Sb, Sn (Cataldo and Wildung, 1978). Metals not required for growth only have two types of dose concentration: luxury consumption, and toxic. Mechanisms for tolerance of these metals appear to be compartmentalization, complexation and metabolic adaptation. Table 1-1 shows background concentrations of several metals of interest in mining (Bes *et al*., 2010; Barrutia *et al*., 2011; Banasova, 2012). Some of these metals are required in small amounts in biotic systems.

Trees have a greater tolerance for metal content in the soil (Pulford and Watson, 2003). Their size makes it possible to spread their root systems to a larger area in comparison to smaller herbs and shrubs. The larger root system is able to seek out areas of less contamination and also attain better nutrition and water sources. *Betula pendula* was found to grow in soil with 29000 µg Pb g^{-1} with had plant tissue of 7000 µg Pb g^{-1} with a pH of 3.83-6.61 (Magua *et al*., 2007).

Table 1-1 Background concentrations in plant tissues with toxic effects (mg kg^{-1}) (based on Reeves *et al*., 1999; Garbisu and Alkorta, 2001; Wang *et al*., 2002; Yang *et al*., 2004; Babula *et al*., 2008; Nagajyoti *et al*., 2010)

	Micronutrients Essential for Plant Growth					Non-essential Elements			
	Cu	Zn	Ni	Co	Mo	Cd	Pb	As	Sb
Mean	14.2	55.3	23.3			0.22	23.1		
Normal	2-250	1-400	0.02-5	0.02-1	0.03-5	0.1-2.4	0.2-20	0.02-7	
Excessive	6-100	400-900	100-200	40-50	50	30-200	300	20	5-10

1.1.1. Motivation for this study

The Hemlo Gold Camp, now owned by Barrick Gold, is slowly ending its production capacity. The Golden Giant Mine was the first to close in 2005, with David Bell Mine following in 2014. Williams open pit and underground operation will be continuing operations for the near future. Closure will involve a variety of procedures which are designed to return the site to a sustainable and functioning natural ecosystem. An important part of this closure process will be the revegetation of impacted areas on the site. Given the highly visible location of Hemlo beside the Trans-Canada Highway near the town of Marathon, Ontario, this aspect of its closure is particularly important.   Metals of concern in the soils at this location have been identified as arsenic (As), molybdenum (Mo) and antimony (Sb). Other locations (Steep Rock Mine near Atikokan, ON; Winston Lake Mine near Schreiber, ON; and Premier's properties near Beardmore, ON) were chosen as we had permission to go onto these properties and they were available by road access. As the mines in this study have been completely disturbed, there is no residual plant roots that can regenerate via cuttings or roots. All the species growing on the sites have started via seeds. The distance of the disturbed area to the surrounding forest ecosystem or the size of the disturbed area would have shown an increased amount of vegetation or species richness on the edges of the site but this did not appear to occur on these soils showing that soil conditions have a large impact on the plants survival and welfare (Thompson *et al*., 1998).

1.1.2. Metals of Concern for the Study

Arsenic is one of the metals considered to be non-essential to plants. Much is known about toxic effects due to its use as a pesticide in many products used to deal with disease, insects and rodents (Mandel and Suzuki, 2002). Plants that have a tolerance for As tend to have mycorrhizal associations to assist in limiting the availability of As to the plant (Fitz and Wendel, 2002). Arsenic uptake into the aboveground portions of plants is mostly due to the P metabolic pathway (Alloway, 1995 and Kumpiene *et al*., 2008). There are three main groups of As: inorganic compound, organic compound and gasses. Of the multitude of As complexes, the inorganic compounds are thought to be the most toxic, with arsenites more toxic than arsenates (Vaclavikova *et al*., 2008). Symptoms of As toxicity in plants include sterility, inhibition of cellular function and cell death (Akter *et al*., 2005).

Excess Mo is not readily evident or easily identified in plants (Gupta and Lipsett, 1982). Mo is one of the elements required for growth so metabolic pathways are the main method of accumulation in the plant (Kaiser *et al*., 2005). In comparison to Pb, Cd, or Ni, Mo has a higher inhibition to plant growth when in excess (Kevresan *et al*., 2001). Bioavailability of Mo increases as the soil pH increases (McGrath *et al*., 2010). If plants with elevated molybdenum content are consumed by ruminant species, molybdenosis can be a toxic result (Raisbeck *et al*., 2006). Mo is the least required element for plants and has the least amount of research of how plants accumulate and utilize Mo (Kaiser *et al*., 2005). Molybdate is the predominate form for uptake and is used for redox reactions within the plant. The uptake mechanism of Mo from the soil is thought to be prokaryotic transport systems similar to S (Self *et al*., 2001).

Antimony is a less common issue for plants. As one of the non-essential metals in plants, Sb is starting to become a concern but As still remains more of a worry. Much research is started due to toxic effects to humans via plant intake (Tschan *et al*., 2009). At one time Sb was thought to be immobile and inactive in the soil but Sb impacts root and shoot growth and chlorophyll synthesis as the main toxic response in plants (Flynn *et al*., 2003). In rice and corn, Sb concentration in tissue directly related to concentration in soils in China (Hammel *et al*., 2000; Pan *et al*., 2011). The primary mechanism for Sb uptake is the competition for site with essential P and Ca metabolites (Alloway, 1995). The chemical forms of Sb (V) and Sb(III) are directly related to its availability and mobility in the soil and plant tissues (Feng *et al*. 2013).

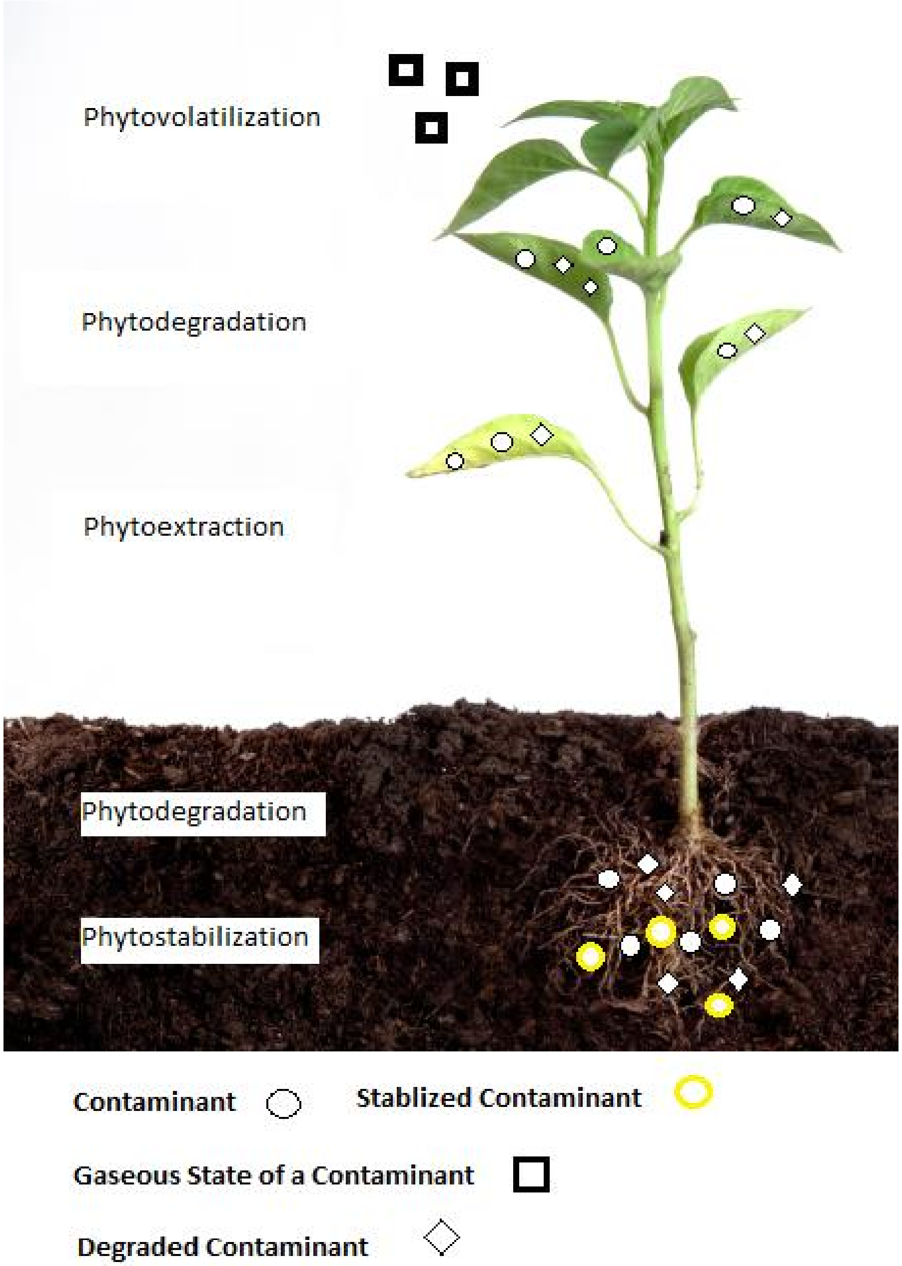


Figure 1-2 Types of terrestrial phytoremediation and the end fate of the contaminants (based on Greipsson 2011)

1.1.3. Phytoremediation Strategies

Within the realm of phytoremediation lies an array of technologies. Depending on the type of contaminant involved, a range of fates can happen to contaminants when various plants grow in the impacted soil. Table 1-2 lists the types of phytoremediation technologies, the fate of the contaminant, which type of contaminant best suited for the process and the maintenance involved in the vegetation (Salt *et al*., 1995; Greipsson, 2011). Visual representation of these processes can be seen in Figure 1-1 and Table 1-2.

Phytoremediation can work on one or multiple contaminants. Types are as follows:

- Phytodegradation - plant contaminants are metabolized into other less toxic substances but this process can only work for certain organic pollutants that can be degraded (Newman and Reynolds, 2004). An example from Denmark shows a variety of herbaceous species can metabolize methyl tertiary butyl ether, a gasoline additive, from the soil (Trapp *et al*., 2003).
- Phytovolatilization - plant converts the organic contaminant into a gaseous state and releases the subsequent products into the atmosphere, (Arnold *et al*. 2007). An example can be found in Ma and Burken (2002) with hybrid poplar that remove trichloroethylene from groundwater and volatalize it through the trunk.

- Phytoextraction - accumulates in the aboveground portions of the plant including leaves, stems, fruit or seeds. The phytoextraction plants can be harvested and refined to use the extracted product (Raskin and Ensley, 2000). Plants used for this process are called hyperaccumulators. The active production of a crop of phytoextraction plants is known as phytomining (Brooks *et al*., 1998). An example from Port Colborne, Ontario, Canada is the use of *Alyssum murale* and *Alyssum corsicum* to clean up Co and Ni (Li *et al*., 2003).
- Phytostabilization - the reduction of movement of the mobility of metals in the root zone. Plants phytostabilize by decreasing water and wind erosion, decreasing metal solubility and lessening bioavailability. For many circumstances, phytostabilization can be the optimal choice for many rehabilitation projects (Raskin and Ensley, 2000). With less continued maintenance, a containment of the contamination and suitability on a variety of pollutants, phytostabilization species should be identified for many circumstances (Mendez and Maier, 2008).

Table 1-2 Types of terrestrial phytoremediation techniques, fate of the contaminant, types of contaminants and whether vegetation is harvested or maintained (based on Greipsson, 2011).

Technology	Contaminant Fate	Contaminant Type	Vegetation
Phytoextraction	Removed	Metals	Harvested
Phytodegradation	Attenuated	Organics	Maintained
Phytovolatilization	Removed	Organics and Metals	Maintained
Phytostabilization	Retained	Organics and Metals	Maintained

Decisions need to be made on what type of plants are used for phytostabilization including well used agronomic species or native species that are well adapted to the area which is contaminated. Since the site is required to be regreened, native plants and domesticated agronomic species are both useful in rehabilitation. The seeding of agronomic grasses and legumes are used on sites as reclamation tools for prevention of erosion. They also provide forage for ruminants and other animal species, fixation of N and improved soil characteristics due to increased organic matter, water holding and addition of other nutrients (Oxenham *et al*., 1966). These species require some fertilization or pH adjustments and can prevent the site from resembling the surrounding forested areas. The native species are well adjusted for climate, are the natural food and habitation species for the wildlife and will blend with the surrounding areas in the future (Cadotte and Lovett-Doust, 2001). But, native species can be hard to source, be expensive, and few can fix nitrogen for soil improvement (Knops *et al*., 2002; Mendez and Maier, 2007). With use of spatially diverse native species identified for phytostabilization, the added benefit is the metal contaminants will not enter into the local food chain as easily.

1.2. Scope and Objectives

Much of this research was undertaken to contribute to a rehabilitation strategy for mine closure and other bioremediation interests in a boreal forest setting. The following objectives were:

- Identification of species of plants suitable for boreal forests in Northern Ontario climate and soil conditions that are potential phytostabilization candidates for As, Mo and Sb. Phytostabilization was emphasized to ensure that the potential contaminants in a mined area do not spread into the surrounding region through the food chain. Plants naturally growing on three closed mines with highly impacted soils were identified, collected, and analyzed for their efficiencies as phytoremediation species.
- Analysis of the soil and plant metal concentration growing at Barrick Hemlo was performed to understand the metal contents of the soil, roots and shoots of plants already growing on the mine site to identify metals of concern in the plant population. A field experiment was established to determine a plan for replanting the mine area using *Populus tremuloides*, *Salix* sp., *Picea glauca*, *Physocarpus opulifolius*, and *Cornus sericea* planted in spring or fall in four different topsoil/woodbark treatments using a mycorrhizal fungus, *Pisolithus tinctorius.* A rehabilitation plan for replanting will be found with a broad range of planting options and low costs.

- A greenhouse experiment of *Betula papryrifera, Trifolium pratense, Trifolium repens, Medicago sativa*, and *Festuca Rubra* in known quantities of As, Mo and Sb was done to study the removal of these plant growth, biomass quantities and health as well as metal contents of the above and belowground parts of the plant. Many mines in Northern Ontario use these species in current rehabilitation practices so I wanted verify their suitability for phytostabilization use.

1.3 Dissertation organization

This thesis dissertation is presented in five parts addressing several aspects of phytostabilization of As, Mo and Sb in mining contaminated soils in Northwestern Ontario. Chapter one provides a general introduction into rehabilitation practices using plants, for phytoremediation and an introduction to phytostabilization, as well as the objectives of this study, and research plan. The next three chapters are the research papers for journals. Chapter two focuses on identifying potential phytostabilization species by exploring closed mines in Northwestern Ontario. Versions of this chapter were presented at the Ontario Mine Reclamation Symposium and Field Trip in Cobalt, June 18-19, 2013 and Peterborough, June 17- 19, 2014 as the research developed. An investigation into current vegetation and soil as well revegetation of Barrick Hemlo comprises chapter three. A version of this chapter was presented at the Canadian Land Reclamation Association/Manitoba Soil Science Society Joint Conference: Land Reclamation and Soil Science - Solutions for a Sustainable Future in Winnipeg, MB June 15-18, 2015 as well as a tour stop on Ontario Mine Reclamation Symposium and Field

Trip in Marathon, ON September 1-2, 2015 . Chapter four deals with a greenhouse study investigating *Betula papryrifera, Trifolium pratense, Trifolium repens, Medicago sativa*, and *Festuca Rubra* as potential phytostabilization species for As, Mo and Sb. This chapter was presented at Ontario Mine Reclamation Symposium and Field Trip in Marathon, ON September 1-2, 2015. Chapter five is a summary of the conclusions and recommendations developed throughout this research. It is followed by an appendix section with additional data, QA/QC procedures, experimental design and posters created throughout this thesis.

2. Plants growing in closed mines of Northwestern Ontario are useful for phytostabilization potential of arsenic, antimony and molybdenum in mine reclamation

2.1. Abstract

We examined plants that have tolerance to metal contaminations and the ability to survive on lands with poor growing conditions, including low water holding capacity, low fertility, and low pH levels. These plants are able to survive, reproduce and thrive under these tough growing conditions. In particular we were interested in plants growing on closed mine sites that were contaminated from arsenic (As), antimony (Sb) and molybdenum (Mo) and that have the phytostabilization potential to keep the contamination contained to the impacted site rather than uptake these metals into their plant tissue and thereby introduce the contaminants into the environment. Soil and plant samples were collected at four mining areas in Northwestern Ontario (Steep Rock Mine, Winston Lake Mine and Premier's properties near Beardmore, ON) and analyzed for their total metal content. While many plants were growing on these sites, changes in colour, size and increased insect and disease pressures indicated that often these plants were stressed by the metal contents of the soil and the poor growing conditions.

Bioconcentration factors and translocation factors between the soil and plant tissue were calculated for over 30 species. A good phytostabilizer was defined as one with a low bioconcentration and translocation index. Species showing good phytostabilization potential included white birch (*Betula papyrifea* Marshall), willow (*Salix* spp.

L*),* trembling aspen (*Populus tremuloides* Michx.), goldenrod (*Solidago canadensis* L), pearly everlasting (*Anapahlis margaritacea* L) and tamarack (*Larix laricina* Du Roi). The most common tree found on these sites was white birch which was sometimes growing symbiotically with the fungus, *Pisolithus tinctorius* (Pers.) , a known metal accumulator. No species were considered to be hyperaccumulators of As, Mo and Sb suggesting that even to survive, metal uptake had to be limited. Management implications to establish specific phytostabilizing species on mine sites with elevated metal levels were considered.

2.2. Keywords

Metals, Rhizosphere, Mining, Boreal, Bioavailability, Phytoremediation, Bioconcentration, Translocation, Contaminated Soils

2.3. Introduction

Northwestern Ontario is a boreal forest region on the Canadian Shield that is rich in mineral resources with active as well as closed and abandoned mines. These areas have the potential to be reclaimed with phytoremediation species to prevent wind and water erosion as well as prevention of metal contamination 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).

Three locations in Northwestern Ontario have been closed with little to no replantings: Steep Rock Mine near Atikokan, ON; Winston Lake Mine near Schreiber, ON; and the Premier properties near Beardmore, ON. Each of these areas was not active for over ten years. They have highly mineralized soils with high metal content of a variety of elements and have had the opportunity for native species to colonize the sites.

Naturally occurring plants on closed mine sites are usually the 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 and potential thrive on elevated metal soils and are useful for reclamation purposes of other industrial sites or as indicators of potential ore bodies to be explored (Whiting *et al*., 2004). Ideal candidate species for rehabilitation should be plants that 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 that are 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 plants are known as accumulators. If the concentration of the contaminant in the plant is extremely high in comparison to the concentration in the soil, these species are known as hyperaccumulators (Kumar *et al*., 1995; Peer *et al*., 2006). Phytostabilizers which do not uptake metals and other contaminants into the above ground tissues of the plant are known as 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, 2009).

Metal pollution on these mine sites is directly influenced by the type of deposit mined, so potential 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. It is important to identify and produce the specific plant species that cater to these contaminated areas (Tordoff *et al*., 2000; Mendez and Maier, 2008).

Both arsenic and Sb are not required for plant growth (Nagajyoti *et al*., 2010). Natural As tolerant plants on contaminated soils have been studied by Craw *et al*. (2007), Antosiewicz *et al*. (2008), Zandsalimi *et al*. (2011), and Bergqvist and Greger (2012). Sb and As were investigated at a Chinese mine by Fu *et al*. (2011) and at a Portuguese mine by Pratas *et al*. (2005). Sb contaminated soils and their impact on plants was studied by Hammel *et al*. (2000), Qi *et al*. (2011) and Pan *et al*. (2011). While all these studies have been done, very little research has been done on their phytostabilization use for replanting metal contaminated areas. Mo is essential to plant growth but is biologically inactive, mechanisms that exist for uptake in plants are closely tied with iron (Bittner, 2014).

This paper explores the phytostabilization potential of plants naturally regenerating on closed mines in Northwestern Ontario. Data will be presented with 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 contamination from 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. It may be possible that 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.

2.3.1. Objectives and Hypothesis

The objectives of the study were i) to identify plants growing on these closed mines, ii) to examine the variation in the soil conditions natural regenerating species experience on these closed mines, and iii) to classify these species as candidates for phytostabilization. Plant species will be identified that are tolerant to metal stress, and provide phytostabilization for mine soils.

2.4. Materials and Methods

2.4.1. Site Description

This study examines three closed mines areas in Northwestern Ontario with no replanting of plants or trees following closure (Figure 2-1 and Table 2-1). Located on the Canadian Shield, these mines sites sampled have been closed or not disturbed for a minimum of 25 years and had restricted road access.

Table 2-1 Mine characteristics of Steep Rock, Winston Lake and Premier Gold

Mine	Main Ore Extracted	Metals of Concern	Vegetation Zone	Frost Free Days	Precipitation (mm)	Latitude	Longitude	Altitude
Steep Rock	Iron	As, Cr, Ni, Se	Boreal forest	109	762.2	48.49	91.37	396.7
Winston Lake	Zinc	Zn	Boreal forest	100	809.6	48.58	87.21	482.1
Premier Gold	Gold	As, Ni, Sb	Boreal forest	101	764.6	49.37	88.2	298.9

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 (Shklanka, 1972). These open pit mines had areas of milling, hydrocarbon and ore storage and many mined rock piles.

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.

Surrounded by granatic terrain, this mine location is on a sliver of metamorphosed sedimentary and volcanic rocks on the west end of the Big Duck Greenstone Belt (LaFrance *et al*., 2004). Operations included an underground shaft, milling and storage areas. The only elevated metal on this location is zinc.

The third area consists of several properties managed by Premier Gold located in the Beardmore/Geraldton greenstone Belt: Northern Empire and Leitch mines. The project area encompasses gold mineralization hosted by quartz veins in metagreywacke. 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 (White, 2011; GEDC, 2005). The areas chosen for sampling were where elevated metal concerns were evident, such as known contaminated soils, tailings, processing sites and ore storage.

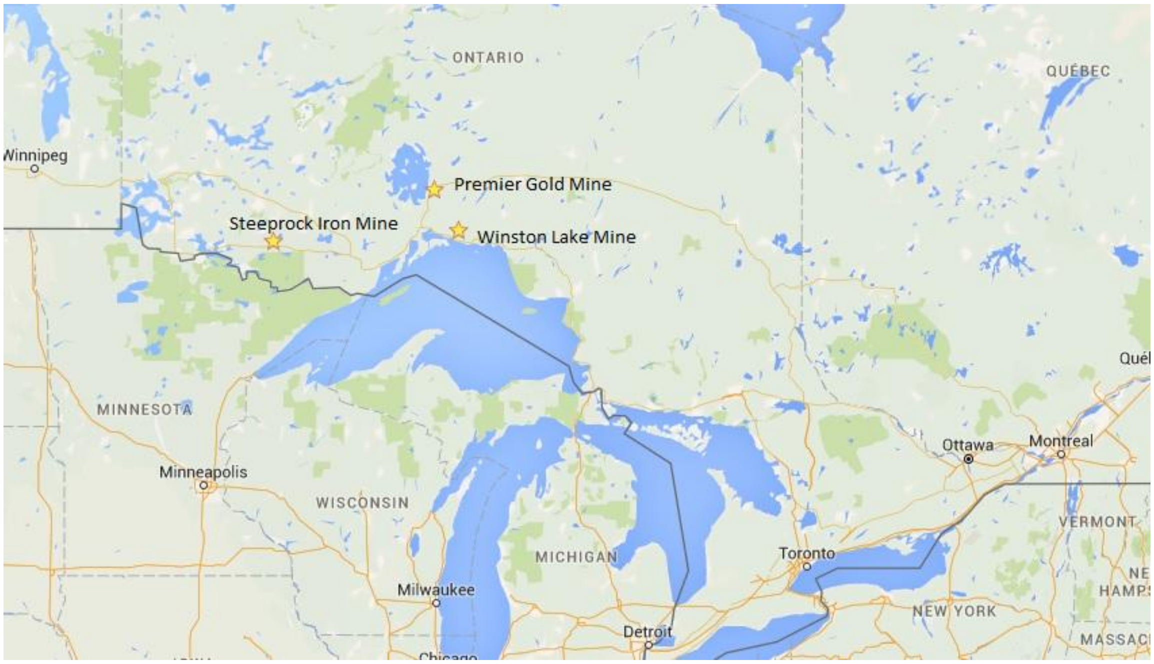


Figure 2-1 Location of the mines studied: Steeprock Iron Mine near Atikokan, ON, Premier Gold Mine in Greenstone, ON and Winston Lake Mine near Schreiber, ON

2.4.2. Field and Sample Preparation

Thirty metre long transects (Figure 2-2) 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 how the species were resistant to the stress of 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).

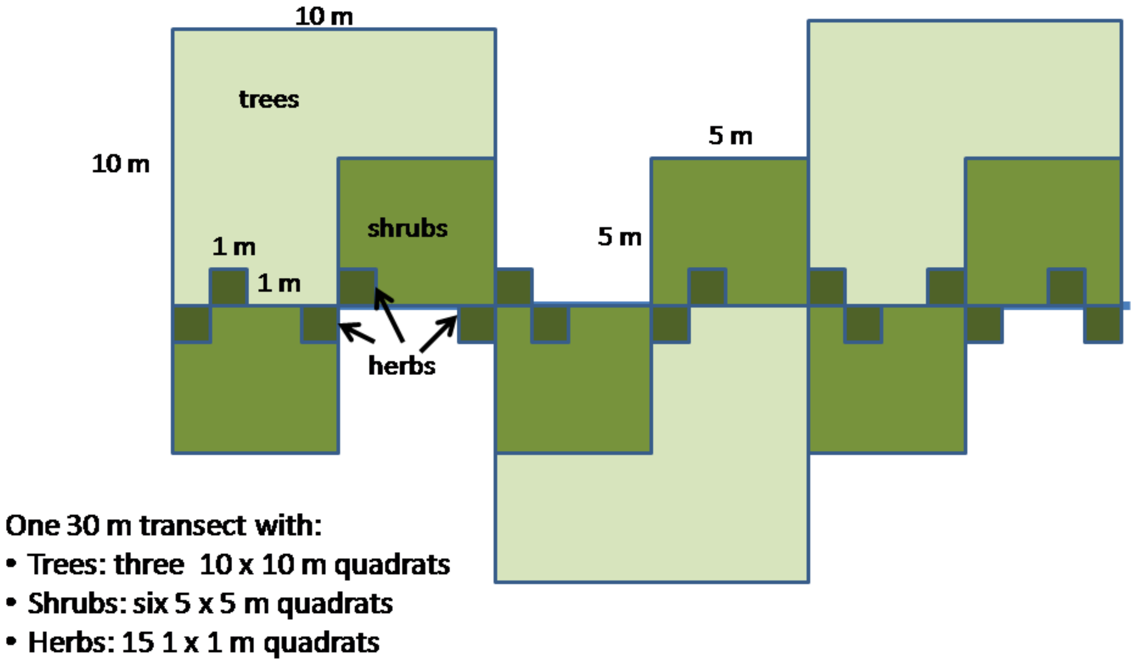


Figure 2-2 Transect diagram of a sample plot of all trees, shrubs and herbs in the plant community

Plant and soil samples were taken along these transects: 3 soil samples per transect with areas with large plant populations and three plant samples per species. Transect number per mine was determined by the size of the mine and number of contaminated areas. Winston Lake had three transects, Steeprock had six transects, and Premier had five transect areas. 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 performed in the plant material encompassed all aboveground plant material at the time of collection 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.

