

**THE USE OF SOIL AMENDMENTS IN THE REVEGETATION
OF SMELTER-IMPACTED SOILS NEAR FLIN FLON, MB/CREIGHTON, SK**

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ABSTRACT

Some areas near Flin Flon, MB and Creighton, SK are devoid of vegetation due to a variety of mining, smelting, forestry activities and forest fires that have occurred since the 1930's. This study investigated the use of soil amendments to enhance revegetation in these areas. The study was comprised of two main components, an *in situ* study and a growth chamber trial. The *in situ* component was conducted to determine the efficacy of soil amendments that could be utilized in a revegetation program. The growth chamber trial examined if the amount of moisture present in the soil would have an influence on the success of vegetation survival and growth.

The *in situ* study was conducted near Flin Flon, MB and Creighton, SK over two growing seasons and consisted of replicated treatments imposed at 12 sites. Tree seedlings [trembling aspen (*Populus tremuloides* Michx.) and jack pine (*Pinus banksiana* Lamb.)] and understory species [tufted hairgrass (*Deschampsia cespitosa* L.) and American vetch (*Vicia americana* Muhl.)] were planted at each site. Each site also received soil amendments; bone meal and meat biochar (BMB), compost, commercial mycorrhizal inoculant (EMF) and, willow biochar (WB) in combination with dolomitic limestone and fertilizer. Each site also had a control that received an application of only dolomitic limestone and fertilizer. The growth chamber trial utilized the same plant species and soil amendments as the field trial with the exclusion of willow biochar.

In general, soil amendments did not influence the survival or growth of the tree seedlings *in situ* or in the growth chamber trial. However, the compost amendment increased survival and growth of the tufted hairgrass significantly in the growth chamber trial and to a lesser extent in the field trial. Compost also positively influenced the pH and base saturation of the soil compared to the other amendments. The mycorrhizal inoculant increased the rate of mortality of tree species in the growth chamber trial. Moisture did not influence the survival and growth of the seedlings or understory species or the efficacy of the amendment treatments in this study.

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1. INTRODUCTION

Mining activities, such as refining and smelting of base metals, commonly result in the development of areas devoid of vegetation due to aerial deposition of pollutants from the mining practices that contribute to forest dieback and soil erosion. This anthropogenic aerial deposition can include sulfur dioxide, heavy metals and can subsequently lead to soil acidification. Government regulations regarding mining activities have become more stringent over time, thereby curbing the rate of emissions but not eliminating the damage to vegetation and soil completely.

Flin Flon, MB and Creighton, SK are neighboring communities that have been affected by mining and smelting activities since the 1930's. The mining and smelting activities were occurring long before significant environmental regulations were in place. Consequently, the area was subject to considerable damage before more stringent regulations were enacted (HudBay Minerals, 2009). Specifically the area is affected by sulfur dioxide (SO₂) deposition resulting in increased acidification of soils. Metal contaminated sites are challenging to revegetate because metals cannot be degraded. Sites are often complex with variable amounts of deposition and more than one type of metal present (Dermont et al., 2008).

In restoration and reclamation projects the end goal can be to reclaim an area back to the original ecosystem or to increase the visual appeal and function (Environmental Protection Agency, 2007). The goal should be predetermined prior to the planning and initiation of the project. The reclamation or remediation of base-metal contaminated sites is often very expensive and difficult due to the expansive areas that are affected. Commonly, *ex situ* methods, that involve removing the contaminated soil from the area, are used. However, an increasingly common approach is to utilize *in situ* methods of remediation that use strategies that alter the form of contaminate in the soil into less available and less toxic forms on-site, commonly through the application of soil amendments and vegetation establishment (Environmental Protection Agency, 2007; Dermont et al., 2008).

The area surrounding Flin Flon/Creighton is dominated by jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* Mill.), white spruce (*Picea glauca* Moench), green alder (*Alnus viridis* Chix.) and trembling aspen (*Populus tremuloides* Michx.) comprising a mixed coniferous forest cover (Hogan and Wotton, 1984; McLaughlan et al., 2010). The area has

experienced vegetation dieback due to logging, forest fires and mining activities. Many soils in the vicinity of the mine are smelter-impacted and have higher levels of metal deposition than those found in the nearby natural forest area. The copper smelter was shut down in June 2010 due to costs of upgrading (HudBay Minerals, 2009). However, during operation of the smelter the area received deposition of airborne contaminants including zinc (Zn), lead (Pb), magnesium (Mg), iron (Fe), copper (Cu), cadmium (Cd) and arsenic (As). These contaminants are present in the highest concentrations in the first 3 km from the smelter stack where the impact of contamination is visually noticeable in the vegetation patterns (Henderson et al., 1998). Vegetation in the smelter-impacted area, where present, is dominated largely by a non-native bent grass (*Agrostis capillaris* L.) (originally identified by Professor Winterhalder of Laurentian University, pers. comm. R. Farrell) which is relatively metal tolerant, and inclusions of some stunted willow (*Salix*), birch (*Betula*) and poplar (*Populus*) trees (Henderson and McMartin, 1995).

There is a strong desire to revegetate Flin Flon/Creighton (The Green Project, 2010). The use of soil amendments has been attempted in areas with similar disturbances (Kumpiene et al., 2008). Some of the benefits of revitalizing land using amendments include providing wildlife habitat, increased evapotranspiration, improved water quality, decreased mobility of contaminants and restored soil health and function (Environmental Protection Agency, 2007). Immobilization of metals can be facilitated by the use of amendments through processes such as adsorption to mineral surfaces and through the formation of complexes with organic ligands, which can lead to improved soil quality and improved potential for revegetation (Kumpiene et al., 2008). The pH of soil in Flin Flon/Creighton is low (3.83 to 5.84) and it is thought to be a contributing factor in vegetation dieback. Thus, application of amendments to increase soil pH may be utilized as an integral component of a successful revegetation strategy in Flin Flon/Creighton.

Based on research conducted in Sudbury, ON which demonstrated the positive effects of spreading dolomitic limestone, The Green Project was established in Flin Flon/Creighton (The Green Project, 2010). The Green Project is comprised of a group of active residents that have been spreading dolomitic limestone throughout the area of Flin Flon/Creighton since 1999 to help decrease the level of soil acidity to aid in revegetation (The Green Project, 2010). Areas have been successful in regaining vegetation from seeds blowing in and establishing, although,

some areas have not been responsive to the liming. These unresponsive areas in Flin Flon/Creighton have been compared to a similar site in Sudbury, ON and the lack of response in Flin Flon/Creighton has been attributed to the different parent materials from which the Flin Flon/Creighton soils developed (Winterhalder, 2000). The first application of dolomitic limestone was tested in Flin Flon/Creighton in 1994 by Professor Keith Winterhalder from Laurentian University, and who also conducted the initial liming research in Sudbury (The Green Project, 2010). Limestone is still currently being spread in Flin Flon/Creighton due to the success it has had over the past 13 years (The Green Project, 2010). The Green Project is run by community volunteers who spread the dolomitic limestone; however, due to safety concerns, volunteers are not allowed access to the mine property, which limits area being treated.

The study described herein was initiated to examine the impact of various soil amendments including bone meal and meat biochar (BMB), willow biochar (WB), municipal compost and a commercial mycorrhizal product (EMF) containing endo- and ectomycorrhizae on the survival and growth of the tree species trembling aspen and jack pine, and the understory species tufted hairgrass (*Deschampsia cespitosa* L.) and American vetch (*Vicia americana* Muhl.), *in situ* with the goal of identifying effective revegetation strategies. The success of the revegetation strategies was evaluated using a variety of measurements including plant root and shoot biomass, shoot heights and basal diameters, percent ground cover and visual measurements of plant health in the field.

Soil collected from a healthy forest system in a similar environment was used as a baseline thereby providing comparative information. Assessing soil properties post-amendment application might help in determining strategies that are likely to promote revegetation. Furthermore, observing changes associated with the addition of different amendments provides a quantitative measure of success of the soil amendments relative to soil properties conducive to supporting revegetation. In this study, soil measurements included pH, base saturation, effective CEC, available metals, total organic carbon, available sulfur, nitrate and ammonium.

The impact of soil moisture on the efficiency of the various amendments was studied in a growth chamber experiment using the same tree and understory species as were used for the *in situ* trial. The same amendments were also studied in the growth chamber trial as in the *in situ* trial with the exception of WB. Different soil moisture levels were imposed and plant growth

was measured to determine if moisture influenced the relative success of the amendments for revegetation using soil collected from Flin Flon/Creighton.

The overall objective of this study was to determine the effectiveness of various organic and microbial amendments to aid in the revegetation of smelter-impacted soils. This study represents a first step in creating a revegetation strategy that could be implemented in the future to improve the visual appearance and possibly return the area to a state similar to that of a non-smelter affected landscape. The specific objectives and hypothesis of this study were to:

Objective 1: Determine which combinations of soil amendment and vegetation species are most viable (increase survival/growth of vegetation, practicality) for *in situ* remediation of smelter-impacted soils.

Hypothesis 1: Sites with higher available heavy metal concentrations will have less vegetation survival and growth.

Hypothesis 2: Soil amendments, dolomitic limestone and fertilizer will increase overall soil pH and base saturations towards a level similar to that of a healthy forest stand.

Hypothesis 3: Amended plots will have decreased plant metal uptake and greater vegetation survival and growth than the unamended control plots.

Objective 2: Identify if moisture is a limiting factor for revegetation in smelter-impacted soils when utilizing soil amendments.

Hypothesis 4: The amount of available soil metal and plant metal uptake will be influenced by the amount of moisture present in the soil.

Hypothesis 5: Vegetation biomass will be influenced by the moisture present in the soil in combination with amendments used.

The thesis is written in “paper format” with the introduction (Chapter 1) that discusses the main concepts behind the project. This is followed by a literature review (Chapter 2) relevant to both research chapters (Chapter 3 and 4). Chapter 3 describes a field trial (objective 1) and chapter 4 focuses on a growth chamber trial (objective 2). Chapter 5 provides a synthesis and conclusion of the entire project. Chapters 6 and 7 are references and appendices, respectively.

2. LITERATURE REVIEW

2.1 Environmental Impact of Smelting Operations

Smelting operations create aerial deposition of SO₂ and trace metals and lead to changes in the landscape environment surrounding the smelting complexes (Rigina and Kozlov, 2000). Although smelting operations occur worldwide, there are a few large smelting complexes in the northern hemisphere in northwest Russia on the Kola Peninsula, in Sudbury, ON and in northwest and southwest Finland that are notable for the environmental impact that these operations have produced. For example, smelting operations created an area in Russia where the area of forest dieback is 600 to 1000 km² due to the inability of the soil system to support plant survival and growth (Rigina and Kozlov, 2000). Forest dieback at Sudbury is estimated to be 170 km² and less than 1 km² in Finland (Kiikkila, 2003). Remediation projects have been established in Finland and Sudbury with the largest scale remediation project conducted in Sudbury, ON (Kiikkila, 2003).

HudBay Minerals Inc. (formerly Hudson Bay Mining and Smelting Co. Ltd.) has operated a mine and associated Zn and Cu processing facility in the vicinity of the communities of Flin Flon, MB and Creighton, SK, Canada since 1930. The original smelting stack was 30 m high and was replaced by a stack that is 251 m in 1974 to meet environmental regulations (Shaw, 1981). These operations released particulate matter through aerial emissions and have contributed to die back of the surrounding boreal forest. As a result the area lacks plant biodiversity and has limited new plant growth. Zinc processing continues to this day, though Cu smelting operations ceased in 2010 (HudBay Minerals Inc., 2009).

Areas that have significant forest dieback such as Kola Peninsula, Sudbury, Harjavalta Finland and Flin Flon/Creighton also have all been influenced by accompanying disturbances. Kozlov and Zvereva (2007) denote accompanying disturbances as other human caused disturbances that have contributed to the overall impact of the mining and smelting activities in non-ferrous metal operations. The landscape of Flin Flon/Creighton had accompanying disturbances of forest logging and forest fires that contributed to the amount of forest dieback (Kozlov and Zvereva, 2007). Logging and forest fires can lead to sequential soil erosion from areas with sloped landscapes like those in Flin Flon/Creighton.

2.1.1 Vegetation

The native vegetation in the area of Flin Flon/Creighton is typical of a mixed coniferous forest. Tree species in the non-impacted surrounding area include white spruce, aspen, birch [*Betula papyrifera* (Marsh) Spach] and black poplar (*P. balsamifera* L.) (Mycock, 2011). Soil metal contamination that is distributed aurally from a point source is highly variable due to wind directions and uneven distribution (MacDonald and Hendershot, 2003). The metal concentration within the soil decreases as the distance from the stack increases (Henderson and McMartin, 1995). At a distance of 37.5 km from the smelter there is little impact to vascular plants and cryptogams whereas close to the smelter, vascular plants and cryptogams are now absent (Scott and Orlandini, 2002). Within 6 km from the smelter, soil is present only in small depressions or rock outcrops representing mineral soil deposits that have occurred as a result of water and wind erosion that has been exacerbated by the lack of stabilizing plant cover (Fig. 2.1) (Scott and Orlandini, 2002).



Figure 2.1 Photograph of the smelter and the surrounding area in Flin Flon displaying areas devoid of vegetation and resulting rock outcrops with soil pockets

2.1.2 Soil pH

Smelter activities frequently result in significant changes in soil pH. The pH of the soil influences the availability of metals in soil: Al, Cu, Ni and Zn are all more toxic where soil pH is less than 5.5 (Environmental Protection Agency, 2007). For example, in Sudbury, the pH of the soil decreased as distance to the smelter decreased (Winterhalder, 2000). However, some studies show the pH of the soil is unaffected by the distance from the smelter when the area sampled is on the same bedrock (Hogan and Wotton, 1984; Derome and Lindroos, 1998; Scott, 2000). Hogan and Wotton (1984) suggested that there were no changes in soil pH during smelting activities in Flon Flon/Creighton due to the ability of Zn deposition to increase the soil pH even though the SO₂-SO₄ inputs from smelting activities are known to decrease soil pH. The loss of vegetation could be attributed more to the atmospheric inputs of base metals and sulfur on plants and soil microbes than the soil pH (Scott, 2000).

2.1.3 Cation exchange

The cation exchange capacity (CEC) of a soil is a measure of the ability of a soil to retain cationic nutrients. Cation exchange capacity and base saturation can be influenced by pH, soil organic matter and erosion. The CEC of the soil can influence the metal uptake by plant roots. As CEC of the soil increases the CEC of the roots also increases and the uptake of metals into the plant increases (Greger, 2004). The CEC in heavy metal contaminated sites can also be affected by the presence of Cu and Ni which bind to cation exchange sites, which in some studies create an underestimation of CEC values (Derome and Lindroos, 1998). A study conducted at the smelter on the Kola Peninsula showed that the CEC decreased with a decreasing amount of organic matter due to lower plant biomass inputs from vegetation loss in areas with higher levels of metal contamination (Lukina and Nikonov, 2001). However, a study conducted by Anderson et al. (2009) found that CEC increased within the contaminated sites compared to the control site and the calcium concentrations varied from high to low between contaminated and control sites, respectively. The difference between the studies suggests that other environmental factors may be of more importance than the amount of contamination present in an area and that contamination may be influencing other factors that can affect CEC such as organic matter inputs from vegetation.

2.2 Revegetation Strategies

Phytotechnologies are green methods of remediation and reclamation that are becoming increasingly common due to their cost effectiveness and ease of application (ITRC, 2009). In 1989 there were only 11 published articles on phytoremediation; as of May 2009 there were 10, 684 published articles (Prasad et al., 2010). Phytotechnology methods are carried out *in situ*, which decreases the amount of disruption that takes place during the reclamation or remediation process compared to *ex situ* methods that ruin the natural on-site properties of the site (Farrell et al., 2010b). One type of phytotechnology is phytostabilization that involves growing vegetation in contaminated areas to contribute to slowing erosion, runoff and increased visual appeal and decreasing dust movement (Frerot et al., 2006). Phytostabilization often includes phytosequestration in which the contaminants are contained or changed into less available forms by utilizing plant species (ITRC, 2009). Benefits of *in situ* remediation include no transportation and no excavation of contaminated soil and ease of application in large sites; however, some limitations include difficulties verifying how efficient the techniques are and site specific conditions can affect the success of the strategy (Dermont et al., 2008).

A number of issues need to be considered when developing an *in situ* remediation plan. Some of the considerations include site selection, seedbed preparation, plant selection, need to irrigate, weed problems, access to sites and managing wildlife (Environmental Protection Agency, 2007). One of the challenges that may restrict revegetation success in a contaminated area is a low number of vegetation propagules present to be able to move back into the disturbed area. Propagules often have been absent for too long in the region; however, *in situ* techniques can mitigate this problem by introducing new or existing species by the use of seed application or tree planting (Helmisaari et al., 2007). Other factors that can contribute to the success or failure of phytotechnologies include physiology of vegetation, moisture, root length, temperature and growing season (ITRC, 2009). Field studies focused on in-field trials around smelting complexes can be difficult to conduct due to the amount of variability between sites. Consequently, many studies are conducted within a laboratory setting to minimize variability and therefore less research has been conducted at a field scale in smelter-impacted areas (Hermle et al., 2006).

Sudbury, ON had similar disturbance characteristics to those found in Flin Flon/Creighton including forest fires, logging and heavy metal contamination. A variety of

different measurements were used in Sudbury, ON to record the native vegetation and newly established vegetation in the area. Measurements including density, percent cover, basal diameter and frequency of the plants were used collectively to form an index to help represent the vegetation of a given area and comparisons were made from one growing season to the next which is of importance for revegetation projects (Amiro and Courtin, 1981). Another study conducted near Sudbury compared a revegetation strategy of using only liming techniques to one that used a combination of liming, seeding of understory species and fertilization (Winterhalder, 1983). The areas that were seeded with understory species had less movement of woody species into the area compared to the unseeded areas. However, seeded areas had 50% more percent cover after two years but a decreased presence of woody species (Winterhalder, 1983). Applying fertilizer when reforesting can improve photosynthetic activity, improve water efficiency, increase stem growth and increase the overall amount of foliage (Kozlowski et al., 1991).

2.2.1 Tree species used in phytoremediation

Trees of the *Populus* species are known to support relatively high absorption, accumulation, storage and degradation of environmental contaminants, making aspen trees a viable option when choosing a species for *in situ* designs in contaminated areas. Trembling aspen trees are, however, not a hyper-accumulating species (Mala et al., 2006). Hyperaccumulator species tend to be small and slow growing which in field studies is not ideal if biomass production is important (Kumar et al., 1995). Trembling aspen can grow in soils that are low in nutrients. Trembling aspen also can have a deep root system, be fast growing, and be resistant to contamination, when nutrient and moisture requirements are met, which are all traits of trees that are favored for phytoremediation practices (Pulford and Watson, 2003). Trembling aspen trees are known as a free growth species meaning that the shoots are partially developed in the buds the previous growing season and then expand and continue to grow in the next season (Kozlowski et al., 1991). Fast growing tree species are particularly favored for revegetation in areas that are devoid of vegetation, as improvements in visual appeal occur more quickly.

Jack pine is a tree species that is native to the Flin Flon/Creighton area and is typical of mixed coniferous forests in the northern hemisphere (Hogan and Wotton, 1984). Jack pine had the greatest survival rate in the Sudbury project for pine species (Winterhalder, 2000). Trees of the *Pinus* genus have a pre-determined growth rate, therefore, the shoots are fully developed as buds during the late part of the previous growing season and then emerge and elongate during the

next growing season (Kozłowski et al., 1991). There have been numerous studies that examined the uptake of metals by species from the *Pinus* genus. *Pinus* genus uptake metals into the needles of the plant as well as the bark, stem and roots (Gratton et al., 2000, Saarela et al., 2005, Walsh and Redente, 2011). Jack pine, white spruce and white pine have been found to be more tolerant to heavy metal accumulation in soil than red pine, black spruce and birch tree species (Patterson and Olson, 1983). Wotton et al. (1986) found that seed survival of jack pine was not affected in soils that were impacted by smelting operations but root growth within 5 km of the stack was found to be inhibited.

2.2.2 Understory species

Tufted hairgrass is a bunch grass species that is capable of growing in areas that are highly disturbed as well as in areas of heavy metal contamination and may therefore colonize areas in which other plants are unable to grow (Winterhalder, 2000). Tufted hairgrass is fast growing and produces large amounts of biomass which makes it a favourable option for projects that want to quickly develop a visual appeal of greening an area (McIntyre, 2003). Tufted hairgrass reportedly grows near Sudbury which has a similar smelting operation and landscape to that of Flin Flon/Creighton (Winterhalder, 2000). Known by many common names including blue-green hairgrass and fescue-leaved hairgrass, the plant grows between 20 and 60 cm in height and can be grown in a wide range of soil types as well as in soils with pH values between 3.5 and 7.5 (Darris and Gonzalves, 2009; St. John et al., 2011).

American vetch is a forb that has purple flowers and can grow between 30 and 60 cm in height. Other common names include purple vetch and American deer vetch (Kirk and Belt, 2010). American vetch is similar to tufted hairgrass in that it has proven ability to grow in disturbed areas and spreads through the root system, establishing new plants. American vetch is also a nitrogen fixing plant species that can be grown in different soil textures and moisture areas (Kirk and Belt, 2010).

2.3 Heavy Metal Contamination

2.3.1 Soil metal concentrations

Both wet and dry heavy metal deposition from smelting complexes is known to negatively affect seedling establishment, growth and plant survival through soil contamination. Deposition can be variable within a small area due to a variety of influencing factors. For example, amount of deposition is influenced by wind direction, precipitation, stack height and

smelting operations (Hutchinson and Whitby, 1977). The amount of available metal in the soil can be influenced by the amount of soil organic matter and pH, and is important as it is available for plant uptake and movement into water systems. Negative correlations between amounts of available metals Ni, Cd, Cu, Pb and Fe and distance from the smelter have been reported with metal levels increasing as distance decreases (Hutchinson and Whitby, 1977; Derome and Lindroos, 1998). This correlation was not found for Zn and Al. This has been attributed, in part, to particle size of the different metals being carried different distances from the smelter (Hutchinson and Whitby, 1977; Derome and Lindroos, 1998). A study conducted at Flin Flon/Creighton generally found there to be no strong directional effects from the smelter for total cation exchange capacity (CEC), calcium (Ca^{2+}), magnesium (Mg^{2+}), aluminum (Al^{3+}), percent base saturation (%BS), total sulfur (TS), carbon to nitrogen ratio (C:N) and pH (Mycock, 2011). Aluminum is considered to be a key element in smelter-impacted sites due to mobility and its phytotoxic affects as it is available for plant uptake in acidic soils (Hutchinson and Whitby, 1977).

2.3.2 Vegetation and metal interactions

Plants growing in areas of metal contamination will often take up metals into their tissues including the stems, leaves, roots and bark, with different plant species differing in their abilities to do so (Kozlov et al., 1995; Saarela et al., 2005). Heavy metals react differently with plants depending on the available metal concentration, soil pH and texture, and presence of other metals. Less is known about how vegetation responds in a natural setting with variant climate and soil factors under mixed metal contamination as compared to controlled laboratory settings (Hagemeyer, 1999; Hermle et al., 2006). Heavy metal concentrations can reduce seedling survival and establishment. Around smelting complexes where there has been SO_2 deposition the soils are often acidic and heavy metals are more soluble under acidic conditions (Patterson and Olson, 1982). The concentration of heavy metals that is damaging to plants varies depending on the species of plant. Some plant species are more sensitive to soil metal concentrations while others have the ability to adapt to higher levels of metals. Generally, deciduous tree species are known to have a higher metal tolerance than coniferous trees with the exception of those of the *betula* genus (Patterson and Olson, 1982). Translocation and storage mechanisms of heavy metals to roots and shoots vary between tree species and metals (Pulford and Watson, 2003).

Copper and Zn are essential elements for plant growth meaning that a certain level is necessary for plant growth and survival; however, these elements can also be toxic to plants at high concentrations (Pahlsson, 1989). Zinc is involved in protein synthesis and nucleic acid and lipid metabolism. Zinc toxicity can affect root and shoot growth by stunting growth, causing chlorosis, decreasing leaf chlorophyll and thus affecting rate of photosynthesis (Pahlsson, 1989). Copper is important for metabolism of the plant as well as seed production and disease resistance (Pahlsson, 1989). Copper toxicity can reduce stem growth and biomass in woody plant species and can cause stunting of root growth and discolouration (Heale and Ormrod, 1982). Studies have also shown that Zn and Cu can inhibit photosynthesis in plants leading to reduced development and growth (Clijsters and Van Assche, 1985).

Aluminum and Cd are non-essential for plant survival and growth. Nickel is also considered a non-essential element; however, it is essential for some plant species but is required at the lowest level of the essential elements for plant survival in those specific species and is toxic at higher levels (Environmental Protection Agency, 2007). Cadmium toxicity can cause plants to have curled and smaller leaves that show signs of chlorosis. In studies using deciduous and coniferous tree species and grass species, biomass was reduced as the amount of Cd applied increased (Pahlsson, 1989). Aluminum toxicity can restrict root growth as well as inhibit the uptake of other nutrients, and is dependent upon the Ca to Al ratio (Environmental Protection Agency, 2007; Wit et al., 2010). Nickel was more toxic than Cu and Co in a study conducted on four different woody species (Patterson and Olson, 1982). In Sudbury, the liming of soil decreased the amount of Ni taken up by at least half indicating that the mobility of Ni is influenced by the pH of the soil (Hutchinson and Whitby, 1977).

2.4 The Use of Soil Amendments in Revegetation

Soil amendments are useful in revegetation projects due to the ability of soil amendments to improve fertility, change the availability of metal contaminants, increase water retention and improve soil stability (Gadapalle et al., 2007). Organic soil amendments improve soil properties mainly by the addition of organic matter to the soil. The type of amendment being used can have varying benefits on the revegetation process depending on the soil type, moisture regime and contaminants present. Amendments are most effective in acidic and low nutrient areas (Park et al., 2011). One of the practical benefits of soil amendments is that they can be used *in situ* compared to having to treat the soil *ex situ*, which is an expensive remediation technique. This

makes using soil amendments a cost effective form of remediation. Amendments such as compost and biochars can be made locally on site if necessary using potential waste materials which also reduces the cost of remediation (Environmental Protection Agency, 2007)

In some studies, application of soil amendments has increased the amount of microbial diversity and activity present in the soil which has been found to be reduced in contaminated soil areas (Farrell et al., 2010a). Farrell and Jones (2010) conducted a greenhouse trial using five different amendments including green waste and municipal derived composts in a heavy metal (As, Cu, Pb, Zn) contaminated soil. Plants had increased root and shoot yield over the control for all of the soil amended treatments. Another study using contaminated mine waste used a variety of organic amendments (greenwaste compost, peat, wood bark) in a greenhouse trial concluded that application of organic amendments increased biomass over the controls as well as led to a reduction in the amount of extractable metals (Zn, Cu and Pb) (Nwachukwu and Pulford, 2009).

2.4.1 Compost

Compost is a common and affordable soil amendment that is very high in organic matter. Compost has been used as a fertilizer and as mulch in remediation of disturbed and contaminated areas as it has positive effects on soil properties. The chemical, physical and biological changes in soil properties associated with the amendments are largely attributed to changes in organic matter content (Borken et al., 2002). Improvement in physical soil properties such as porosity, stability, water content and erosion can occur when compost is used. Compost can reduce the impact of heavy metal contamination by immobilization or reduction of the heavy metals in the soil (Park et al., 2011).

Compost can increase root and shoot biomass of plants grown in contaminated soil but success is dependent on a specific case by case basis (Farrell et al., 2010b). The properties of the compost are important to ensure that the amount of heavy metal in the compost is not going to become detrimental to the environment in which the compost is being applied (Pinamonti et al., 1997). Applying municipal compost to areas of contamination can reduce solubility and leaching of Cu, Pb and Zn by providing binding sites for these heavy metals in the organic matter (Brown, 2003; Paradelo et al., 2011).

Compost can reduce the availability of heavy metals through binding to the organic matter; however, the positive effects of the addition of the organic matter in the compost may decrease as the organic matter decomposes over time. Benefits over the long term still exist as

compared to unamended control sites (Borken et al., 2002). Other site conditions can also reduce the effectiveness of compost. Factors such as salt concentrations and combinations of metals within the soil were found to decrease binding of Zn, Cu and Pb (Nwachukwu and Pulford, 2008).

2.4.2 Biochar

Applying commercially produced biochar as a soil amendment on metal contaminated areas is a fairly new concept. Biochar is a charred material that is made by pyrolysis of different types of materials under low temperatures for a set period of time (Kloss et al., 2011). Biological materials used for this process include, but are not limited to wood (e.g. willow and spruce), railway ties, cereal straw, fish meal and bone meal and meat. The temperature used influences the end properties of the biochar including the EC, pH, ash content, C, P and N contents (Kloss et al., 2011). However, the main influencing factor for the composition of biochar is the starting product used to create the biochar. For example, biochar made from animal bi-products have much higher P content than biochar produced from wood products (Chan and Xu, 2009). This makes comparing biochars between studies more difficult as products can vary depending on initial product sources.

Applying biochar can increase the soil pH, increase water retention capacity, increase bulk density, improve cation exchange capacity and reduce the leaching of nutrients from the soil (Beesley and Marmiroli, 2011; Karami et al., 2011; Ennis et al., 2012). Many organic amendments decompose over time so reapplication is necessary; however, biochar has been found to be stable, have increased nutrient retention and have greater resistance to microbial decomposition making it a longer lasting soil amendment option compared to greenwood and compost (Chan and Xu, 2009). A laboratory study was conducted comparing green waste compost and biochar in an area contaminated with Zn, Cd, As and Cu; both of the amendments were found to have a positive effect on the plant growth in the area but biochar was able to better reduce the bioavailability in the area that had multiple contaminants (Beesley et al., 2010). Biochar has been found to be effective in areas that have organic and inorganic contamination (Beesley et al., 2011).

There are many different possible methods of applying biochar including application as liquid slurry, deep banded rows, uniformly mixing with topsoil or top dressing (Blackwell et al.,

2009). It is not known how quickly the biochar moves downwards into soil but biochar has been found at depths suggesting that it does move vertically downwards (Blackwell et al., 2009).

2.4.3 Fungal amendment

Mycorrhizal fungi develop a symbiotic relationship with plant rooting systems and help the host plants gain nutrients and moisture from the soil. Ectomycorrhizal fungi (EMF) and arbuscular mycorrhizal fungi (AMF) have different mechanisms of interacting with the plant. Ectomycorrhizal fungi form symbiotic relationships around the outside of the root and AMF form within the cells of the root (Bundrett et al., 1996). Tree species commonly interact with EMF, and grasses and forbs form association with AMF due to the difference in symbiotic associations. There are commercially developed inoculant products of EMF/AMF that can be added to landscapes. Some vegetation species, however, can form relationships with both AMF and EMF (Hoeksema et al., 2010).

Inoculation with EMF have resulted in positive effects on plant growth, including improved phosphorus and water uptake, increased soil stability and increased plant tolerance in polluted or contaminated sites (Dodd and Thomson, 1994; Wilkinson and Dickinson, 1995). Fungi may be resistant to metal contamination as a result of the fungi having a short life cycle. The short life cycle would allow for genetic changes to occur rapidly and adapt to a heavy metal contaminated soil to help protect the plant from the heavy metal environment (Wilkinson and Dickinson, 1995). The affect that mycorrhizal colonization has on plants in heavy metal contaminated areas can be influenced by the concentrations of metal, fungi species and pH and is not always successful in mitigating toxicity (Shetty et al., 1994).

2.5 Moisture in Revegetation of Smelter-impacted Soils

When considering the use of phytotechnologies it is important to consider the availability of soil moisture for plant use. It is also important that the amount of moisture present in an area is adequate for the plant species chosen for the project (ITRC, 2009). The moisture present in an area can affect the availability of metals in the soil, the amount of metal found within the plant and the plant biomass. In a study comparing the effects of moisture levels on both hyperaccumulating plant species and non-hyperaccumulating species, availability of metals in the soil, the metal in the plant and the plant biomass all increased as the amount of moisture increased (Angle et al., 2003). In this study it was also found that the moisture had no influence on the availability of Zn to plants but extractable Ni decreased as soil moisture increased (Angle

et al., 2003). A one-year *ex situ* study conducted using willow and poplar trees found that height, stem diameter and plant biomass all decreased in a high water regime compared to a lower water regime in an soil impacted by heavy metals from sludge waste (Guidi and Labrecque, 2010). Seedlings, however, need radicle growth to be able to access available moisture in order to survive in areas of low moisture. Heavy metals in soil can reduce the radicle length of plants which can contribute to moisture stress of young plants (Patterson and Olson, 1982).

2.5.1 Precipitation patterns in Flin Flon/Creighton

The area of Flin Flon/Creighton receives an average annual precipitation of 463.1 mm. The summer months average between 40.9 mm to 75.5 mm of rainfall. The monthly average temperatures throughout the year in Flin Flon/Creighton range from -20.4°C to 18.5°C (Environment Canada, 2011). These types of conditions can potentially influence plant growth and success due to the variability from one month to the next and are important to consider when setting up a field study within the area. It is unknown if the amount of moisture is a factor in the revegetation process of the smelter-impacted areas in Flin Flon/Creighton.

3. THE USE OF SOIL AMENDMENTS FOR *IN SITU* REVEGETATION OF SMELTER-IMPACTED SOILS

3.1 Preface

A field experiment was conducted to examine the impact of various soil amendments on the survival and growth of vegetation in smelter-impacted soils *in situ*. The amendments were chosen on the basis of a preliminary growth chamber screening experiment, conducted as part of a larger study, in which several amendments were screened for efficacy. Success was measured in the field by the survival and growth of the vegetation, change in soil properties (pH and base saturation) and the influence the amendments had on uptake of metals into the plant biomass. In doing this it becomes possible to choose the most effective field amendment for a potential revegetation plan in Flin Flon/Creighton.

3.2 Abstract

An *in situ* trial was established in Flin Flon/Creighton in July 2011 consisting of 12 sites chosen based on conditions that were representative of the larger landscape. At these 12 sites, 60 plots (5 plots/site) were established and soil amendments (microbial inoculant, compost, bone meal and meat biochar and willow biochar) were applied to examine the impact of soil amendments on vegetation survival in smelter-impacted soils. All 12 sites also received an application of dolomitic limestone and fertilizer at the time of planting. The survival and growth of tree species (trembling aspen and jack pine) and understory species (tufted hairgrass) was used as a measure of success of the soil amendments. Although the amendments had been chosen on the basis of a prescreening bioassay in which all amendments enhanced growth and survival under controlled growth chamber conditions, no significant enhancements of survival or growth were detected in the field study. The soil amendments had some influence on the soil physical properties but the soils were not uniformly affected across all 12 sites.

3.3 Introduction

The impact of heavy metal contamination on soils and plant growth has been studied in depth by many different researchers although studies are commonly conducted in controlled laboratory settings and less commonly studied *in situ* (Hermle et al., 2006). In developing a revegetation strategy for an area such as Flin Flon/Creighton it is important to take into consideration the environment of the area including climate, topography, the geographic extent of the contamination and local vegetation. Often as metal concentrations in an area increase so do vegetation mortality rates; however, some understory and some tree species have the ability to store metals in the root or shoot biomass depending on the type and availability of the metal present (Patterson and Olson, 1982). Trees such as trembling aspen (*Populus tremuloides* Michx.) are able to rapidly transition into areas and grow quickly to help improve the visual appeal of an area (Pulford and Watson, 2003). Consequently, they are good candidates for revegetation programs. The addition of soil amendments to soil can alter the physical and chemical properties of the soil by changing the bioavailability of the metals and/or increasing water retention, among other effects (Borken et al., 2002). Soil amendments that are locally available for use should be selected for field trials so there is potential to use them in subsequent landscape-scale reclamation projects (ITRC, 2009).

3.4 Materials and Methods

A field study was conducted to assess the impact of various soil amendments on the revegetation of smelter-impacted soils near Flin Flon, MB/Creighton, SK. The experiment included 12 experiment sites in and around Flin Flon/Creighton. The sites were established to represent the diverse areas that are devoid of vegetation that potentially could be included in a subsequent landscape-scale reclamation program, if it were to occur. The tree species used at each site were jack pine (*Pinus banksiana* Lamb.) and trembling aspen and the understory species were tufted hairgrass (*Deschampsia cespitosa* L.) and American vetch (*Vicia americana* Muhl.). Each plant species was grown under one of five treatments. The treatments included an unamended control and four amendments, namely municipal compost, willow biochar, bone meal and meat biochar and a commercial mycorrhizal product. The trial ran for the duration of 14 months and trees and understory were destructively harvested upon completion. Throughout the trial survival and growth rates were recorded. Soil samples pre-plant growth and post-plant growth were collected for further laboratory analysis.

3.4.1 Site selection and characterization

Figure 3.1 shows the relationship of the 12 study sites to the smelting stack which was the emission source for the pollution. The sites are located in different soil types, elevations, distances from the smelter and varying distances from other vegetation. Sites were randomly selected. The amount of soil present (i.e., area and depth) within the area was very important as the soil needed to be deep enough in which to plant tree plugs, as well as be accessible by foot. A healthy mixed wood boreal forest that is out of the range of contamination from the smelting complex, located near Sherridon, MB, was used as a comparison for the soil properties. Sherridon was used as a reference site for previous projects done in the Flin Flon/Creighton area (Bentz, 2013).