2.4.3. Laboratory analysis

Analyses were done at the Lakehead University Environmental Laboratory (LUEL) according to the LUEL (2012) Quality Assurance/Quality Control (QA/QC) protocols. A blank sample was run at the beginning of each tested parameter, then a QA/QC sample, and followed by a repeat of the next field sample. This was repeated for every ten field samples.

The moisture content of soil was determined by gravimetry on a separate aliquot of sample. A 2.0 g aliquot of soil was weighed and then dried in a drying oven at 70^o C overnight and the dry weight determined. Percent moisture was the oven dried weight divided by wet weight. Soil samples were dried in a drying oven at 70^o C prior to analysis for total metals for up to 72h checking every 12 hours. Both soil and plant samples were homogenized to pass through a 2 mm mesh. For total concentration of metals, a 0.2 g aliquot for soil and a 0.25 g g aliquot for plant tissue were allowed to predigest in teflon express microwave digestion tubes overnight in a 3:1 ratio of concentrated HNO_3:HCl acids. The samples were then digested in a MARS 5 microwave digestion oven for 45 minutes at 175^o C. Samples were removed and diluted to 25 ml with distilled deionized water (DDW) and 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 were determined by the VarianPro Inductively Coupled Argon Plasma Spectrometer (ICP-OES). Replicate, QC , and lab blank samples were measured during each batch of samples. pH and conductivity of soil were measured in a 1:1 ratio by volume of dry sample to DDW on a Mettler Model Seven Multi equipped with a conductivity cell and a pH probe. Loss on ignition was used as an estimate of organic matter for soil by placing 2.0 g of soil into a crucible and then ashing it overnight at 550^{o} C in a muffle furnace. Organic matter was calculated as ash weight – dry weight divided by dry weight.

2.4.4. 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 mean species richness and density at all stand levels among the three mines were examined using one way analysis of variance (ANOVA). The model for the this can be stated as:

$$y_{ij}=m+a_i+\epsilon_{ij}$$

The equation indicates that the *j*th data value, from level *i*, is the sum of three components: the common value (grand mean), the level effect (the deviation of each level mean from the grand mean), and the residual (what's left over) (Christensen, 1996).

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. All plots were included in the analyses as none were identifed as outliers. Rarely occurring herbaceous species (those that occurred in only one of the 13 transects) were removed to reduce noise in the data set (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. Raw stress and stress-I values are reported for each outcome. Cluster analysis using Ward's linkage and Squared Euclidean distance was performed to confirm separation of the species.

iii. In order to distinguish the mines based on soil characteristics, 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 $P < 0.05$.

iv. For plant metal data, aboveground and belowground metal concentrations were summarized using the means for each mine. Translocation factor (TF) and bioconcentration factor (BCF) were calculated using the total metal concentrations (dry weight) in the aboveground biomass, belowground biomass and the soil. TF is the metal in the aboveground biomass/metal in the root biomass while the BCF is the metal in the aboveground tissue/the metal content of the soil. A TF value higher than 1 is considered an accumulator of metals (Deng *et al*., 2004; Juarez-Santillan *et al*., 2010), while a hyperaccumulator is a plant with a TF value above 10 (Ashraf, 2011). BCF is the representation of the metal accumulation efficiency and can show the bioaccumulation of the metal in the food chain. If the value of BCF is higher than 1, the plant can be classified as a phytoextraction species (Zhang *et al*., 2002; Santillan et al., 2010; Dowdy and McKone, 1997).

2.5. Results

2.5.1. 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 2-2. The transect data showing the stand structure of the plants present at each mine is shown by Table 2-3, with the proportion of herbs, shrubs and trees. 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, which was closed the longest, there are either older trees and very little understory with very few herbaceous species or areas with no trees, some shrubs and sparse herbs. At Steep Rock, soils and surrounding water have vivid multicoloured hues with very low populations of unhealthy herbs (Figure 2-3). 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.

Figure 2-3 White birch, *Betula papyrifera*, growing at Steep Rock Mine with mychorrizal fungus, *Pisolithus tinctorius* (see the square)

Table 2-2 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 (Figure 2-4). 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 2-5). 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 shrubs 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 Figure 2-4: 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 2-4. Some of the plant species with leaves had highly chlorotic and the chlorophyll content was below the detection limit on 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.

Table 2-3 Plant Density m^{-2} of plants found growing on the mines WL - Winston Lake, S - Steeprock, and P - Premier.

			WL1	WL2	WL3	S1	S2	S3	S4	S5	S6	P1	P2	P3	P4	P5
Herbs	Balsam fir	*Abies balsamea*								0.27						
	Balsam poplar	*Populus balsamifera*				0.67						0.53	1.13		0.60	
	bearberry	*Arctostaphylos uva-ursi*								0.27						
	White birch	*Betula papryrifera*	0.07			6.20									1.47	1.80
	Birds foot trefoil	*Lotus corniculatus*		0.53		0.80		13.33	0.53	5.87						
	Lowbush blueberry	*Vaccinium angustifolium*	0.53													
	Cattail	*Typha spp.*												0		
	Cedar	*Thuja occidentalis*													0.07	8.33
	Oxe eye daisy	*Leucanthemum vulgare*			0.13	0.13						1.13	0.07		1.20	
	Dandelion	*Taraxacum officinale*		0.07	0.27							1.00	0.13			
	Horsetail	*Equisetum spp.*	6.67		3.73								18.67	0	24.20	2.33
	Goldenrod	*Solidago canadensis*	3.73	0.60	5.33				4.27			0.53	1.00		0.87	1.20
	False melic grass	*Schizachne purpurascens*	4.87	14.00	12.67	0.33			5.87	0.07		62.80	1.60		0.47	25.20
	Hawkweed	*Hieracium canadense Michx*	0.67	17.00	0.60	12.27			10.00	3.13		0.47	1.40		6.87	7.00
	Heartleaved aster	*Symphyotrichum cordifolium*							0.07			2.27	1.33		1.33	
	pepperweed	*Lepidium densiflorum*											0.07			
	Pearly everlasting	*Anaphalis margaritacea*	3.60	17.40	6.80								0.07			
	Plantain	*Plantago lanceolata*													0.07	
	Trembling aspen	*Populus tremuloides*								0.07						
	Evening primrose	*Oenothera biennis*		5.33		0.07										
	Raspberry	*Rubus idaeus*	0.33													
	Red clover	*Trifolium pratense*				5.00				0.13						
	Sedge	*Carex brunnescens*	1.27		0.07	0.47				0.13				0	0.47	
	Sow thistle	*Sonchus arvensis*							0.07							
	Wild strawberry	*Fragaria vesca*							1.93	0.73						0.60
	Sweet clover	*Melilotus albus*				0.13										
	Tamarack	*Larix laricina*														0.80
	Vetch	*Vicia americana*				0.07										
	White clover	*Trifolium repens*		0.67												0.533333
	White pine	*Pinus strobus*							0.07							

Table 2-2 Plant Density m^{-2} of plants found growing on the mines WL - Winston Lake, S - Steeprock, and P - Premier. (Continued)

				WL1	WL2	WL3	S1	S2	S3	S4	S5	S6	P1	P2	P3	P4
Shrub	White pine	*Picea glauca*											1.13			0.80
	Willow	*Salix spp.*				0.33				0.07		0.13			1.13	0.53
	yarrow	*Achillea millefolium*		0.13	1.00	0.07	0.73			0.07				0.13		
	Balsam poplar	*Populus balsamifera*			0.50			7.00			7.17	8.67		1.33		5.5
	White birch	*Betula papryrifera*	13.17	5.83	3.33	36.33	12.67		3.83				0.50		1.83	4.33
	Lowbush blueberry	*Vaccinium angustifolium*		0.17												
	Cedar	*Thuja occidentalis*							0.17							5.50
	Jack pine	*Pinus banksiana*				0.17										
	Trembling aspen	*Populus tremuloides*	5.00	0.17	0.67	1.33	0.17		0.67			0.17				
	Red pine	*Pinus resinosa*						1.33		0.17						
	White spruce	*Picea glauca*	2.00	0.17	0.17				0.83						0.17	2.83
Trees	Tamarack	*Larix laricina*														3.33
	White pine	*Pinus strobus*				0.33										
	Willow	*Salix spp.*	9.00	0.83	1.83	0.83	0.33		1.33			2.17	1.00		1.17	1.33
	White birch	*Betula papryrifera*				1.00		23.00		28.67	4.33					
	Jack pine	*Pinus banksiana*					0.33									
	Trembling aspen	*Populus tremuloides*					0.67				0.33					
	Red pine	*Pinus resinosa*				0.33		0.33								
	White pine	*Pinus strobus*							1.67	2.67						
	White spruce	*Picea glauca*							1.00							
	Willow	*Salix spp.*								1.00						

Table 2-4 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

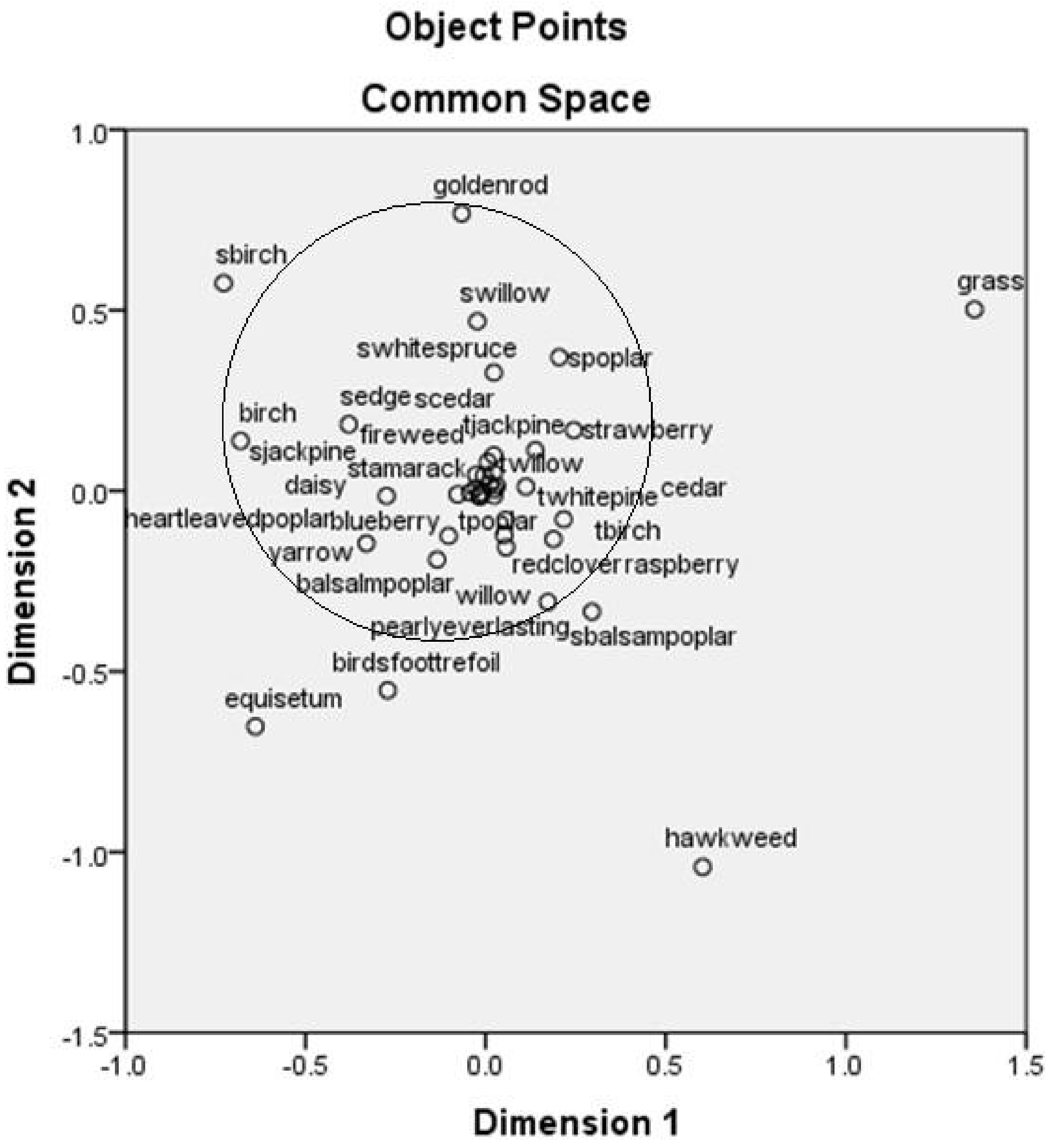


Figure 2-4 A two-dimensional ordination plot derived from non-metric multi-dimensional scaling (NMS) of 13 transects using herbaceous species composition and abundance data.

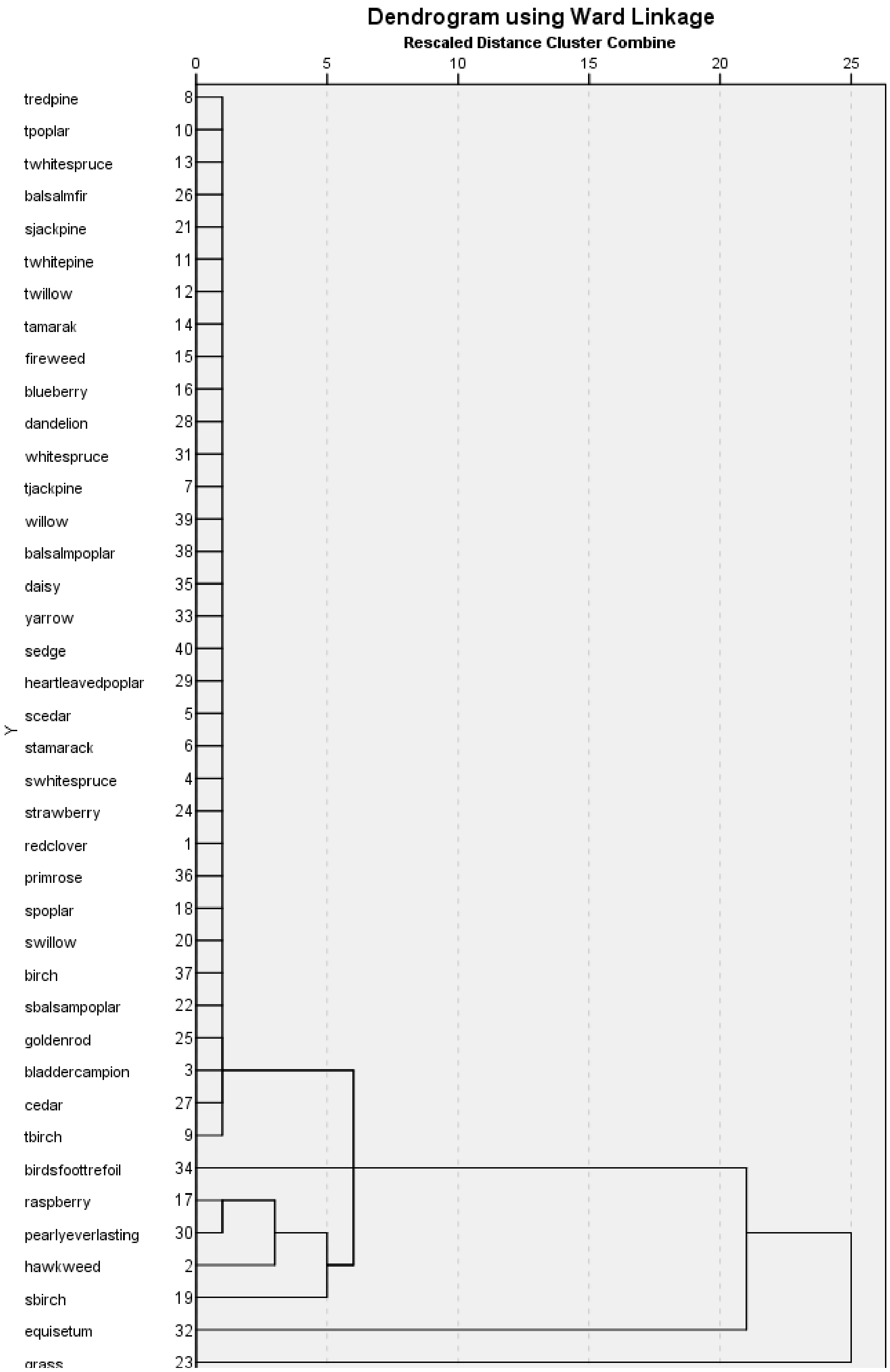


Figure 2-5 Hierarchical cluster analysis of 13 transects using herbaceous species composition and abundance data.

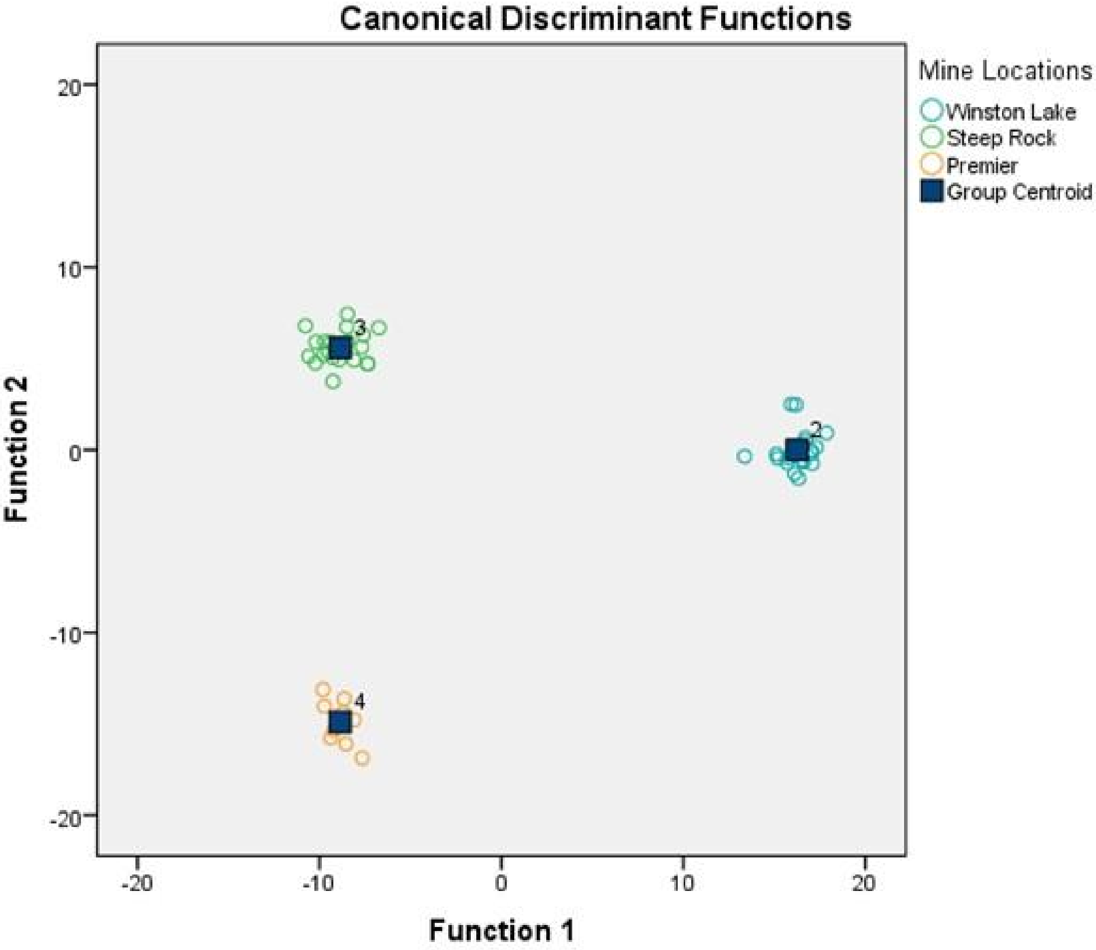


Figure 2-6 Discriminant Function Analysis Plot with all the soil variables at the mine locations. Standardized discriminating function 1 Fe -4.08, Ni - 1.70, K -1.38, Cr -1.29, Co and Pb +1.85, V +2.27, As, +2.28, P +2.34. Function 2 Mg -0.94, Cr -0.92, Co -0.84

Table 2-5 Soil chemistry characteristics of the studied areas on the three mines includes total metal concentrations in mg kg^{-1}, moisture (%), conductivity (us cm^{-1}), bulk density (g cm^{-3}), 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	347.35	157.86	139.79	101.31	235.71	229.09
Bulk Density	.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
Beryllium	.04	0.00	.55	.26	.17	.08
Calcium (%)	2.2	1.2	1.7	4.5	0.19	0.18
Cadmium	.04	0.00	.18	.51	2.60	1.92
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

2.5.2. Soil Characterization

A summary of the soil analysis of each of the studied mines is given in Table 2-5. Soil values were highly variable and had wide ranges of metal concentrations. Metals with elevated concentrations in the soil samples were As, Mo and Sb at Steep Rock and Premier sites. Winston Lake had elevated amounts of zinc 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 2-6). 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.

2.5.3. Metal Bioaccumulation in Plant Tissues

While all metals were analyzed, more focus was placed on As, Mo, and Sb as these are metals of concern in surface soils for many closed mining operations in Northwestern Ontario. The summary of the soil and plant As, Sb and Mo concentrations is seen in Table 2-6,Table 2-7, and Table 2-8 as well as the translocation factors and bioconcentration factors. Winston Lake had no registerable levels of Sb and

Mo in the plant tissue and levels of As at 1 mg kg^{-1} in the aboveground tissue and up to 9 mg kg^{-1} in the roots. Plants grown at Steeprock Mine had no to very low levels of Sb and Mo in their tissues. Sb was found in the roots at the Premier locations up to 16 mg kg^{-1} in balsam poplar and in horsetail. in the aboveground tissue at 5 mg kg^{-1}. Very little Mo was found in plants at Premier except for the grass species false melic grass, which had 24 mg kg^{-1} in the aboveground tissue and 85 mg kg^{-1} in the roots. As was found in many of the plant species grown at Premier and Steeprock locations. Highest levels were found in false melic grass at Premier with levels of 180 mg kg^{-1} in the leaves and stems, and 2129 mg kg^{-1} in the roots. The levels for the TF and BCF at the Premier mines are considered to be non accumulating considering the amount of As in the soil, except for tamarack with a TF of 1.53. Meanwhile plants accumulating As at Steeprock are mullien (*Verbascum thapsus* L) (TF 1.41), white pine (TF 9.17) and white spruce (TF 2.35).

2.6. Discussion

2.6.1. 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 majority of the plant species could be classified as competitive, stress tolerant and ruderal (CSR) according to Grime (1977). 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, but the species found on each of the mines showed a tolerance to heavily disturbed soils and have adapted to these site conditions. 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. This group seems to be separated by the age of the stand as the trees from the older sites are at the top of the cluster analysis grouping and the shrubs and herbs in the lower part.

Some measure of plant health was taken with the measurement of chlorophyll. Other research has shown that stress from addition of sewage sludge and metaliferous water can contribute to lower chlorophyll concentrations in *Typha* sp. (Manios et al., 2003). Paivoke and Simola (2001) found that chlorophyll content in *Pisum sativum* increased with the addition of arsenate, while Mascher *et al.* (2002) found a decrease of chlorophyll with the addition of arsenate in *Trifolium pratense*. Increased zinc, as is found in Winston Lake soils, has been shown to improve chlorophyll content (Wang and Jin, 2005). Molybdenum has been known to influence chlorophyll synthesis in plants but is difficult to quantify through chlorophyll content (Kaiser *et al*., 2005). Pan *et al*. (2011) found that antimony shows a reduction of chlorophyll with higher concentrations in the soil.

Table 2-6 Mean concentrations, translocation factors and bioconcentration factors of arsenic in soils and plant species of mines in Northwestern Ontario (in mg kg^{-1} dry weight) Missing values are below detection limit.

		Premier					Steeprock					Winston Lake				
		Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF
Balsam poplar	*Populus balsamifera*	19	749	1443	.18	.02										
White birch	*Betula papyrifera*	14	382	4804	.18	.05	1	12	330	.11	.00	1	2		1.23	
Birdsfoot trefoil	*Lotus corniculatus*						1	42	334	.02	.01	2	4		1.91	
Cedar	*Thuja occidentalis*	41	134	781	.36	.16	0	13	197	0.00	0.00					
Goldenrod	*Solidago canadensis*	31	2820	1283	.11	.03	15	33	222	.34	.14	2	4		.62	
False melic grass	*Schizachne purpurascens*	180	2129	1869	.08	.10	31	65	212	.57	.16	1				
Horsetail	*Equisetum spp*	155		3394		.07						1				
	Verbascum thapsus						24	46	415	1.41	.06					
Pearly everlasting	*Anaphalis margaritacea*	13	95	5028	.14	.01						1	9		.50	
Trembling aspen	*Populus tremuloides*						4	7	218	.22	.03	2	2		1.31	
Evening primrose	*Oenothera glazioviana*						0	27	407			2	3		.93	
Wild carrot	*Daucus carota*						1	14	299	.24	.02					
Red clover	*Trifolium pratense*						21	36	398	.59	.05					
Red pine	*Pinus resinosa*						2	9	307	0.00	.01					

Table 2-5 Mean concentrations, translocation factors and bioconcentration factors of arsenic in soils and plant species of mines in Northwestern Ontario (in mg kg^{-1} dry weight) Missing values are below detection limit. Continued

		Premier					Steeprock					Winston Lake				
		Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF
Sedge	*Carex gynocrates*	22		1876		.02						3	3			
Sow thistle	*Sonchus oleraceus*	50	797	1819	.18	.03										
Sweet clover	*Melilotus officinalis*						1	19	298	.01	.00					
Tamarack	*Larix laricina*	23	280	1057	1.53	.05										
White pine	*Pinus strobus*						24	15	318	9.17	.41	0				
White spruce	*Picea glauca*	29	3642	1369	.25	.02	31	25	281	2.65	.16	1	3			
Wild strawberry	*Fragaria vesca*	4		187		.02	6		311		.04					
Willow	*Salix spp.*	15	269	4309	.14	.01	7	5	290	0.00	.02	1	4		.59	
yarrow	*Achillea millefolium*											1	3		1.69	

Table 2-7 Mean concentrations, translocation factors and bioconcentration factors of molybdenum in soils and plant species of mines in Northwestern Ontario (in mg kg^{-1} dry weight) Missing values are below detection limit.