3.4.2 Plot design

At each of the 12 study sites there were five experimental plots (0.5 m x 0.5 m) established with a total of 60 plots for the entire study. Plot size was constrained by the area available. Each of the 60 plots received a base treatment of 75 g of slow release fertilizer (20-17-10) and 4 g of dolomitic limestone that had been crushed and sieved to 0.5 mm. The amendment treatments were bone meal and meat biochar (BMB), willow biochar (WB), a commercial combination of endomycorrhizal and ectomycorrhizal inoculant (EMF) and compost

(Table 3.1). The compost was a class A product meaning there was no significant amounts of heavy metals. The untreated control received only the slow release fertilizer and the dolomitic

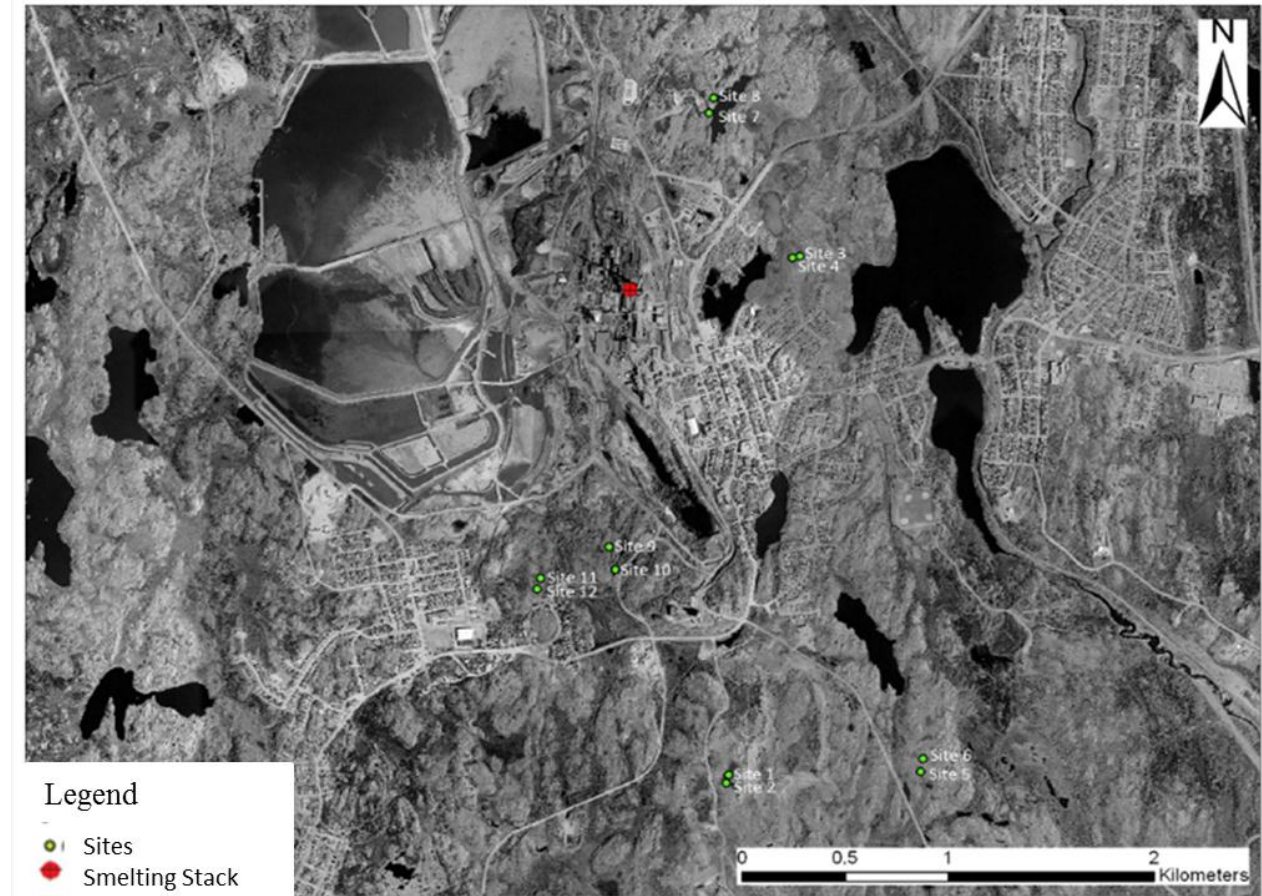


Figure 3.1 Aerial photograph of Flin Flon/Creighton displaying 12 field sites indicated by green circles and smelting stack indicated by a red circle. Direction and distance from the smelting stack is indicated.

limestone. The amendments were chosen based on success from a preliminary trial conducted as a component of a larger research project (Gord Andersoff, pers. comm.). The BMB, WB and compost amendments were applied to the surface of each plot. Amendments were not incorporated into the soil because mixing in the amendments, if applied on a large scale, would not be practical in Flin Flon/Creighton due to the variability and extent of area that would need to be treated.

The BMB, WB and compost application rate was calculated based on the plot area, 2.5 cm depth, amendment bulk density and 10% w/w. The quantity of each amendment applied varied depending upon bulk density: 900 g of compost ($36,000 \text{ kg ha}^{-1}$); 160 g of WB

(6,400 kg ha⁻¹); and 340 g (13,600 kg ha⁻¹) of BMB.

Table 3.1. Description of amendments used in field trial, including source, type of amendment, pH and Al, Cd, Cu and Zn concentrations in the amendment.

Amendment	Trade Name	Source	Amendment Type	pH	Metal Concentrations			
					Al	Cd	Cu	Zn
Bone meal and meat Biochar; BMB	--	Titan Clean Energy, Saskatoon, SK	Organic	8.39	0	0	0	0
Willow Biochar; WB*	--	Titan Clean Energy, Saskatoon, SK	Organic	9.28	0	0	0.01	0
Compost ;	--	City of Saskatoon Compost Depot, Saskatoon, SK	Organic	6.98	0	0	0.03	0
Mycorrhizal Inoculant; EMF ††	Myke [®] Pro Landscape	Premier Tech Biotechnologies Ltd., Quebec, Canada	Fungal ‡	6.73	0	0	1.00	21

† Mycorrhizal inoculant (Zn) was analyzed by ALS Laboratories, Inc., Saskatoon, SK. All other amendments were analyzed, as part of current project, at the University of Saskatchewan.

‡ Fungal content (approximate spores g⁻¹) Ectomycorrhizal fungi: *Pisolithus tinctorius* (100,000), *Scleroderma cepa* (7500), *S. citrinni* (7500), *Rhizopogon roseolus* (3750), *R. subscaerelescens* (3750), *R. villosulus* (3750), *R. vulgaris* (3750), *Laccaria laccata* (2250). Endomycorrhizal fungi: *Glomus intradadices* (15)

The Mycorrhizal inoculant used was a commercial product with active endomycorrhizae (*Glomus intraradices*) and ectomycorrhizae (*Pisolithus tinctorius*, *Scleroderma cepa*, *S. citrinni*, *Rhizopogon roseolus*, *R. subscaerelescens*, *R. villosulus*, *R. vulgaris*, *Laccaria laccata*) (MykePro Mycorrhizal inoculant, Premier Tech Biotechnologies, Riviere-du-Loup, QC, Canada). The manufacturer's recommended rate of application for the EMF product was 28.4 mL per 10.16 cm² area which is equivalent to 40 g per understory area (0.25 m x 0.5 m) as a surface amendment. In addition, 5 g was put directly into each of the holes where the tree plug was planted.

Each experimental unit receiving one amendment measured 0.5 m x 0.5 m, and was divided into four equal quadrants. Each quadrant was planted to one of four plant species. The understory quadrants were combined and planted to a mix of the two species treatments. Each tree species was planted separately in the remaining quadrants (Fig. 3.2). The planting density was higher than typical in a revegetation program to ensure replication was possible in the smaller areas that were restricted by the amount of soil present.

Both tree species were planted as one-year-old rooted seedlings of jack pine (~18 cm tall) and trembling aspen (>50 cm tall). Tree seedlings were acquired from Tree Time Services Inc. (Edmonton, AB, Canada) as fresh stock (which had not been frozen). There were four jack pine and four trembling aspen plugs planted in each of the 60 plots. The plugs were planted so the top of the root ball was flush with the soil surface. The understory species were seeded at a rate of 3.6 kg ha⁻¹ for the tufted hairgrass (Olge et al., 2010) and 36 kg ha⁻¹ for American vetch (Kirk and Belt, 2010). The plant species were chosen based on being native to the area, past success in Sudbury, ON project, fast growing and ability to survive in contaminated areas. The location of each amended plot at each site was recorded. At the time of planting each of the plots was moistened with 7.5 L of water. Soil was sampled at each location (0.5 m depth or until bedrock was reached).

3.4.3 Field measurements

At the time of planting in late June 2011 the basal diameter of each of the tree seedlings was measured using a digital caliper, and seedling heights were recorded. Each of the seedlings in the plot was tagged with a different color of plastic ribbon and planted in a recorded arrangement so that measurements could be made throughout the growing season for each individual tree. The sites were re-visited in July 2011 and September 2011 and the tree diameters and heights were recorded. The survival rate was also recorded for each of the plots in July 2011 and September 2011. The estimated percent ground cover was recorded for each of the plots in July and September based on a visual estimate of the amount of the 0.25 m x 0.5 m understory area that was covered in understory vegetation. Prior to harvesting the plots in August 2012 the overwinter survival rate was recorded as well as the heights and diameter of the tree species.

At the time of harvest, soil samples to a depth of 15 cm (or to bedrock of less than 15 cm) were collected from each experimental plot and from the Sherridon reference site. Soil from Sherridon was collected in July 2012 to be used as a comparison of a healthy forest stand to the

soils in the experimental sites. Sherridon soil was collected to a 15-cm depth excluding the leaf litter layer.

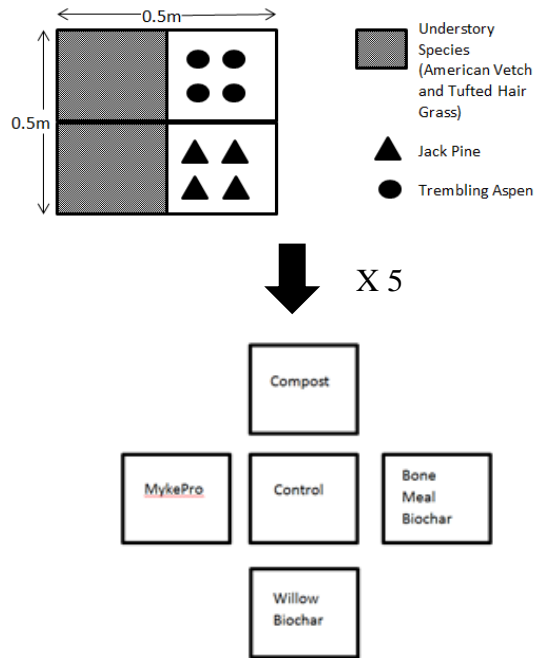


Figure 3.2 Plot configuration at each of the 12 sites showing the arrangement of the four soil amendments and control areas. The upper part of the figure shows the configuration of each of the five plots including four plugs of each tree species as well as half of the plot designated to understory species.

The precipitation and temperature for the years 2011 and 2012 when the field trial occurred were recorded and compared to the long-term averages (1981-2010) by month (Table 3.2) (Environment Canada, 2011). Above normal precipitation occurred in July and August of 2011. Due to an extreme rainfall event on July 20th in 2011, where the area received 72 mm of precipitation in a 24 h period, the applied amendments on sites 2, 3, 4, 6, 7, 9 and 11 were washed away due to the slope positions of these sites. These sites were re-amended on July 24th, 2011. The temperatures during the field season were around normal and represented what is typical of Flin Flon/Creighton.

Table 3.2. Monthly (years 2011 and 2012) and average (years 1981 to 2010) monthly temperatures and precipitation recorded for Flin Flon, MB.

Month	Precipitation (mm)			Temperature (°C)		
	Average	2011	2012	Average	2011	2012
January †	-	-	-	-18.7	-22.0	-16.4
February †	-	-	-	-14.9	-17.8	-13.4
March †	-	-	-	-7.6	-14.0	-5.9
April †	-	-	-	1.8	1.0	0.8
May	39.3	37.0	7.4	9.4	8.8	9.2
June	69.3	84.6	94.4	15.8	15.3	15.6
July	77.9	149.4	66.0	18.9	18.6	21.0
August	63.7	141.0	59.2	17.5	18.4	17.8
September	64.2	14.8	40.6	10.8	13.9	11.5
October †	-	-	-	3.0	5.3	1.4
November †	-	-	-	-8.0	-7.4	-10.0
December †	-	-	-	-16.2	-12.2	-20.6

† Environment Canada does not record mm of precipitation in winter months, (only cm of snow on the ground for the Flin Flon station), therefore data is not included in table.

3.4.4 Plant preparation for laboratory analysis

In August 2012, at final harvest, the trees and understory vegetation were excavated and brought to the University of Saskatchewan where the soil was washed from the roots and the biomass was weighed. For the trees, roots were separated from the shoots then dried and re-weighed separately. For the understory biomass the roots were not separated from the shoots because the root biomass in most cases was not sufficiently large enough to use for analysis so analysis was done on the whole plant. At the time of washing, a 5 g (fresh weight) sample of root was subsampled from each tree species from each plot to complete ectomycorrhizal counts. Roots were stored in distilled water at room temperature (~20°C) and were analyzed within 48 h (section 3.4.5.3). The dry root and shoot biomass was recorded for both tree species and the understory. The shoots, roots from the trees were bulked and finely ground using a plant matter grinder for laboratory analysis. The understory was ground as a whole plant sample using a coffee grinder due to the small amount of biomass in some plots.

3.4.5 Laboratory analysis

3.4.5.1 Soil characteristics

Soil pH was measured on 1:2 (w/w) soil:water extracts using a Symphony meter and Symphony™ Gel 2-in-1 pH electrode (VWR International, USA). Soils were extracted with double-deionized water (Hendershot et al., 2008). Soils at the time of planting and the time of harvest were air dried and ground and analyzed using the C632 Carbon Determinator (Leco Corporation, St. Joseph, Missouri USA) to determine percent total carbon (TC) through combustion at 1100°C.

To determine cation exchange capacity elements (Ca, K, Mg, Na and Al), soil was analyzed using a procedure adapted from Meyer and Arp (1994), Schoning and Brummer (2008) and Skinner et al. (2001). The method was consistent with previous research done within the larger Flin Flon project by Mycock (2011) and Bentz (2013). Briefly, air-dried soil (2 g) was agitated with 80 mL of 1M NH₄Cl for 24 h at 150 rpm in a 200 mL polypropylene container. The solution was filtered through a Whatman #42 filter (Whatman, Piscataway, NJ). Standards (1 to 20 µg/L) created in 1M NH₄Cl were analyzed with samples and blanks using the Microwave plasma-atomic emission spectrometer (Agilent 4100 MP-AES, Australia). Available ammonium (NH₄⁺) and nitrate (NO₃⁻) were extracted from 5.0 g air-dried ground soil with 50 mL of 2M KCl and agitated for 1 h at 142 rpm (Hendershot et al., 2008). Extract was filtered through VWR 454 (VWR International, USA) filter paper into vials and analyzed on an auto analyzer (WestCo Scientific Instruments, Inc., USA).

3.4.5.2 Metal analysis (soil and plant)

Available metals (Al, Cd, Cu, Ni) and S were extracted from the soil samples based on the adapted extraction method of Wightwick et al. (2010). Air-dried soil (2 g) was weighed into a 250 mL polypropylene container to which 200 mL of 0.01M CaCl₂ extraction reagent was added. Containers were agitated at 300 rpm for 12 h at room temperature (20 °C). Bottles were left to settle (1 h) prior to being filtered through a millipore (2.5 µm) vacuum filter (Millipore Corporation, Billerica, MA). Available metal in the extract was measured using the Microwave plasma-atomic emission spectroscopy (Agilent 4100 MP-AES, Australia).

Elements (Al, Cd, Cu, Ni, and Zn) in plant tissues were analyzed using a procedure adapted from Ippolito and Barbarick (2000) and Lesniewicz and Zyrnicki (2000). Ground plant sample (1 g ± 0.05 when sufficient plant matter was available) was measured into 100 mL glass

digestion tubes and 6 mL HNO₃ was added and heated to 90 °C for 75 min for digestion to occur. Hydrogen peroxide (5 mL) was added and the mixture was left to digest for 30 min. After cooling, the solution was brought up to 25 mL using distilled water and gravity filtered through a Whatman #5 filter. Metals were measured in solution using microwave plasma–atomic emission spectroscopy (MAP-AES 4100; Agilent Technologies, Mississauga, ON, Canada); solutions were syringe filtered to 0.1 µm (Whatman, Piscataway, NJ).

3.4.5.3 Ectomycorrhizal colonization

Tree roots were examined for ectomycorrhizal colonization according to the method developed by Bundrett et al. (1996) using a gridline intersect method. Roots (5 g) collected at the time of harvest are laid across a 9 cm petri dish with 0.5 cm grid lines. Counts were performed using a 10x dissecting microscope on the number of times a mycorrhizal root tip crossed a line in comparison to the amount of times a non-mycorrhizal tip crossed over a line. A minimum of 100 crosses were counted to ensure results were representative of the sample. The root samples used were dried and weighed to give an estimate of the percent of colonization present in the entire root ball of each tree.

3.4.6 Statistical analysis

Statistical analysis was performed using IBM® SPSS© Statistics (Version 20). None of the results for the field trial were normally distributed or had homogeneous variances (Levene's test; $p \leq 0.05$) or normality (Shapiro-Wilk test; $p \leq 0.05$). The results did not meet criteria for homogeneity of variance or normality using log transformations. Non-parametric statistics were utilized for data analysis. The Kruskal-Wallis ($p \leq 0.05$) test was used to analyse data and means separation was performed using the Games-Howell test ($p \leq 0.05$). Correlations between variables (plant biomass, soil available metals, plant metals and baseline soil properties) were determined using Pearson Product Moment Correlation.

3.5 Results

3.5.1 Initial soil characteristics

Initial soil characterization (Table 3.3) before the establishment of field plots and for the Sherridon reference site was important to quantify the changes in soil properties throughout the duration of the field trial and relate vegetation response to the amendments and soil characteristics. The reference site, which was far enough away from the smelting complex as to not be affected by the smelting activities, had a well-established healthy mixed-wood coniferous forest stand containing jack pine and a understory with bearberry (*Arctostaphylos uva-ursi* L.) and grey reindeer lichen (*Cladina rangiferina* L.). The soil at the reference site was an Eluviated Dystric Brunisol. Comparing initial site characterization to the reference site can help develop ideas of what some of the measurable components may be that are influencing the success or failure of vegetation establishment and growth at the 12 study sites within the affected smelting area.

The pH of the 12 study sites (Table 3.3) ranged from 3.83 at site 9 to 5.84 at site 12 and the reference site had a pH of 4.15. The CEC_e for the reference site was 3.21 cmol_ckg⁻¹. The experimental sites had a range of CEC_e of 2.08 cmol_ckg⁻¹ at site 2 to 8.98 cmol_ckg⁻¹ at site 11. Exchangeable Na, Ca, Mg, Al and K were used to calculate the base saturation values. Exchangeable Na, Ca, and Mg in most cases were higher in the reference site than in the 12 study sites. The reference site therefore also presented a higher base saturation than the 12 sites. The Ca:Mg ratio for the reference site was 3:1 and for the experimental sites ranged from 2:1 to 12:1.

In the 12 sites available metal concentration range of Cu was 8.82 to 1181.18 mg kg⁻¹, Zn 5.12 to 663.27 mg kg⁻¹, Cd < 0.10 to 7.58 mg kg⁻¹ and Al 3.36 to 58.48 mg kg⁻¹ (Table 3.3). Typically, the smelter-affected soils had higher levels of Cu, Zn and Cd than the reference site. Available Al was higher in the reference site than in the 12 study sites with the exception of site 3 which had almost two times the amount of Al present compared to the reference site at the beginning of the field trial. Nickel levels in all sites, including the reference site, were below the detection limit of 0.05 mg kg⁻¹ and therefore Ni was not quantified. Total carbon was lower in the Sherridon site than in the 12 sites except sites 1 and 2. The available S ranged from 477 mg kg⁻¹ at sites 2 and 6 to as high as 2250 mg kg⁻¹ in site 4. The amount of NO₃⁻ was higher in all sites.

Table 3.3. Physical and chemical characteristics and metal concentrations in soils from the Sherridon site (reference) and 12 study sites. Sampled in 2011 at the initiation of the study.