		Premier					Steeprock					Winston Lake				
		Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF
Balsam poplar	*Populus balsamifera*	0	7	105			0	0	61		0.00	0				
White birch	*Betula papyrifera*	3	7		1.33		0	0			0.00	0				
Birdsfoot trefoil	*Lotus corniculatus*						0	0			0.00	5				
Cedar	*Thuja occidentalis*	0					0	0			0.00					
Goldenrod	*Solidago canadensis*	0					1					0				
False melic grass	*Schizachne purpurascens*	24	85		.28		0	9				0				
Horsetail	*Equisetum spp*	4		105								0				
Mullien	*Verbascum thapsus*						0									
Pearly everlasting	*Anaphalis margaritacea*	0		105								0				
Trembling aspen	*Populus tremuloides*						0	0			0.00	0				
Evening primrose	*Oenothera glazioviana*						2	8				0				
Wild carrot	*Daucus carota*						0									
Red clover	*Trifolium pratense*						0									
Red pine	*Pinus resinosa*						0	0			0.00					
sedge	*Carex gynocrates*															

Table 2-6 Mean concentrations, translocation factors and bioconcentration factors of molybdenum in soils and plant species of mines in Northwestern Ontario (in mg kg^{-1} dry weight) Missing values are below detection limit. Continued

		Premier					Steeprock					Winston Lake				
		Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF
Sow thistle	*Sonchus oleraceus*	1		105								0				
Sweet clover	*Melilotus officinalis*	0	7	105												
Tamarack	*Larix laricina*						0	0			0.00					
White pine	*Pinus strobus*	0		105												
White spruce	*Picea glauca*						0					0				
Wild strawberry	*Fragaria vesca*	0					1			0.00	0.00	0				
Willow	*Salix spp.*	0					0									

2.6.2. 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). While As and Sb are immobile within the soil profile, the majority of their possible distribution into the surrounding environments would be due to anthropogenic sources or through uptake via plant metabolism. 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.

pH had a large influence on the metal and nutrient availability in these mine soils. Acid soils can increase the solubility of metals such as As and Sb which then increases the bioavailibility of the metal to the plant (Marin *et al*., 1993). Molybdenum is the only nutrient required for plant growth that has higher bioavailability at higher pH levels (Goldberg and Forster, 1998). 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). Solubility of these metals decreases with the increase in pH. Interaction between these ions is also more complex at high pH conditions.

2.6.3. Metal Bioaccumulation in Plant Tissues

Hyperaccumulation and extraction definition can vary depending on the metal investigated (Vassilev, 2004). Shoot metal concentrations can be quite variable considering the variability of the metal concentration in the soil as well as the metal concentration that is bioavailable. Plants that grow in normal soils typically accumulate less than 3 mg kg^{-1} of non-essential metals (Ruiz-Chancho *et al*., 2008; Kabatas-Pendias and Mukherjee, 2007). In our study, most of the plants at the Winston Lake mine follow this statistic, while plants at the other two mines accumulate higher concentrations while growing in As contaminated soil. Plants growing on uncontaminated soils accumulate 0.00001 to 0.2 mg Sb kg^{-1} (Bowen, 1979). Most plants in this study follow this pattern except horsetail with a level of 5 mg Sb kg^{-1}. Levels of Mo found in representative plant species is 1 to 2 mg Mo kg^{-1} (Kabatas-Pendias, 2010). False melic grass at the Premier mines was found to have 10 times this amount in this investigation, while the other plants are in or close to the normal range.

Table 2-8 Mean concentrations, translocation factors and bioconcentration factors of antimony in soils and plant species of mines in Northwestern Ontario (in mg kg^{-1} dry weight) Missing values are below detection limit.

		Premier					Steeprock					Winston Lake				
		Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF
Balsam poplar	*Populus balsamifera*		13	1362			0	0	1031		0.00					
White birch	*Betula papyrifera*		9	1761			0		324		0.00					
Birdsfoot trefoil	*Lotus corniculatus*						0	0	327		0.00					
Cedar	*Thuja occidentalis*			857			0	0	2215		0.00					
Goldenrod	*Solidago canadensis*		10	1656				5	2575							
False melic grass	*Schizachne purpurascens*		6	1376				6	3166							
Horsetail	*Equisetum spp*	5		1639		.01										
Pearly everlasting	*Anaphalis margaritacea*								527							
Trembling aspen	*Populus tremuloides*			1662												
Evening primrose	*Oenothera glazioviana*						0	0	464		0.00					
Red clover	*Trifolium pratense*								443							

Table 2-7 Mean concentrations, translocation factors and bioconcentration factors of antimony in soils and plant species of mines in Northwestern Ontario (in mg kg^{-1} dry weight) Continued

		Premier					**Steeprock**					**Winston Lake**				
		Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF	Shoot	Root	Soil	TF	BCF
Red pine	*Pinus resinosa*								1696							
Sedge	*Carex gynocrates*								347							
Sow thistle	*Sonchus oleraceus*						0	0	206		0.00					
Sweet clover	*Melilotus officinalis*		15	1471												
Tamarack	*Larix laricina*						0	0	1135		0.00					
White spruce	*Picea glauca*		9	836												
Wild strawberry	*Fragaria vesca*								406							
Willow	*Salix spp.*		12	1524			0		725	0.00	0.00					
Yarrow	*Achillea millefolium*			3665					1312							

None of the plants at the sites can be considered as hyperaccumulators of As, or Mo as the TF and BCF were not over 10 (Table 2-6 and Table 2-7). Since none of the plant species had any appreciable amounts of Sb, it will not be discussed here, as all plant species in this study could be classified as potential Sb phytostabilizers, baring more research into their growth on high Sb concentration soils. Other studies also show very little bioaccumulation of Sb at other mine sites (Hammel *et al*., 2000; Fu *et al*., 2010; Qi *et al*., 2011). Plants that can be classified as hypertolerant include yarrow, white spruce, tamarack and mullien for As and yarrow, sweet clover, red clover and alfalfa for Mo. If the quantities of Mo accumulated are above 5 mg kg $^{-1}$ these plants are a concern if ruminant animals graze them (Blakley, 2014). Species with TF values less than one are considered possible tolerant plants. As tolerant plants are balsam trembling aspen, white birch, birdsfoot trefoil, cedar, goldenrod, false melic grass, pearly everlasting, trembling aspen, primrose, red clover, sow thistle and willow while for Mo the plants are white birch birdsfoot trefoil, golden rod, false melic grass, trembling aspen, white spruce and willow . Phytostabilization candidates with TF values of less than 0.1 include red pine for both As and Mo, while sweet clover excludes As and cedars exclude Mo. The species that fall in the tolerant and phytostabilization categories could be considered the best species for phytostabilization as they do not accumulate appreciable amounts of As and Mo that would promote harm to the food chain. Pearly everlasting was only found at Winston Lake, a former zinc mining operation and showed to be a hyperaccumulator of Zn as did goldenrod.

2.6.4. Management Implications

Succession dictates that in the southern boreal forest the dominant species of conifers such as white pine and white spruce will follow the initial growth of deciduous species such as white birch and aspen (Bergeron, 2000). In the case of these metal contaminated and disturbed areas, this succession does not happen if left with no assistance as the disturbance to the soil influences the natural seed bank and very few dominant conifers are disbursed from far distances providing the ideal circumstances for invasive species (Perkins *et al*., 2011). This study showed that very few of the trees were evident at a size and quality that compare with the surrounding areas that are disturbed due to logging or fire (Table 2-2). While the older site of Steeprock did have trees, there were some areas with little to no understory species. Some transects had a monoculture of plants so as to outcompete and prevent the natural succession of trees from colonizing the degraded land.

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 assist plant survival and growth. The addition of some topsoil or organic amendments improves soil moisture and nutrient availability (Helmisaari et al., 2007). These could include woodbark, composts or another local waste source (Brown and Naeth, 2014).

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. Minimal inputs could be used so as to reduce the future requirements for fertilizer and pesticides. 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). 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. If the plants are accumulating more than 5 mg Mo kg^{-1} , supplementation of the site could use 1-5% copper sulfate salts to prevent control negative impacts on ruminant animals (Blakley, 2014). 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.

2.7. 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.

Results of the study can provide insight into the land reclamation practices following mining operations in the boreal forest regions in Northwestern Ontario. Recreating an environment that mimics the surrounding, untouched areas can be a challenge but there are plant species candidates for reclamation purposes including phytostabilizers. Vegetation from these mine regions can be integrated into closure plans to create a self-sustainable environment with plant species that are tolerant to low levels of fertilization and elevated metal concentration. By incorporating species such as white birch, willow, trembling aspen, goldenrod, pearly everlasting, and tamarack with good rooting habits and diverse growth habits, plants can be used to manage soil contamination and mimics the untouched forest surrounding the mines. While the species might not be performing at their top health potential as seen in the chlorophyll content, these phytostabilizer species are improving the areas by preventing erosion and improving the soil conditions, while providing a safe habitat for wildlife to eat and inhabit.

3. Planting of Native Trees on a Former Gold Mine in Northwestern Ontario Using Mycorrhizae and Woodbark

3.1. Abstract

The David Bell Mine Marathon, Ontario ceased production on May 16, 2014 leaving areas of land that require replanting. Plant site pads that were constructed with mine rock may cause possible issues in the revegetation due to metal uptake. Buildings are being demolished, stockpiled topsoil replaced, and seeding with various grasses as well as transplanting trees are being done to the site. All of these actions should encourage colonization of volunteer species of plants from the surrounding area. In order to make the revegetation of Barrick's former gold mine a success, we investigated the seeded and natural vegetation on the site for metal accumulation as well as studied the establishment of 5 different tree and shrub species (trembling aspen (*Populus tremuloides)*, willow (*Salix* sp.), white spruce (*Picea glauca)*, common ninebark (*Physocarpus opulifolius)*, and dogwood (*Cornus sericea)*) planted in fall or spring into overburden mixed with 4 levels of woodbark (0, 6, 12, and 25%) and with the addition of the mycorrhizal fungus, dog turd fungus (*Pisolithus tinctorius)*. While the levels of As, Mo and Sb exceeded the values for industrial soil in the Canadian Soil Quality Guidelines, many of the plants growing on the site did not phytoaccumulate these elements into their aboveground tissues. A negative concern is the plants in the family Fabaceae which could phytoextract Mo and impact ruminant grazing species. The addition of woodbark improved soil nutrition and bulk density. Each of the species planted had a survival of over 70% except for the spring planted *Salix* sp. *Pisolithus*

tinctorius did not appear to improve the growth of any of the tree species. The addition of the woodbark did improve the initial growth of the trees. Little difference in planting times shows that planting could be done in spring or fall. Volunteer species of plants have also been successful at colonizing the experimental site with uniform coverage over the entire area.

3.2. Keywords

Arsenic, Molybdenum, Metals, Phytostabilization, Rhizosphere, Mining, *Pisolithus tinctorius*, Bioavailability

3.3. Introduction

In Canada, the majority of gold deposits are found in the Canadian Shield with much of the production from open pit and hard rock underground mines. Most of this area is covered with the Boreal forest and areas should be revegetated following mining operations, preferably with plants native to the region (Ontario, 2012). Dispersal of other metal contaminants to the surrounding area can occur through mining processes. Mine tailings, effluent and mined rock can contain elevated concentrations of metals (Wang and Mulligan 2006). Buildings must be demolished and leveled and overburden removed during the opening of the mine should be leveled over the impacted areas. Stockpiled overburden can be misplaced on the site, dispersed through rocky terrain, or damaged with little organic matter and low bioactivity (Harris, 2003). Trees and seeding mixes are planted in the graded areas. Many of the replantings focus on coniferous trees as these are widely used for replanting forests in these regions and are typical climax species in these forests (Peters, 1984; Peters, 1988).

Soils play a large role in the life of many organisms. They influence plant growth, contain resources for humans, mediate atmospheric regulation, create a living habitat for many life forms and contribute to water purification (Binkley and Giardina, 1998). Soil and plant health systems are a combination of drainage, compaction, pH, fertility, competition/weeds, residue, plant rotation/biodiversity, and cover (Menard, 2014). Some species grow well on metaliferous soils but the concern is the impact of the elevated metal content of the plants on the surrounding wildlife ecology. If plants uptake an elevated amount of metals, potential negative effects could occur higher in the food chain. Metallophytes that amass metals in the aerial tissues above normal conditions can be classified as phytoextraction species also known as accumulators or hyperaccumulators (Peer *et al*., 2006). Phytostabilization or excluder plant species restrict uptake of metals or prevent the transport into the aboveground portions of the plant body (Raskin, 2000).

Seed and cuttings from tree and shrub species are inexpensive methods used to propagate and replant large tracts of land in the region. Several tree species show promise for replanting mines. White Spruce (*Picea glauca*) is a conifer frequently used to replant areas following forest fire and logging as it is found throughout northern temperate and boreal forest in mixed stands (Freedman, 1995) and is propagated by seed.

3.3.2. Plant species investigated

Trembling Aspen (*Populus tremuloides*), a deciduous tree species, is also widespread in NWO, frequently in stands with white spruce. It grows on burnt areas, recently disturbed ground and on a variety of soil types and site conditions (Lillies *et al*., 2010; Nevel *et al*., 2011; Pinno *et al*., 2012).

Red Osier Dogwood (*Cornus sericea*) is a shrub with ornamental winter stems, found throughout NWO on many areas that are recently disturbed soils on a range of habitats from dry to wet (Renault *et al*., 2001; Renault *et al*., 2004).

Willows (*Salix* sp.) are a family of shrubs found in many forest habitats, usually mixed with coniferous stand on drier soil types and are frequently used to control erosion (Pulford *et al*., 2002; Pulford and Watson, 2003; Mickovski, 2008).

Common ninebark (*Physocarpus opulifolius*) are a species of shrub with ornamental fall foliage found on rocky and disturbed areas in eastern North America, used in control for erosion but rarely found west past the 90^{o} meridian (Dirr, 1997).

3.3.3. Soil amendment

Composted woodbark is the leftover bark, sawdust and wood chips from sawmill production. As a soil amendment, woodbark is valuable because it decays very slowly, causes higher survival rates for tree transplants and encourages root growth (Helmisaari *et al*., 2007; Brown and Naeth, 2014). There is potential for the antifungal chemicals in pine bark that reduce rot and diseases of plants when incorporated into the soil (Alfredsen *et al*., 2008). These pine bark exudates have high water permeability and low to medium water retention, which are beneficial to fine soils, prevent weeds, and moderate soil temperature (Robinson 1988, Hoitink and Boehm 1999). On the other hand, mulches can tie up the microbial activity in the soil causing nitrogen deficiencies in plants, and also induce nutrient cycling in soils similar to natural ecosystems (Tukey and Schoff, 1963; Roe, 1998; Tiquia *et al*., 2002). The pH of the softwoodbark tends to be the same as peat (Gartner *et al*., 1973). Papermill sludge can benefit plant growth on neutral/alkaline mine soils by potentially improving soil structure (Green and Renault, 2008).

Microbes can be an important component of bioremediation through immobilization, remediation and detoxification (Gadd, 2010). Arbuscular mycorrhizal fungi have the ability to create a mutual symbiosis with various species providing direct links between the soil and roots (Khade and Adholeya, 2007). The mycorrhizal fungus *Pisolithus tinctorius* was found at Steep Rock Mine in Atikokan, Ontario (Mol, 2013). This fungus is used to promote growth of several tree species to increase plant health and earlier establishment success. This symbiosis helps to better tolerate soil conditions such as extreme pH, metal contents, low fertility, low organic matter and drought (Marx, 1977; Colpaert, 2011). While it is known to withstand elevated amounts of Al, Fe, Cu, and Zn, little is known about *Pisolithus tinctorius* impact on soils with elevated concentrations of As, Mo and Sb (Tam, 1995). The bioremediation techniques using fungi rely on the correct matching of plant species, metal contamination and fungal species (Turnau *et al*., 2012).

3.3.4. Objectives and Hypothesis

Objectives of this research are aimed at identifying phytoremediation plant species that can be used to plant mining sites and the methods used to increase planting success. This study was carried out at the former Golden Giant mine in Hemlo, ON near Lake Superior. First an investigation of the plants growing at the mine site was performed to determine the accumulation of metals from the soil to classify these as phytostabilizer, accumulator or hyperaccumulator species. Then a planting of five different species *Populus tremuloides*, *Salix* sp., *Picea glauca*, *Physocarpus opulifolius*, and *Cornus sericea* was established on a section of the property with freshly applied topsoil and woodbark. Time of planting, addition of fungus, and the amount of woodbark in the topsoil were assessed in the replanting process. Measures of success included survival, tree height, and chlorophyll content. The deciduous trees will have a higher survival, height and health compared to the white spruce. Soil health will be improved with the addition of a lower content of woodbark.

3.4. Materials and Methods

3.4.1. Study Area

Currently known as a part of Barrick Gold Hemlo, the former Golden Giant mine is a closed gold mine located in the Hemlo mining camp on the north shore of Lake Superior, near the town of Marathon, Ontario (Figure 3-1). As one of three gold mines established on the Hemlo deposit, 6 million ounces of gold were removed over the lifespan of the mine from 1985 to 2006 (Dawson, 2004). The other operations on the site, include the Williams and David Bell mines. Williams Mine is still in operation and has milling, processing and tailings facilities where ores are fed to a standard grind, leach and carbon-in-pulp extraction mill. Mined rock from the operations were spread over the site to smooth the previous landscape. Elevated amounts of As, Mo and Sb can be found in the soils on the mine property (personal communication, Shane Hayes, Barrick Hemlo, 2012).

Figure 3-1 Map of the location of the study site, Barrick Hemlo Gold Mine

3.4.2. Plant and Soil Collection for Phytoremediation Classification

Random soil and vegetation sampling was done throughout the vegetated areas that were previously impacted on mine site at the former Golden Giant Mine. Focus was placed on plant species with significant root systems and substantial aboveground biomass. Plant samples were identified, collected, separated into above and belowground parts, air dried and ground to be used for laboratory analysis. Soil samples were obtained from the 0-30 cm part of the soil profile, sieved to remove large debris and air dried for laboratory analysis.

3.4.3. Site Preparation for Tree Planting

The site of this experiment was located on a level area measuring 25 m by 50 m. A split plot design was used for the tree experiment (Figure 3-2). Site preparation used a backhoe to place 0.5 m of soil cover on the area. Stockpiled topsoil was mixed the softwood woodbark sourced from a nearby sawmill White River Forest Products in White River, ON in four blends:

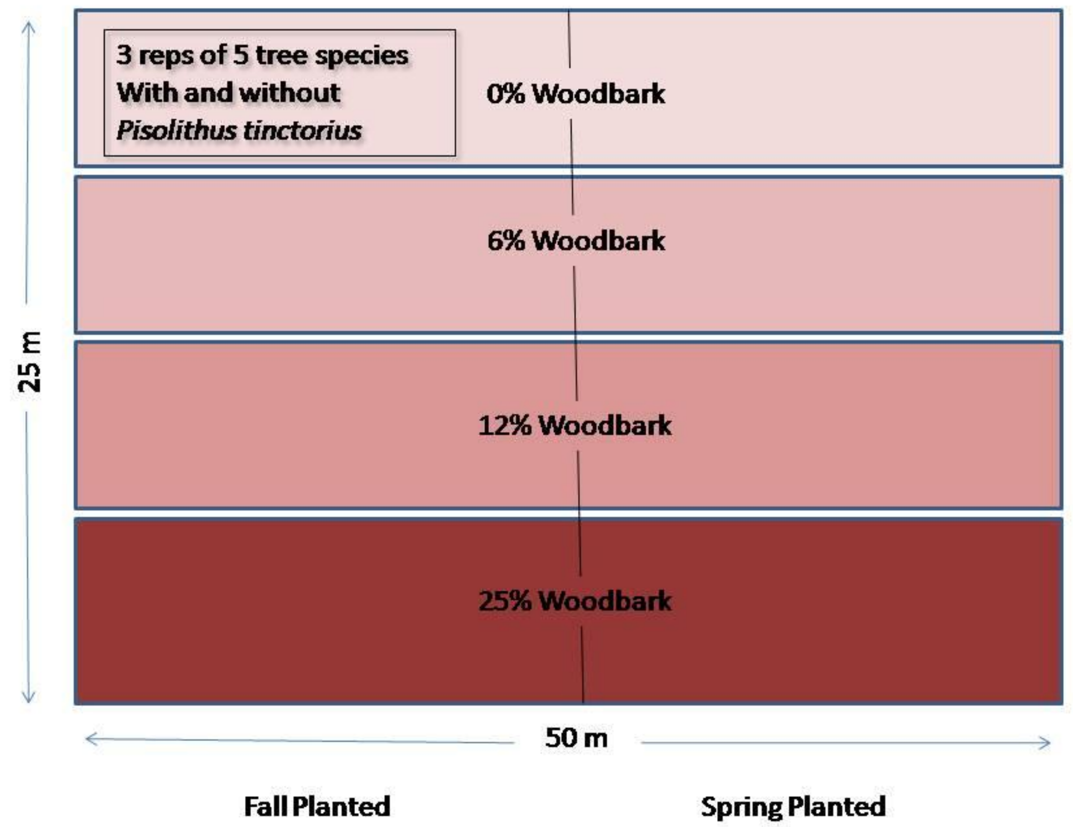


Figure 3-2 Split plot design with blocks of soil treatments (0, 6, 12, 25% woodbark) and planting dates (fall or spring)

100% topsoil, 94% topsoil and 6% woodbark, 88% topsoil and 12% woodbark, and 75% topsoil and 25% woodbark. Tree species were chosen from species found in the near vicinity of the mine: Trembling aspen (*Populus tremuloides*), Willow (*Salix* sp.), White Spruce (*Picea glauca*), Ninebark (*Physocarpus opulifolius*), and Red Osier Dogwood (*Cornus sericea*). Bare-rooted plants were used for all experiments. Trembling aspen, willow, and dogwood were all rooted cuttings while the white spruce and ninebark were grown in 60 cell plugblock trays from Beaver Plastics. Trees were planted at 2 m spacing (equivalent of 2500 seedlings per hectare), with the plot surrounded by a buffer of non-experimental seedlings planted at the same density. Half of the trees were inoculated with *Pisolithus tinctorius* and half of the trees were planted without any added fungus. Half of the trees were planted in Fall of 2012 and the other half were planted in the Spring of 2013. Weed control was not attempted and volunteer plant species were allowed to grow on the site.

3.4.4. Data Collection from the Planted Site

Five soil samples were taken randomly throughout each of the four soil mixtures to a depth of 15 cm prior to planting the trees. Each sample was sieved to remove rocks and a representative sample was placed in a plastic bag for laboratory analysis. Tree height (from the base of the tree to the uppermost bud) and survival (binary measurement of alive versus dead) was measured at planting and in the Spring and Fall each year following. In order to quantify plant stress, chlorophyll content was chosen as a way to measure a response to stress of the plants between the treatments (Walters 2005). Chlorophyll was measured using a CCM-300 Chlorophyll Content Meter from Opti-Sciences in the spring following leaf opening and fall just prior to leaf colour change of 2014 (Gitelson *et al*., 1999). Leaf area of volunteer plant species on each soil treatment was analyzed using 10 photos and Assess 2.0 (Lamari, 2008). Assess 2.0 is a computer program that performs a rapid measurement of leaf area, percent disease, root length, lesion count, and percent ground cover with imported photographs.

3.4.5. Laboratory Analysis

Analyses were done at the Lakehead University Environmental Laboratory (LUEL) according to the LUEL (2012) Quality Assurance/Quality Control (QA/QC) protocols. A blank sample was run at the beginning of each tested parameter, then a QA/QC sample, and followed by a repeat of the next field sample. This was repeated for every ten field samples.

Soil and plant samples were dried in a drying oven at 70^{o} C prior to analysis for total metals. Both soil and plant samples were homogenized to pass through a 2 mm mesh. A 0.2 g aliquot for soil and a 0.25 g aliquot for plant tissue were allowed to predigest in teflon express microwave digestion tubes overnight in a 3:1 ratio of concentrated HNO_3:HCL acids. The samples were then digested in a MARS 5 microwave digestion oven for 45 minutes at 175^{o} C. Samples were removed and diluted to 25 ml with distilled deionized water (DDW) and 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 were determined by the VarianPro Inductively Coupled Argon Plasma Spectrometer (ICP-OES). Replicate, QC , and lab blank samples were measured during each batch of samples. pH and conductivity of soil were measured in a 1:1 ratio of dry sample to DDW on a Mettler Model Seven Multi equipped with a conductivity cell and a pH probe. The moisture content of soil was determined by gravimetry on a separate aliquot of sample. A 2.0 g aliquot of soil was weighed and then dried in a drying oven at 100^{o} C overnight and the dry weight determined. Percent moisture was the oven dried weight divided by wet weight. Loss on ignition was used as an estimate of organic matter for soil by placing

2.0 g of soil into a crucible and then ashing it overnight at 550^{o} C. Organic matter was calculated as ash weight – dry weight divided by dry weight.

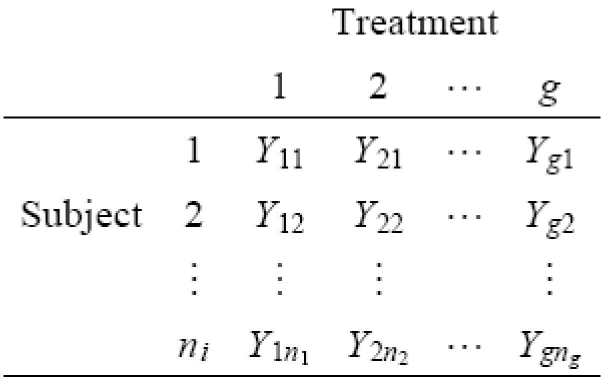
3.4.6. Data Analysis

Data analysis proceeded in the following steps using SPSS version 23 (IBM Corp, 2015):

i. Metal concentration by dry weight of the soil and plant material were used to calculate Translocation Factor (TF) and Bioconcentration Factor (BCF). TF is the metal in the aboveground biomass/metal in the root biomass while the BCF is the metal in the aboveground tissue/the metal content of the soil. A TF value higher than 1 is considered an accumulator of metals (Deng *et al*., 2004; Juarez-Santillan *et al*., 2010), while a hyperaccumulator is a plant with a TF value above 10 (Ashraf, 2011). BCF is the representation of the metal accumulation efficiency and can show the bioaccumulation of the metal in the food chain. If the value of BCF is higher than 1, the plant can be classified as a phytoextraction species (Zhang *et al*., 2002; Juarez-Santillan *et al*., 2010; Dowdy and McKone, 1997).

ii. Soils chemical variation among the topsoil/woodbark treatments were investigated for homogeneity and distribution. No transformation was needed before it was analyzed using one-way analysis of variance with a posthoc test of least significant difference (LSD). Statistical significance was defined as $P < 0.05$.

iii. General tree health rating (1 as poor to 5 as healthy based on vigour, leaf colour, insect infestation, amount of leaves and branches and height) and survival was analyzed using univariate ANOVA with LSD as a posthoc test to see if the treatments differed from each other in terms of a) timing of planting in spring or fall, b) presence or absence of added *Pisolithus tinctorius* and c) four levels (0, 6. 12. 25%) of woodbark added to topsoil. Statistical significance was defined as $P < 0.05$. The univariate equation can be shown like Figure 3-3.



		Treatment			
		1	2	$\cdots$	g
Subject	1	Y_{11}	Y_{21}	$\cdots$	Y_{g1}
	2	Y_{12}	Y_{22}	$\cdots$	Y_{g2}
	$\vdots$	$\vdots$	$\vdots$		$\vdots$
	n_i	Y_{1n_1}	Y_{2n_2}	$\cdots$	Y_{gn_g}

Figure 3-3 Univariate Analysis of Variance (ANOVA)

The columns correspond to the responses to g different treatments or from g different populations. And, the rows correspond to the subjects in each of these treatments or populations.

- Y_{ij} = Observation from subject j in group i
- n_i = Number of subjects in group i
- $N = n_1 + n_2 + \ldots + n_g$ = Total sample size (Anderson 2001).

iv. Tree Height (cm) and chlorophyll content (mg m^{-2}) data were log transformed and tested using repeated measures ANOVA with LSD to see if tree growth in the treatments differed from each other in terms of a) timing of planting in spring or fall, b) presence or absence of added *Pisolithus tinctorius* and c)four levels (0, 6. 12. 25%) of woodbark added to topsoil. Statistical significance was defined as $P < 0.05$. The model can be shown for this method (also known as split plot ANOVA) as:

$$Y_{ijk}=\mu+\alpha_i+\beta_{j(i)}+\tau_k+(a\tau)_{ik}+\epsilon_{ijk}$$

Yijk=μ+αi+βj(i)+τk+(aτ)ik+ϵijk

Using this linear model we assume that the data for treatment *i* for tree *j* at time *k* is equal to an overall mean μ plus the treatment effect α_i, the effect of the tree within that treatment $\beta_{j(i)}$, the effect of time τ_k, the effect of the interaction between time and treatment $(\alpha\tau)_{ik}$, and the error ε_{ijk}.

Such that:

- μ = overall mean
- α_i = effect of treatment *i*
- $\beta_{j(i)}$ = random effect of tree *j* receiving treatment *i*
- τ_k = effect of time *k*
- $(\alpha\tau)_{ik}$ = treatment by time interaction
- ε_{ijk} = experimental error (Weinfurt 2000)

3.5. Results

3.5.1. Characteristics of the existing soil on the former Golden Giant Mine

The soil metal contents in the former mine site are summarized in Table 3-1. Existing soils of the former Golden Giant mine surface generally had a neutral pH with some areas with extreme acidity (pH< 4.5). Soil conductivity was low and not considered saline or sodic. There were high amounts of Ca 1.6%, K 1849 mg kg^{-1}, Na 204 mg kg^{-1} and P 548 mg kg^{-1} as compared to normal levels of agricultural soils in Ontario (Legg 2012). Soils over the mine mined rock exceed the Canadian Soil Quality Guidelines for As, Sb and Mo. As averaged 23 mg kg^{-1} but some samples reached 101 mg kg^{-1}. The concentration of Sb in the soil was also elevated with some samples reaching 86 mg kg^{-1}. The average Mo in the soil was 76 mg kg^{-1} with values going as elevated as 406 mg kg^{-1}. Other metals of concern on closed mines such as Cr, Cu, Pb, and Zn were low with averages of 24 mg kg^{-1} , 23 mg kg^{-1}, 10 mg kg^{-1} , and 69 mg kg^{-1}, respectively.

Table 3-1 Soil Chemistry of the existing soil of the former gold mine compared to Canadian Soil Quality Guidelines and the typical agricultural Soils in Ontario. Units for metals are in mg kg^{-1} unless stated (Legg, 2012; CCME 2014)

	Mean	Standard Deviation	Canadian Soil Quality Guidelines	Ontario Agricultural Topsoil Range
Aluminum	7250	645		
Arsenic	23	7	12	
Barium	504	120		
Calcium (%)	1.67	0.34		0.10-0.40
Cobalt	1		300	
Chromium	24	2	87	
Copper	23	8	91	
Iron (%)	1.72	0.19		
Potassium	1849	275		80 to 250
Magnesium	7834	92		
Manganese	185	19		
Molybdenum	76	30	40	
Sodium	204	28		less than 200
Nickel	15	2	50	
Phosphorus	548	35		10 to 60
Lead	10	2	600	
Antimony	13	6	40	
Strontium	33	3		
Titanium	856	59		
Vanadium	42	10	130	
Zinc	69	24	360	
pH	7	1	6 to 8	
Conductivity (us/cm)	199.2	126.2	0 to 450	

3.5.2. Metal concentration in plants collected on the former Golden Giant Mine

Sampling of the former Golden Giant mine yielded 13 plant species. Some plants exhibited obvious deformities. For example, *Picea glauca* showed loss of needles and vibrant red and yellow colouring occured in the *Medicago sativa* leaves (Figure 3-4). *Larix laricina* and *Betula papyrifera* showed no visual signs of stress. The concentrations of As, Mo and Sb in the plants found on the site are presented in Table 3-2. No hyperaccumulators were found in the plants sampled on the mine as the TF values of the plant species was below 10. None of the plants moved any Sb from the soil into the aboveground parts of the plant. Higher levels of Mo were found in *Lotus corniculatus* (70 mg kg^{-1}) , and *Meliolotus officinalis* (98 mg kg^{-1}). *Medicago sativa*, *Anaphalis margaritacea,* and *Achillea millefolium* have Mo TF values above 1 which indicates they are accumulators of Mo.

Figure 3-4 Unknown Visual Toxicity/Deficiency Symptoms in *Medicago sativa* and *Picea glauca*

Table 3-2 Translocation Factor (TF), Bioaccumulation Factor (BCF) and Mean Levels of Arsenic, Molybdenum and Antimony in plant shoots, roots and soils on the former gold mine (mg kg^{-1})

	Plant Species	As					Mo					Sb				
		Shoots	Roots	Soil	TF	BCF	Shoots	Roots	Soil	TF	BCF	Shoots	Roots	Soil	TF	BCF
Alfalfa	*Medicago sativa*	0	3	18	0	0	8	27	31	.45	2.42	0	0	11	0	0
White birch	*Betula papyrifera*	1	5	24	.18	.03	4	16	73	.53	.39	0	3	13	0	0
Birdsfoot trefoil	*Lotus corniculatus*	0	10	25	0	0	37	76	52	.35	.86	0	0	15	0	0
Goldrod	*Solidago canadensis*	0	13	41	0	0	1	25	186	.49	.08	0	7	30	0	0
Horsetail	*Equisetum* spp	0	0	58	0	0	9	0	78	0	.11	0	0	43	0	0
Pearly everlasting	*Anaphalis margaritacea*	0	0	60	0	0	12	10	239	1.33	.12	0	0	48	0	0
Trembling aspen	*Populus tremuloides*	0	1	27	.37	.10	1	6	93	.31	.22	0	1	18	0	0
Sweet clover	*Melilotus officinalis*	0	2	35	0	.01	33	21	89	1.97	1.32	0	0	24	0	0
Tamarack	*Larix laricina*	3	0	30	0	.23	2	0	72	0	.09	0	0	13	0	0
White spruce	*Picea glauca*	1	3	36	.38	.08	2	18	90	.33	.05	0	2	24	0	0
Wild strawberry	*Fragaria vesca*	0	0	30	0	0	0	0	77	0	0	0	0	14	0	0
Willow	*Salix* spp.	0	9	34	.15	.02	1	24	115	.09	.05	0	4	23	0	0
Yarrow	*Achillea millefolium*	0	0	52	0	0	9	10	204	1.20	.04	0	0	43	0	0

3.5.3. Soil characteristics of topsoil/woodbark treatments

Soil samples were analyzed for metal contents, bulk density and pH. The summary of the soil metal concentrations is shown in Table 3-3. pH values were neutral. These soils are not considered saline or sodic and had low conductivity. All of the metals fall within the normal range for soils according to the Canadian Soil Quality Guidelines for industrial soils. As the quantity of woodbark increased in the topsoil treatments, there was a significant increase ($P < 0.05$) in % moisture, C/N ratio, and organic matter, significantly. Otherwise the addition of the woodbark diluted the other metal components of the topsoil with Pb and Sn having a statistically significant decrease ($P < 0.05$). There was also a significant decrease ($P < 0.05$) in pH, and bulk density as the level of woodbark added to the topsoil increased.

Table 3-3 Total concentration of metals, % moisture, conductivity, C/N ratio, loss on ignition and bulk density of the topsoil and woodbark treatments on the former gold mine*.

	75% Topsoil 25% Woodbark		88% Topsoil 12% Woodbark		94% Topsoil 6% Woodbark		100% Topsoil 0% Woodbark	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
% Moisture	20.29_{a}	2.22	16.73_{b}	1.39	15.76_{b}	.68	14.24_{b}	1.18
Conductivity (us/cm)	118.8_{a}	37.5	137.2_{a}	34.9	157.0_{a}	18.3	156.4_{a}	27.7
Bulk Density (g/cm^3)	$.59_{a}$	.05	$.71_{b}$	.07	$.75_{b,c}$	.02	$.80_{c}$	.02
Aluminum (%)	0.78_{a}	0.06	0.90_{a}	0.09	0.83_{a}	0.026	0.91_{a}	1.2
Arsenic	3.21_{a}	2.19	4.07_{a}	1.61	4.17_{a}	2.71	2.64_{a}	4.20
Barium	137.05_{a}	49.42	119.84_{a}	37.76	126.67_{a}	24.87	111.90_{a}	17.86
Beryllium	$.21_{a}$	.02	$.22_{a}$	.03	$.21_{a}$	.01	$.22_{a}$	.02
Calcium (%)	0.50_{a}	0.11	0.50_{a}	0.098	0.54_{a}	0.10	0.44_{a}	0.078
Cadmium	$.28_{a}$	.16	$.19_{a}$	.18	$.23_{a}$	.21	$.07_{a}$	.15
Cobalt	8.17_{a}	2.98	8.95_{a}	1.40	8.43_{a}	1.28	8.61_{a}	1.16
Chromium	15.88_{a}	1.86	17.19_{a}	.95	16.72_{a}	.56	18.06_{a}	2.26
Copper	11.87_{a}	.59	12.47_{a}	.94	12.58_{a}	.50	13.27_{a}	1.50
Iron (%)	1.28_{a}	0.15	1.45_{a}	0.11	1.37_{a}	0.082	1.52_{a}	0.19
Potassium (%)	0.12_{a}	0.025	0.14_{a}	0.034	0.13_{a}	0.017	0.15_{a}	0.069

Table 3-3 Total concentration of metals, % moisture, conductivity, C/N ratio, loss on ignition and bulk density of the topsoil and woodbark treatments on the former gold mine*. Continued

	75% Topsoil 25% Woodbark		**88% Topsoil 12% Woodbark**		**94% Topsoil 6% Woodbark**		**100% Topsoil 0% Woodbark**	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Magnesium (%)	0.66_{a}	0.052	0.74_{a}	0.061	0.72_{a}	0.032	0.77_{a}	0.15
Manganese	242.75_{a}	47.99	279.33_{a}	44.17	284.63_{a}	61.02	351.27_{a}	161.05
Sodium	217.96_{a}	42.42	226.74_{a}	53.82	244.34_{a}	61.52	244.79_{a}	36.13
Nickel	11.55_{a}	1.31	12.81_{a}	1.21	12.17_{a}	1.25	13.11_{a}	1.22
Phosphorus	315.46_{a}	12.84	338.30_{a}	16.32	342.23_{a}	26.99	352.84_{a}	44.01
Lead	3.12_{a}	.93	$3.62_{a,b}$	.77	$4.27_{a,b}$	1.10	5.20_{b}	1.42
Sulphur	571.61_{a}	160.91	740.38_{a}	229.67	612.23_{a}	90.87	583.17_{a}	228.49
Silicon	146.85_{a}	41.90	143.71_{a}	33.05	168.82_{a}	25.96	171.39_{a}	15.57
Tin	$.00_{a}$	0.00	11.91_{b}	7.13	18.29_{b}	2.72	15.65_{b}	9.09
Strontium	12.90_{a}	2.01	12.19_{a}	2.13	12.71_{a}	1.63	10.82_{a}	1.53
Titanium	841.53_{a}	124.00	945.85_{a}	80.95	856.50_{a}	46.45	954.16_{a}	165.78
Vanadium	26.67_{a}	3.24	30.14_{a}	2.35	27.93_{a}	1.77	32.09_{a}	5.87
Zinc	32.47_{a}	3.49	30.13_{a}	2.15	28.62_{a}	1.59	28.50_{a}	3.04
Loss on Ignition	9.6_{a}	2.1	5.7_{b}	1.7	4.8_{b}	.6	4.1_{b}	1.0
C/N Ratio	23.26_{a}	2.43	16.82_{b}	2.75	15.07_{b}	1.57	14.39_{b}	0.84

*Total concentrations of metals without units are expressed in units of mg kg^{-1}. Units for other parameters as shown. Values within rows with the same letters (a,b,c) are not significantly different at $P < 0.05$.

3.5.4. Tree survival and early growth of tree planting trial

Averaged across all the treatments, the survival for the trees can be seen in Figure 3-5 Table 3-4, with the majority of trees having higher than 70% survival except for the spring planted willows with a survival of 42%. Spring planted ninebark had 100% survival. Fall planted dogwood and ninebark, as well as spring planted white spruce and trembling aspen all had a survival of 92%. Presence of *Pisolithus tinctorius* significantly aided the survival of *Picea glauca* and it had a significant negative impact on *Cornus sericea.* No significant impact of the fungus was seen on the other plant species. Survival significantly increased with the addition of some woodbark in comparison with the treatment that was 100% topsoil but tended to show a decline in survival at the higher proportion of woodbark to topsoil especially in the *Salix* spp.

Table 3-4 Survival (%) of trees grown in year three at four levels (0, 6. 12. 25%) of woodbark added to topsoil, planting in either fall or spring and planted with or without *Pisolithus tinctorius*

Plant Species		***Cornus sericea***	***Populus tremuloides***	***Physocarpus opulifolius***	***Picea glauca***	***Salix* sp.**	***Treatment Mean***
Woodbark (%)	0	66.7	83.3	83.3	58.3	41.7	66.7_x
	6	91.7	100.0	91.7	100.0	66.7	90.0_y
	12	91.7	83.3	100.0	100.0	66.7	88.3_y
	25	83.3	83.3	100.0	100.0	58.3	85.0_y
Timing	Fall	87.5	79.2	87.5	87.5	83.3	85.0_x
	Spring	79.2	95.8	100.0	91.7	33.3	80.0_x
Pisolithus tinctorius	no	91.7	91.7	95.8	87.5	58.3	85.0_x
	yes	75.0	83.3	91.7	91.7	58.3	80.0_x
Species mean		83.3_a	87.5_a	93.8_a	89.6_a	58.3_b	82.5

Values within rows with the same letters (a,b) are not significantly different at P < 0.05. For each treatment, values within columns with the same letters (x, y) are not significantly different at P < 0.05.

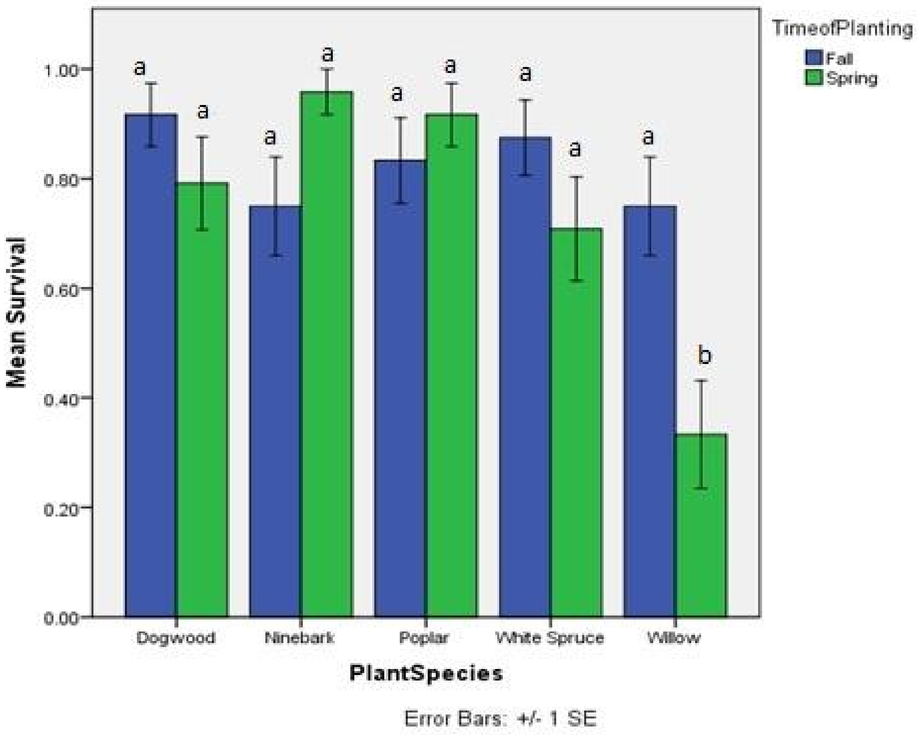


Figure 3-5 Mean survival of *Cornus sericea*, *Physocarpus opulifolius, Populus tremuloides*, *Picea glauca*, and *Salix* sp. at fall or spring planting times. Values with the same letters (x, y) are not significantly different at P < 0.05.

Table 3-5 Height (cm) of trees grown in year three at four levels (0, 6. 12. 25%) of woodbark added to topsoil, planting in either fall or spring and planted with or without *Pisolithus tinctorius*

Plant Species		***Cornus sericea***	***Populus tremuloides***	***Physocarpus opulifolius***	***Picea glauca***	***Salix* sp.**	***Treatment Mean***
Woodbark (%)	0	36	84	62	16	29	46_x
	6	36	109	59	28	42	55_x
	12	39	74	66	26	45	50_x
	25	32	86	68	28	32	49_x
Timing	Fall	44	96	53	24	54	54_x
	Spring	28	80	74	25	20	45_y
Pisolithus tinctorius	no	42	89	66	24	38	52_x
	yes	30	87	61	24	36	48_x
Species mean		36_a	88_b	64_c	24_d	37_a	50

Values within rows with the same letters (a,b,c,d) are not significantly different at P < 0.05. For each treatment, values within columns with the same letters (x, y) are not significantly different at P < 0.05.

In general the addition of the mychorrizal fungus, *Pisolithus tinctorius,* did not show a benefit to any of the height of the trees (*F*= 0.248, *P*=0.604) (Table 3-5) or chlorophyll content (*F*= 1.398, *P*=0.240) (Table 3-6). There was no visual evidence of the fungus around the tree roots where it was inoculated. Time of planting did have a significant impact on height (*F*= 14.031, *P*<0.050) and chlorophyll content ((*F*= 6.631, *P*<0.050) of the trees with *Cornus sericea*, *Populus tremuloides*, and *Salix* sp showing a greater height and decreased chlorophyll content with fall planting and *Physocarpus opulifolius* had a higher height and lower chlorophyll content in the spring. No impact of planting time was seen on *Picea glauca*. The different combinations of topsoil and woodbark showed no significant impact on tree growth (*F*=0.845, *P*=0.604) or chlorophyll content (*F*= 1.248, *P*=0.261).

Table 3-6 Chlorophyll Content (mg m^{-2}) of trees grown in year three at four levels (0, 6. 12. 25%) of woodbark added to topsoil, planting in either fall or spring and planted with or without Pisolithus tinctorius

Plant Species		***Cornus sericea***	***Populus tremuloides***	***Physocarpus opulifolius***	***Picea glauca***	***Salix* sp.**	**Treatment Mean**
Woodbark	0	395	415	421	245	362	376_x
	6	495	364	489	201	465	395_x
	12	356	406	549	214	428	388_x
	25	415	474	442	259	499	408_x
Time of Planting	Fall	416	342	411	210	427	361_x
	Spring	418	471	535	246	488	426_y
Pisolithus tinctorius	no	388	432	465	223	512	398_x
	yes	452	391	490	233	377	387_x
Species Mean		417_a	413_a	477_b	228_c	445_d	392

Values within rows with the same letters (a,b,c,d) are not significantly different at P < 0.05. For each treatment, values within columns with the same letters (x, y,z) are not significantly different at P < 0.05.

Health ratings of the trees (Table 3-7) showed a significant impact with time of planting (F= 6.007, P<0.05), and woodbark content (F= 2.640, P<0.05) but no significant impact of the *Pisolithus tinctorius* (F= 0.619, P=0.649). Fall planted trees for all species except *Physocarpus opulifolius* and *Picea glauca* had a better visual appearance. *Picea glauca* showed no difference between fall and spring planting while *Physocarpus opulifolius* had a better appearance with spring planting. All tree species showed a visual benefit with the addition of some woodbark to the topsoil in comparison with the control. No evidence of mammal browsing was noted in the plots, but there was much evidence of insect damage evident by galls and eaten leaves which was also common in trees surrounding the mine.

Table 3-7 Health rating of trees grown in year three at four levels (0, 6. 12. 25%) of woodbark added to topsoil, planting in either fall or spring and planted with or without Pisolithus tinctorius

Plant Species		***Cornus sericea***	***Populus tremuloides***	***Physocarpus opulifolius***	***Picea glauca***	***Salix* sp.**	**Treatment Mean**
Woodbark	0	2	3	3	1	1	2_x
	6	3	3	3	3	2	3_y
	12	3	2	4	3	2	3_y
	25	2	3	4	4	2	3_y
Time of Planting	Fall	3	3	3	3	3	3_x
	Spring	2	2	4	3	1	2_y
Pisolithus tinctorius	no	3	3	4	3	2	3_y
	yes	2	3	3	3	2	3_y
Species Mean		3_a	3_a	4_b	3_a	2_c	3

Values within rows with the same letters (a,b,c,d) are not significantly different at P < 0.05. For each treatment, values within columns with the same letters (x, y) are not significantly different at P < 0.05.

A natural reseeding of vegetation on the experimental area occurred during the course of this project with a wide variety of species growing in an uniform manner over the entire plot. These plants do not appear to be inhibiting the growth of the planted trees. The ground cover of the naturally regenerating plants growing on the topsoil/woodbark treatments varied significantly (F= 5.668, P<0.05) as seen in Figure 3-6.

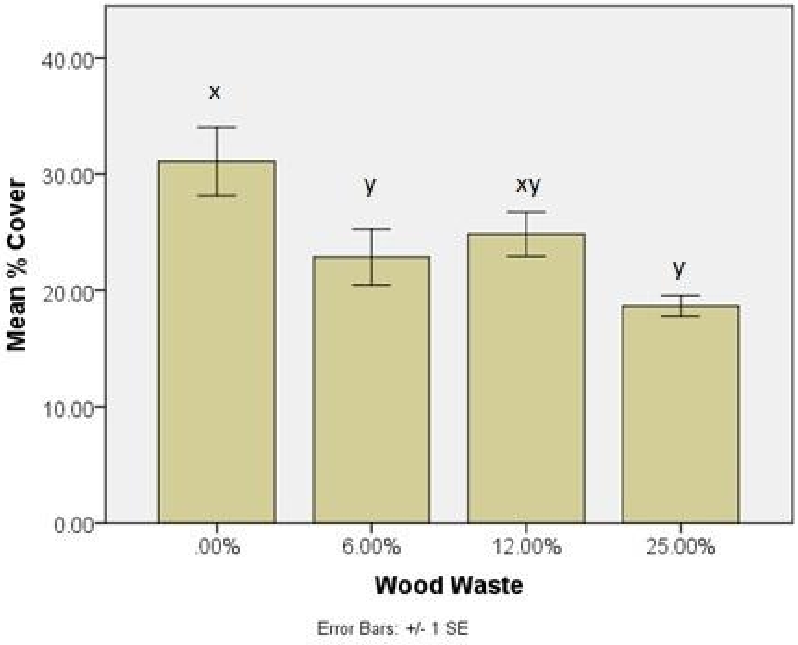


Figure 3-6 The amount of natural ground cover regenerating on four levels of woodbark (0, 6, 12, 25%) mixed in topsoil.

3.6. Discussion

3.6.1. Characteristics of the existing soil on the former Golden Giant Mine

Soil characteristics on the former Golden Giant property present a challenge for replanting the site to emulate the surrounding area. These anthropogenic soils are not developed and layered with natural processes as in the boreal forest but are man-made, created through mining processes which can vary depending on technology available and ore type and quality. Poor soil nutrition can contribute to the lack of soil stability, poor soil exploration by roots, nutrient cycling, and degraded soil ecosystems (Mummey *et al*., 2001; Ma *et al*., 2003; Wong *et al*., 2003; Freitas *et al*., 2004). The availability of As in the soil matrix is dependent on several factors including Fe/P, salts, pH, clay content, and soil moisture (Vega *et al*., 2006, and Moreno-Jimenez *et al*., 2012). Lower pH increases the availability of As in the soil, while the pressence of Fe/P, organic matter and clay can decrease the availability. Mo interactions with the soil matrix rely on pH, the presence of reducing and complexing agents, and soil moisture (Vega *et al*., 2006). Wet soil increases the availability of Mo (Kubota *et al*., 1963). Sb has low solubility rates and low mobility in the soil due to low pH, and the presence of Cd and Zn interfering in uptake(Hammel *et al*., 2000). For soils with elevated concentrations of metals such as As, Mo and Sb, toxicity could impede the growth of the plants, except for the phytoremediation candidates. However the presence of these metals does not mean that they are in a bioavailable form, readily toxic to plants. Other factors such as soil microbes, temperature, metal speciation, and nutrient status will impact the way that plants respond to the mine soil. The pH at this site (Table 3-3) is within the range that provides maximum nutrient availability (Harris *et al*., 1996). Mo becomes more phytoavailable as the pH increases with the highest availability between pH 7.8-8 which could be influencing the amount of Mo in the plants at this site (Gupta and Lipsett, 1982). As seen in Table 3-3, the levels of P, K, and Ca are all higher than typical agricultural soils in Ontario so more fertilizer is not recommended to improve the fertility of the soil for plant growth (Reid, 2006). Soil N was not measured as it is soluble in water and highly mobile in the soil. Further testing on plant tissue N would help with understanding the nutrient levels of N at this location. With the ample soil content of essential macronutirents, symptoms seen in some of the plant species on the mine site is due to elevated metal concentrations or other soil characteristics.

3.6.2. Metal concentration in plants collected on the former Golden Giant Mine

Some topsoil areas of the mine showed elevated levels of metals, others showed low levels and all areas with poor soil conditions so the identification of an array of healthy looking native phytostabilizing plants is key to establishing vegetation. According to Schwitzguebel *et al*. (2009), plants act as "green livers" to clean the environment, remediate damage caused by industrial practices and to prevent further degradation of the soil and water. Thirteen species in this study were analyzed for metal content in the aboveground and belowground tissue (Table 3-2). The plants evaluated were common to the site and collected from a completely resurfaced area during mine construction. While there were higher than above recommended levels of the As and Sb in the soil, none of the plants showed high levels of these metals in their aboveground plant tissues. Sb did not register any measured amounts in the shoots but accumulated up to 7 mg Sb kg^{-1} in the roots of *Solidago canadensis*. *Solidago canadensis* also accumulated the highest content of As in the roots with a level of 13 mg As kg^{-1}. Species growing in the highest levels of As were also going in the highest levels of Sb with no recorded accumulation in the roots or their shoots are *Equisetum* spp, *Anaphalis margaritacea* and *Achillea millefolium*.