Soil Property (n=2)	Site												
	Reference (Sherridon)	1	2	3	4	5	6	7	8	9	10	11	12
pH	4.15	4.48	4.46	4.34	4.19	4.06	4.30	4.32	4.10	3.83	4.15	4.11	5.84
TC (%)	0.93	0.74	0.73	3.91	8.96	8.62	6.48	25.73	13.09	10.83	1.64	2.05	6.45
Texture	SL	SL	CL	SL	LS	SCL	SL	SCL	SCL	SC	SCL	CL	CL
NO ₃ ⁻ (mg kg ⁻¹)	0.02	0.07	0.45	0.17	1.31	0.76	0.74	1.19	0.94	0.62	0.13	0.15	0.07
NH ₄ ⁺ (mg kg ⁻¹)	0.48	0.32	0.77	0.93	1.77	1.58	1.26	3.07	2.51	2.63	0.47	0.69	1.33
CEC _e [†] † (cmol _c kg ⁻¹)	3.21	2.32	2.08	3.37	2.94	5.51	5.71	6.41	7.71	7.05	3.81	8.98	6.68
Exchangeable Ca (cmol _c kg ⁻¹)	0.58	0.04	0.02	0.21	0.07	0.36	0.18	0.12	0.05	0.08	0.03	0.10	0.09
Exchangeable Mg (cmol _c kg ⁻¹)	0.16	0.02	0.01	0.06	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.07	0.06
Exchangeable K (cmol _c kg ⁻¹)	0.07	0.02	0.02	0.04	0.07	0.07	0.11	0.12	0.17	0.06	0.20	0.20	0.24
Exchangeable Na (cmol _c kg ⁻¹)	1.50	0.10	0.04	0.17	0.13	0.03	0.19	0.14	0.18	0.24	0.33	0.34	0.33
Base Saturation (%)	72.14	7.62	4.87	4.49	9.87	8.85	8.82	6.32	5.41	5.81	14.80	7.82	10.79
Available S (mg kg ⁻¹)	899	477	506	700	2250	629	477	654	635	971	772	600	981
Available Cu (mg kg ⁻¹)	1.20	12.88	9.27	285	1181	22.65	15.08	407	259	57.67	8.82	31.23	52.56
Available Zn (mg kg ⁻¹)	2.14	80.24	45.71	73.31	525	328	13.64	663	86.53	47.53	5.2	22.66	39.40
Available Cd (mg kg ⁻¹) ‡	-	-	-	0.17	7.58	3.90	0.09	3.44	0.82	0.55	-	-	-
Available Ni (mg kg ⁻¹) ‡	-	-	-	-	-	-	-	-	-	-	-	-	-
Available Al (mg kg ⁻¹)	32.78	3.52	3.36	58.48	15.54	7.22	6.58	7.73	15.95	15.13	8.93	15.71	16.48

CL = clay loam; LS = loamy sand SC= sandy clay; SCL = sandy clay loam; SL = sandy loam

† CEC_e= effective cation exchange capacity

‡ Blanks spaces indicate that levels were below detection limits

in comparison to the Sherridon site; similarly, NH_4^+ was also higher in all 12 sites compared to the Sherridon site with the exception of sites 1 and 11 that were lower.

3.5.2 Vegetative survival and growth

The survival and growth of the tree and understory species were variable among sites (Fig. 3.3). There was variation between the types of sites that were selected and the local vegetation present at the time of planting as well as the position of the site in the landscape (i.e., rock outcrop soil pocket compared to larger general soil area) which could account for variation between the success at the different sites. Visual observations allow for an assessment of health that may not be detected through height, basal diameter and biomass measurements, alone. Visual assessments confirmed that sites 1, 2, 6, 9, 10, 11 and 12 had more vegetation survival and growth for the tree species as well as the understory species over the course of the trial than the other sites. Visual assessment included observations of color and overall health of tree seedlings.

There were no strong relationships between soil available metal and change in growth for trembling aspen and jack pine species (Fig. A.1.). However, there was an apparent relationship between increased growth of trembling aspen and jack pine in relationship to the sites that had lower available metal concentrations (Al, Cd, Cu, Ni) compared to sites that had higher concentrations of available metals (Fig. 3.4). Sites 3 and 4 were almost completely dead by the time of harvest, whereas sites 5 and 8 were not completely dead but did not have the same understory survival and the trees did not show the same amount of growth as some of the other sites. Site 7 had a small amount of understory present but the trees were showing signs of necrosis as can be seen by the darker orange color of the jack pine trees in the photograph (Fig. 3.3).

Site 3



Site 10



Figure 3.3 Example photographs of successful sites (site 10) and sites that had minimal survival and growth (Site 3) planted in Flin Flon/Creighton at the time of planting (top) and prior to harvest (bottom).

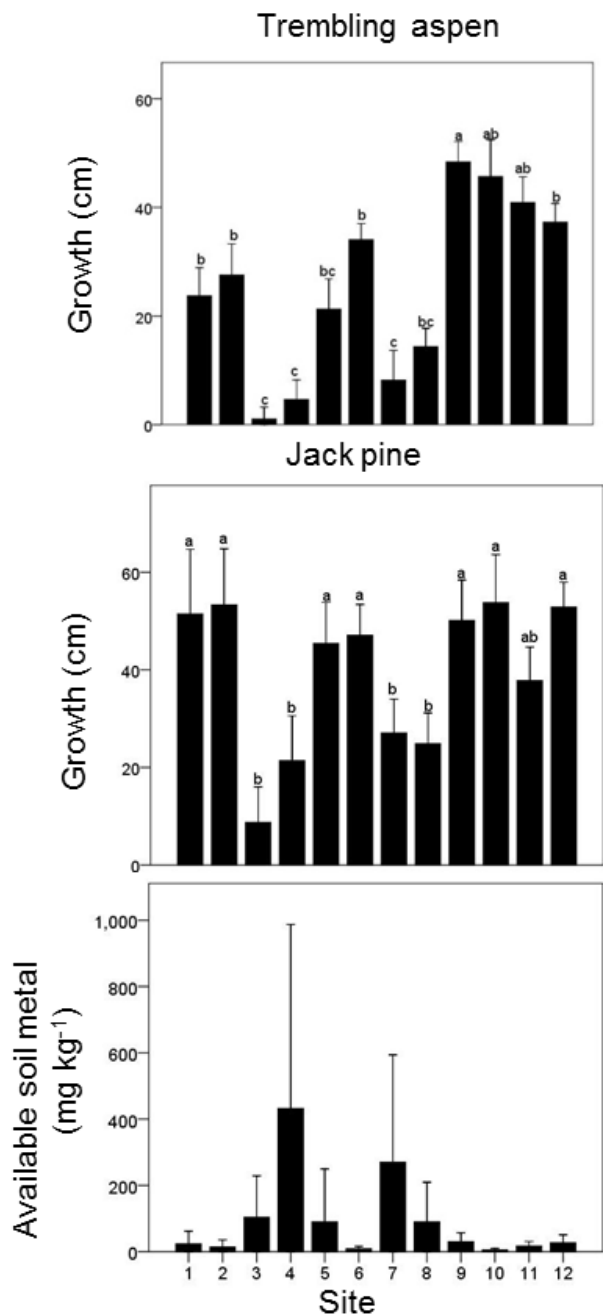


Figure 3.4 Growth (change in height from time of planting to harvest) (cm) of trembling aspen and jack pine and the available soil metal concentration (averaged Al, Cd, Cu, Ni, Zn). Letters represent significant ($p \leq 0.05$) differences in growth between sites based on Kruskal-Wallis and Games-Howell tests. No significant differences ($p > 0.05$) in available soil metal among sites detected. Error bars represent ± 2 standard errors of the mean.

Trembling aspen biomass (root, shoot and total) was negatively correlated with soil available metals (Table B.1) and all correlations were significant with the exception of Zn, for which root biomass was negatively correlated to available soil Zn ($r = -0.26$ to $r = -0.49$; $p \leq 0.05$). Smelter deposited metals (Cd, Cu, Zn) in trembling aspen roots generally correlated to the TC, CECe, NO_3^- , NH_4^+ and soil available S. The non-smelter metals (Al and Ni) in trembling aspen roots correlated to NO_3^- and available S. Trembling aspen shoot metals Cu and Zn generally correlated significantly to soil available metals, TC, NO_3^- , NH_4^+ and available soil S. Trembling aspen shoot Cd did not correlate to any of the measured soil properties. Soil available Al behaved differently from the other soil available metals and did not correlate with any of the biomass metals except for root Al and Cd.

Jack pine biomass (root, shoot and total) was negatively correlated to the soil available metals (Table B.2). Generally, soil available metals were ($r = -0.47$ to $r = -0.58$; $p \leq 0.05$,) correlated to metals in the biomass (root and shoot) of jack pine trees. However, available soil Al was not correlated to the metals in the biomass of jack pine trees. The soil properties, NO_3^- and available soil S, were correlated to the root and shoot biomass metals for jack pine.

There were no significant ($p > 0.05$) correlations between biomass (total, root and shoot), root or shoot metals (Cd, Cu, Zn, Al, Ni) and the soil pH, ectomycorrhizal colonization for trembling aspen and jack pine (data not shown).

No differences were detected in biomass of tufted hairgrass grown with or without amendments (Fig. 3.5) ($p > 0.05$), although there was a trend of increased biomass where compost was applied compared to other amendments. An *in situ* experiment could be conducting using a compost and tufted hairgrass combination to examine if there is the potential for significant revegetation. American vetch survived and grew in five of the 12 sites in the first year of the field trial but comprised less than 5% vegetative cover in any individual plot. Overwinter survival rate was zero.

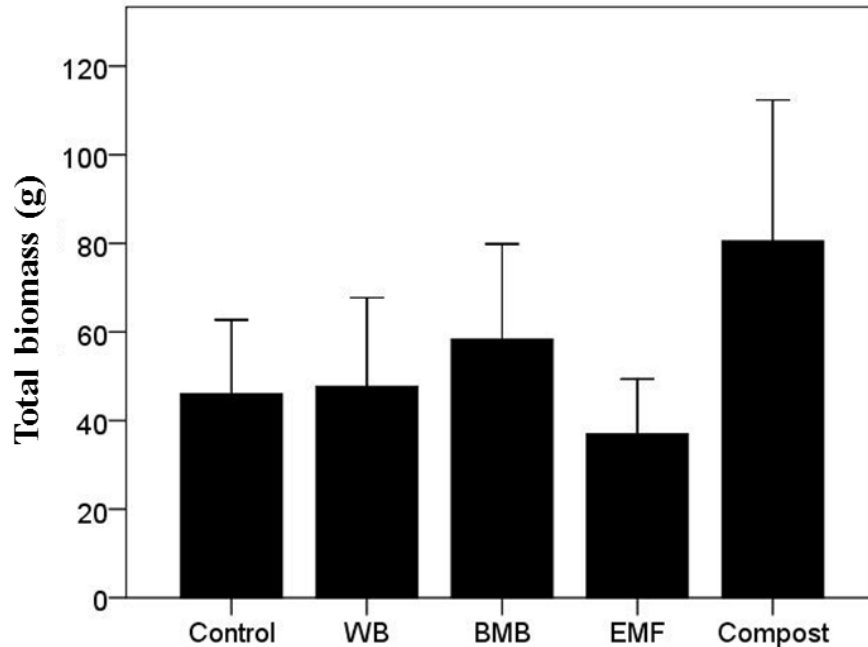


Figure 3.5 Biomass (g) of tufted hairgrass grown with soil amendments [ectomycorrhizal fungi inoculant (EMF), compost, willow biochar (WB), bone meal and meat biochar (BMB)] and control. No significant ($p > 0.05$) differences were detected between amendments based on Kruskal-Wallis and Games-Howell tests. Error bars represent ± 2 standard errors of the mean.

Similar, to the tree species, the understory total biomass (tufted hairgrass) was ($r = -0.27$ to $r = -0.32$; $p \leq 0.05$) negatively correlated to the amount of available soil metal (Al, Cd, Cu, Zn) (Table B.3). There was no correlations between the amount of metals present in the biomass and total biomass of tufted hairgrass except for Ni where there is a significant correlation with total biomass ($r = 0.40$; $p \leq 0.01$). The soil CECe and soil pH did not correlate to the tufted hairgrass biomass or to the metal concentrations of the biomass (data not shown).

3.5.3 Change in base saturation, pH, and available soil metal

All of the base saturations of all soils increased from the initial levels (i.e., pre-amendment and pre-planting) to the final sampling time (i.e., after 14 months) (Fig. 3.6). The magnitude of the increase was generally less under the EMF amendment than the other amendments. Site 1 had a smaller increase in percent base saturation under all amendments than the other sites, except for site 6 which had minimal increase in the control treatment. The control had an increase in base saturation for all sites indicating that the liming and fertilizing of the soil can increase the percent base saturation without further amendments being added.

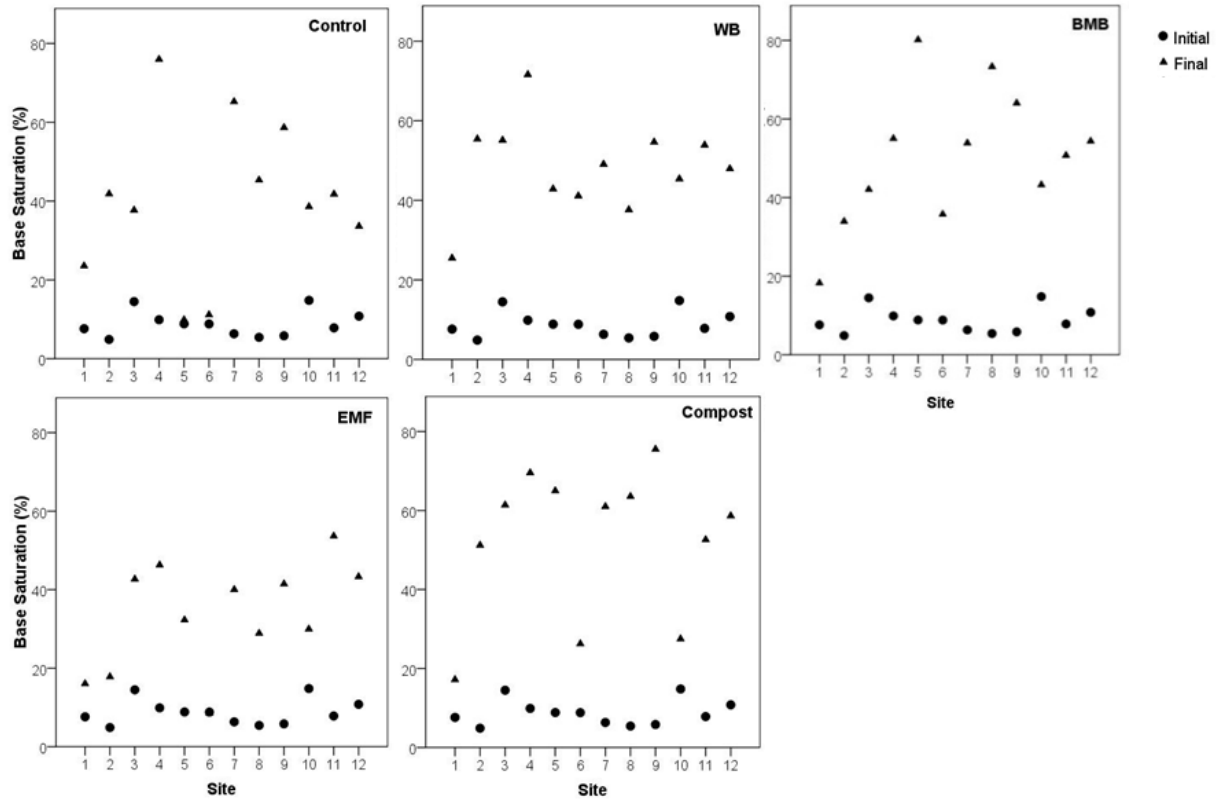


Figure 3.6 Initial soil base saturation and final soil base saturation (%) plotted for each site (12) in Flin Flon/Creighton under each amendment [ectomycorrhizal fungi inoculant (EMF), compost, willow biochar (WB) and bone meal and meat biochar (BMB)] and control.

The amendment treatments and the controls plots did not have uniform increases in soil pH at all sites from planting to harvest (Fig. 3.7). Some of the sites had a reduction in soil pH from the time of planting to harvest. Soil pH increased in more plots (6 of 12) under the compost amendment than under the other amendment treatments. Soil pH in the EMF plots increased in the fewest number of plots (2 of 12) for the amendment treatment plots. The control treatment had only one site that experienced an increased pH throughout the field trial. The effect of the addition of fertilizer on the change in pH is unknown for this experiment due to it being a slow release product but could have potentially decreased the pH in some sites.

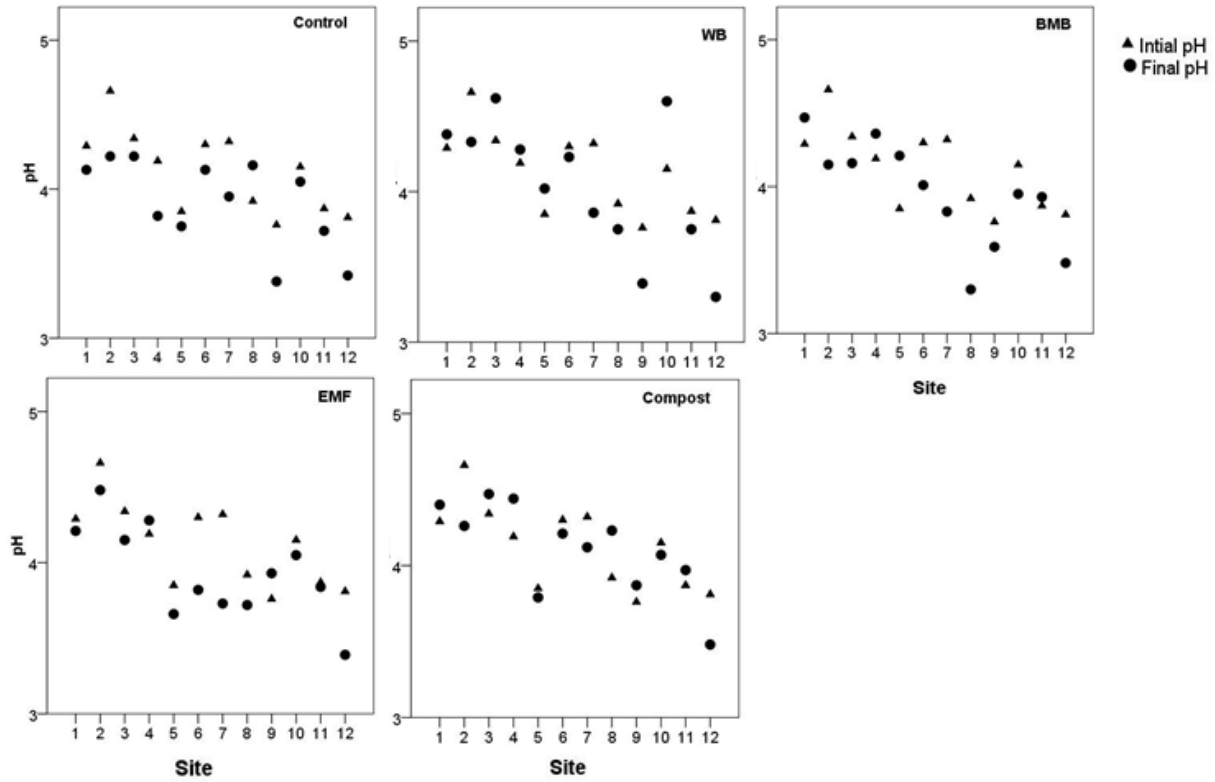


Figure 3.7 Initial soil pH and final soil pH values plotted for each field site (12) in Flin Flon/Creighton under each amendment [ectomycorrhizal fungi inoculant (EMF), compost, willow biochar (WB) and bone meal and meat biochar (BMB)] and control.