Molybenum seems to be the most phytoavailable metal in these soils (Table 3-2). Of concern are the levels of Mo in *Lotus corniculatus , Meliolotus officinalis*, *Medicago sativa*, *Anaphalis margaritacea* and *Achillea millefolium* as they are higher than the 5 mg kg^{-1} recommended for ruminant feeding and could have negative impact on the health of the grazing wildlife in the region (Blakley, 2013). Even though there were elevated metal concentrations, only the *Meliolotus officinalis* and *Medicago sativa* showed a BCF above 1 so concentration of Mo in the aboveground plant tissue was higher than the level of Mo found in the soil.

Compared to other regions, the former Golden Giant Mine exhibits lower levels of As, Mo and Sb in the soil (Chapter 2). It is believed that the concentration of As and Sb in the soil are independent of the As and Sb concentrations in the plants (Qi *et al*., 2011; Zandsalmi *et al*., 2011; Madejon *et al*., 2002; Bech *et al*., 1997; de Koe, 1994). According to Meharg and Hartley-Whitaker (2002), arsenates can substitute for phosphates in plant metabolism. This impacts the plant ability to create ATP and carry out normal metabolism (Finnegan and Chen, 2012). Craw *et al*. (2007) states that typically Sb content of plants is one thousandth of the As content. Mo behaves in a different manner as it is considered the least required element for plant growth (Kaiser *et al*., 2005). Mo bioavailability to plants is somewhat related to the solubility of the chemical species of Mo but soil characteristics also influence the uptake (McGrath et al., 2010). Soil testing alone cannot predict the growth habits of the plant species on a contaminated site and individual plant species react to these elements with various processes for exclusion.

3.6.3. Soil characteristics of topsoil/woodbark treatments

In general, results showed that the application of woodbark with the top soil did not increase the metals in the topsoil (Table 3-3). Quality of the woodbark is variable depending on the source and the age of the bark and can have a high C/N ratio which binds the available N and prevents availability for plant growth. Well aged and decomposed woodbark have organic contaminants that disappear but trace metals could remain (Gomez, 1998). Lead and tin significantly decreased with increased addition of woodbark. Replaced topsoil is better for establishment and as a growth medium for trees due to physical and chemical properties compared to mined rock from the mine (Schoenholtz *et al*., 1992; Larson *et al*., 1995; Kost *et al*., 1998; Casselman, 2006). Grant and Koch (2007) found that replacing the topsoil was the most important step in providing a functional habitat that mimics the surrounding area. With the addition of the woodbark in the topsoil treatments, the organic matter increased (Table 3-3). The level was over double in the 25% woodbark/75% topsoil treatment as it was in the 100% topsoil. As stated by Hudson (1994), as the organic matter increases by 1-6%, so does the available water capacity up to 25%. For mineral soil, the addition of organic matter will enable a better establishment of the planted trees and a higher ability to handle stress. Soil organic matter also decreases the mobility and bioavailability of elements by sorption, chelation and sequestration but their impacts are transient and highly variable (Impellitteri and Allen, 2007). Soils that increase water holding capacity and nutrient availability improve success of the trees planted on the site (Casselman, 2006). An increase in 1% of organic matter can also increase the nutrient content in the soil, providing a fertilizer source for plants, such as 11208 kg C ha^{-1},1120 kg N ha^{-1}, 112 kg P ha^{-1}, 112 kg K ha^{-1} and 112 kg S ha^{-1} (Hoorman, 2010). For periods of hot dry weather such as in the summer months, this increased moisture holding capacity could aid in the water available to the trees and maintain a more even growth pattern. With no evidence of metal concentrations of an excessive nature in the soil in the area of the topsoil treatments (Table 3-3), no sampling of the plant tissues was done to determine metal content and their phytostabilization potential.

3.6.4. Tree survival and early growth of tree planting trial

Early success seems to be the key to establishment of trees on a reclaimed mine. Survival was above 60% for all of the tree species and treatments except for the willow especially those planted in the spring and in the highest level of woodbark (Figure 12 and Table 3-4). *Pisolithus tinctorius* is beneficial to use with *Picea glauca* but seemed to have either no impact or a negative effect on height in the four other species in this experiment (Table 3-5). Ectomycorrhizal fungi depend strongly on soil nutrition and can have a negative growth effect depending on the nutrient status of other saprophitic microbes (Koide and Kabir, 2001). Nursery grown plugs of *Picea glauca* are slower growing in comparison to the surrounding bush (Stiell, 1976). No trees were lost following the first year of growth. Younger seedlings are more susceptible to stresses of soil conditions compared to trees of 10 months (Ma *et al*., 2003).

Competition from other volunteer herbs caused a dense cover in treatments when lower amounts of woodbark was added to the topsoil (Figure 3-6). Helmisaari *et al*. (2007) found that mulching enhanced the growth of native trees such as *Pinus sylvestris*, *Betula* sp., and *Salix* sp. but it also increased the population volunteering herbs. Mulches such as the woodbark can contribute to fertility and moisture retention of the soil, bind the metals in the organic matter matrix and provide a cover to prevent establishment of blown in seed of volunteer plant species. No effects of the woodbark can be seen on the growth of the trees but other studies showing differences in height on mulched vs unmulched sites were analysed after a minimum ten year period of growth (Angel *et al*., 2006; Helmisaari *et al*., 2007).

3.6.5. Potential Management Practices

A number of actions can be taken to replant these mines and this variety could be the key to the success of the revegetation. The species planted and colonized on the experimental sites should mimic the native vegetation surrounding the mine and provide diversity for wildlife habitat and forage, as well as seed bank establishment. As there was excellent survival and growth of the various species planted in this experiment, different timings in planting could spread out the labour needs and could also spread the risk of drought, flooding, frost and other hard growing conditions. The species of trees used could also be changed depending on supply and cost as long as the species chosen are found in the area. If mulch is costly and in limited supply, the woodbark could be restricted to the area around each planted tree to allow for fertilization and prevention of competition while allowing other areas to be vegetated by blown in seed from the areas surrounding the mine. These plants would be acclimated to the climate and have a potential tolerance for possible elevated metal contents of the mine soil.

Further areas to investigate include alternate species of plants and fungi. Potential future research should investigate more local trees and shrubs, especially white birch, *Betula papryfiera*. As we sourced the *Pisolithus tinctorius* from a commercial source in United States, conceivably it would be better to cultivate a strain from a northern location, with a closer climatic condition to this mine. Also, as this was a short term investigation, this site could be revisited in the future to see if the continued success changes with time.

3.7. Conclusion

Native metallophyte communities growing on metal contaminated soils are a beneficial resource for creating strategies for phytotechnologies (such as phytostabilization or phytoextraction) and determining a process for replanting the former Golden Giant Mine. The soils on the existing mine site have a neutral pH with higher amounts of As, Mo and Sb. While the plants species growing on the site are not extracting appreciable amounts of As or Sb, several species including *Lotus corniculatus, Meliolotus officinalis* , *Medicago sativa*, *Anaphalis margaritacea*, and *Achillea millefolium* are removing Mo from the soil.

The results of my planting experiment show impacts of the timing of planting, the effect of adding *Pisolithus tinctorius* and the results of adding woodbark. Conclusions that can be drawn from this experiment is that:

- Timing of planting is less important to tree survival and early growth. This could provide more opportunities to spread out the timeline for scheduling replanting.
- *Pisolithus tinctorius* assists the survival of *Picea glauca* but not the deciduous species. As *Picea glauca* is a slower growing species compared to the others tested, assistance for survival and early growth should improve long term success.

- Woodbark acts as a mulch to prevent competition from volunteer plant species and could assist in the continued success of the trees planted through moisture availability, increased organic matter and improved bulk density.

Successful reforestation of a closed mine relies on understanding soil conditions, ameliorating identified soil concerns and planting trees in the least stressful manner. Plantings for mine closure should include a mix of deciduous and coniferous tree species with different timings of plantings as well as a the minimal addition of a source of locally found organic matter. Benefits of this type of plan include a spread of the workload, increase planting success, lessen impacts from weather conditions (drought, frost and excess rainfall), increase stress tolerance, and diversify plant life for wildlife habitat and esthetics. Plantings could also be done as seeding, cuttings or seedlings so as to decrease plant costs as well as labour costs of planting and maintenance.

4. A greenhouse experiment to determine the growth and phytostabilization potential of agronomic plant species used for remediation

4.1. Abstract

Phytostabilization species that moderate the release of heavy metals into the surrounding environment while having excellent plant growth creates great planting options for mine reclamation. A controlled greenhouse study was performed to investigate plant growth and the uptake of As, Sb and Mo by plant species commonly used in replanting open soils of roadsides and mines in Ontario. Playground sand was used as a growth medium to simulate the mine mined rock soils with 0, 0.1, 0.2, 0.4, 0.8 and 1.6 g L^{-1} of As, Mo and Sb, separately. Five species were grown in the experiment: white birch (*Betula papryrifera),* red clover *(Trifolium pratense),* white clover *(Trifolium repens),* alfalfa *(Medicago sativa)*, and creeping red fescue (*Festuca Rubra)*. Parameters measured were root and shoot length, root and shoot weight, leaf area, chlorophyll content, and metal content. As was excluded from all the plant species at most concentrations with a low translocation factor into the shoots. Mo showed a higher translocation factor in the legumes and red fescue and should be monitored for impacts to the ecosystem. Plant species can uptake Sb at the higher levels tested. White birches showed the least impact from the metal content of the soil on all growth parameters for all the metals tested and accumulated the least metals in their aboveground growth.

4.2. Keywords

plant uptake, mine tailings, ground cover, metals, arsenic, molybdenum, antimony

4.3. Introduction

Plants used to replant a closed mine can impact the ecosystem in the area for many years. Ideal species candidates should be low cost and focused on both long term and short term impacts to the soil and wildlife in the region (Bradshaw, 1997). Areas need to be planted to prevent air and water erosion, to improve soil conditions by increasing organic matter and increase the available N, for wildlife habitat and beautification purposes (Wong, 2003). In the past few years, many of the plants for reclamation purposes, stabilization and green cover have been agronomic species (Tordoff,, 2000). While these species can be used for nitrogen fixation, and are inexpensive and readily available, they can require irrigation, harvesting, fertilizing and reduce colonizing success of native plant populations (CLRA, 2015). Also herbaceous species can prevent other tree species from naturally regenerating in the area planted and compete with other species such as hand planted trees or naturally regenerating native species especially when there were large disturbances to the ecosystem (Wagner, 2000; Haeussler *et al*., 2004). Native species are already adapted to the area and to provide wildlife habitat and nutrition. These species are adapted to the climate but can be harder to source as seeds or cuttings in addition to being costly compared to the agronomic plants (personal communication, Derek Rodgers, Pickseed Canada 2015).

Phytoremediation approaches to the treatment of mine soils can be classified as phytostabilization and phytoextraction. Classification is based on the metal content of the plant tissue and the soil. Translocation factor (TF) and bioconcentration factor (BCF) are calculated using the total metal concentrations (dry weight) in the aboveground biomass, belowground biomass and the soil. TF is the metal in the aboveground biomass/metal in the root biomass while the BCF is the metal in the aboveground tissue/the metal content of the soil. Phytostabilization species are classified as having a BCF and TF less than 1 (Mendez and Maier, 2008). A TF value higher than 1 is considered an accumulator of metals (Deng *et al*., 2004; Juarez-Santillan *et al*., 2010), while a hyperaccumulator is a plant with a TF value above 10 (Ashraf, 2011). BCF is the representation of the metal accumulation efficiency and can show the bioaccumulation of the metal in the food chain. If the value of BCF is higher than 1, the plant can be classified as a phytoextraction species (Zhang *et al*., 2002; Santillan *et al*., 2010; Dowdy and McKone, 1997).

For a plant species to be successful at phytostabilization, the root systems should be able to restrict and contain a high amount of available metals and prevent their movement into the aboveground tissues of the plant and into the surrounding soil. They should also be an actively growing, perennial, have a vast root system and tolerant to climate stresses. Much of their success of bioremediation depends on several factors including bioavailabilty of metals via pH levels and soil organic matter content. (Towers and Paterson, 1997; McBride *et al*., 1997).

Naturalized agronomic species have many benefits for the degraded soils and could have potential for phytostabilization. Legume species such as *Trifolium pratense, Trifolium repens,* and *Medicago sativa*, have nitrogen fixing properties, and extensive rooting habits to prevent erosion and increase soil stability. Legumes have the ability to withstand a variety of drought and high moisture conditions, and provide a palatable feed for wildlife. Perennial grass species such as *Festuca Rubra,* is considered a soil stabilizer that can handle most soil types and has a dense fibrous rooting system. Also tolerant to a range of pH and salinity in soils, this long lasting perennial grass performs better in soils with adequate moisture, especially in the spring.

Native species are well adapted to the climate and conditions in these closed mine areas. Paperbark white birch, *Betula papryrifera,* is frequently found as the main tree species in many early succession forests and on closed mines in Northern Ontario. *B. papyrifera* is a perennial which tends to grow in areas of full sun and plentiful moisture, prefers highly drained soil, can withstand a variety of pH conditions and grows rapidly (Jones and Hutchenson, 1986; Safford, 1990). Their roots fan out in a shallow dense mat with the majority of the roots in the top 60 cm of the soil; no deep or taproot formation is evident (Safford, 1990). Seedlings of white birch are known to be more tolerant to concentrations of other metals such as Zn and Cu and the effect increases with the pressence of mycorrhizas (Denny and Wilkins, 1987; Colpaert and Van Assche, 1993; Utraianen *et al*., 1997).

Some problem metals in closed northwestern Ontario mines include, As, Mo and Sb. There are naturally occurring in the ore deposits in Northwestern Ontario. As they are not readily mobile in the soil via water, much of their spread occurs through erosion, uptake by plant species and movement of the soil mechanically. In Chapter 1, I investigated potential phytostabilization plant species found on three mining areas. All plant species had a large variation in metal content of the plant tissues in comparison with their soil metal content. *Betula papryrifera* accumulated up to 27 mg As kg^{-1} in soils containing 14031 mg As kg^{-}1, while *Schizachne purpurascens*, a grass species, had 180 mg As kg^{-1} in soils with 1869 mg As kg^{-1}. The leguminous species of *Medicago sativa, Trifolium repens* and *Melilotus albus* found on the mines accumulate 15 to 191 mg Mo kg^{-1} in soils containing 78 to 406 mg Mo kg^{-1} . None of the species found naturally on mine soils accumulate an appreciable amount of Sb in soils containing 11 to 3819 mg Sb kg^{-1}.

Remediation of mine soils with elevated metal content could be performed using phytoremediation species. There is little research on commonly used plant species for replanting to determine their type of phytoremediation. Species chosen for this investigation are commonly found or planted on closed or abandoned mines.

4.3.1. Objectives and Hypothesis

The objectives of this research was i) to evaluate plant growth of *Betula papryrifera*, *Festuca Rubra, Trifolium pratense, Trifolium repens,* and *Medicago sativa* in sand spiked with As, Mo or Sb at low to moderate levels$^{\cdot}$ and ii) to evaluate the metal content of the roots and aboveground portions of these plants as well as classify their performance as phytostabilization or phytoextraction species. Each of these species should be excellent candidates for phytostabilization.

4.4. Materials and Methods

4.4.1. Greenhouse Study

A randomized complete block design greenhouse experiment was performed with 6 treatments and 5 replications for As, Mo and Sb using five species: *Betula papryrifera* , *Trifolium pratense, Trifolium repens, Medicago sativa*, and *Festuca Rubra* . Seeds for *Betula papryrifera* originated in Timmins, ON as provided from Dr Han Chen (Lakehead University Natural Resources Department) and grown for transplanting as seedlings into the contaminated soils. Seeds for the *Trifolium pratense, Trifolium repens,* and *Medicago sativa* (Thunder Bay Coop and Farm Supplies) and *Festuca Rubra var. rubra* seed (Boles Feeds, Thunder Bay, Ontario) were germination tested and showed above 95% viability. Seedlings of white birch and seeds of the other four species were planted in 1L polypropylene containers containing 500 mL of Quikrete playsand. Pots were not perforated to prevent loss of added nutrients and metals. Fertilization was added as McCown Woody plant medium from Sigma Aldrich at 1.5 g L^{-1}. Sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$) was used as the As source while ammonium molybdate ($(NH_4)_2MoO_4$) was the Mo species and antimony potassium tartrate ($C_8H_4K_2O_{12}Sb_2$) was the Sb species. Metals were added at 0, 0.1, 0.2, 0.4, 0.8 and 1.6 g L^{-1} . Plants were watered as needed when less than 1 cm of water was seen in the bottom of the pot using distilled and deionized water to maintain an even water supply. Distilled and deionized water was used to reduce the influence of metals in the typical urban water supply. There was no further fertilizer added over the growth period. The pots were grown in greenhouse conditions from July to August for 32 days where temperature ranged from 15 C night to 30 C day, air humidity was 50 to 75% and the light/dark cycle was 16h/8h. After 32 days, pots were photographed for leaf area, plants were removed from the pot, and aboveground biomass was separated from belowground biomass to be weighed after air drying at room temperature of 20^{o}C. Soil from each pot was also collected for metal analysis after being thoroughly mixed.

4.4.2. Measurements

Lengths of roots and shoots of the potted plants in the greenhouse were recorded at the end of the trial. Chlorophyll content can be a sign of a plants response to environmental conditions (Walters 2005). Chlorophyll was recorded on a weekly basis for the white birch and at the end of the trial for the other four species using a CCM-300 Chlorophyll Content Meter (Gitelson *et al*., 1999). Leaf area was calculated for each experimental pot using Assess 2.0. Dry weight of each pots root and shoot biomass were recorded. Since there was limited biomass produced, plants were combined for each treatment for total metal analysis.

4.4.3. Analysis of Soil and Plant Samples

Analyses were done at the Lakehead University Environmental Laboratory (LUEL) according to the LUEL (2012) Quality Assurance/Quality Control (QA/QC) protocols. A blank sample was run at the beginning of each tested parameter, then a QA/QC sample, and followed by a repeat of the next sample. This was repeated for every ten field samples.

After separation into shoots and roots, plant samples were dried in a drying oven at 100^{o}C and homogenized to pass through a 2 mm mesh prior to analysis for Sb, As and Mo. Initial analyses indicated that a larger weight of plant sample than normally used (0.25 g) was required to reach the detection limit of the analytical method. However, this required the bulking of the shoot and roots for the plants in each treatment. A 0.5 g aliquot of plant tissue were allowed to predigest in teflon express microwave digestion tubes overnight in a 3:1 ratio of concentrated HNO_3:HCL acids. The samples were then digested in a MARS 5 microwave digestion oven for 45 minutes at 175^o C. Samples were removed and diluted to 25 ml with distilled deionized water (DDW) and concentrations of Sb, As, and Mo were determined by the VarianPro Inductively Coupled Argon Plasma Spectrometer (ICP-OES). Replicate, QC , and lab blank samples were measured during each batch of samples.

4.4.4. Statistical Analysis

All statistical analysis was performed using SPSS 22 package for Windows. First the data was explored to determine its distribution and variance. Since the distribution was normal and there was a homogeneity of variances, no further transformation was performed. Next, all variables were analyzed using ANOVA. LSD was performed as a post hoc test. Significance was set at $p<0.05$. Translocation factor (TF) and bioaccumulation factor (BCF) were calculated for each plant species at each metal concentration level in the soil. TF is the metal content in the aboveground biomass to the metal content in the root biomass. BCF is the metal concentration in the aboveground biomass to the metal content in the soil.

4.5. Results

4.5.1. Plant Growth

During the growth period, there were no visual leaf symptoms in any of the plant species for any of the metals tested (Figure 4-2, Figure 4-3, and Figure 4-4). Each of the plant species looked consistantly healthy. Table 4-1, Table 4-2, and **Table 4-3** show the growth parameters of the plants including heights and weights of the roots and shoots of each of the plants. The healthy looking plants among treatments can be seen in Figure 4-2, Figure 4-3, and Figure 4-4 where the plants in the control are hard to differentiate from the highest level of metal added.

As showed some minor growth reduction to some species studied (Table 4-1). White birch and red fescue had no significant differences between the levels of As concentrations. Both alfalfa and white clover showed a significant decline in their growth parameters as the concentration of As increased, except for root weight in alfalfa and chlorophyll content in white clover. As had a significant impact on the roots of red clover with a reduction in length and weight as the concentration increased. There was an increase in root weight with a small addition of As but the highest concentration of As has similar root characteristics as the control.

Mo impacted all the plant species in a similar manner (Table 4-2). White clover had the most growth parameters with a significant decrease except for chlorophyll content. Alfalfa, and red clover showed higher plant growth at lower concentrations of Mo but were not significantly different from the control values. For the alfalfa only the root length had a significant decrease as the Mo increased. White birches showed very little significant difference in growth among the Mo concentrations but for the increase in leaf area at middle concentrations of Mo. Red fescue grew significantly taller and greener as the concentration of Mo increased.

Small amounts of Sb aided plant growth but started to show some decrease in growth characteristics as the highest concentration(Table 4-3). In white birches, Sb made no significant difference in growth except for the weight of the roots decreased as the Sb concentration increased. Alfalfa, red clover and white clover acted in a similar manner to the Mo as well with a significant increase in growth at lower concentrations of S. Controls for the legumes were not significantly different from the

Table 4-1 Growth characteristics of alfalfa, white birch, red clover, red fescue and white clover exposed to 6 levels of As

Species		**Concentration (mg L^{-1})**					
		Control	As 10 mg L^{-1}	As 20 mg L^{-1}	As 40 mg L^{-1}	As 80 mg L^{-1}	As 160 mg L^{-1}
alfalfa	*Shoot Weight (g)	$.1874_{a,b}$	$.2706_{a,b}$	$.1628_{a,b}$	$.3714_{a}$	$.2188_{a,b}$	$.0366_{b}$
	Root Weight(g)	.664	1.154	.684	.991	.956	.349
	*Shoot Length (cm)	$4.9_{a,b}$	$5.1_{a,b}$	$5.0_{a,b}$	5.6_{a}	4.7_{b}	$4.2_{b,c}$
	*Root Length (cm)	$12_{a,b}$	13_{a}	$12_{a,b}$	10_{b}	$10_{b,c}$	$10_{a,b}$
	*Chlorophyll Content (mg m^2)	379_{a}	$341_{a,b}$	$366_{a,b}$	270_{b}	$301_{a,b}$	386_{a}
	*% Leaf Area	$5.89_{a,b}$	$8.98_{a,b}$	$6.01_{a,b}$	11.85_{a}	$6.93_{a,b}$	3.12_{b}
white birch	Shoot Weight (g)	.4994	.7374	.7964	.5360	.6280	.4674
	Root Weight(g)	1.071	.858	.748	.912	.567	.325
	Shoot Length (cm)	4.7	7.2	6.2	4.5	7.2	8.4
	Root Length (cm)	9	8	8	8	7	5
	Chlorophyll Content (mg m^2)	289	292	325	342	252	304
	% Leaf Area	3.28	4.21	3.68	3.25	3.21	3.05
red clover	Shoot Weight (g)	.1746	.1238	.1706	.1454	.0986	.1050
	*Root Weight(g)	$.187_{a,b}$	$.450_{a}$	$.281_{a,b}$	$.178_{a,b}$	$.025_{b}$	$.211_{a,b}$
	Shoot Length (cm)	3.8	4.1	4.0	4.0	3.3	3.7
	*Root Length (cm)	$7_{a,b}$	8_{a}	8_{a}	6_{b}	$6_{b,c}$	$7_{a,b}$
	Chlorophyll Content (mg m^2)	285	272	260	316	357	319
	% Leaf Area	3.74	3.47	4.01	3.42	1.24	1.35
red fescue	Shoot Weight (g)	.0842	.0776	.0878	.0772	.0860	.0866
	Root Weight(g)	.301	.753	.874	1.047	.977	1.132
	Shoot Length (cm)	8.7	10.2	11.4	11.8	10.4	10.3
	Root Length (cm)	8	9	7	7	7	9
	Chlorophyll Content (mg m^2)	316	259	299	365	327	372
	% Leaf Area	2.13_{a}	2.85_{a}	3.92_{a}	3.27_{a}	4.25_{a}	3.76_{a}
white clover	*Shoot Weight (g)	$.0594_{a,b}$	$.0736_{a,b}$	$.0638_{a,b}$	$.1278_{a}$	$.0472_{b}$	$.0172_{b,c}$
	*Root Weight(g)	$.504_{a}$	$.135_{b}$	$.194_{a,b}$	$.487_{a,b}$	$.181_{a,b}$	$.231_{a,b}$
	*Shoot Length (cm)	$3.0_{a,b}$	$3.0_{a,b,c}$	3.3_{a}	3.3_{a}	$2.6_{b,c}$	2.3_{c}
	*Root Length (cm)	9_{a}	$6_{b,e}$	$8_{a,c}$	8_{c}	$7_{b,c,d}$	6_{e}
	Chlorophyll Content (mg m^2)	316	259	299	365	327	372
	*% Leaf Area	$3.32_{a,b}$	$3.21_{a,b}$	4.36_{a}	4.30_{a}	1.24_{b}	$1.79_{a,b}$

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

Table 4-2 Growth characteristics of alfalfa, white birch, red clover, red fescue and white clover exposed to 6 levels of Mo

Species		Concentration (mg/L)					
		Control	Mo 10 mg L^{-1}	Mo 20 mg L^{-1}	Mo 40 mg L^{-1}	Mo 80 mg L^{-1}	Mo 160 mg L^{-1}
alfalfa	Shoot Weight (g)	.13	.09	.035	.06	.19	.19
	Root Weight(g)	.41	.28	.16	.17	.45	.45
	Shoot Length (cm)	4.02	4.02	3.04	3.68	4.08	3.49
	*Root Length (cm)	13.74$_{a,b,c}$	16.57$_{a}$	15.88$_{a}$	15.51$_{a,b}$	11.15$_{b,c}$	10.65$_{c}$
	Chlorophyll Content (mg m^2)	408	423	378	352	370	350
	% Leaf Area	5.09	4.73	2.53	3.13	8.55	7.62
white birch	Shoot Weight (g)	.68	.57	.76	.64	.76	.55
	Root Weight(g)	.58	.66	.62	.98	.82	.70
	Shoot Length (cm)	9.99	9.72	9.37	9.72	11.64	7.68
	Root Length (cm)	14.77	15.13	15.30	12.58	12.90	14.35
	Chlorophyll Content (mg m^2)	316	359	375	341	404	362
	*% Leaf Area	8.99$_{a,b}$	17.88$_{a}$	13.10$_{a,b}$	12.94$_{a,b}$	10.38$_{a,b}$	7.36$_{b}$
red clover	*Shoot Weight (g)	.17$_{a,b}$	.23$_{a}$	.17$_{a,b}$	.077$_{b}$	.15$_{a,b}$	.18$_{a,b}$
	Root Weight(g)	.43	.56	.44	.29	.46	.48
	Shoot Length (cm)	3.84	3.81	3.67	3.24	3.80	3.80
	Root Length (cm)	10.27	12.54	11.54	11.21	10.61	10.25
	Chlorophyll Content (mg m^2)	383	307	389	330	281	351
	*% Leaf Area	5.96$_{a,b}$	9.39$_{a}$	5.87$_{a,b}$	4.12$_{b}$	5.61$_{a,b}$	7.00$_{a,b}$
red fescue	Shoot Weight (g)	.084	.050	.047	.057	.043	.059
	Root Weight(g)	.30	.041	.050	.051	.057	.11
	*Shoot Length (cm)	8.66$_{a}$	9.93$_{a,b}$	10.02$_{a,b}$	12.66$_{b}$	10.62$_{a,b}$	12.13$_{b,c}$
	Root Length (cm)	7.75	8.17	8.54	8.24	10.23	9.19
	*Chlorophyll Content (mg m^2)	372$_{a}$	502$_{a,b}$	581$_{b}$	661$_{b,c}$	584$_{b,d}$	633$_{b,e}$
	% Leaf Area	2.13	3.06	3.02	3.83	2.49	3.61
white clover	*Shoot Weight (g)	.17$_{a}$	.15$_{a,b}$	.053$_{a,b}$	.035$_{b}$	.10$_{a,b}$	.094$_{a,b}$
	*Root Weight(g)	.42$_{a,b}$	.75$_{a}$	.18$_{b}$	.14$_{b,c}$	.39$_{a,b}$	.35$_{a,b}$
	*Shoot Length (cm)	3.63$_{a}$	2.785$_{a,b}$	2.77$_{a,b}$	2.10$_{b}$	2.65$_{b,c}$	2.30$_{b,d}$
	*Root Length (cm)	9.87$_{a,c}$	10.26$_{a}$	9.63$_{a,c}$	6.97$_{b}$	9.47$_{a,c}$	8.26$_{b,c}$
	Chlorophyll Content (mg m^2)	245	248	241	252	257	191
	% Leaf Area	4.49	4.54	2.57	2.00	3.90	4.19

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

highest concentrations. Red fescue had significantly greener leaves and longer roots with the addition of some Sb.