3.5.4 Plant metals

There were no increases in growth (cm) of either tree species (trembling aspen or jack pine) associated with any soil amendment ($p > 0.05$) (Fig. 3.8).

The uptake of metal (Al, Cu, Ni, Zn, Cd) into the biomass of the trembling aspen and jack pine trees was not influenced by the application of amendments (Fig. 3.9 and Fig. 3.10). The vegetation planted under the control treatment did not have significantly different metal uptake from the plants grown under amendments ($p > 0.05$).

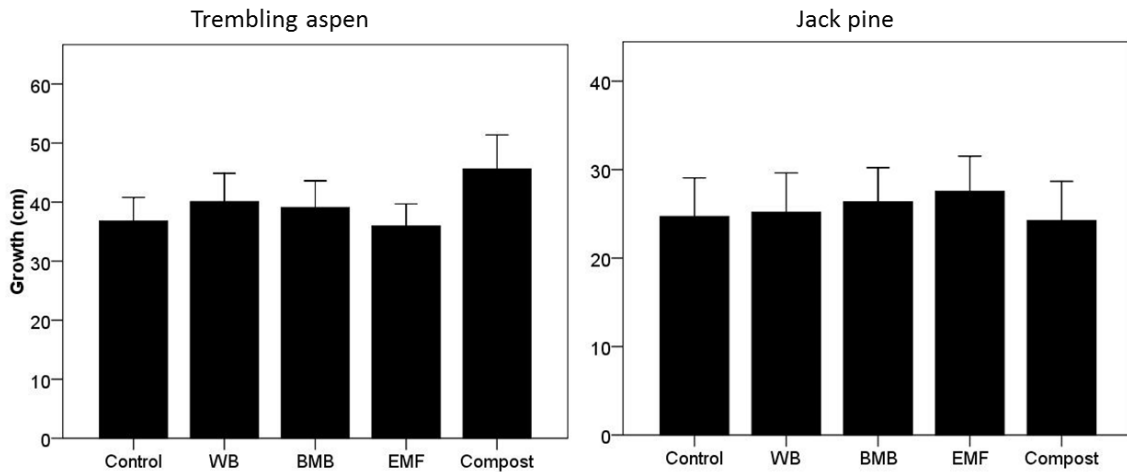


Figure 3.8 Growth (cm) of tree species trembling aspen and jack pine from amended [ectomycorrhizal fungi inoculant (EMF), compost, willow biochar (WB), bone meal and meat biochar (BMB)] and control field sites. No significant ($p > 0.05$) differences between amendments based on Kruskal-Wallis and Games-Howell tests. Error bars represent ± 2 standard errors of the mean.

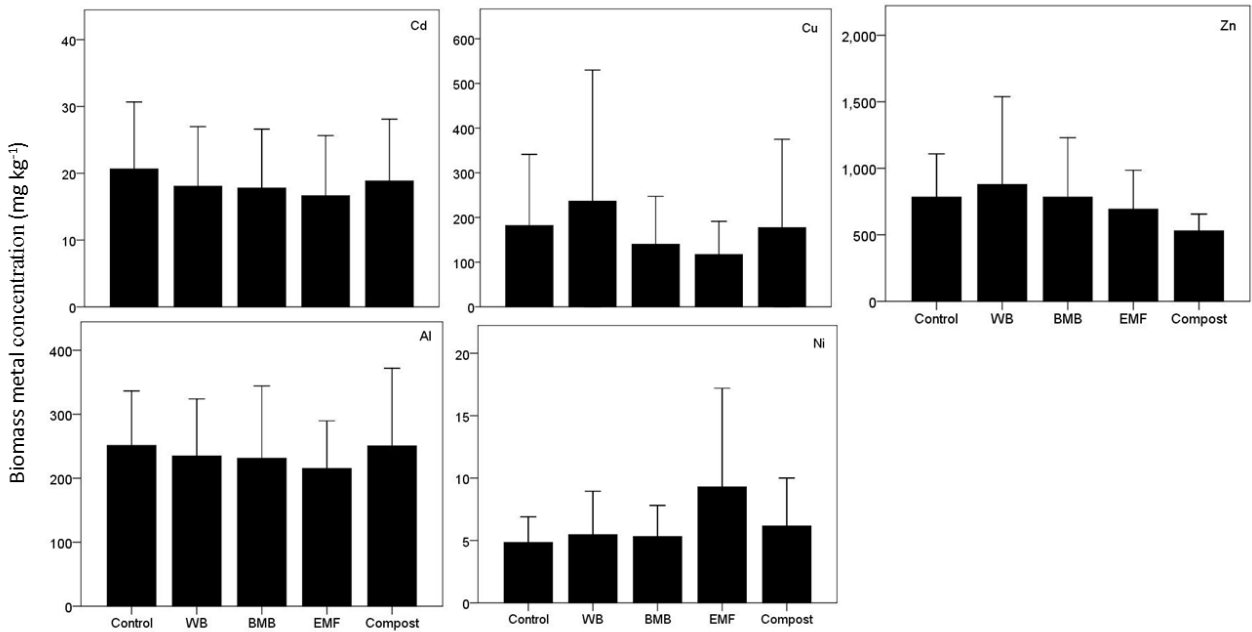


Figure 3.9 Trembling aspen biomass metal concentrations (Cd, Cu, Zn, Al and Ni) from control and amended [ectomycorrhizal fungi (EMF), compost, willow biochar (WB), bone meal and meat biochar (BMB)] sites. No significant ($p > 0.05$) differences between amendments based on Kruskal-Wallis and Games-Howell tests. Error bars represent ± 2 standard errors of the mean.

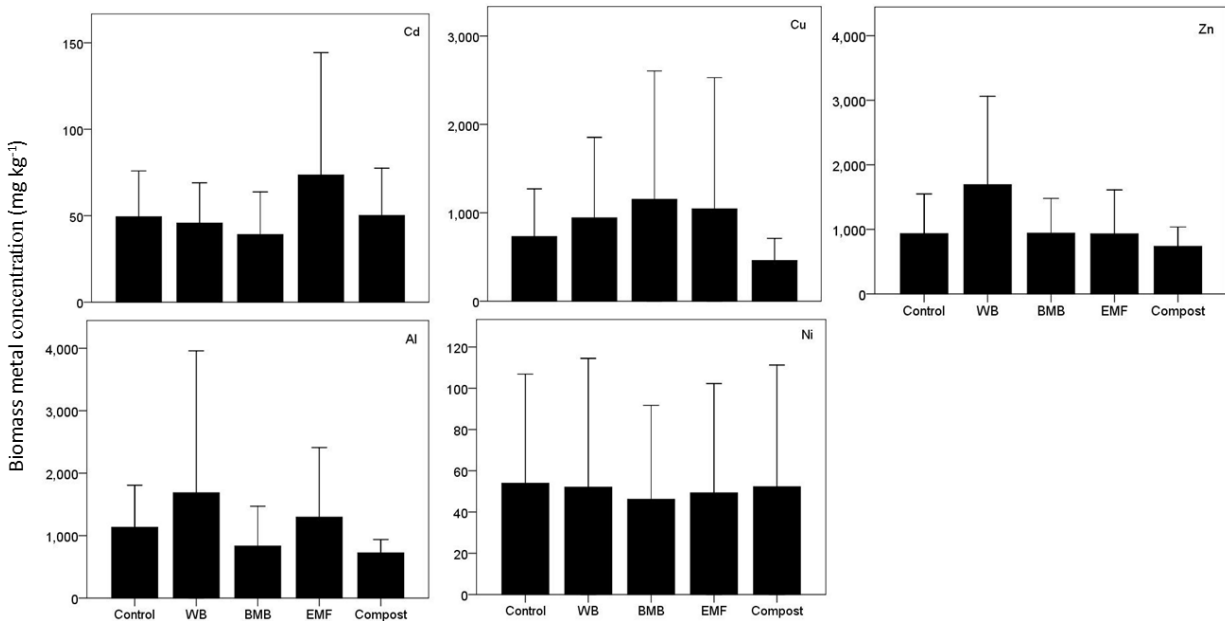


Figure 3.10 Jack pine biomass metal concentrations (Cd, Cu, Zn, Al and Ni) from control and amended [ectomycorrhizal fungi (EMF), compost, willow biochar (WB), bone meal and meat biochar (BMB)] sites. No significant ($p > 0.05$) differences between amendments based on Kruskal-Wallis and Games-Howell tests. Error bars represent ± 2 standard errors of the mean.

3.6 Discussion

The study sites represented a varying range of landscapes and distances from the smelter stack. The result was different soil characteristics between the 12 field sites. Others have reported that the cation exchange capacity of Dystric Brunisols, determined by assessing the exchangeable cation capacity via extraction using NH_4NO_3 had a range of 2.93 to 9.55 $\text{cmol}_c\text{kg}^{-1}$ for the eluviated layer of the profile and a range of 1.41 to 5.20 $\text{cmol}_c\text{kg}^{-1}$ for horizons at greater depths (Borge, 1997). The CECe range of the reference and field sites in our study, was from 2.08 to 8.98 $\text{cmol}_c\text{kg}^{-1}$, and thus are within the range of typical CECe values for Dystric Brunisolic soils for the soils at the initial time of planting. As expected, the Ca, Mg, Na and K values for the field study soils also fell into the same ranges as found in the research conducted by Borge (1997). The Ca:Mg ratio should be no greater than 20:1 as it can create a Mg deficiency for plants (Borge, 1997). This risk can be minimized by using dolomitic limestone such as the limestone applied in Flin Flon/Creighton. None of the sites at the initial time of planting had a high Ca:Mg ratio. Generally, the TC content was higher in the study sites compared to the Sherridon reference site. Mycock (2011) accounted for variation in TC by peaty phase soils that are underlain by mineral soils.

In Sudbury, jack pine had a survival rate of 78% after seven years of growth and annual average height increase of 41 cm (Winterhalder, 2000). In Flin Flon/Creighton the survival rate of jack pine at the end of the field trial was 77% with an average height increase of 32.3 cm for the 240 jack pine trees planted under all amendments. The growth of jack pine and trembling aspen was expected to be the highest in the first growth season as both species growth are influenced, at least in part, by bud formation in the previous year. The one year-old seedling plugs planted in the first growing season in Flin Flon/Creighton had been grown in ideal conditions in a nursery; therefore, the bud formation did not have the same environmental stresses that would be imposed on bud formation on these trees once planted and growing in Flin Flon/Creighton for a complete season. Also, because the roots are transplanted in soil from the nursery and need time to grow and adapt to the soil in Flin Flon/Creighton, outside of the original transplanted root ball, more damage may occur subsequent to the first growing season. Visible injury and retarded growth and development are two of the responses of stress in plants identified by Kozłowski et al. (1991). The visible injury and retarded growth and development between the time of planting and the time of harvest were clearly identified in the photographs in

Fig. 3.3 for some of the sites. One of the benefits of planting a variety of species in the area as tree plugs or understory establishment is it creates a seed source for other areas to establish (Winterhalder, 2000).

The correlations between growth responses and soil characteristics (Table B.1 and Table B.2) generally were weak, ranging from $r = 0.25$ to $r = 0.81$. In some instances, these relationships were reflected in positive correlations between soil metal and corresponding shoot metal uptake, particularly for Cu and Zn. Significant, although weak, correlations between variables such as soil Zn and shoot Cu suggest that some relationships were not necessarily causal, but occurred because soil Zn and Cu levels varied together as a consequence of the mode of deposition. This was particularly evident in terms of correlations between levels of metals in the root tissue and soil metal levels. In general, these correlations were relatively strong and highly significant.

There were no significant differences in growth for the trees or the understory under the different soil amendments. This data suggests that no additional benefits were achieved beyond those associated with applying dolomitic limestone and fertilizer. Additionally, surface applying amendments may have limited efficacy compared to mixing them into the soil which would allow more immediate effects to the root zone of the plants, which is a challenge commonly encountered with *in situ* remediation (Environmental Protection Agency, 2007).

The reference site, which was located a considerable distance away from the 12 field sites in Flin Flon/Creighton, was classified as a Dystric Brunisol due, in part, to the pH being less than 5.5 and supported a well-established forest stand, common for these types of soils in Canada. The pH of the A horizon in a typical Dystric Brunisol typically ranges from 3.5 to 3.7 and increases with increased depth in the profile. The C horizon below 25 cm may have a pH higher than 5.5 as Brunisolic soils can have a calcareous parent material (Smith et al., 2011). The pH is not considered to be the main cause of vegetation dieback around Flin Flon/Creighton and the low pH values are not a direct result of smelter deposition affecting the soil (Scott, 2000). The pH data from this study supports this contention because the Sherridon reference site has a pH value (4.15) which is within the range of pH values from the study sites (3.83 to 5.84) that were devoid of vegetation closer to the smelter. However, one consideration is that very few of the Flin Flon/Creighton sites developed exhibited soil horizons that were similar to the observed typical horizon formation at the reference site due to past erosion activity. The plants therefore

may not have the same rooting zone in which to establish and grow, as would be the case in an established forest stand.

The pH of the soil was expected to increase from the start of the field trial to the completion of the field trial due to the addition of dolomitic limestone, fertilizer and amendments; however, there were no trends observed among the sites that supports this expectation. Winterhalder (2000) experienced similar results with the pH of the soil not increasing one year after liming in Sudbury. The pH in some areas of Sudbury actually decreased initially but saw the increase in pH occur after a few years (Winterhalder, 2000). The delayed pH response was attributed to plants changing the total base levels over time (Winterhalder, 2000). The process of changing the total base levels is described as a cation pump which, in the presence of neutralizing species (such as trembling aspen), can create and maintain a more neutral forest floor than pine species which will increase the acidity of the forest floor through biological activities (Aber, 1987). The effects of a cation pump system may potentially be limited in Flin Flon/Creighton due to the shallow soil profiles and farther experimentation would need to be conducted to assess potential limitations. Sites 2 and 12 originally had the highest pH values of 4.48 and 5.84, respectively, and had a decrease in pH throughout the field trial under all of the amendments.

A study looking at chemical properties of forest soils reported a base saturation range of 44.1 to 58.2 % (Schmidt et al., 1996). The percent base saturation was higher in Flin Flon/Creighton for the reference site in comparison to the pre-planted site soils. The same observation was made by Schmidt et al. (1996) that base saturation decreased after an area was disturbed. Therefore the lower base saturation was expected from Flin Flon/Creighton due to soil being disturbed and smelter-impacted.

The base saturation of all sites increased from the initial time of planting to the time of harvest. This increase was expected as a consequence of the extent of disturbance in the area, however, the increase in base saturation was not consistent with the soil pH as base saturation will generally increase as pH increases. In this experiment, there was not uniform increase in pH across all sites, as was observed for base saturation. This suggests that there were other factors involved with the increase of base saturation created by the amendments, liming, fertilizer and plant growth. One of the known benefits of amendments is the addition of organic matter to the soil which can lead to an increased base saturation. Although this study did not measure soil

organic matter directly, this contention is supported by the observation that EMF, which did not add appreciable organic matter, had less of an impact on base saturation than the other soil amendments. In contrast, the control sites generally had increased base saturation although no other amendment was applied to those areas.

The practicality of applying the amendment was a component of this field research because it is important to consider feasibility when developing a remediation plan. The WB amendment was not well suited for field application because it is relatively light and the majority applied was lost from the site by wind or water within 30 d after planting. The other three amendments were unaffected by heavy rains and wind in most cases. The EMF for the trees needed to be placed in the transplant hole compared to the other amendments that are amendable to surface application.

3.7 Conclusion

The reference soil from Sherridon, which supports a healthy forest stand, was used as a standard to which certain soil properties for the Flin Flon/Creighton area were compared. Comparisons indicate that decreased available soil metal content needs to be achieved along with an increased base saturation and pH of the soil to achieve revegetation goals. Building on this concept, it is suggested that the compost amendment increased pH in the greatest number of sites and increased base saturations to levels closer to that found in the reference site. Compost also had the best understory establishment in many of the sites. These observations suggest that compost application may be one of the most beneficial for Flin Flon/Creighton revegetation in areas currently devoid of vegetation, when used in combination with dolomitic limestone and fertilizer.

The variable results between sites indicate that there are challenges in the revegetation of Flin Flon/Creighton due to the past influence of the activities within the area and that further research is necessary. The relationship between increased soil available metals and decreased plant growth indicated that this may be one of the greater factors influencing the revegetation of Flin Flon/Creighton. Of particular interest is the relationship between soil available Cu and biomass Cu in the tree species. The time frame for this project was relatively short and it would be interesting to examine the results of a project similar to this to see if the depth of soil in Flin Flon/Creighton will influence tree survival over time.

4. THE EFFECT OF DIFFERENT MOISTURE REGIMES ON THE EFFICACY OF SOIL AMENDMENTS USED TO ENHANCE PLANT SURVIVAL AND GROWTH IN SMELTER-IMPACTED SOILS

4.1 Preface

The influence of moisture on the success of a revegetation project in Flin Flon/Creighton is of interest. Different moisture regimes may influence the efficacy of different amendment treatments. The same amendments that were used in the field trial (not including willow biochar) of this study (Chapter 3) were used in a growth chamber trial to determine if soil moisture affects the success of amendments intended to enhance plant survival and growth. Success was measured by assessing the survival and growth of vegetation, ectomycorrhizal fungi colonization, levels of plant uptake of metals, and residual soil metal levels. In doing this, it will be known if moisture in combination with amendments will affect the success of a revegetation project in Flin Flon/Creighton.

4.2 Abstract

A growth chamber trial was set up at the University of Saskatchewan to determine the effect of moisture on the revegetation of smelter-impacted field soils from Flin Flon/Creighton, treated with different amendments. Soil amendments [microbial inoculant (EMF), compost and bone meal and meat biochar (BMB)] were selected based on use from a field trial. Each pot had one 1-yr- old seedling [trembling aspen (*Populus tremuloides*. Michx.) or jack pine (*Pinus banksiana*. Lamb.)] and two understory species [tufted hairgrass (*Deschampsia cespitosa* L.) and American vetch (*Vicia americana* Muhl.)] as seeds planted in it. Two different moisture regimes (low and high) were established and measures of plant survival and growth were used to determine the impact of the different moisture regimes. Other measurements used to assess the influence of moisture were soil and plant metal concentrations and ectomycorrhizal fungal colonization in the roots of the plants. It was generally found that moisture had no influence on biomass, production, amendment success, or soil and biomass metal concentrations. There was significantly more growth of understory species (tufted hairgrass) when compost and BMB were applied compared to the control or the commercial mycorrhizal inoculant.

4.3 Introduction

Soil moisture is an important factor to be considered in remediation projects because the successful establishment of tree species and efficacy of amendments can be influenced by available moisture in site areas. Because examining moisture relationships can be difficult on sites with variable terrain it is more practical to the relationships in the controlled setting of a growth chamber where the temperature and moisture levels can be regulated. Organic soil amendments are known to enhance water retention and therefore the relationship between contaminated soil, soil amendments and vegetation may provide valuable information about the likely success of an amendment in a proposed revegetation plan. Ectomycorrhizal fungi are also known to increase the ability of a plant to adapt to different moisture levels (Dodd and Thomson, 1994; Wilkinson and Dickinson, 1995). Soil moisture can influence the survival and growth of vegetation. Metal mobility and the uptake into plants can be influenced by soil moisture (Angle et al., 2003). A growth chamber experiment was conducted to examine the influence of different levels of moisture on survival and growth of two tree species and two understory species treated with different amendment applications.

4.4 Materials and Methods

4.4.1 Soil collection and plant preparation

Soils were collected from Flin Flon/Creighton in August 2011 from a variety of site areas. Sites 1, 2, 3, 4, 7 and 8, described in Chapter 3 (Fig. 3.1), were selected because the volume of soil available at those sites was large enough to support a growth chamber experiment and the sites were accessible by road to facilitate soil collection. Soils were collected to a 15-cm depth where possible; where soils were shallower than 15 cm, the soil was collected until bedrock was reached. Some soils were collected in areas where bent grass (*Agrostis capillaris*) was established. In these areas the bent grass was removed by hand prior to soil collection but some dormant seeds were collected along with soil samples. Soils were air dried and bulked for the growth chamber experiment. The soil was sieved to 5 mm and thoroughly mixed using a drum barrel mixer. Soil also was collected from a reference site (Sherridon, MB) which was located far enough away from the smelter complex to not be visually affected by the smelting operation. Sherridon soil is classified as an Eluviated Dystric Brunisol. Soil from Sherridon was collected to a 15-cm depth and was analyzed as a comparison of a healthy forest stand soil compared to the smelter-impacted soils that were used in the growth chamber trial.

The shipment of 1-yr-old seedlings (trembling aspen and jack pine) was received in September of 2011 from Tree Time Services Inc. (Edmonton, Alberta, Canada). The trees were headed into dormancy and were put through the winter season planted outdoors in topsoil in sunken pots for easy extraction in spring. The trees were then slowly warmed to 20°C over a period of 7 d to bring them out of dormancy and were planted in the growth chamber in early spring of 2012.

4.4.2 Experimental design

There were a total of 96 15-cm diameter pots, containing 1450g of air dried soil (± 10 g in the root ball), used in the growth chamber trial. Amendments used in the growth chamber trial were a commercial ectomycorrhizal inoculant (EMF), municipal compost, and bone meal and meat biochar (BMB) (Table 3.1). Each pot received fertilizer, crushed dolomitic limestone and an amendment, with the exception of the control pots which received only the fertilizer and crushed dolomitic limestone application. The amount of fertilizer used was 2.74 g of slow-release fertilizer (20-17-10) and 0.15 g of crushed dolomitic limestone per pot. The municipal compost and BMB application rates were calculated based on the pot diameter (15 cm), 2.5 cm

soil depth, amendment bulk density and 10% w/w, and were intended to simulate field application rates.

The mycorrhizal inoculant was a commercial product with active endomycorrhizae (*Glomus intraradices*) and ectomycorrhizae (*Pisolithus tinctorius*, *Scleroderma cepa*, *S. citrinni*, *Rhizopogon roseolus*, *R. subscaerelescens*, *R. villosulus*, *R. vulgaris*, *Laccaria laccata*) ingredients (Myke[®] Pro Landscape, Premier Tech Biotechnologies, Riviere-du-Loup, Quebec, Canada). The manufacturers' rate of application for the EMF product was 28.4 mL per 10.16 cm² area which equaled 1.5 g per understory area as a surface amendment. An additional 5 g was put directly into each of the holes where the tree plug was planted (manufacturers' rate chosen based on height and diameter of seedlings). Each pot had one tree species (trembling aspen or jack pine) as a 1-year old seedling and both understory species [Tufted hairgrass (0.0055g) and American vetch (0.044g)] as seeds planted in it.

The trial ran for 90 d and had two different watering treatments used with six replicates of each tree species (trembling aspen and jack pine), with each amendment and a control. The trees were grown with an 18 h daylight/6 h night cycle. The growth chamber had a day temperature of 24 °C and a night temperature of 18 °C. Half of the pots were watered at a low watering regime and half the pots were watered at a high application rate. The low watering regime was achieved by allowing the pots to dry down to 35 % field capacity and then wetting them back to 100 %. For the high watering regime, the pots dried down to 75 % field capacity and then rewetted back up to 100 % field capacity. The pots were re-randomized at the time of watering. The soil for each pot was weighed at the time of planting and the weights at 100 % field capacity were determined.

4.4.3 Growth measurements

Each seedling (combined soil, root and shoot) was weighed individually before being planted. The root and shoot biomass and understory weights were recorded at the time of harvest (90 d). The height and basal diameter of the tree seedling shoots were measured at 60 d and 90 d. Chlorophyll readings were taken using a SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc.) for each trembling aspen tree at 60 d and 90 d. The meter takes a reading of how much light is able to pass through the leaf tissue. Thirty measurements were recorded from each tree at 60 d and 90 d.

At time of harvest (90 d) a 5 g (wet weight) sample of each tree root mass was taken and stored in distilled water at 20°C until ectomycorrhizal counts were completed. The leaf area of each trembling aspen tree was determined using the LI-3100C area meter (LI-COR Inc.) and recorded at the time of harvest. The plant matter (root and shoot separately) was ground to 2 mm for laboratory analysis.

Visual observations of the health of the trees were recorded at 0, 30, 60 and 90 d. Visual observations included the colour of leaves for trembling aspen and needles for jack pine; wilted leaves and leaves/needles that were experiencing necrosis were recorded based on percent compared to the visually healthy leaves/needles. The percentage of the surface area of the soil covered by understory was also recorded at 0, 30, 60 and 90 d.

4.4.4 Laboratory analysis

A soil sample from before the trial began at 0 d (baseline) was analyzed for pH, total carbon (TC), base saturation, exchangeable cation exchange capacity (CECe), NH_4^+ , exchangeable Ca, Mg, K, Na, and available S, Al, Cd, Cu, and Zn. The analyses performed on the soil samples were completed as previously described (Section 3.4.5.1). The metal analysis performed on the plant biomass was conducted as for the field trial (Section 3.4.5.2). A sample of trees (trembling aspen and jack pine) were randomly selected at day 0 to be analyzed to provide baseline data of the metal concentrations in the biomass prior to being grown in smelter-impacted soils. Mycorrhizal analysis was completed for all plants at the time of harvest, as previously described (Section 3.4.5.3).

4.4.5 Statistical analysis

Statistical analysis was performed using IBM® SPSS© Statistics (Version 20). All data were tested for homogeneity of variance using Levene's test ($p \leq 0.05$), and normality using the Shapiro-Wilk test ($p \leq 0.05$) because the data did not meet the criteria for homogeneity of variance nor normality, nor could the data be transformed to meet the criteria. Non-parametric statistics were utilized. Data were analysed using the Kruskal-Wallis test ($p \leq 0.05$) and means separation using the Games-Howell test ($p \leq 0.05$).

4.5 Results

4.5.1 Baseline soil characteristics

Initial soil characteristics and metal concentration prior to planting are reported in Table 4.1. In comparison to the Sherridon site the soil utilized in the growth chamber trial has a lower pH, base saturation and CECe and higher available metal concentrations, more sulfur and higher levels of NO_3^- and NH_4^+ .

Table 4.1. Chemical characteristics and metal concentrations in soils for bulked growth chamber soil.

Soil Property (n=2)	Growth chamber soil	Sherridon reference site soil
pH	3.98	4.15
	----- % -----	
Total Carbon	4.85	0.93
Base Saturation	15.0	72.14
	----- ($\text{cmol}_c\text{kg}^{-1}$) -----	
CECe †	2.48	3.21
Exchangeable Ca	0.12	0.58
Exchangeable Mg	0.03	0.16
Exchangeable K	0.07	0.07
Exchangeable Na	0.02	1.50
	----- (mg kg^{-1}) -----	
NH_4^+	1.05	0.48
Available S	1410	898.8
Available Al	41.3	32.78
Available Cd	7.30	-
Available Cu	210	1.20
Available Zn	730	2.14

† effective cation exchange capacity

4.5.2 Change in available soil metal

Figure 4.1 reports the change in available soil metal from initial time of planting (0 d) to harvest (90 d) for each treatment. The initial available soil metal content was determined before amendments were added. There was a decrease in available metal in the soil over 90 d but there were no significant ($p > 0.05$) differences between amendments or watering regimes. Planting different tree species similarly had no detectable significant impact on soil metal concentrations ($p > 0.05$) (data not shown).

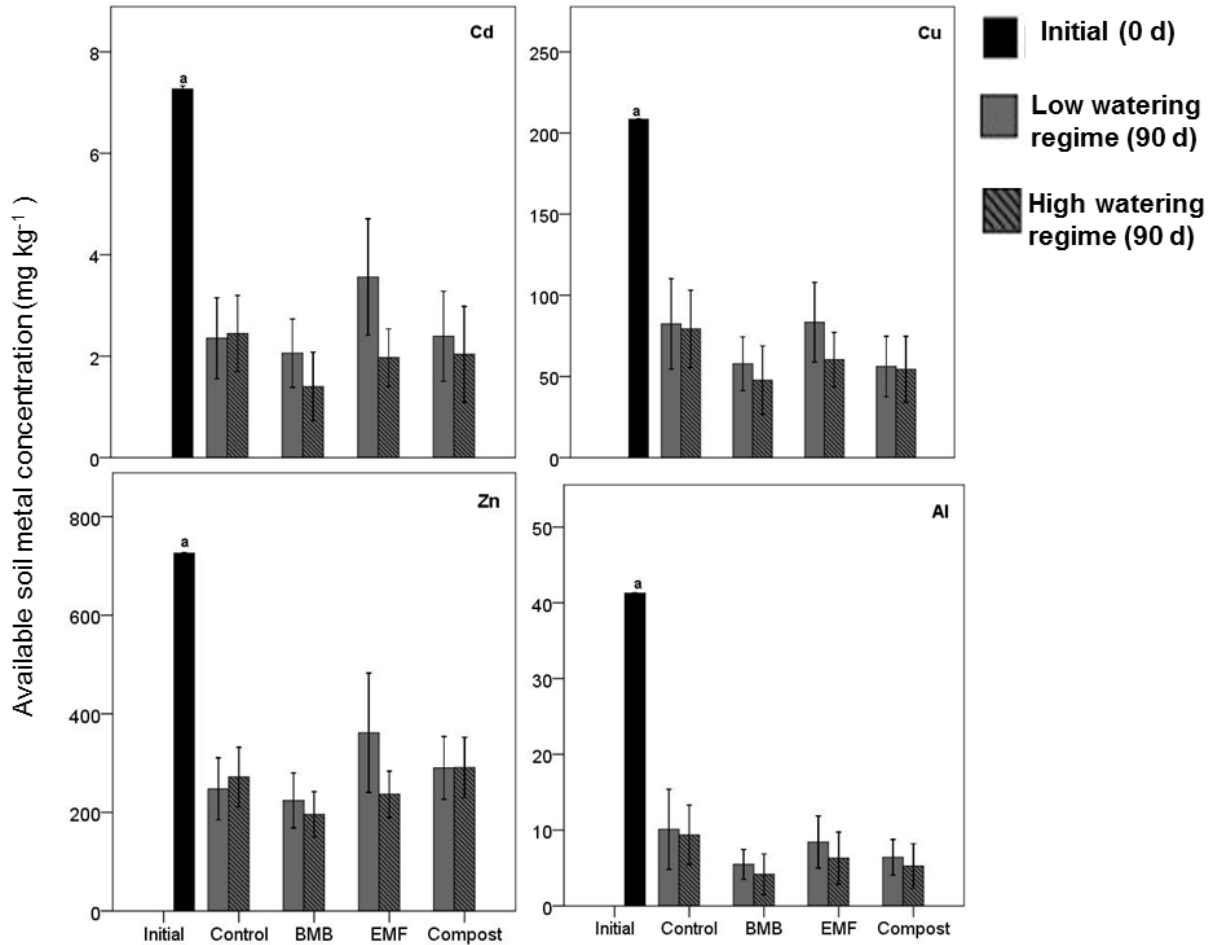


Figure 4.1 Available soil metal concentrations (Cd, Cu, Zn, Al) at the initiation of the study (0 d) and 90 d under each treatment [ectomycorrhizal fungi (EMF), compost, control and bone meal biochar (BMB)] and high and low watering regimes. Letters represent significant ($p \leq 0.05$) differences between amendments based on Kruskal-Wallis and Games-Howell test. Error bars represent ± 2 standard errors of the mean.

4.5.3 Ectomycorrhizal colonization

Ectomycorrhizal colonization of tree roots was determined at 90 d (Fig. 4.2). Colonization was enhanced in trembling aspen by application of EMF relative to all other amendments. Application of EMF similarly enhanced colonization of jack pine relative to the compost treatment, although no other significant differences were detected. Jack pine had no differences between the treatments at a high water regime.

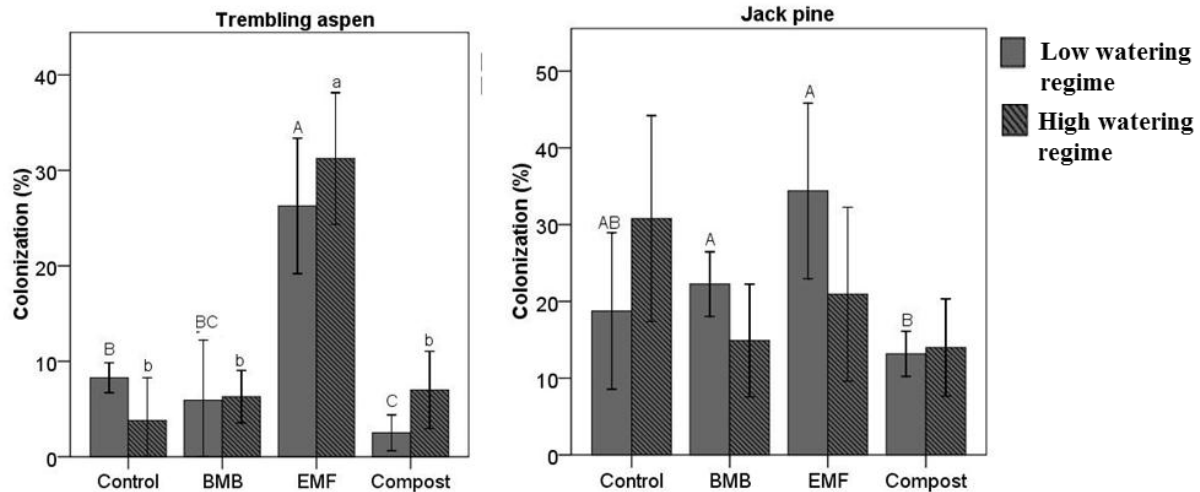


Figure 4.2 Percent colonization of ectomycorrhizal fungi in roots of trembling aspen and jack pine in the high (n=6) and low watering regime (n=6) after 90 d growth with amendments. Letters above bars represent significant differences ($p \leq 0.05$) based on Kruskal-Wallis and Games-Howell test. Lower case letters represent low watering rate and upper case letters represent high watering rates. Error bars represent ± 2 standard errors of the mean.

4.5.4 Survival and growth of understory and tree species

Biomass of roots and shoots of trembling aspen were not affected by amendment ($p > 0.05$) (Fig. 4.3). None of the amendments significantly enhanced shoot growth of jack pine relative to the control, whereas application of EMF resulted in a significant reduction in shoot growth. Similarly, application of EMF reduced root biomass relative to the control, which did not differ significantly from any other treatment (Fig. 4.3). The differences between high and low watering regimes on biomass production were analyzed and no differences ($p > 0.05$) were detected for the Kruskal-Wallis and Games Howell tests (data not shown).

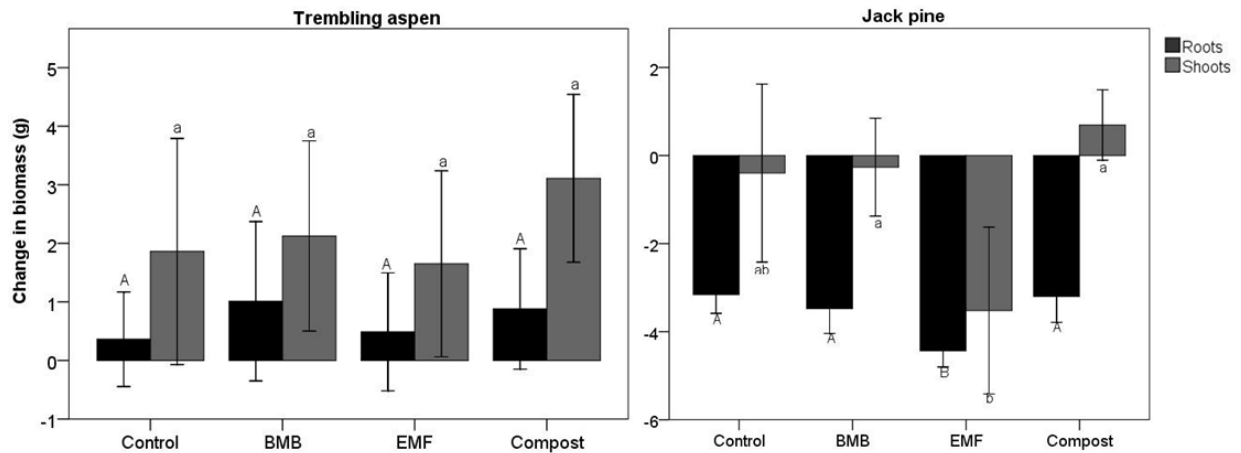


Figure 4.3 Average weight of roots (n=12) and shoots (n=12) of trembling aspen and jack pine grown with amendments [bone meal and meat biochar (BMB), ectomycorrhizal fungi inoculant (EMF), compost] and control averaged across watering regimes. Letters above the bar represent significant differences ($p \leq 0.05$) based on the Kruskal-Wallis and Games-Howell test. Capital letters apply to roots while lower case letters apply to the shoots. Error bars represent ± 2 standard errors of the mean.

Necrosis was observed (Fig. 4.4) in the leaves of the trembling aspen planted under the EMF treatment starting at 30 d and became more severe through the duration of the growth chamber trial (Table 4.2). Chlorosis and necrosis was observed among all treatments at 90 d but was first observed in the EMF treatment and was most prevalent in the EMF treatment at the time of harvest with greater than 70% of the plants showing signs (Table 4.2). Surprisingly, the chlorophyll readings did not indicate any differences ($p > 0.05$) in the amount of chlorosis and necrosis between treatments. There was no difference between treatments or watering regimes in the total leaf area at 90 d for trembling aspen ($p > 0.05$) (data not shown).