4.3.2. Metal Content of Plants

Figure 4-1 displays the heights and metal content of the roots and shoots. As the soil concentrations of As, Mo and Sb increases, visual examination of the plants do not translate in the metal concentration of the plant tissues.

Low concentrations of As were found in the roots and shoots of the five plant species (Table 4-4). Many of the species had a consistent level of As at all concentrations tested. For all plants, the amount of As found in the shoots ranged from 0.85 mg As kg^{-1} to 3.91 mg As kg^{-1}. The roots contained 1.49 mg As kg^{-1} to 7.47 mg As kg^{-1}. No pattern was seen in levels in comparison to the As content of the soil. White clover did not have enough biomass for a sample at the 160 mg As L^{-1} concentration.

The effect of Mo was similar for each plant species (Table 4-5). As the concentration of Mo increased in the soil, it increased in the roots as well as the shoots. But the concentration of Mo found in the aboveground portions of the plants differs considering the species. At the highest concentrations the white clover had 70.26 mg Mo kg^{-1} in the shoots at 80 mg Mo L^{-1} and not enough biomass available for a sample at the 160 mg Mo L^{-1}. The next highest levels was found in red fescue 30.83 mg Mo kg^{-1} in the shoots. Least was found in white birch at 15.6 mg Mo kg^{-1}. Alfalfa shoots contained 24.13 mg Mo kg^{-1} and red clover shoots contained 17.84 mg Mo kg^{-1}.

Sb did not start registering in the plant tissues until a concentration in the soil of 40 mg Sb L^{-1} (Table 4-6). No Sb was detected in the roots or shoots of the red fescue plants. White birches started showing 0.45 mg Sb kg^{-1} in the roots at the 40 mg Sb L^{-1} soil concentration, alfalfa showed 0.46 mg Sb kg^{-1} in the roots. At the 80 mg Sb L^{-1} soil concentration, red clover registered 0.65 mg Sb kg^{-1} in the shoots and 0.64 mg Sb kg^{-1} in the roots. White clover had Sb in the roots 0.68 mg Sb kg^{-1} and in the shoots 1.5 mg Sb kg^{-1} at the soil concentration of 80 mg Sb L^{-1}. There was not enough biomass for a sample at the 160 mg Sb L^{-1} for red clover, red fescue and white clover.

Table 4-3 Growth characteristics of alfalfa, white birch, red clover, red fescue and white clover exposed to 6 levels of Sb

Species		**Concentration (mg/L)**					
		Control	**Sb 10 mg L^{-1}**	**Sb 20 mg L^{-1}**	**Sb 40 mg L^{-1}**	**Sb 80 mg L^{-1}**	**Sb 160 mg L^{-1}**
alfalfa	*Shoot Weight (g)	$.065_a$	$.32_b$	$.21_{a,b}$	$.19_{a,b}$	$.40_b$	$.22_{a,b}$
	*Root Weight(g)	$.36_a$	1.09_b	$.65_{a,b}$	$.51_a$	$.91_{a,b}$	$.44246_a$
	Shoot Length (cm)	3.52	4.87	3.64	3.30	4.53	4.35
	*Root Length (cm)	$13.87_{a,b}$	15.66_a	$14.97_{a,b}$	9.56_b	$13.85_{a,b}$	$12.50_{a,b}$
	Chlorophyll Content (mg m^2)	246	298	295	231	319	273
	% Leaf Area	5.08	11.37	8.16	8.17	10.14	7.67
white birch	Shoot Weight (g)	.55	.56	0.61	.46	.53	.44
	Root Weight(g)	.71	.61	.66	.48	.54	.40
	Shoot Length (cm)	10.99	11.41	9.83	8.99	9.68	9.30
	Root Length (cm)	10.27	14.48	14.07	11.43	12.16	11.01
	Chlorophyll Content (mg m^2)	420	366	430	360	413	384
	% Leaf Area	26.62	36.63	24.43	28.67	27.90	23.42
red clover	Shoot Weight (g)	.16	.064	.17	.27	.38	.068
	*Root Weight(g)	$.27_{a,b}$	$.54_a$	$.53_a$	$.44_{a,b}$	$.45_{a,b}$	$.11_b$
	Shoot Length (cm)	4.16	4.23	3.44	3.46	3.40	2.66
	Root Length (cm)	11.57	11.73	12.04	10.29	9.76	9.20
	Chlorophyll Content (mg m^2)	257	205	241	239	224	244
	% Leaf Area	4.38	8.68	6.70	8.54	9.56	1.70
red fescue	Shoot Weight (g)	.084	.038	.024	.201	.036	.022
	Root Weight(g)	0.3	.19	.12	.11	.076	.16
	Shoot Length (cm)	8.66	10.12	9.44	9.42	10.72	10.28
	*Root Length (cm)	7.75_a	12.15_b	12.12_b	$9.98_{a,b}$	$10.73_{a,b}$	12.20_b
	*Chlorophyll Content (mg m^2)	372_a	615_b	590_b	583_b	612_b	$538_{a,b}$
	% Leaf Area	2.13	1.84	1.08	1.11	.81	.96
white clover	*Shoot Weight (g)	$.054_a$	$.21_b$	$.21_b$	$.13_{a,b}$	$.072_a$	$.082_{a,b}$
	*Root Weight(g)	$.11_a$	$.47_a$	$.45_a$	$.49_a$	$.34_a$	$.23_a$
	*Shoot Length (cm)	2.54_a	3.95_b	$3.23_{a,b}$	$3.31_{a,b}$	2.65_a	2.84_a

*Root Length (cm)	8.08_a	10.44_b	10.60_b	10.48_b	9.79_b	10.10_b
*Chlorophyll Content (mg m^2)	276_a	$191_{a,b}$	128_b	$138_{b,c}$	$153_{a,b}$	$145_{a,b}$
% Leaf Area	1.51	4.54	4.53	5.16	4.57	3.73

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

4.5.2. Bioaccumulation

None of the five plant species is in the hyperaccumulator range with a TF above 10. White clover plants are in the hypertolerant range (TF values 1 to 10) at the higher As soil concentration levels with TF values of 1.15 and 2.03. All of the other plants in the As treatments were tolerant with a TF below 1 or excluders with a TF below 0.1 (Table 4-4). Plants in the Mo treatments are considered hypertolerant with TF between 1 and 10 with the exception of white birch which can be classified as tolerant with TF falling below 1 (Table 4-5). In the Sb treatments, red fescue was an excluder with no registerable TF. Alfalfa, white birch, and red clover are considered tolerant of Sb and white clover is Sb hypertolerant (Table 4-6). None of these plant species bioconcentrated the metals in the aboveground plant from the soil, with the highest BCF value of 0.84 in white clover.

Table 4-4 As concentration (mg kg $^{-1}$) and translocation factor (TF) in alfalfa, white birch, red clover, red fescue and white clover at 6 different treatments of As

Species	Variable	Control	As 10 mg L^{-1}	As 20 mg L^{-1}	As 40 mg L^{-1}	As 80 mg L^{-1}	As 160 mg L^{-1}
alfalfa	Root As	2.55	2.71	2.09	3.01	2.74	2.69
	Shoot As	1.13	0.91	0.85	1.28	1.30	2.00
	TF	0.44	0.34	0.41	0.43	0.47	0.74
white birch	Root As	1.56	1.87	1.98	1.69	2.74	2.80
	Shoot As		0.93			1.68	2.61
	TF		0.50			0.61	0.93
red clover	Root As	2.55	7.47	2.80	3.69	4.63	2.58
	Shoot As	0.84	0.93	1.61	1.42		1.43
	TF	0.33	0.12	0.58	0.38		0.55
red fescue	Root As	6.99	4.00	5.33	3.15	5.30	6.38
	Shoot As	3.50	2.77	3.04	3.34	2.94	3.91
	TF	0.50	0.69	0.57	1.06	0.55	0.61
white clover	Root As	1.49	2.04	2.37	1.97	1.87	2.52
	Shoot As	2.10	1.53	2.07	2.28	3.80	
	TF	1.41	0.75	0.87	1.16	2.03	

Table 4-5 Mo concentration (mg kg $^{-1}$) and translocation factor (TF) in alfalfa, white birch, red clover, red fescue and white clover at 6 different treatments of Mo

species	Variable	Control	Mo 10 mg L^{-1}	Mo 20 mg L^{-1}	Mo 40 mg L^{-1}	Mo 80 mg L^{-1}	Mo 160 mg L^{-1}
alfalfa	Root Mo	1.03	1.61	2.1	3.34	7.34	12.01
	Shoot Mo	1.56	3.73	8.26	7.97	14.58	24.13
	TF	1.51	2.32	3.93	2.39	1.99	2.01
white birch	Root Mo	0.95	1.82	3.18	4.22	10.85	17.22
	Shoot Mo	1.56	3.67	4.29	5.63	4.77	15.6
	TF	1.64	2.02	1.35	1.33	0.44	0.91
red clover	Root Mo	1.43	2.18	2.39	4.56	7.4	12.61
	Shoot Mo	3.99	6.83	8.00	15.71	17.89	17.84
	TF	2.79	3.13	3.35	3.45	2.42	1.41
red fescue	Root Mo			4.51	7.01	8.03	15.55
	Shoot Mo		7.42	8.13	12.92	18.45	30.83
	TF			1.80	1.84	2.30	1.98
white clover	Root Mo	1.33	1.71	5.57	7.75	15.52	29.08
	Shoot Mo	6.04	8.42	12.89	24.35	29.77	70.26
	TF	4.543	4.92	2.31	3.14	1.92	2.42

4.6. Discussion

4.6.1. Plant Growth

With low As, Mo and Sb soil contamination, each of the species grown had good growth characteristics and are candidates for reclamation with very little visual differences seen in this experiment (Figure 4-2, Figure 4-3, and Figure 4-4). Classification of these plants as a certain type of phytoremediation candidates by sight is difficult due to no visual symptoms of toxicity, so plant tissue analysis must be done to determine type of phytoremediation classification. Each of these species does appear to maintain a continuous green cover but further testing is needed to ensure no metal uptake from the soil (Mendez and Maier, 2008). Chlorophyll data supports the idea that the plants have a high tolerance at various levels of As, Mo and Sb. Plant growth might be inhibited by the soil properties in this sand growth medium rather than the metal content of the soil (Friezl *et al*., 2003). Sb stress can be seen with the lower chlorophyll content of the leaves (Table 4-3)(Craw *et al*., 2007). White clover can tolerate these soils with As, Mo and Sb but has a smaller growth habit and may be less desirable as a candidate species for phytostabilization in comparison to the other legumes, red clover and alfalfa. Plants with a fibrous root system such as white birch or red fescue could help with preventing erosion while tap rooted systems of legumes like clovers and alfalfa could be used to add nutrients to the degraded soils through nitrogen-fixation (Alexander, 2000).

4.6.2. Metal Content of Plants

Although a species could be classified as a good reclamation species, it is the metal content of the plant that classify as a certain type of phytoremediation. Each of the species grown in this experiment performed well in all the concentrations of As, Mo and Sb so all could be used for phytoremediation (Table 4-1, Table 4-2, and Table 4-3). Red fescue, alfalfa, and white clover were consistent in the uptake of As in both the roots and the shoots at all As concentrations tested (Table 4-4). The type of root system does not translate to the amount of metal can be removed from the soil. There is variation in both fibrous roots systems such as red fescue and the tap rooted systems of the legumes (Alexander, 2000). While the red fescue in this study had the highest As root and shoot concentrations compared to the four other species tested, Antosiewicz *et al*. (2008) found that another grass species, *Calamagrostis arundinaca,* removed 40% of the As in the soil. Craw *et al*. (2007) determined that 1/1000 of Sb/As relationship occured in plant metal uptake, although poor soil nutrition has a greater impact on the potential for accumulation as the metals can replace the less available P. In this experiment, Sb was only found at the higher concentrations tested (Table 4-6). Sb uptake in shoots of plants typically leveled out at 2.2 mg kg $^{-1}$ so our results fall in line with Hammel *et al*. (2000). Mo content in the plant tissues in all species increased as the concentration of Mo in the soil increases across all five species investigated (Table 4-5).

Table 4-6 Sb concentration (mg kg $^{-1}$) and translocation factor (TF) in alfalfa, white birch, red clover, red fescue and white clover at 6 different treatments of Sb

Species	Variable	Control	Sb 10 mg L^{-1}	Sb 20 mg L^{-1}	Sb 40 mg L^{-1}	Sb 80 mg L^{-1}	Sb 160 mg L^{-1}
alfalfa	Root Sb					0.46	0.90
	Shoot Sb						0.74
	TF						0.82
white birch	Root Sb				0.45	0.64	2.09
	Shoot Sb					0.33	1.10
	TF					0.52	0.53
red clover	Root Sb					0.65	
	Shoot Sb					0.64	
	TF					0.98	
red fescue	Root Sb						
	Shoot Sb						
	TF						
white clover	Root Sb						0.68
	Shoot Sb						1.50
	TF						2.21

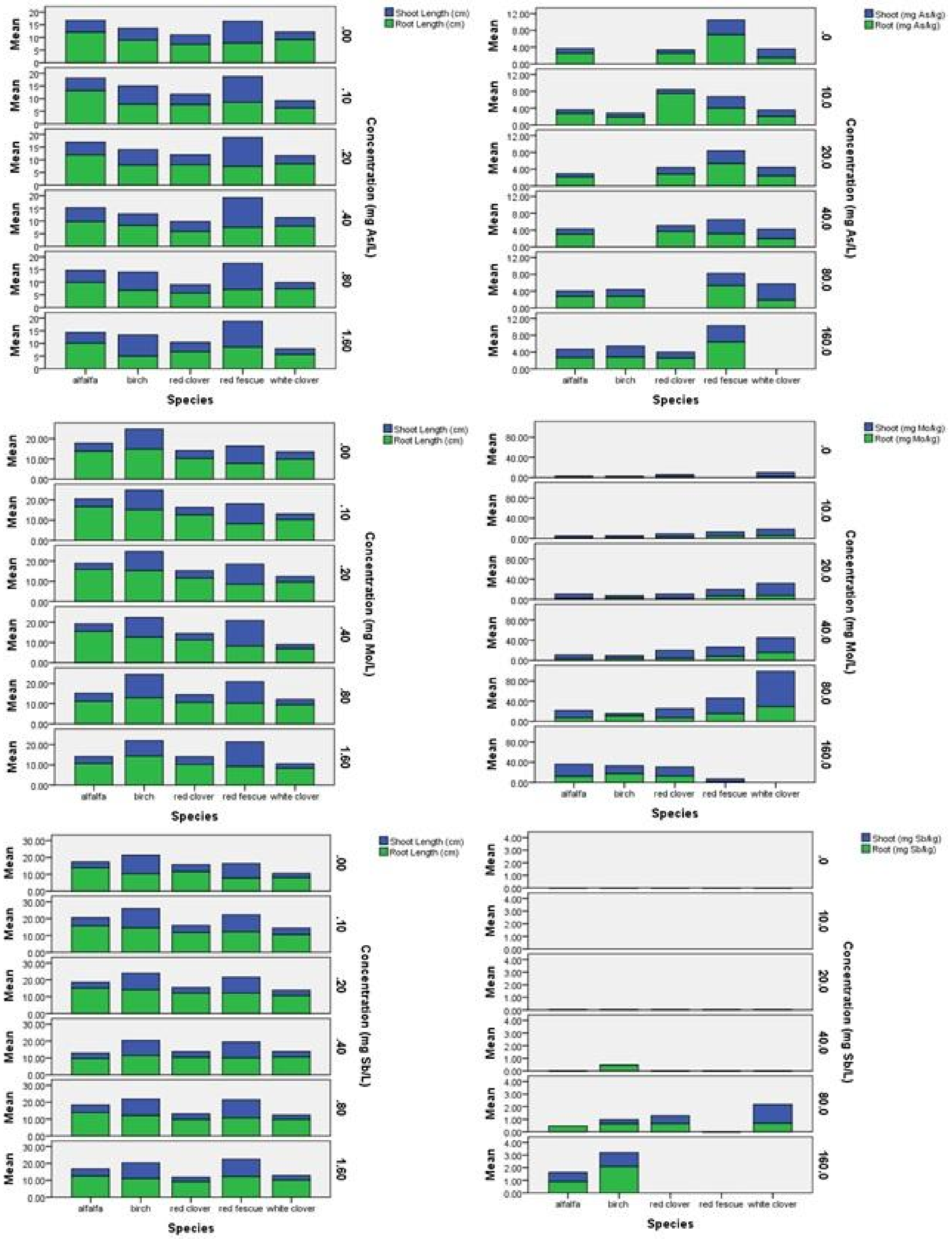


Figure 4-1 Heights (cm) and Metal Content (mg kg $^{-1}$) of Shoots and Roots of As, Mo and Sb at all soil/metal concentrations in the pot study. ANOVA results for heights can be seen in Tables 1-3. No ANOVA was performed for metal content.

4.6.3. Bioaccumulation

Although bioaccumulated metals are not necessarily lethal, they can produce sublethal impacts on the region (Luoma and Rainbow, 2005). This study (Table 4-4) tends to show an increase in the TF as the soil concentration of As increased for some species tested, but some of the species did have TF values that went up and down. Species in the family Fabaceae, which includes alfalfa, white clover and red clover, have been known accumulators of As and it concentrates in the edible portions of the plant (Ramirez-Andreotta et a,l 2013). Low amounts of As can actually stimulate growth but is not considered to be an essential element required for growth (Liebig *et al*., 1959; Lepp, 1981; Carbonell *et al*., 1998; Fitz and Wentzel, 2002). As does appear to have the ability to biomagnify in higher trophic levels (Huq *et al*., 2001; Barwick and Maher, 2003). However, other cases do not seem to show this As magnification effect (Wagemann *et al*., 1978; Kubota *et al*., 2000). The TF values in the Mo experiment did

Figure 4-2 Alfalfa grown with no added Sb (M) and with 160 mg Sb/L (R)

not show the same increase with soil concentration like the As samples (Table 4-5). Mo is considered to have higher uptake in pea plants in comparison to other heavy metals of Pb, Cd and Ni but has the least toxic effects (Kevresan *et al*., 2001). As there were so few results detected from the Sb samples tested in this study, it is difficult to comment on the trend of TF values in relation to soil concentrations (Table 4-6). This could mean that the plants do not accumulate Sb or that the cells of the plant are being impacted by the Sb and preventing Sb accumulation. Sb toxicty from plants is very rare even when grown in soil with elevated concentrations of the metal (Tschan *et al*., 2009; Pratas *et al*., 2005). He and Yang (1999) commented that the form of Sb should be considered over the total concentration in the soil; antimony potassium tartrate used in this study is one of the forms of concern and would show a worst case scenario.

4.6.4. Management Implications

Management decisions need to occur on a case by case basis with an analysis of the metals and plant species present. Testing of the soil and plants growing in the contaminated soils need to be done to create a plan for reclamation. Species that are known to accumulate toxic levels of these metals could be harvested and disposed in a safe manner or herbicides could be used to control their growth. Grazing done by ruminant species such as deer which prefer species such as small legumes and grasses can have Mo toxicity symptoms if ingested in quantities larger than 10 ppm but can be blocked with modest Cu supplementation on a 10 to 1 ratio Cu:Mo (Raisbeck et al. , 2006). Copper salt blocks could be distributed in areas that have the potential to impact the ruminants. Gymnosperms can show a high accumulation in their aboveground plant tissues as such current preferred replanting species of conifers has the potential to accumulate large quantities of As in comparison to angiosperm trees such as white birch (Bergqvist and Greger, 2012).

While this research investigated the impact of low levels of As, Mo, and Sb on *Betula papryrifera, Trifolium pratense, Trifolium repens, Medicago sativa*, and *Festuca Rubra*, there are other research questions that should be answered. Other plants commonly found in the reclamation seed mixes should be tested including other grasses to see if their growth habit is the same as *Festuca Rubra*.

Figure 4-3 White birch trees grown in 6 concentrations of Molybdenum. From left to right 0, 10, 20, 40, 80, 160 mg Mo/L

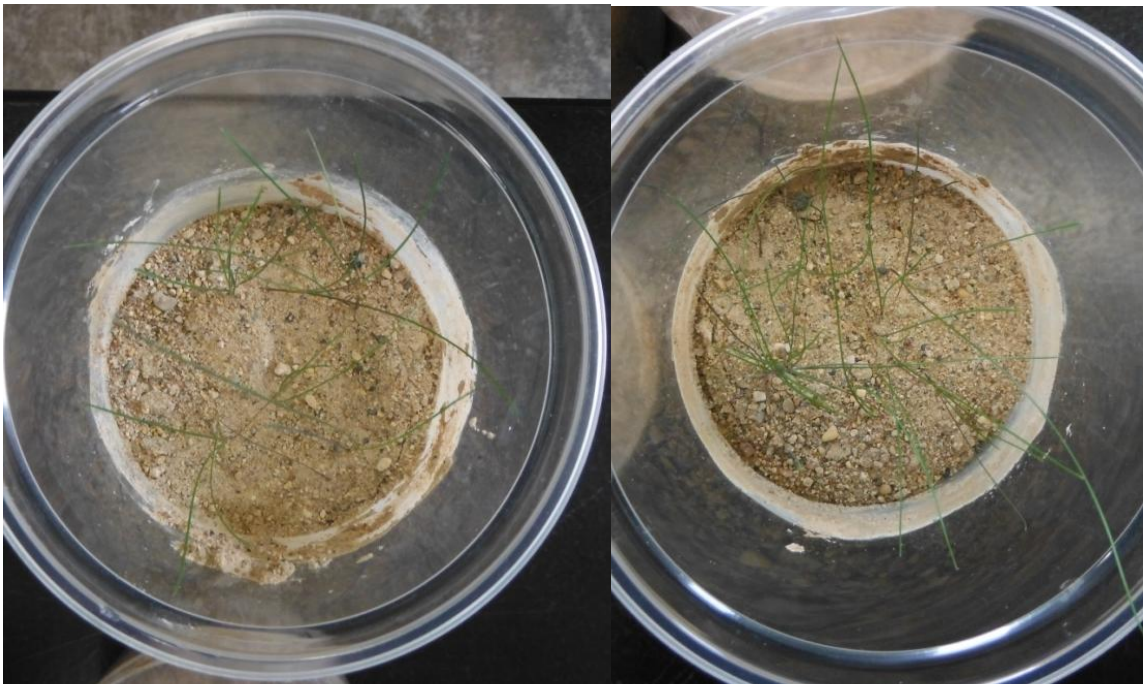


Figure 4-4 Red Fescue grown with no added As (left) and with 160 mg As/L (right)

Overall, each of these commonly used mine rehabilitation species has a tolerance for these metals at low to medium levels of As, Mo and Sb. While each of these agronomic species can have phytostabilization potential, their impact on the long-term ecology of the region should be investigated. Investigation of these species with varying amounts of organic amendments, lower water requirements, and seeding rates could enhance the understanding of the plants under difference stress levels. Also the impact of other biotic characteristics could improve the ability of these plants to retain these metals, such as bacterial and fungal colonies in these soils.

4.7. Conclusions

All plants performed well and would be good candidates for phytostabilization. Evidence points to signs of stress at the highest concentrations such is seen in the As impact on white clover. As was excluded from most plants as each species tended to have a low translocation factor and little uptake into the aboveground portions by any species. Legumes uptake Mo even at very low levels as does red fescue as it is a required element. It should be monitored for biomagnification impacts to the ecosystem. White birch accumulated the least Mo. Sb uptake can happen at levels of starting at 80 or 160 mg L^{-1} depending on the species planted. The use of phytostabilization plants seems to be an ideal method to provide a stable plant cover for light metal contaminated areas. Higher concentrations of As, Mo and Sb should be used to see more visual effects and to provide the higher limits of their tolerance to these metals. Addition of organic matter and other amendments need to be evaluated to see if it buffers effects of elevated metal content soils. Competition between metals can also impact plant growth, metabolism and uptake so these results may not translate into actual processes in field conditions with different types of soils.

5. Conclusions and Recommendations

5.1. Conclusions

With the increasing concerns on the environmental impacts of mining, mine closure should incorporate phytoremediation into their plans, particularly phytostabilization. Phytostabilization plant species are a resource in mine closure and reclamation of mine soils with high metal contents. Their effects will depend on the type of metal contamination and the metal's solubility and mobility within the environment. For elements such as As, Mo and Sb which are immobile within soils, phytostabilization is an option for containing the metals within a known contaminated location. A summary of findings can be found in Table 5-1.

The first study in chapter 2 investigated three closed mines in Northwestern Ontario for native plant species with phytostabilization potential for As, Mo and Sb in soils with different contamination levels. Plant species found naturally growing on the mine were tabulated and tested for their metal content in the above and belowground portions of the plant. Several species showed good phytostabilization potential for one or more metal contaminants including white birch *Betula papyrifea*, willow *Salix spp.,* trembling aspen *Populus tremuloides*, goldenrod *Solidago canadensis*, pearly everlasting *Anapahlis margaritacea* and tamarack *Larix laricina*. The most common tree found on these sites was white birch *Betula papyrifea* which was sometimes growing symbiotically with the fungus, *Pisolithus tinctorius*, a known metal accumulator at Steeprock Mine. None of the species could be classified as hyperaccumulators of As, Mo and Sb.