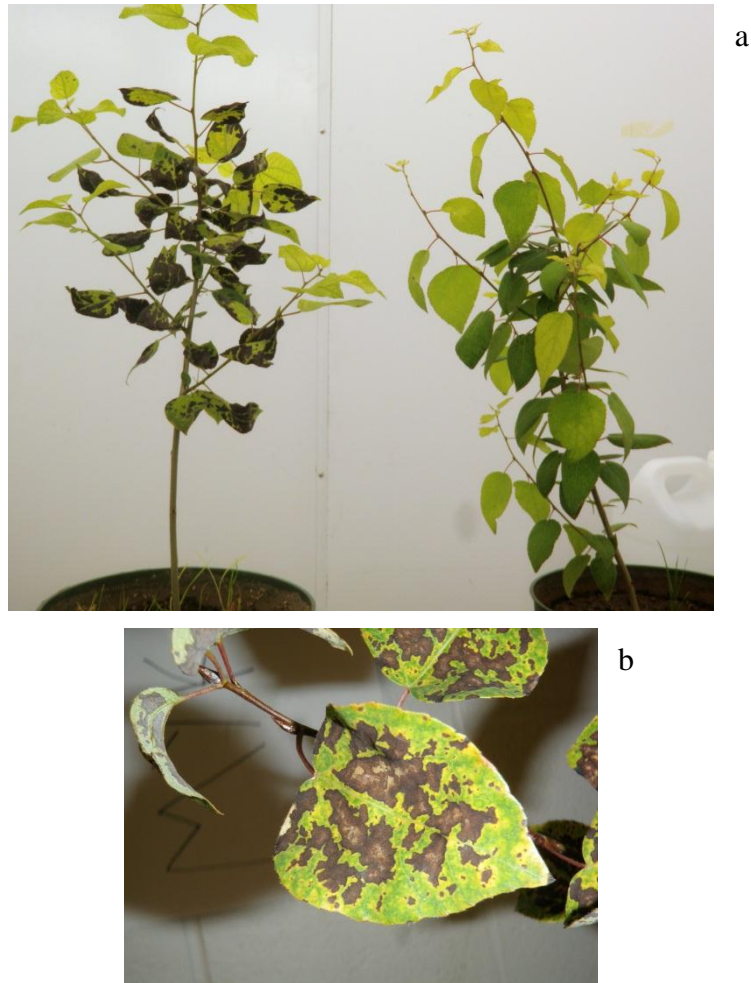


Figure 4.4 Photographs of trembling aspen in growth chamber trial a) left tree planted under ectomycorrhizal fungi inoculant (EMF) amendment showing signs of necrosis at 45 d compared to a tree typical of those growing under three other treatments (compost, BMB, control); b) Trembling aspen leaf displaying necrosis grown with the EMF amendment at 90 d.

Jack pine trees subject to the low watering regime, amended with EMF, compost or the control, yellowed and then turned orange within 60 d as signs of necrosis leading to the death of the trees. At 90 d, for both high and low watering regimes, jack pine displayed signs of necrosis with at least 70 % of the plants affected except for the compost treatment receiving the low water regime, that had slightly less observed damage (between 50 to 70 % of plants showing signs of necrosis) (Fig. 4.5). Jack pine grown with BMB amendment and the low watering regime compost and control did not show signs of stress until 90 d.

Table 4.2. Plants showing signs of necrosis throughout the growth chamber trial at 0, 30, 60 and 90 days for trembling aspen and jack pine species. – none, + 0 to 10 %, ++ 10 to 30 %, +++ 30 to 50 %, ++++ 50 to 70 %, +++++>70 %. Data provided for control, bone meal and meat biochar (BMB), ectomycorrhizal fungi inoculant (EMF) and compost amendments.

Days	Control	BMB	EMF	Compost
---Trembling Aspen - High watering regime---				
0	-	-	-	-
30	-	-	+++	-
60	-	-	+++	-
90	++++	+++++	+++++	++++
---Trembling Aspen - Low watering regime---				
0	-	-	-	-
30	++	-	++++	-
60	++	-	++++	++++
90	++++	+++++	+++++	+++++
--- Jack Pine - High watering regime---				
0	-	-	-	-
30	-	-	-	-
60	-	-	+++	-
90	+++++	+++++	+++++	+++++
--- Jack Pine - Low watering regime---				
0	-	-	-	-
30	-	-	-	-
60	++++	-	+	++
90	+++++	+++++	+++++	++++



Figure 4.5 Photographs of jack pine in growth chamber trial grown with ectomycorrhizal fungi inoculant (EMF), compost, bone meal and meat biochar (BMB) and control from left to right a) low watering regime; b) high watering regime.

Amendments did not affect the growth of bent grass (Fig. 4.6). Bent grass was found in the growth chamber trial because the seeds were dormant in the bulk soil collected and seeds germinated during the trial. Tufted hairgrass had significantly ($p \leq 0.05$) more growth under the compost and BMB treatments than the control or EMF treatments (Fig. 4.6 and Fig. 4.7). The success is also shown visually in Fig. 4.7, in which the compost and BMB are displaying a healthy understory community in comparison to the control and EMF treatments. Similarly, the understory associated with jack pine was most successful under the BMB and compost amendments (Fig. 4.5). American vetch had no survival under any of the conditions.

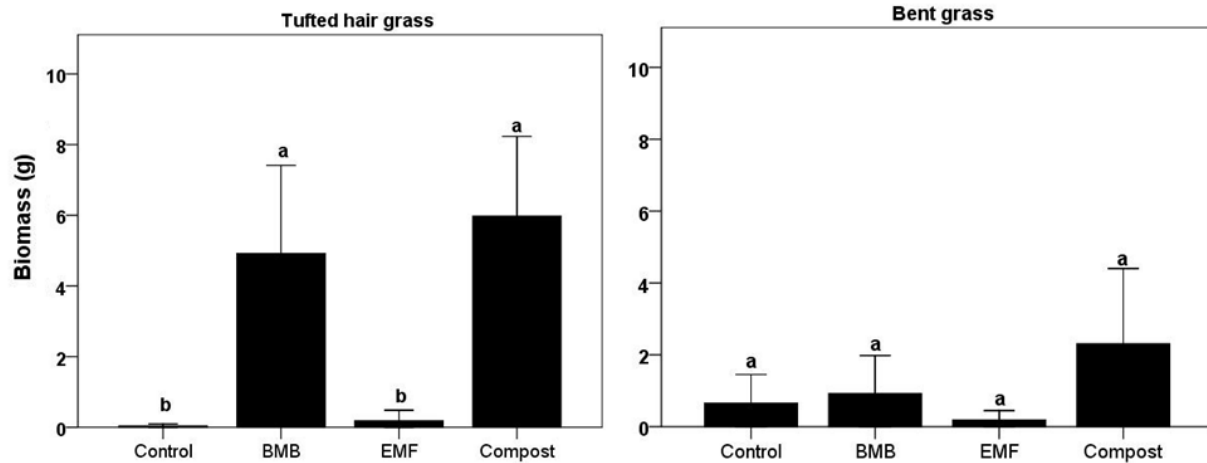


Figure 4.6 Average biomass of understory species bent grass (n=24) and tufted hairgrass (n=24) grown with amendments, ectomycorrhizal inoculant (EMF), compost, bone meal and meat biochar (BMB) and control. Letters above bars represent significant differences ($p \leq 0.05$) based on Kruskal-Wallis and Games-Howell test. Error bars represent ± 2 standard errors of the mean.



Figure 4.7 Photograph of tufted hairgrass from the growth chamber trial. Treatments from left to right are ectomycorrhizal inoculant (EMF), compost, bone meal and meat biochar (BMB) and control grown with trembling aspen.

4.5.5 Plant metals

Few significant differences between amendments for the low and high water regimes were detected for metal concentration in shoots and roots of trembling aspen (Fig. 4.8 and Fig. 4.9) or jack pine (Fig. 4.10 and Fig. 4.11). There were no significant differences in plant metal

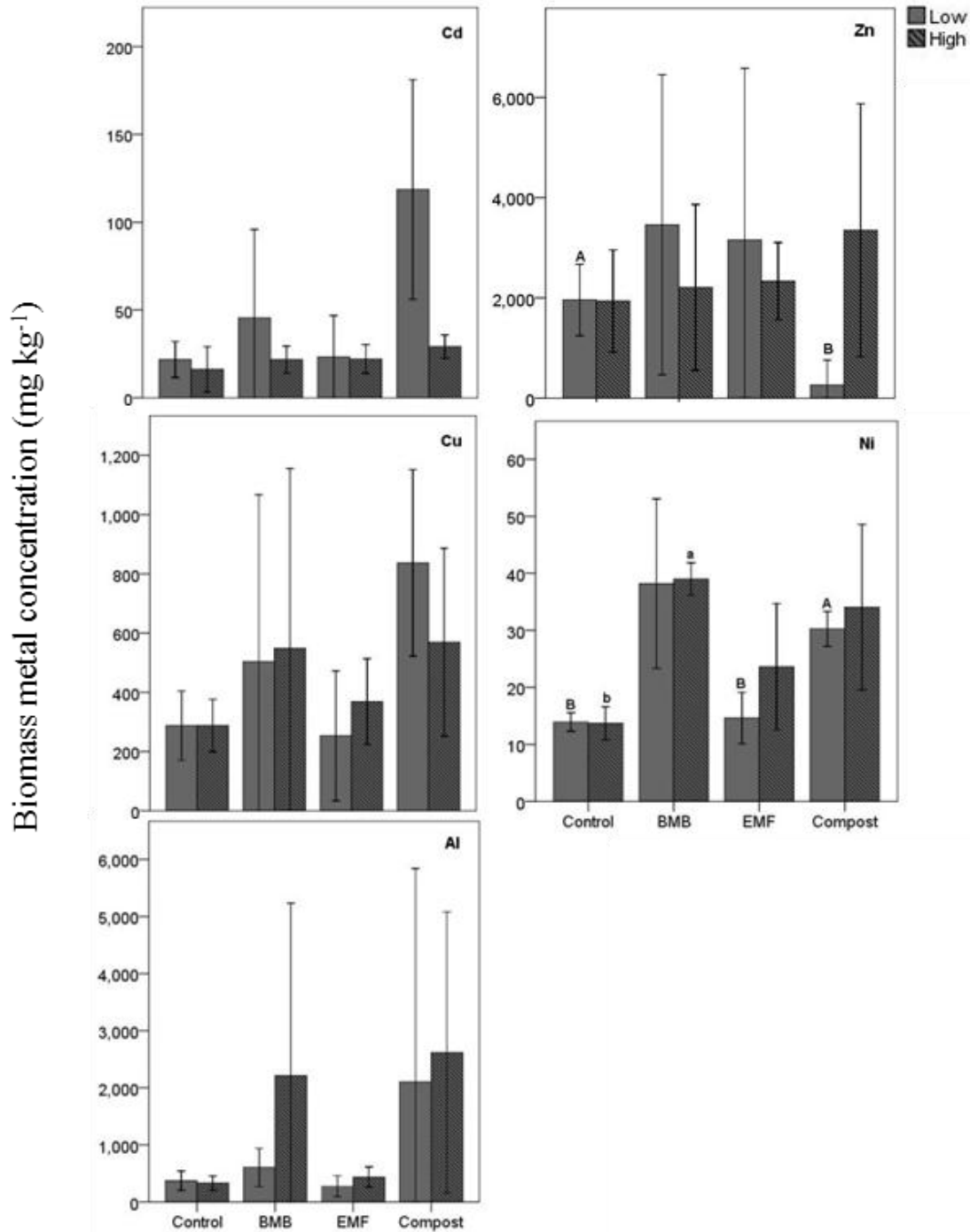


Figure 4.8 Metal concentration (Al, Cd, Cu, Ni, Zn) in roots for trembling aspen with amendments [ectomycorrhizal fungi inoculant (EMF), compost, control and bone meal and meat biochar (BMB)] and high and low watering regimes. Lower case (high) and capital (low) letters represent significant ($p \leq 0.05$) differences between amendments based on Kruskal-Wallis and Games-Howell test. Error bars represent ± 2 standard errors of the mean. Note differences of scale on y-axis.

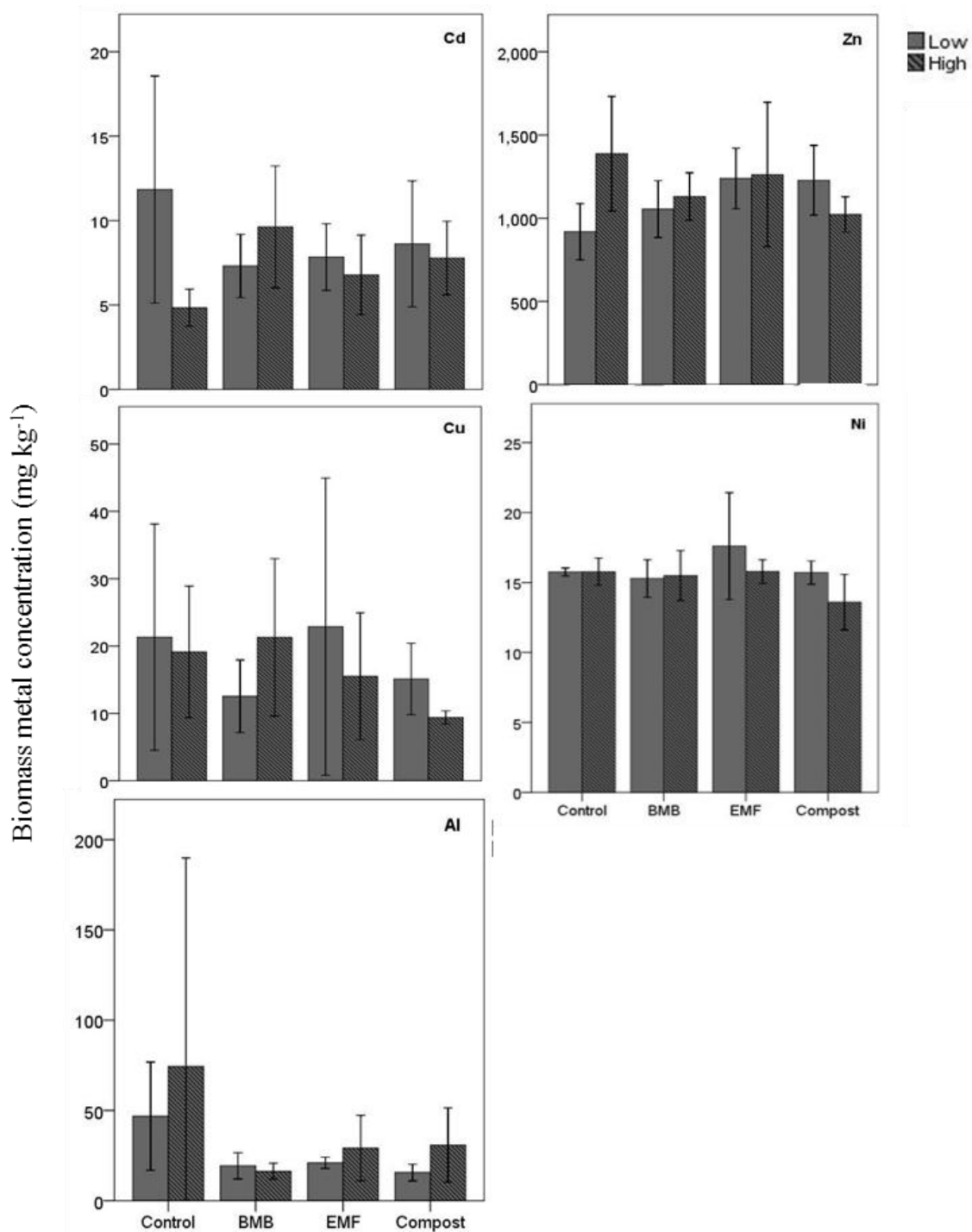


Figure 4.9 Metal concentration (Al, Cd, Cu, Ni, Zn) in shoots for trembling aspen with amendments [ectomycorrhizal fungi inoculant (EMF), compost, control and bone meal and meat biochar (BMB)] and high and low watering regimes. Lower case (high) and capital (low) letters represent significant ($p \leq 0.05$) differences between amendments based on Kruskal-Wallis and Games-Howell test. Error bars represent ± 2 standard errors of the mean. Note differences of scale on y-axis.

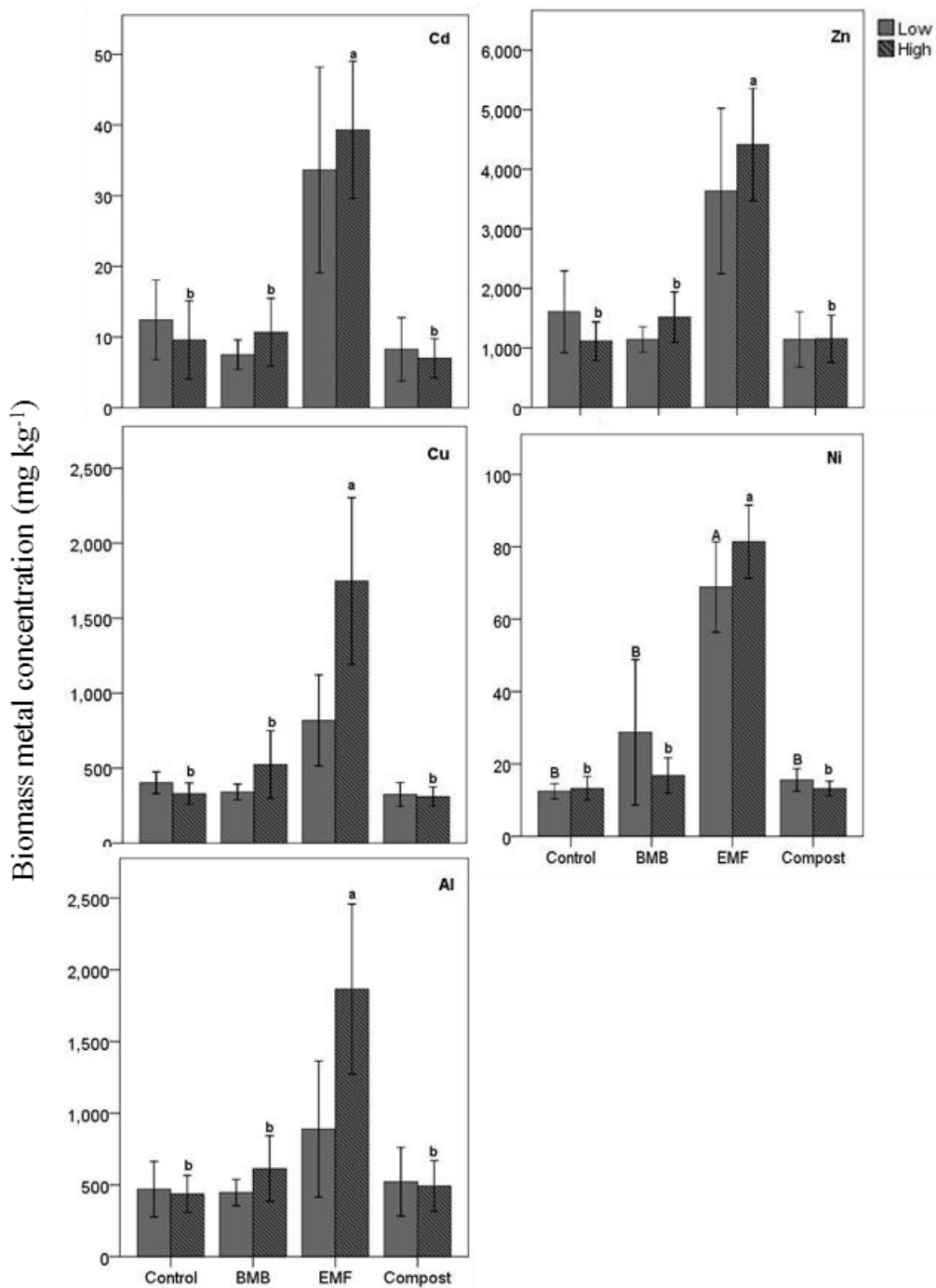


Figure 4.10 Metal concentration (Al, Cd, Cu, Ni, Zn) in roots for jack pine with amendments [ectomycorrhizal fungi inoculant (EMF), compost, control and bone meal and meat biochar (BMB)] and high and low watering regimes. Lower case (high) and capital (low) letters represent significant ($p \leq 0.05$) differences between amendments based on Kruskal-Wallis and Games-Howell test. Error bars represent ± 2 standard errors of the mean. Note differences of scale on y-axis.