Chapter 3 examined the plants growing at Barrick Hemlo and their phytostabilization potential. The current soils and plants growing on the mine location were collected and analysed for metal content. A field trial was planted in spring and fall with 5 species of trees/shrubs (*Populus tremuloides*, *Salix* sp., *Picea glauca*, *Physocarpus opulifolius*, and *Cornus sericea)*, in four different topsoil/woodbark treatments and in the presence of a mychorrhizal fungus, *Pisolithus tinctorius*. Some of the plant species had concerning amounts of Mo in their leaf and stem tissues, while the majority of plant species did not accumulate excess metals in their aboveground tissues. The addition of woodbark improve some soil qualities including bulk density, organic matter and diluted some of the metals as well as had a mulching effect to prevent volunteer species. Each of the species planted in the field trial had a rate of survival above 70% with the exception of *Salix* sp. The addition of *Pisolithus tinctorius* did not seem to impact plant growth in the first few years of the trial.

A greenhouse study of five potential phytostabilization species of As, Mo and Sb was conducted for Chapter 4. *Betula papryrifera, Trifolium pratense, Trifolium repens, Medicago sativa*, and *Festuca Rubra* were grown in low concentrations of As, Mo and Sb and growth parameters and metal content were measured. Each of the elements was translocated into the plants but not hyperaccumulated. White birches accumulated the least of these three metals. White clover was impacted the most by the metals. Mo could biomagnify in the legume species if the soil conditions are alkaline.

Table 5-1 A summary of findings in mine rehabilitation in boreal forest regions

	Chapter 2	Chapter 3	Chapter 4
Approach	Exploration of plants and soils at three types of closed mines to investigate metallophillic species	Examination of possible phytoremediation plants on a gold mine with a trial planting of 5 species with woodbark and mycorrhizal fungus	Greenhouse experiment of 5 species to investigate impacts of As, Mo and Sb
Key findings	• a variety of plants were the same at each type of mine • soil characteristics varied completely • potential phytostabilization candidates were identified	• Pt Fungus assisted the growth of white pine • timing of planting is less important • woodbark acts as a mulch,organic matter source, nutrient source and dilutor of metals	• little visual differences between the treatments • Plants can take up Sb at higher Sb soil concentrations • White clover was impacted the most compared to the other species tested
Possible Phytostabilization candidates	As, Mo and Sb - white birch, willow, trembling aspen, goldenrod, pearly everlasting, and tamarack	As, Mo and Sb - tamarak and birch	As, Mo and Sb - birch
Possible Phytoextraction candidates	Zn - Pearly everlasting and goldenrod	Mo - Sweet clover, alfalfa	Mo - red fescue, red clover, white clover and alfalfa
Recommendations	• soil conditions need some help prior to planting - pH, organic matter, drainage, compaction, etc • a variety of plants can be planted to create microclimates and support other life • minimal maintenance should be required after initial planting	• addition of 6% woodbark helps with plant competition • planting can be spread to either spring or fall • Pt fungus could be used with white spruce but not the other species	• test at higher concentrations of As, Mo and Sb to determine upper limits • addition of organic matter to the soil could aid in plant growth

5.2. Recommendations

Due to the variability of metal uptake and the plant response to metals, there is no one solution that can be implemented for phytoremediation purposes. There needs to be flexible guidelines that coincide with a variety of soil conditions, native plant species, and end use possibilities. Different metal speciation, pH, organic matter content and water holding capacity of the soil can change the way these metals are available via water, plants or soil movement. Closure plans need to incorporate a combination of plant species, with a focus on succession species rather than the climax conifer species so as to mimic the natural ecosystems in the boreal forest.

Another part of this equation is to improve soil health. The addition of woodbark improved the soil qualities of bulk density and organic matter while decreasing the competition of volunteer herbs. Organic matter can also bind the available metals so as to decrease the toxic symptoms of the plants, while adding nutrients for plant growth. Mining communities could create organic bark collection systems so as to accumulate a close supply of compost for closure activities. These could include yard waste, wood ash, food waste and other common household wastes, but more research should be done to determine their success as a soil amendment.

5.3. Future Research

Other mining operations should investigate plants growing on the elevated metal soils that occur due to mining processes. With more plant species investigated, more metals can be investigated and less metals can bioaccumulate in the food chain and watersheds. Further work needs to be done to understand the effects of multiple metal contaminations on plants and the impact of soil amendments to plant growth and uptake. More commonly used plants for mine replanting should also be tested to determine their style of phytoremediation: phytoextraction or phytostabilization.

Another area of investigation would be to look at the microbial community associated with these plants. Microbes, including bacteria and fungi, are a crucial part of the soil ecosystem providing transformation of the redox states, chemical structure, solubility and bioavailability of metals. They also interact with plants in the rhizosphere to adsorb metals, increase water availability, increase nutrient absorption, and improve soil structure. These communities should be explored so as to improve bioremediation processes and identify potential species for use in bioremediation.

6. References

Akter, K.; Owens, G.; Davey, D. & Naidu, R. 2005. Arsenic Speciation and Toxicity in Biological Systems, *in* GeorgeW. Ware; LiliaA. Albert; D.G. Crosby; Pim Voogt; O. Hutzinger; JamesB. Knaak; FosterL. Mayer; D.P. Morgan; DouglasL. Park; RonaldS. Tjeerdema; DavidM. Whitacre; RaymondS.H. Yang & FrancisA. Gunther, ed., 'Reviews of Environmental Contamination and Toxicology', Springer New York, , pp. 97-149.

Alfredsen, G.; Solheim, H. & Slimestad, R. 2008. 'Antifungal effect of bark extracts from some European tree species', *European Journal of Forest Research* **127**(5), 387-393.

Alloway, B. 1995. *Heavy metals in soils*, Springer Science & Business Media.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral ecology*, *26*(1), pp.32-46.

Angel, P. N.; Graves, D. H.; Barton, C.; Warner, R. C.; Conrad, P. W.; Sweigard, R. J. & Agouridis, C. 2006. Surface mine reforestation research: Evaluation of tree response to low compaction reclamation techniques, *in* 'Proceedings, American Society of Mining and Reclamation', pp. 45--58.

Antosiewicz, D.; EscudÄ•-Duran, C.; Wierzbowska, E. & SkÅ‚odowska, A. 2008. 'Indigenous Plant Species with the Potential for the Phytoremediation of Arsenic and Metals Contaminated Soil', *Water, Air, & Soil Pollution* **193**, 197-210.

Arnold, C. W.; Parfitt, D. G. & Kaltreider, M. 2007.Phytovolatilization of Oxygenated Gasoline-Impacted Groundwater at an Underground Storage Tank Site Via Conifers *International Journal of Phytoremediation, 9*, 53-69.

Ashraf, M.; Maah, M. & Yusoff, I. 2011. 'Heavy Metals accumulation in plants growing in ex tin mining catchment', *Int. J. Enivron. Sci Tech.* **8**(2), 401-416.

Bagatto, G. & Shorthouse, J. D. 1999. 'Biotic and abiotic characteristics of ecosystems on acid metalliferous mine tailings near Sudbury, Ontario', *Canadian Journal of Botany* **77**(3), 410-425.

BanÃ¡sovÃ¡, V.; ÄŽuriÅ¡ovÃ¡, E.; NadubinskÃ¡, M.; GurinovÃ¡, E. & ÄŒiamporovÃ¡, M. 2012. Natural Vegetation, Metal Accumulation and Tolerance in Plants Growing on Heavy Metal Rich Soils, *in* Erika Kothe & Ajit Varma, ed., 'Bio-Geo Interactions in Metal-Contaminated Soils', Springer Berlin Heidelberg, , pp. 233-250.

Barrutia, O.; Artetxe, U.; HernÃ¡ndez, A.; Olano, J. M.; GarcÃa-Plazaola, J. I.; Garbisu, C. & Becerril, J. M. 2011. 'Native Plant Communities in an Abandoned Pb-Zn Mining Area of Northern Spain: Implications for Phytoremediation and Germplasm Preservation', *International Journal of Phytoremediation* **13**(3), 256-270.

Barwick, M. & Maher, W. 2003. 'Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia', *Marine Environmental Research* **56**(4), 471--502.

Bech, J.; Poschenrieder, C.; Llugany, M.; Barceló, J.; Tume, P.; Tobias, F.; Barranzuela, J. & Vásquez, E. 1997. 'Arsenic and heavy metal contamination of soil and vegetation around a copper mine in Northern Peru', *Science of the Total Environment* **203**(1), 83--91.

Bergeron, Y. 2000. 'Species and stand dynamics in the mixed woods of Quebec's southern boreal forest', *Ecology* **81**(6), 1500-1516.

Bergqvist, C. & Greger, M. 2012. 'Arsenic accumulation and speciation in plants from different habitats', *Applied Geochemistry* **27**(3), 615 - 622.

Bes, C.; Mench, M.; Aulen, M.; Gaste, H. & Taberly, J. 2010. 'Spatial variation of plant communities and shoot Cu concentrations of plant species at a timber treatment site', *Plant and Soil* **330**, 267-280.

Binkley, D. & Giardina, C. 1998. 'Why do tree species affect soils? The warp and woof of tree-soil interactions', *Biogeochemistry* **42**(1-2), 89--106.

Bittner, F. 2014. 'Molybdenum metabolism in plants and crosstalk to iron', *Frontiers in plant science* **5**.

Blakley, B. R. 2014. 'Overview of Molybdenum Poisoning', Merck Veterinary Manual.

Bossdorf, O.; Auge, H.; Lafuma, L.; Rogers, W. E.; Siemann, E. & Prati, D. 2005. 'Phenotypic and genetic differentiation between native and introduced plant populations', *Oecologia* **144**(1), 1--11.

Bowen, H. J. M. & others 1979. *Environmental chemistry of the elements.*, Academic Press..

Bradshaw, A. 1997. 'Restoration of mined landsâ€"using natural processes', *Ecological Engineering* **8**(4), 255 - 269.

Brenner, F. 1979. 'Soil and plant characteristics as determining factors in site selection for surface coal mine reclamation', *Environmental Geochemistry and Health* **1**, 39-44.

Brooks, R. R.; Chambers, M. F.; Nicks, L. J. & Robinson, B. H. 1998. Phytomining *Trends in Plant Science , 3*, 359 - 362.

Brown, R. L. & Naeth, M. A. 2014. 'Woody Debris Amendment Enhances Reclamation after Oil Sands Mining in Alberta, Canada', *Restoration Ecology* **22**(1), 40--48.

Buckley, J.; Gates, W. P. & Gibbs, D. T. 2012. 'Forensic examination of field GCL performance in landfill capping and mining containment applications', *Geotextiles and Geomembranes* **33**, 7--14.

Cadotte, M. W. & Lovett-Doust, J. 2001. 'Ecological and taxonomic differences between native and introduced plants of southwestern Ontario', *Ecoscience*, 230--238.

Canadian Council of Ministers of the Environment. 2014. 'Canadian Environmental Quality Guidelines'.

Canadian Land Reclamation Association. 2015. 'Ontario Chapter', http://www.clra.ca/default.aspx?page=17.

Canadian Land Reclamation Association and others. 2015. 'Land Reclamation and Soil Science: Solutions for a Sustainable Future, 2015: Conference Proceedings', .

Carbonell, A.; Aarabi, M.; DeLaune, R.; Gambrell, R. & Patrick Jr, W. 1998. 'Arsenic in wetland vegetation: availability, phytotoxicity, uptake and effects on plant growth and nutrition', *Science of the Total Environment* **217**(3), 189--199.

Casselman, C. N.; Fox, T. R.; Burger, J. A.; Jones, A. T. & Galbraith, J. M. 2006. 'Effects of silvicultural treatments on survival and growth of trees planted on reclaimed mine lands in the Appalachians', *Forest Ecology and Management* **223**(1), 403--414.

Cataldo, D. & Wildung, R. 1978. 'Soil and plant factors influencing the accumulation of heavy metals by plants.', *Environmental Health Perspectives* **27**, 149.

Chen, B.-C.; Lai, H.-Y. & Juang, K.-W. 2012. 'Model evaluation of plant metal content and biomass yield for the phytoextraction of heavy metals by switchgrass', *Ecotoxicology and Environmental Safety* **80**(0), 393 - 400.

Christensen, Ronald. 1996. "One-way ANOVA." In *Plane Answers to Complex Questions*, pp. 79-93. Springer New York.

Colpaert, J. V. & van Assche, J. A. 1993. 'The effects of cadmium on ectomycorrhizal Pinus sylvestris L.', *New Phytologist*, 325--333.

Craw, D.; Rufaut, C.; Haffert, L. & Paterson, L. 2007. 'Plant Colonization and Arsenic Uptake on High Arsenic Mine Wastes, New Zealand', *Water, Air, & Soil Pollution* **179**, 351-364.

De Koe, T. 1994. 'Agrostis castellana and Agrostis delicatula on heavy metal and arsenic enriched sites in NE Portugal', *Science of the total environment* **145**(1), 103--109.

Deng, H.; Ye, Z. & Wong, M. 2004. 'Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China', *Environmental Pollution* **132**(1), 29--40.

Denny, H. J. & Wilkins, D. 1987. 'Zinc tolerance in Betula spp.', *New Phytologist* **106**(3), 545--553.

Dickinson, N. M.; Baker, A. J.; Doronila, A.; Laidlaw, S. & Reeves, R. D. 2009. 'PHYTOREMEDIATION OF INORGANICS: REALISM AND SYNERGIES', *International Journal of Phytoremediation* **11**(2), 97-114.

Dirr, M. 1997. *Dirr's Hardy trees and shrubs: an illustrated encyclopedia*, Timber Press.

Dowdy, D. L. & McKone, T. E. 1997. 'Predicting plant uptake of organic chemicals from soil or air using octanol/water and octanol/air partition ratios and a molecular connectivity index', *Environmental toxicology and chemistry* **16**(12), 2448--2456.

Erakhrumen, A. & Agbontalor, A. 2007. 'Phytoremediation: an environmentally sound technology for pollution prevention, control and remediation in developing countries', *Educational Research and Review* **2**(7), 151--156.

Feng, R.; Wei, C.; Tu, S.; Ding, Y.; Wang, R. & Guo, J. 2013. 'The uptake and detoxification of antimony by plants: a review', *Environmental and experimental botany* **96**, 28--34.

Feng, R.; Wei, C.; Tu, S.; Wu, F. & Yang, L. 2009. 'Antimony accumulation and antioxidative responses in four fern plants', *Plant and Soil* **317**, 93-101.

Finnegan, P. M., & Chen, W. (2012). Arsenic Toxicity: The Effects on Plant Metabolism. *Frontiers in Physiology*, *3*, 182. http://doi.org/10.3389/fphys.2012.00182

Fitz, W. J. & Wenzel, W. W. 2002. 'Arsenic transformations in the soil–rhizosphere–plant system: fundamentals and potential application to phytoremediation', *Journal of biotechnology* **99**(3), 259--278.

Flynn, H. C.; Meharg, A. A.; Bowyer, P. K. & Paton, G. I. 2003. 'Antimony bioavailability in mine soils', *Environmental Pollution* **124**(1), 93--100.

Freedman, B. 1995. *Environmental ecology: the ecological effects of pollution, disturbance, and other stresses*, Academic Press.

Freitas, H.; Prasad, M. & Pratas, J. 2004. 'Plant community tolerant to trace elements growing on the degraded soils of SÃ£o Domingos mine in the south east of Portugal: environmental implications', *Environment International* **30**(1), 65 - 72.

Friesl, W.; Lombi, E.; Horak, O. & Wenzel, W. W. 2003. 'Immobilization of heavy metals in soils using inorganic amendments in a greenhouse study', *Journal of Plant Nutrition and Soil Science* **166**(2), 191--196.

Fu, Z.; Wu, F.; Mo, C.; Liu, B.; Zhu, J.; Deng, Q.; Liao, H. & Zhang, Y. 2011. 'Bioaccumulation of antimony, arsenic, and mercury in the vicinities of a large antimony mine, China', *Microchemical Journal* **97**(1), 12--19.

Gadd, G. M. 2010. 'Metals, minerals and microbes: geomicrobiology and bioremediation', *Microbiology* **156**(3), 609-643.

Garbisu, C. and Alkorta, I., 2001. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment.*Bioresource technology*, *77*(3), 229-236.

Gartner, J.; Still, S. & Klett, J. 1973. The use of bark waste as a substrate in horticulture, *in* 'I Symposium on Artificial Media in Horticulture 37', pp. 2003--2012.

Gitelson, A. A.; Buschmann, C. & Lichtenthaler, H. K. 1999. 'The Chlorophyll Fluorescence Ratio F735/F700 as an Accurate Measure of the Chlorophyll Content in Plants', *Remote Sensing of Environment* **69**(3), 296 - 302.

Glick, B. R. 2010. 'Using soil bacteria to facilitate phytoremediation', *Biotechnology Advances* **28**(3), 367 - 374.

Goldberg, S. and Forster, H.S., 1998. Factors affecting molybdenum adsorption by soils and minerals. *Soil Science*, *163*(2), 109-114.

Gomez, A. 1998. 'The evaluation of compost quality', *TrAC Trends in Analytical Chemistry* **17**(5), 310--314.

Grant, C. & Koch, J. 2007. 'Decommissioning Western Australia's First Bauxite Mine: Co-evolving vegetation restoration techniques and targets', *Ecological Management & Restoration* **8**(2), 92--105.

Green, S., & Renault, S. 2008. Influence of papermill sludge on growth of Medicago sativa, Festuca rubra and Agropyron trachycaulum in gold mine tailings: a greenhouse study. *Environmental pollution*, *151*(3), 524-531.

Greenstone Economic Development Corporation. 2015. 'Northern Empire mine - Beardmore'.

Greipsson, S. 2011. 'Phytoremediation', *Nature Education Knowledge* **2**(1), 7.

Grime, J. 1977. 'Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory', *American naturalist*, 1169--1194.

Gupta, U. C. & Lipsett, J. 1982. 'MOLYBDENUM IN SOILS, PLANTS', *Advances in agronomy* **34**, 73.

Haeussler, S.; Bedford, L.; Leduc, A.; Bergeron, Y. & Kranabetter, J. M. 2002. 'Silvicultural disturbance severity and plant communities of the southern Canadian boreal forest', *Silva fennica* **36**(1), 307--327.

Hammel, W.; Debus, R. & Steubing, L. 2000. 'Mobility of antimony in soil and its availability to plants', *Chemosphere* **41**(11), 1791 - 1798.

Harris, J. 2003. 'Measurements of the soil microbial community for estimating the success of restoration', *European Journal of Soil Science* **54**(4), 801--808.

Harris, J. A.; Birch, P.; Palmer, J. P. & others 1996. *Land restoration and reclamation: principles and practice.*, Addison Wesley Longman Ltd.

Hazama, K.; Nagata, S.; Fujimori, T.; Yanagisawa, S. & Yoneyama, T. 2015. Concentrations of metals and potential metal-binding compounds and speciation of Cd, Zn and Cu in phloem and xylem saps from castor bean plants (Ricinus communis) treated with four levels of cadmium. *Physiologia Plantarum, Blackwell Publishing Ltd,* 154, 243-255

He, M. & Yang, J. 1999. 'Effects of different forms of antimony on rice during the period of germination and growth and antimony concentration in rice tissue', *Science of The Total Environment* **243-244**(0), 149 - 155.

Helmisaari, H.; Salemaa, M.; Derome, J.; Kiikkila¨, O.; Uhlig, C. & Nieminen, T. M. 2007. 'Remediation of Heavy Metal–Contaminated Forest Soil Using Recycled Organic Matterand Native Woody Plants', *J. Environ. Qual.* **36**, 1145–1153.

Hoitink, H. & Boehm, M. 1999. 'Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon', *Annual review of phytopathology* **37**(1), 427--446.

Hoorman, J. J. & Islam, R. 2010. 'Understanding Soil Microbes and Nutrient Recycling', *Ohio State University Extension Publication* **SAG-16-10**.

Hosney, M. & Rowe, R. 2014. 'Performance of three GCLs used for covering gold mine tailings for 4 years under field and laboratory exposure conditions', *Geosynthetics International* **21**(3), 197--212.

Hudson, B. D. 1994. 'Soil organic matter and available water capacity', *Journal of Soil and Water Conservation* **49**(2), 189--194.

Huq, S. I.; Ara, Q. A. J.; Islam, K.; Zaher, A. & Naidu, R. 2001. 'The possible contamination from arsenic through food chain', *Groundwater arsenic contamination in the Bengal Delta Plain of Bangladesh*, 91--6.

Impellitteri, C. A. & Allen, H. E. 2007. *Effect of humic substances on attenuation of metals: Bioavailability and mobility in soil. In 'Natural attenuation of trace element* *availability in soils'.(Eds R Hamon, M McLaughlin, E Lombi) pp. 89–112*, CRC Press, Taylor and Francis: Boca Raton, FL.

Jana, U.; Chassany, V.; Bertrand, G.; Castrec-Rouelle, M.; Aubry, E.; Boudsocq, S.; Laffray, D. & Repellin, A. 2012. 'Analysis of arsenic and antimony distribution within plants growing at an old mine site in Ouche (Cantal, France) and identification of species suitable for site revegetation', *Journal of environmental management* **110**, 188--193.

Jones, M. D. & Hutchinson, T. C. 1986. 'The effect of mycorrhizal infection on the response of Betula papyrifera to nickel and copper', *New Phytologist* **102**(3), 429--442.

Jones, O.; Welander, M.; Waller, B. J. & Ridout, M. 1996. 'Micropropagation of adult birch trees: production and field performance', *Tree physiology* **16**(5), 521--525.

Juárez-Santillán, L. F.; Lucho-Constantino, C. A.; Vázquez-Rodriguez, G. A.; Cerón-Ubilla, N. M. & Beltrán-Hernández, R. I. 2010. 'Manganese accumulation in plants of the mining zone of Hidalgo, Mexico', *Bioresource technology* **101**(15), 5836--5841.

Kabata-Pendias, A. 2010. *Trace elements in soils and plants*, CRC press.

Kabata-Pendias, A. & Mukherjee, A. B. 2007. *Trace elements from soil to human*, Springer Science & Business Media.

Kaiser, B. N.; Gridley, K. L.; Ngaire Brady, J.; Phillips, T. & Tyerman, S. D. 2005. 'The Role of Molybdenum in Agricultural Plant Production', *Annals of Botany* **96**(5), 745-754.

Kevresan, S.; Petrovic, N.; Popovic, M. & Kandrac, J. 2001. 'Nitrogen and protein metabolism in young pea plants as affected by different concentrations of nickel, cadmium, lead, and molybdenum', *Journal of Plant Nutrition* **24**(10), 1633-1644.

Khade, S. W. & Adholeya, A. 2007. 'Feasible Bioremediation through Arbuscular Mycorrhizal Fungi Imparting Heavy Metal Tolerance: A Retrospective', *Bioremediation Journal* **11**(1), 33-43.

Khan, A.; Kuek, C.; Chaudhry, T.; Khoo, C. & Hayes, W. 2000. 'Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation', *Chemosphere* **41**(1-2), 197 - 207.

Knops, J. M. H.; Bradley, K. L. & Wedin, D. A. 2002. 'Mechanisms of plant species impacts on ecosystem nitrogen cycling', *Ecology Letters* **5**(3), 454--466.

Koide, R. T. and Kabir, Z. (2001), Nutrient economy of red pine is affected by interactions between *Pisolithus tinctorius* and other forest-floor microbes. *New Phytologist*, 150: 179–188.

Kost, D. A.; Vimmerstedt, J. P. & Brown, J. H. 1998. 'Topsoiling, ripping, and fertilizing effects on tree growth and nutrition on calcareous minesoils', *Forest ecology and management* **103**(2), 307--319.

Kubota, J.O.E., Lemon, E.R. and Allaway, W.H., 1963. The effect of soil moisture content upon the uptake of molybdenum, copper, and cobalt by alsike clover. *Soil Science Society of America Journal*, *27*(6), 679-683.

Kumar, P. N.; Dushenkov, V.; Motto, H. & Raskin, I. 1995. 'Phytoextraction: the use of plants to remove heavy metals from soils', *Environmental Science & Technology* **29**(5), 1232--1238.

Kumpiene, J.; Lagerkvist, A. & Maurice, C. 2008. 'Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments â€“ A review', *Waste Management* **28**(1), 215 - 225.

Lafrance, B.; DeWolfe, J. C. & Stott, G. M. 2004. 'A structural reappraisal of the Beardmore Geraldton Belt at the southern boundary of the Wabigoon subprovince, Ontario, and implications for gold mineralization', *Canadian Journal of Earth Sciences* **41**(2), 217--235.

Lakehead University Environmental Laboratory (LUEL). 2012. Water, Soil and Plant Tissue Protocols. Lakehead University, 65pp.

Lamari, L. 2008. *ASSESS 2.0: Image analysis software for disease quantification*, APS Press.

Larson, M. M.; Kost, D. A. & Vimmerstedt, J. P. 1995. 'Establishment of trees on minesoils during drought and wet years', *International Journal of Surface Mining and Reclamation* **9**(3), 99--103.

Legg, J. 2012. 'Topsoil Report Ranges', Agrifood Laboratories, Online.

Lepp, N. W. 1981. *Effect of heavy metal pollution on plants: Effects of trace metals on plant function*, Springer Science & Business Media.

Li, Yin-M; Chaney, Rufus L.; Brewer, Eric P.; Angle, J. Scott & Nelkin, J. 2003. Phytoextraction of Nickel and Cobalt by Hyperaccumulator Alyssum Species Grown on Nickel-Contaminated Soils *Environmental Science & Technology, 37*, 1463-1468.

Liebig Jr, G.; Bradford, G. & Vanselow, A. 1959. 'Effects of arsenic compounds on citrus plants in solution culture.', *Soil Science* **88**(6), 342--hyhen.

Lilles, E.; Purdy, B.; Macdonald, S. & Chang, S. 2012. 'Growth of aspen and white spruce on naturally saline sites in northern Alberta: Implications for development of boreal forest vegetation on reclaimed saline soils', *Canadian Journal of Soil Science* **92**(1), 213-227.

Luoma, S. N. & Rainbow, P. S. 2005. 'Why is metal bioaccumulation so variable? Biodynamics as a unifying concept', *Environmental Science & Technology* **39**(7), 1921-1931.

Ma X, & Burken J. 2002. VOCs fate and partitioning in vegetation: use of tree cores in groundwater analysis. *Environ Sci Technol* 36:4663-4668

Ma, Y.; Dickinson, N. & Wong, M. 2003. 'Interactions between earthworms, trees, soil nutrition and metal mobility in amended Pb/Zn mine tailings from Guangdong, China', *Soil Biology and Biochemistry* **35**(10), 1369--1379.

Ma, Y.; Prasad, M.; Rajkumar, M. & Freitas, H. 2011. 'Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils', *Biotechnology Advances* **29**(2), 248 - 258.

Madejón, P.; Murillo, J.; Maranon, T.; Cabrera, F. & López, R. 2002. 'Bioaccumulation of As, Cd, Cu, Fe and Pb in wild grasses affected by the Aznalcóllar mine spill (SW Spain)', *Science of the Total Environment* **290**(1), 105--120.

Magurran, A. E. 2013. *Ecological diversity and its measurement*, Springer Science & Business Media.

Majumder, A. & Jha, S. 2012. Hairy Roots: A Promising Tool for Phytoremediation, *in* T. Satyanarayana & Bhavdish Narain Johri, ed., 'Microorganisms in Environmental Management', Springer Netherlands, , pp. 607-629.

Mandal, B. K. & Suzuki, K. T. 2002. 'Arsenic round the world: a review', *Talanta* **58**(1), 201--235.

Manios, T., Stentiford, E.I. and Millner, P.A., 2003. The effect of heavy metals accumulation on the chlorophyll concentration of Typha latifolia plants, growing in a substrate containing sewage sludge compost and watered with metaliferus water. *Ecological Engineering*, *20*(1), 65-74.

MarguÃ, E.; Queralt, I.; Carvalho, M. & Hidalgo, M. 2007. 'Assessment of metal availability to vegetation (Betula pendula) in Pb-Zn ore concentrate residues with different features', *Environmental Pollution* **145**(1), 179 - 184.

Marin, A. R.; Masscheleyn, P. H. & Patrick, W. H. 1993. Soil redox-pH stability of arsenic species and its influence on arsenic uptake by rice *Plant and Soil, 152*, 245-253

Marx, D. H. 1977. 'Tree host range and world distribution of the ectomycorrhizal fungus Pisolithus tinctoriu', *Canadian Journal of Microbiology* **23**(3), 217-223.

Mascher, R., Lippmann, B., Holzinger, S. and Bergmann, H., 2002. Arsenate toxicity: effects on oxidative stress response molecules and enzymes in red clover plants. *Plant Science*, *163*(5),961-969.

McBride, M.; Sauve, S. & Hendershot, W. 1997. 'Solubility control of Cu, Zn, Cd and Pb in contaminated soils', *European Journal of Soil Science* **48**(2), 337--346.

McCune, B.; Grace, J. B. & Urban, D. L. 2002. *Analysis of ecological communities*, Vol. 28, MjM software design Gleneden Beach, OR.

McGrath, S.; MicÃ, C.; Zhao, F.; Stroud, J.; Zhang, H. & Fozard, S. 2010. 'Predicting molybdenum toxicity to higher plants: Estimation of toxicity threshold values', *Environmental Pollution* **158**(10), 3085 - 3094.

McIntyre, T. 2003. Phytoremediation of heavy metals from soils'Phytoremediation', Springer, , pp. 97--123.

Meharg, A. A. & Hartley-Whitaker, J. 2002. 'Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species', *New Phytologist* **154**(1), 29--43.

Menard, O. 2015. 'Soil Life''2015 Year of Soils - Ontario Soil and Crop Improvement Association Annual Meeting'.

Mendez, M. & Maier, R. 2008. 'Phytoremediation of mine tailings in temperate and arid environments', *Reviews in Environmental Science and Biotechnology* **7**, 47-59.

Mendez, M. O. & Maier, R. M. 2007. 'Phytostabilization of Mine Tailings in Arid and Semiarid Environments - An Emerging Remediation Technology', *Environ Health Perspect* **116**(3), 278-283.

Mengel, K.; Kosegarten, H.; Kirkby, E. A. & Appel, T. 2001. *Principles of plant nutrition*, Springer Science & Business Media.

Mickovski, S.; Hallett, P.; Bengough, A.; Bransby, M.; Davies, M.; Sonnenberg, R.; Dazzi, C.; Costantini, E. & others 2008. The effect of willow roots on the shear strength of soil., *in* '5th International Congress of the European Society for Soil Conservation" Soils changing in a changing World: the soils of tomorrow", Palermo, Italy, 25-30 June 2007.', pp. 247--262.

Mol, C. 2013. 'Make it green: Phytostabilization of mines using boreal forest plants''2013 Ontario Mine Reclamation Symposium and Field Trip'.

Moreno-Jiménez, E.; Esteban, E. & Pecalosa, J. M. 2012. The fate of arsenic in soil-plant systems'Reviews of environmental contamination and toxicology', Springer, , pp. 1--37.

Mulligan, C.; Yong, R. & Gibbs, B. 2001. 'Remediation technologies for metal-contaminated soils and groundwater: an evaluation', *Engineering geology* **60**(1), 193--207.

Mummey, D. L.; Stahl, P. D. & Buyer, J. S. 2002. 'Soil microbiological properties 20 years after surface mine reclamation: spatial analysis of reclaimed and undisturbed sites', *Soil Biology and Biochemistry* **34**(11), 1717--1725.

Nagajyoti, P.; Lee, K. & Sreekanth, T. 2010. 'Heavy metals, occurrence and toxicity for plants: a review', *Environmental Chemistry Letters* **8**(3), 199--216.

Nevel, L. V.; Mertens, J.; Staelens, J.; Schrijver, A. D.; Tack, F. M.; Neve, S. D.; Meers, E. & Verheyen, K. 2011. 'Elevated Cd and Zn uptake by aspen limits the phytostabilization potential compared to five other tree species', *Ecological Engineering* **37**(7), 1072 - 1080.

Newman, L. A., & Reynolds, C. M. (2004). Phytodegradation of organic compounds. *Current Opinion in Biotechnology, 15*(3), 225-230.

Nouri, J.; Karbassi, A. & Mirkia, S. 2008. 'Environmental management of coastal regions in the Caspian Sea', *International Journal of Environmental Science & Technology* **5**(1), 43-52.

Ontario Ministry of Northern Development and Mines. 2012. 'Voluntary rehabilitation of mine hazards'.

Ontario Ministry of Northern Development and Mines & Smith, P. 1986. *Harvest from the rock: A history of mining in Ontario*, Macmillan of Canada.

Oppelt, E. T. 2000. 'Introduction to Phytoremediation'(EPA/600/R-99/107), Technical report, National Risk Management Research LaboratoryOffice of Research and DevelopmentU.S. Environmental Protection AgencyCincinnati, Ohio 45268.

Oxenham, J. R. & others 1966. 'Reclaiming derelict land.', *Reclaiming derelict land.*

Päivöke, A.E. and Simola, L.K., 2001. Arsenate toxicity to Pisum sativum: mineral nutrients, chlorophyll content, and phytase activity. *Ecotoxicology and Environmental Safety*, *49*(2), 111-121.

Pan, X.; Zhang, D.; Chen, X.; Bao, A. & Li, L. 2011. 'Antimony Accumulation, Growth Performance, Antioxidant Defense System and Photosynthesis of Zea mays in Response to Antimony Pollution in Soil', *Water, Air, & Soil Pollution* **215**, 517-523.

Peer, W.; Baxter, I.; Richards, E.; Freeman, J. & Murphy, A. 2006. Phytoremediation and hyperaccumulator plants, *in* MarkusJ. Tamas & Enrico Martinoia, ed., 'Molecular Biology of Metal Homeostasis and Detoxification', Springer Berlin Heidelberg, , pp. 299-340.

Perkins, L. B.; Leger, E. A. & Nowak, R. S. 2011. 'Invasion triangle: an organizational framework for species invasion', *Ecology and evolution* **1**(4), 610--625.

Peters, T. Sheehan, P.J.; Miller, D. B. G. & Bourdeau, P., ed., 1984. *Effects of Pollutants at the Ecosystem Level*, John Wiley & Sons Ltd, chapter Case 7.8 Rehabilitation of Mine Tailings: A Case of Complete Ecosystem Reconstruction and Revegetation of Industrially Stressed Lands in the Sudbury Area, Ontario, Canada, pp. 403-421.

Peters, T. 1988. Mine tailings reclamation. Inco Limited's experience with the reclaiming of sulphide tailings in the Sudbury area, Ontario, Canada'Environmental Management of Solid Waste', Springer, , pp. 152--165.

Pilon-Smits, E. 2005. 'Phytoremediation', *Annu. Rev. Plant Biol.* **56**, 15--39.

Pinno, B. D.; LandhÃusser, S. M.; MacKenzie, M. D.; Quideau, S. A. & Chow, P. S. 2012. 'Trembling aspen seedling establishment, growth and response to fertilization on contrasting soils used in oil sands reclamation', *Canadian Journal of Soil Science* **92**(1), 143-151.

Pratas, J.; Prasad, M.; Freitas, H. & Conde, L. 2005. 'Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation', *Journal of Geochemical Exploration* **85**(3), 99 - 107.

Pulford, I.; Riddell-Black, D. & Stewart, C. 2002. 'Heavy metal uptake by willow clones from sewage sludge-treated soil: the potential for phytoremediation', *International Journal of Phytoremediation* **4**(1), 59--72.

Pulford, I. & Watson, C. 2003. 'Phytoremediation of heavy metal-contaminated land by trees: a review', *Environment International* **29**(4), 529 - 540.

Qi, C.; Wu, F.; Deng, Q.; Liu, G.; Mo, C.; Liu, B. & Zhu, J. 2011. 'Distribution and accumulation of antimony in plants in the super-large Sb deposit areas, China', *Microchemical Journal* **97**(1), 44 - 51.

Ramirez-Andreotta, M. D.; Brusseau, M. L.; Artiola, J. F. & Maier, R. M. 2013. 'A greenhouse and field-based study to determine the accumulation of arsenic in common homegrown vegetables grown in mining-affected soils ', *Science of The Total Environment* **443**(0), 299 - 306.

Raskin, I. & Ensley, B. D., ed. 2000. *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*, John Wiley & Sons Ltd.

Reeves, R.D., Baker, A.J.M., Borhidi, A. and Berazain, R., 1999. Nickel hyperaccumulation in the serpentine flora of Cuba. *Annals of Botany*, *83*(1), 29-38.

Reid, K. 2006. *Soil Fertility Handbook*, Ontario, Ministry of Agriculture, Food and Rural Affairs.

Renault, S.; Croser, C.; Franklin, J. A. & Zwiazek, J. J. 2001. 'Effects of NaCl and

Na2SO4 on red-osier dogwood (Cornus sericea Michx) seedlings', *Plant and soil* **233**(2), 261--268.

Renault, S., MacKinnon, M., & Qualizza, C. 2003. Barley, a potential species for initial reclamation of saline composite tailings of oil sands.*Journal of environmental quality*, *32*(6), 2245-2253.

Renault, S.; Szczerski, C.; Sailerova, E. & Fedikow, M. 2004. 'Phytoremediation and revegetation of mine tailings and bio-ore production: progress report on plant growth in amended tailings and metal accumulation in seedlings plantedat Central Manitoba (Au) minesite' (NTS 52L13), Technical report, Manitoba Industry, Economic Development and Mines, Manitoba Geological Survey.

Robinson, D. 1988. 'Mulches and herbicides in ornamental plantings', *HortScience (USA)*.

Roe, N. E. 1998. 'Compost utilization for vegetable and fruit crops', *HortScience* **33**(6), 934--937.

Ruiz-Chancho, M. J.; López-Sánchez, J. F.; Schmeisser, E.; Goessler, W.; Francesconi, K. A. & Rubio, R. 2008. 'Arsenic speciation in plants growing in arsenic-contaminated sites', *Chemosphere* **71**(8), 1522--1530.

Safford, L.; Bjorkbom, J. C. & Zasada, J. C. 1990. 'Betula papyrifera Marsh. paper birch', *Silvics of North America* **2**(654), 158.

Salt, D. E.; Blaylock, M.; Kumar, N. P.; Dushenkov, V.; Ensley, B. D.; Chet, I. & Raskin, I. 1995. 'Phytoremediation: A Novel Strategy for the Removal of Toxic Metals from the Environment Using Plants', *Nat Biotech* **13**(5), 468--474.

Schoenholtz, S. H.; Burger, J. A. & Kreh, R. E. 1992. 'Fertilizer and organic amendment effects on mine soil properties and revegetation success', *Soil Science Society of America Journal* **56**(4), 1177--1184.

Schwitzguebel, J.; Kumpene, J.; Comino, E. & Vanek, T. 2009. 'From green to clean: a promising and sustainable approach towards environmental remediation and human health for the 21st century', *Agrochimica* **53**(4), 209-237.

Self, W. T.; Grunden, A. M.; Hasona, A. & Shanmugam, K. T. 2001. 'Molybdate transport', *Research in microbiology* **152**(3), 311--321.

Shilev, S.; FernÃndez, A.; Benlloch, M. & Sancho, E. 2006. Sunflower growth and tolerance to arsenic is increased by the rhizospheric bacteria Pseudomonas fluorescens, *in* Jean-Louis Morel; Guillaume Echevarria & Nadezhda Goncharova, ed., 'Phytoremediation of Metal-Contaminated Soils', Springer Netherlands, , pp. 315-318.

Smith, E.; Naidu, R.; Alston, A. M. & others 1998. 'Arsenic in the soil environment', *Advances in agronomy* **64**, 149--195.

Smith, F. & Underwood, B. 2000. 'Mine closure: the environmental challenge', *Mining Technology* **109**(3), 202--209.

Stiell, W. & others 1976. *White spruce: artificial regeneration in Canada*, Canadian Forestry Service, Department of the Environment.

Tam, P. C. F. 1995. 'Heavy metal tolerance by ectomycorrhizal fungi and metal amelioration by Pisolithus tinctorius', *Mycorrhiza* **5**, 181-187.

Thompson, I. D.; Flannigan, M. D.; Wotton, B. M. & Suffling, R. 1998. 'The effects of climate change on landscape diversity: an example in Ontario forests', *Environmental Monitoring and Assessment* **49**(2-3), 213--233.

Tiquia, S. M.; Lloyd, J.; Herms, D. A.; Hoitink, H. A. & Michel, F. C. 2002. 'Effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure determined by analysis of TRFLPs of PCR-amplified 16S rRNA genes', *Applied Soil Ecology* **21**(1), 31--48.

Tordoff, G.; Baker, A. & Willis, A. 2000. 'Current approaches to the revegetation and reclamation of metalliferous mine wastes', *Chemosphere* **41**(1-2), 219 - 228.

Towers, W. & Paterson, E. 1997. 'Sewage sludge application to land–a preliminary assessment of the sensitivity of Scottish soils to heavy metal inputs', *Soil use and management* **13**(3), 149--155.

Trapp S, Yu X, Mosbaek H. 2003. Persistence of methyl tertiary butyl ether (MTBE) against metabolism by Danish vegetation. *Environ Sci Pollut Res Int* 10:357-360.

Tschan, M.; Robinson, B. H. & Schulin, R. 2009. 'Antimony in the soil plant system: a review', *Environ. Chem.* **6**(2), 106--115.

Tukey, R. & Schoff, E. 1963. Influence of different mulching materials upon the soil environment, *in* 'Proc. Am. Soc. Hort. Sci', pp. 68--76.

Turnau, K.; GawroÅski, S.; Ryszka, P. & Zook, D. 2012. Mycorrhizal-Based Phytostabilization of Zn Pb Tailings: Lessons from the Trzebionka Mining Works (Southern Poland), *in* Erika Kothe & Ajit Varma, ed., 'Bio-Geo Interactions in Metal-Contaminated Soils', Springer Berlin Heidelberg, , pp. 327-348.

United Nations Environment Programme. 2002. 'Phytoremediation: An Environmentally Sound Technology for Pollution Prevention, Control and Redmediation -An Introductory Guide To Decision-Makers'.

Utriainen, M. A.; Kãrenlampi, L. V.; Kãrenlampi, S. & Schat, H. 1997. 'Differential tolerance to copper and zinc of micropropagated birches tested in hydroponics', *New*

Phytologist **137**(3), 543--549.

Vaclavikova, M.; Gallios, G. P.; Hredzak, S. & Jakabsky, S. 2008. 'Removal of arsenic from water streams: an overview of available techniques', *Clean Technologies and Environmental Policy* **10**(1), 89--95.

Vassilev, A.; Schwitzguebél, J.-P.; Thewys, T.; van der Lelie, D. & Vangronsveld, J. 2004. 'The Use of Plants for Remediation of Metal-Contaminated Soils', *TheScientificWorldJOURNAL* **4**, 9-34.

Vega, F. A.; Covelo, E. F. & Andrade, M. 2006. 'Competitive sorption and desorption of heavy metals in mine soils: influence of mine soil characteristics', *Journal of Colloid and Interface Science* **298**(2), 582--592.

Wade, G. & Thompson, R. 1990. 'Establishment of Native Plant Species from Forest Topsoil Seedbanks on a Borrow Area in Kentucky"1990 Mining and Reclamation Conference and Exhibition'.

Wagner, R. G. 2000. 'Competition and critical-period thresholds for vegetation management decisions in young conifer stands', *The Forestry Chronicle* **76**(6), 961--968.

Walters, R. G. 2005. Towards an understanding of photosynthetic acclimation. *Journal of experimental botany*, *56*(411), 435-447.

Wang, H. and Jin, J.Y., 2005. Photosynthetic rate, chlorophyll fluorescence parameters, and lipid peroxidation of maize leaves as affected by zinc deficiency. *Photosynthetica*, *43*(4), 591-596.

Wang, S. & Mulligan, C. N. 2006. 'Occurrence of arsenic contamination in Canada: sources, behavior and distribution', *Science of the Total Environment* **366**(2), 701--721.

Weinfurt, K.P., 2000. Repeated measures analysis: ANOVA, MANOVA, and HLM.

Whiting, S.; Reeves, R. D.; Richards, D.; Johnson, M. S.; Cooke, J. A.; Malaisse, F.; Paton, A.; Smith, J. A. C.; Angle, J. S.; Chaney, R. L. & others 2004. 'Research priorities for conservation of metallophyte biodiversity and their potential for restoration and site remediation', *Restoration Ecology* **12**(1), 106--116.

Wong, H. K. T.; Gauthier, A. & Nriagu, J. O. 1999. 'Dispersion and toxicity of metals from abandoned gold mine tailings at Goldenville, Nova Scotia, Canada', *The Science of The Total Environment* **228**(1), 35 - 47.

Wong, M. 2003. 'Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils', *Chemosphere* **50**(6), 775 - 780.

Yang, X.E., Long, X.X., Ye, H.B., He, Z.L., Calvert, D.V. and Stoffella, P.J., 2004.

Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (Sedum alfredii Hance). *Plant and Soil*,*259*(1-2), 181-189.

Young, I. W., Naguit, C., Halwas, S. J., Renault, S., & Markham, J. H. 2013. Natural revegetation of a boreal gold mine tailings pond. *Restoration Ecology*, *21*(4), 498-505.

Zandsalimi, S.; Karimi, N. & Kohandel, A. 2011. 'Arsenic in soil, vegetation and water of a contaminated region', *Int. J. Enivron. Sci Tech.* **8**(2), 331-338.

Zhang, Z.; Shu, W.; Lan, C. & Wong, M. 2001. 'Soil seed bank as an input of seed source in revegetation of lead/zinc mine tailings', *Restoration Ecology* **9**(4), 378--385.

7. Appendix: Google Maps of the Mine Sites

Figure 7-1 Barrick Hemlo

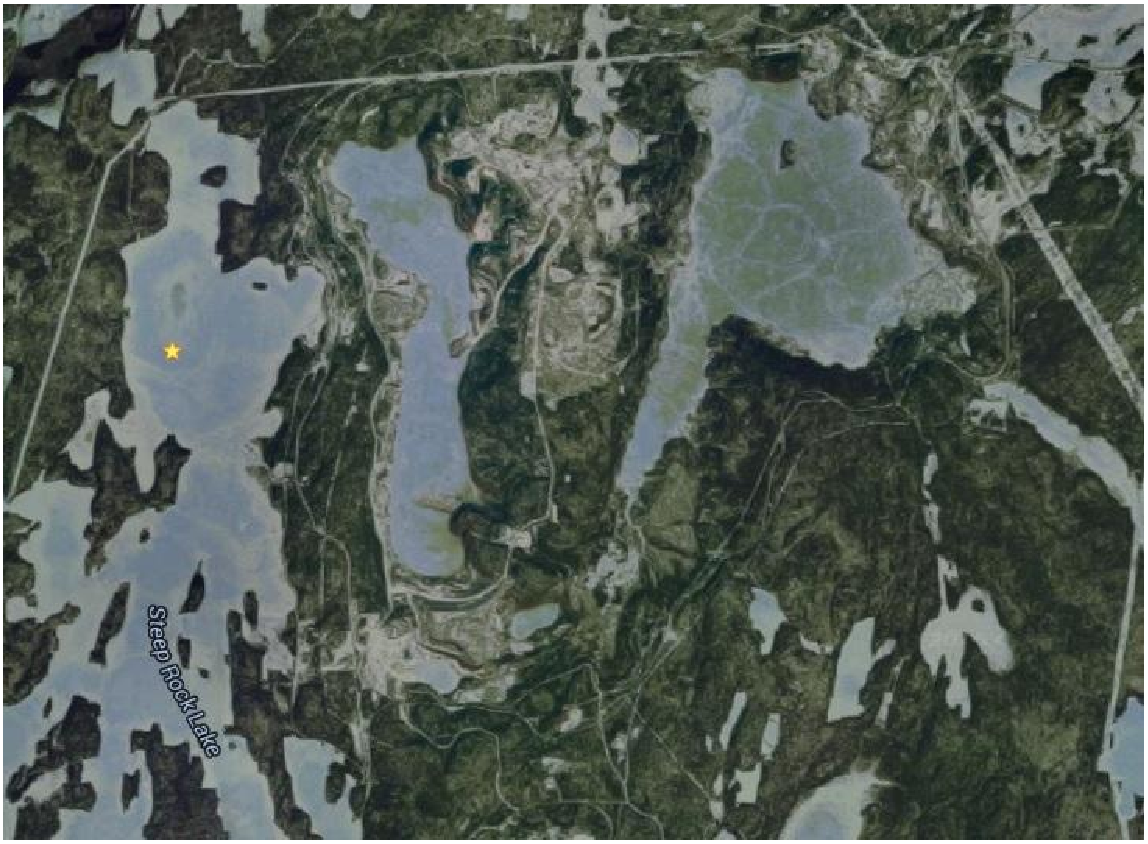


Figure 7-2Steep Rock Mine

Figure 7-3 Winston Lake Mine

Figure 7-4 Premier - Empire Lake Mine Area

Figure 7-5 Premier - Leitch Mine

8. Appendix: Pictures of Transects in Chapter 2

Figure 8-1 Transect WL1

Figure 8-2 Transect WL2

Figure 8-3 Transect WL3

Figure 8-4 Transect S1

Figure 8-5 Transect S2

Figure 8-6 Transect S3

Figure 8-7 Transect S4

Figure 8-8 Transect S5

Figure 8-9 Transect S6

Figure 8-10 Transect P1

Figure 8-11 Transect P2

Figure 8-12 Transect P3

Figure 8-13 Transect P4

Figure 8-14 Transect P5

9. Appendix: Progressive Pictures of the Trees Planted for Chapter 3

9-1 Prior to Planting at Barrick Hemlo, ON Summer of 2013

9-2 Cuttings planted at Barrick Hemlo, ON September 2013

9-3 Cuttings planted at Barrick Hemlo, ON June 2014

9-4 Site planted with cuttings at Barrick Hemlo, ON September 2014

9-5 Cuttings planted at Barrick Hemlo, ON June 2015

9-6 Cuttings planted at Barrick Hemlo, ON September 2015

10. Appendix: Bioconcentration factor

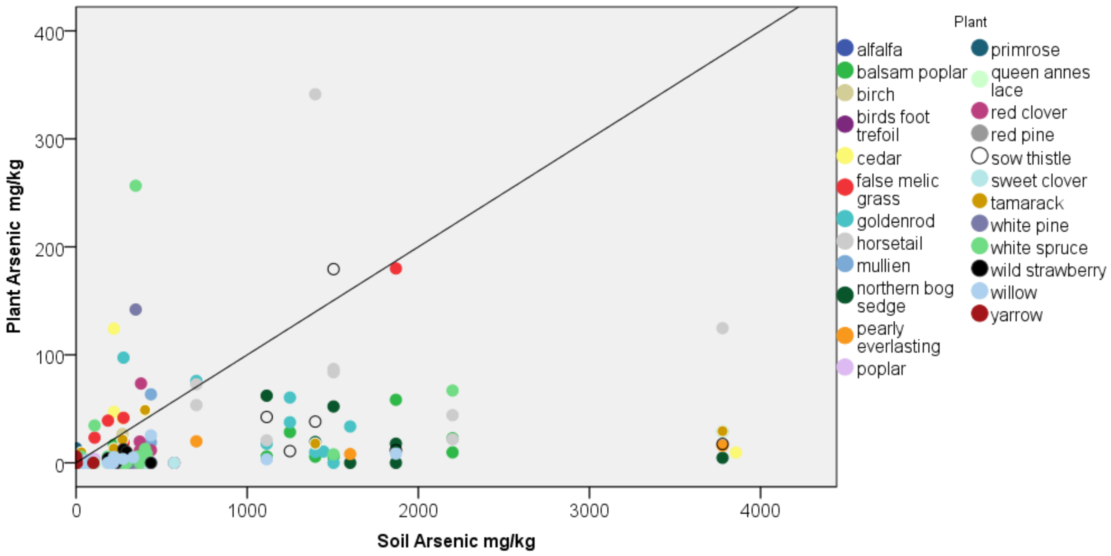


Figure 10-1 As in plant tissue and soil as a visual representation of the bioconcentration factor

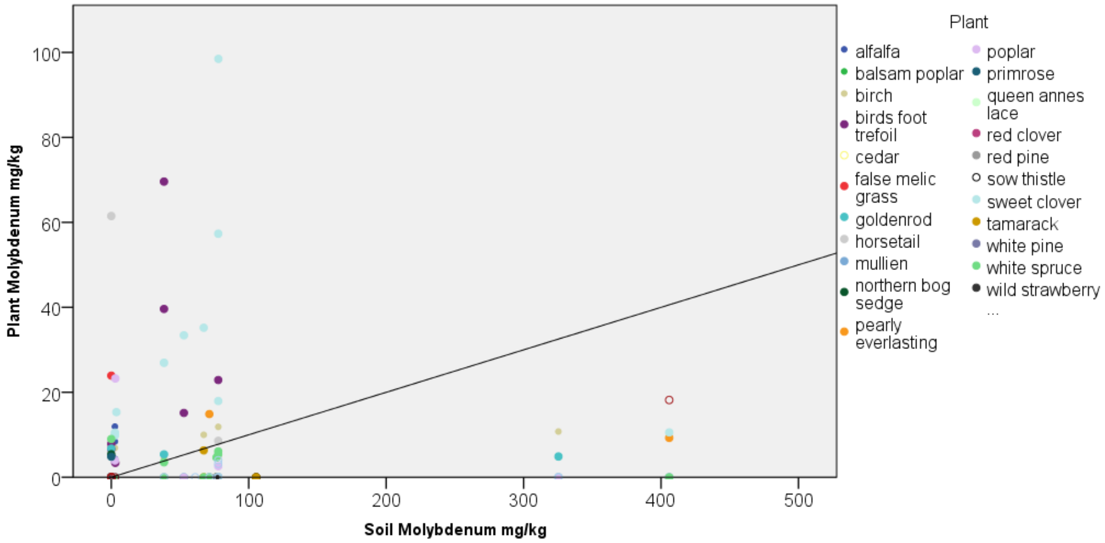


Figure 10-2 Mo in plant tissue and soil as a visual representation of bioconcentration factor