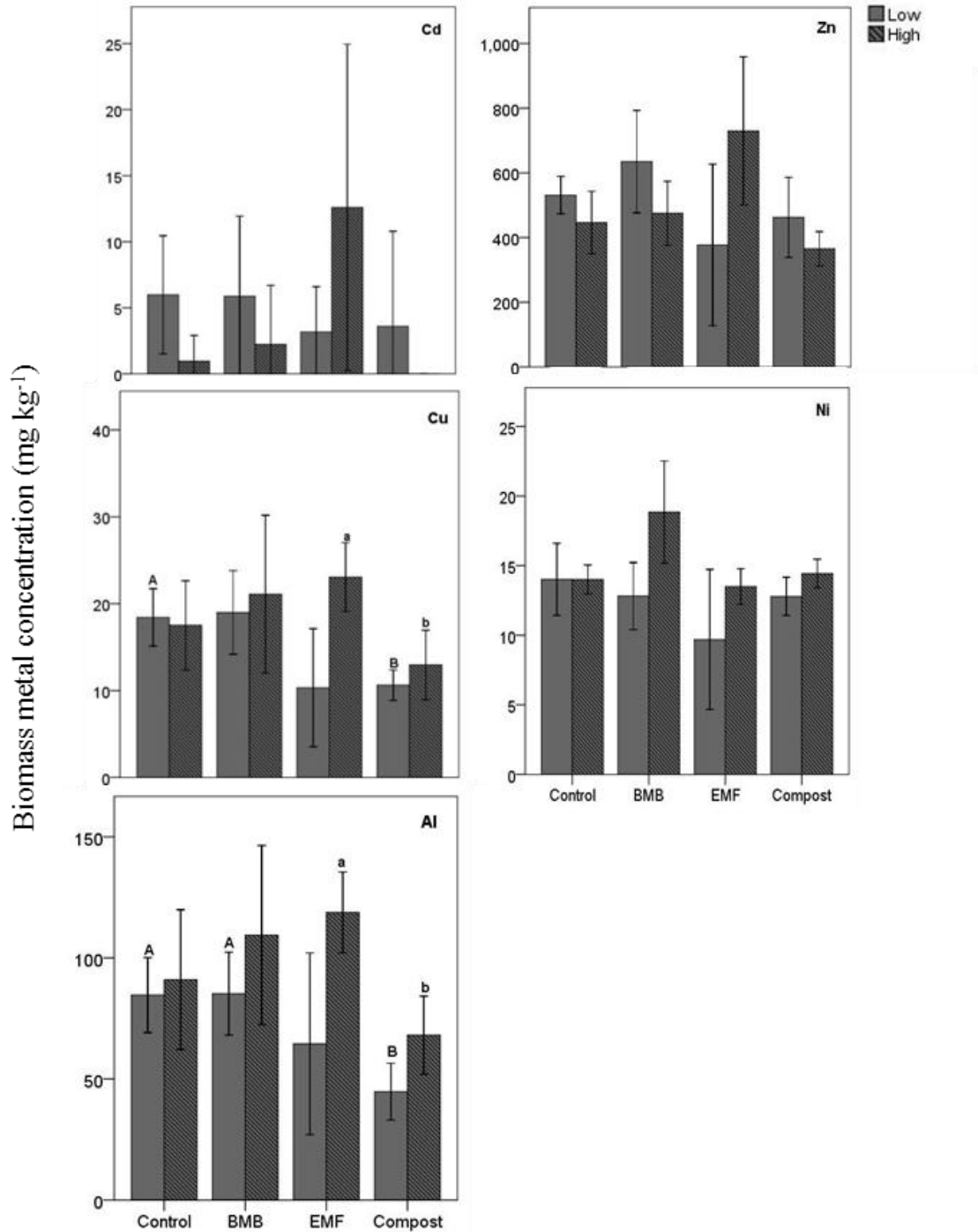


Figure 4.11 Metal concentration (Al, Cd, Cu, Ni, Zn) in shoots for jack pine with amendments [ectomycorrhizal fungi inoculant (EMF), compost, control and bone meal and meat biochar (BMB)] and high and low watering regimes. Lower case (high) and capital (low) letters represent significant ($p \leq 0.05$) differences between amendments based on Kruskal-Wallis and Games-Howell test. Error bars represent ± 2 standard errors of the mean. Note differences of scale on y-axis.

concentrations between the low and high watering regimes ($p > 0.05$) (data not shown). The only differences between amendments for trembling aspen ($p \leq 0.05$) (Fig. 4.8) were observed for Ni in roots where trees grown under the EMF and control amendments had reduced metal levels compared to the compost and BMB amendments. The Zn concentration in roots significantly differed between the compost treatment and the control treatment, with the control having greater metal concentration levels. Trembling aspen roots typically had greater metal concentrations for each metal than the shoots.

Significant differences between amendments were detected more frequently for jack pine than for trembling aspen (Fig. 4.10 and Fig. 4.11). All metals (Al, Cd, Cu, Ni, Zn) had increased concentrations in roots in the high watering regime where EMF was applied compared to the other amendments and the control. Nickel levels in roots exposed to the low watering regime also were higher in the roots exposed to EMF than the other amendments and the control. Similarly, greater metal concentrations were observed in the roots of jack pine compared to the shoots.

4.6 Discussion

It is difficult to establish the exact roles of amendment and plants in a system that is complex with multiple soil metals and other management factors (tree, understory, amendment, dolomitic limestone and fertilizer) present concurrently. It was expected that the amount of moisture present would influence the rate of uptake and movement of available metals in the soil in the growth chamber trial thereby influencing the efficacy of the amendments. There were reductions of available metals in soil from 0 d to 90 d ($p \geq 0.05$), however, no significant differences occurred between amendments or water regimes. This could indicate that all the amendments resulted in less available metal in the soil through mechanisms such as binding or plant uptake. However, since there were no differences detected between the control that received only application of dolomitic limestone and fertilizer it could be that the amendments were having no effect on available soil metal and that effects were a result of the dolomitic limestone and fertilizer application. Interestingly, the compost and BMB amendments which had success with understory survival and growth did not have less available soil metals at the end of the trial compared to the EMF and control amendments. This could indicate that the amount of available soil metal was not a factor in the survival and growth of the understory.

In both trembling aspen and jack pine trees there was higher concentration of metals in the below ground biomass (roots) than the aboveground biomass (shoots), indicating that metals are being taken up into the roots and stored within root tissues at a higher concentration rather than being translocated into the stems and leaves of the plant. There were no significant differences in biomass metal between the high and low watering regimes under any amendment or for any metal. It was expected that there would be a change in the response of biomass metal concentrations due to different soil moistures.

Trembling aspen had significantly more Ni uptake into the roots under the compost and BMB amendments than under the control and EMF amendments and significantly less uptake of Zn under the compost amendment than the control treatment. The properties of compost could be causing decreased availability of Zn and increased availability of Ni to the plants.

Generally, there were no significant differences for biomass (roots and shoots) of jack pine or trembling aspen with the high and low watering regimes. In comparing amendments, jack pine trees grown with the EMF amendment lost significantly ($p \geq 0.05$) more root and shoot biomass throughout the duration of the trial than the trees grown with the other amendments. Jack pine species lost biomass over the course of the trial with the exception of shoots grown with compost. Trembling aspen had increased biomass with each amendment and the control. Trembling aspen had no significant differences ($p < 0.05$) between amendments. The lack of difference in the total leaf area at 90 d between treatments indicated that there was not a reduction or increase of leaf area for trembling aspen and suggests that the soil did not cause a reduction in the trees ability to leaf out but that the damage was in the continued survival of the tree.

Bent grass was not affected by the different amendments. Bent grass is an invasive species commonly found in Flin Flon/Creighton. It already survives in the soil on site without the aid of fertilizer and/or amendments. There were no significant differences for understory biomass between the high and low watering regimes. It was expected that there would be a change in plant survival and growth due to drought stress (low watering regime) or saturation (high watering regime). There was significant ($p \geq 0.05$) biomass increase for the tufted hairgrass under the compost and BMB amendment in comparison with the EMF and control treatments.

Studies using either birch trees or trembling aspen trees with ectomycorrhizal colonization used in a heavy metal contaminated soil found that the fungi minimized the uptake

of metals into the aboveground part of the plant (Bojarchzuk and Kieliszewska-Rokicka, 2010; Langer et al., 2012). This was not consistent for trembling aspen or jack pine grown with the EMF amendment. Trembling aspen or jack pine did not have significantly lower shoot metal concentrations in comparison to the control pots. Langer et al. (2012) observed that the protection capacities of mycorrhizal fungi may be limited in young trees by toxic environment and subsequent nutrient deficiencies that may be present in environments that are less than ideal such as areas like Flin Flon/Creighton. Several studies suggest that the main factor of metal uptake and plant success is the metal concentration of the soil before remediation as well as selecting metal tolerant tree genotypes (Shetty, 1994; Hartley, 1999).

Increased ectomycorrhizal colonization was observed for the EMF amendment for trembling aspen under both high and low water regimes compared to the compost amendment and control. Increased colonization also was observed compared to BMB, but differences were not statistically significant. There was also an increase in colonization under the low watering regime for EMF compared to compost for jack pine. This was expected as the EMF provides inoculant for the tree species as spores applied in the commercial product used. The expected consequential result of increased colonization would be increased nutrient and water uptake by the plant which would consequently could lead to increased plant survival and growth particularly in situations where there are not ideal growing conditions (Auge, 2001; Sylvia 2005). Rousseau et al. (1994) applied ectomycorrhizal fungi to *Pinus taeda* L. and found that the inoculation increased the overall surface area of the roots resulting in increased nutrient and water uptake. This was not observed in this study as jack pine (roots and shoots) and understory species had less biomass survival and growth under the EMF amendment in comparison to the other amendments. Alternatively, increased colonization might lead to a greater uptake of metals. Heggo and Angle (1990) found, in soybeans, that ectomycorrhizal response to heavy metals present in soil is related to the metal concentration initially in the soil. Soils that initially have high levels of heavy metals reduce the amount of colonization of the fungi and then then the foliar concentration of metals increases and where the colonization is not reduced there is less metal uptake into the plant (Heggo and Angle, 1990). Trembling aspen (i.e., both roots and shoots) did not have increased metal uptake from colonization. Observed necrosis for trembling aspen grown with the EMF amendment compared to other amendments and the control was expected to be related to increased metal uptake. Jack pine roots, however, had significantly

increased metal concentrations for every metal under the high watering regime and the EMF amendment and for Ni under the low watering regime. A study conducted using *Pinus sylvestris* grown with ectomycorrhizal fungi found that Cd was the most toxic to the trees and the multiple metal sites had lower toxicity than the single metal trials, due to metal binding. However, there was an overall reduction in plant growth when grown in the contaminated sites (Hartley et al., 1999). Necrosis was observed in the jack pine trees under the EMF amendment at 60 d and was thought to be a product of metal intolerance in the trees in the trial.

It would have been interesting to analyze plant samples from the affected EMF colonized trembling aspen compared to trembling aspens grown under other amendments at 30 and 60 d for metals to observe any effect of early uptake due to colonization of ectomycorrhizae. The effect of early uptake may not be clearly observed at the time of harvest, as many of the trees had significant necrosis by the end of the trial; however, EMF displayed earlier signs of necrosis compared to all other treatments, suggesting that colonization enhanced early metal uptake and toxicity.

4.7 Conclusion

Moisture regime had no effect on the amount of biomass survival and growth under with the trees or the understory species or on the amount of metals in the biomass. The pots that had EMF amendment applied had earlier onset of necrosis (high watering regime) and significantly more loss of biomass for the jack pine individuals compared to the other amendments. Necrosis also was observed earlier in the low watering regime for the trembling aspen. Generally, trees developed some levels of necrosis by the end of the 90 d growth trial. Therefore, none of the amendments in combination with the watering regimes provided a viable amendment option for a revegetation program. The jack pine seedlings lost biomass (roots and shoots) throughout the growth chamber trial under all treatments, with the exception of the shoots under the compost amendment. Trembling aspen had increase although not significant in all treatments throughout the growth chamber trial indicating that trembling aspen has more potential for revegetation practices in Flin Flon/Crieghton.

One area of important findings was the increased growth and survival of the understory species under the BMB and the compost treatments compared to the control and EMF treatments. This indicates that in terms of understory growth, the BMB and compost amendments are the most viable option for revegetation in Flin Flon/Creighton and their sequential success is not influenced by soil moisture. Since no American vetch survived, it is not a species that should be selected for a remediation project in Flin Flon/Creighton.

5. SYNTHESIS AND CONCLUSIONS

Flin Flon/Creighton has many areas that are devoid of vegetation due to past smelting, mining and forestry activities. The overall objectives of this study, as part of a larger study being conducted in Flin Flon/Creighton, were to determine if there is a combination of soil amendments and vegetation that would be viable and practical for *in situ* revegetation in Flin Flon/Creighton. An extension of that objective was to identify whether soil moisture would affect the efficacy of the amendments for remediation in Flin Flon/Creighton.

A relationship was found between available heavy metal concentrations of the soil and the growth of the tree species in the field. Typically, as the concentration of available soil metals increased a decrease in the amount of tree growth was observed. One goal in remediating this area would be to decrease the amount of available metals present in the soils, to concentrations similar to those that were observed in the reference (Sherridon) soils, in order to promote plant establishment and growth. In particular, focusing on the areas in Flin Flon/Creighton where survival and growth was minimal in the field trial. Other features of the soil that were examined were the differences in the soil pH and base saturation between Sherridon and the Flin Flon/Creighton sites. Compost was the most successful amendment in increasing soil pH and base saturation towards values similar to that of Sherridon. The other amendments (i.e. WB, BMB and EMF) and control did not have the same influence on the pH and base saturation values.

Generally, there were no significant differences in metal uptake into plant biomass between any of the amendments in the field trial. However, in the field trial there were significant correlations ($r=0.66$ to $r = 0.86$) between soil Cu and biomass (root and shoot) Cu. There was also a significant negative correlation between total biomass of the tree species and available soil Cu. This indicates that there was increased uptake of Cu into the roots and shoots of the plant and decreased plant biomass where there was increased available soil Cu. Pahlsson (1989) concluded that vascular plants generally are species affected when Cu is between 15 and 25 mg kg⁻¹ dry weight in the plant shoot biomass. The values in field study exceeded 100 mg kg⁻¹ Cu in both plant species but was measured for total biomass (root and shoot) Cu and root Cu is known to display higher concentrations than the shoot biomass. A better comparison may be made between the soil concentrations of Cu where Pahlsson (1989) found that soil Cu

concentrations between 100 to 200 mg kg⁻¹ were found to disturb the growth of plants. The soil Cu levels in the 12 study sites ranged from 8.82 to 1181.18 mg kg⁻¹ therefore some sites had levels of Cu that could disturb plant growth. The amount of Cu taken up into the plants in these sites would also be influenced by a variety of other factors (i.e. availability of metals, interactions with other metals and exposure time). Visual symptoms of Cu toxicity in plants include small chlorotic leaves, dropped leaves, stunted growth and discoloration (Heale and Ormrod, 1982; Pahlsson, 1989). Trees in the field trial were observed with these symptoms but given the multi-metal contamination and growing conditions in Flin Flon/Creighton it cannot be concluded that these symptoms were a direct result of only Cu toxicity.

It was expected that soil moisture would influence the amount of plant biomass, the amount of available metal in the soil and the amount of metal in the plant biomass (Guidi and Labrecque, 2010). Angle et al. (2003) studied hyperaccumulating plant species and non-hyperaccumulating plant species and found increased plant biomass and metal in the plant as the amount of moisture increased in both types of species. There were two moisture regimes examined in the growth chamber trial of this study and there were no significant differences between the high and low watering regimes for plant biomass, plant survival or metal concentrations under individual amendments. As a result it can be concluded that the moisture present in Flin Flon/Creighton will not normally be a major concern for *in situ* revegetation assuming there are no extreme drought or saturation events.

Survival and growth of the tree species (trembling aspen and jack pine) and understory species (tufted hairgrass and American vetch) were expected to increase under soil amendments in comparison to the control. In the growth chamber trial, generally for all amendments, the jack pine trees lost biomass and the trembling aspen trees gained biomass, although not significantly ($p > 0.05$). This indicates that, of the two tree species, trembling aspen is a more viable option. The reason for decreased biomass is not known but can likely be attributed to increased necrosis in the jack pine trees compared to the trembling aspen trees. In contrast, the understory species had better survival and growth when grown with compost or BMB in the growth chamber trial. No amendment significantly increased growth of any of the plant species in the field but there was a trend of increased growth of tufted hairgrass grown with compost.

Application of EMF or only dolomitic limestone and fertilizer (control) limited the growth and establishment success of tufted hairgrass. Species diversity is important for trying to

restore the area to a natural forest ecosystem or for visual appeal. Sudbury, ON had success in revegetating areas with tree species but was not reported as having successful understory establishment (Santala, 2014). Secondary studies using vegetation mats, transplanted from a forest community that was not smelter-affected, have been conducted to try to increase understory survival and diversity (Santala, 2014). These observations of the Sudbury, ON projects increased interest in having understory species as a part of the revegetation plan for Flin Flon/Creighton. The vegetation mats implemented in Sudbury, ON were found to be more successful in areas where there was greater than 60% canopy cover already existing (Santala, 2014). Therefore, these mats may not be successful in Flin Flon/Creighton for the sites examined in this study as the sites had no pre-existing canopy cover. American vetch was initially selected for its ability to fix nitrogen and to grow in a variety of soil textures and moistures (Kirk and Belt, 2010). The field trial baseline study concluded that there was more ammonium and nitrate present in the study sites than in the Sherridon reference site indicating that the nitrogen fixing capabilities of American vetch may not be of an importance for Flin Flon/Creighton. American vetch is not a viable understory species for Flin Flon/Creighton as it had no survival in either of the experiments (growth chamber or field trial). Different understory species should be examined for remediation of Flin Flon/Creighton to create species diversity in the understory.

In the field trial there were sites with trees that appeared to be surviving and healthy at the time of harvest. The growth chamber trial trees had a high rate of mortality by 90 d. It could be suggested that these results were influenced by the bulked growth chamber trial soil that had a greater amount of available soil metal present as well as lower pH and CECe values than the majority of the field sites used in the field study. However, the increased understory biomass under BMB and compost treatments in the growth chamber still provides valuable information on the potential use of these amendments in an *in situ* remediation plan.

There was indication that the rate of necrosis was higher under the EMF amendment in comparison to the other amendments utilized in the growth chamber component of this study which indicates that EMF is not a favourable choice for use in a revegetation plan for Flin Flon/Creighton. Further research should be conducted on the tree species with ectomycorrhizal fungi in heavy metal contaminated soils. A review of literature on the ability for EMF to ameliorate metal toxicity in trees grown in temperate forests found that there were a number of factors influencing the success of this mechanism, but overall that EMF do have the ability to

improve plant survival and growth in heavy metal contaminated areas (Jentschke and Godbold, 2000). Influencing factors included the strain or species of EMF, the type and concentration of metal and its speciation in the rhizosphere (Jentschke and Godbold, 2000). Therefore, further research should be conducted to examine if a different strain or species of EMF would be successful for improving the survival and growth of trees grown in Flin Flon/Creighton. Finding a beneficial combination of plant species and EMF strain may be useful for revegetation as was done in Portugal where *Erica andevalensis* was found to be successfully growing near a pyrite mine in an area with acidic and metal contaminated soils due to the symbiotic relationship with EMF species *Hymenoschyphus ericae* (Turnau et al., 2007). It would also be interesting to examine the metal uptake into the biomass at intervals prior to the completion of 90 d in relation to the amount of colonization as the amount of colonization and metal uptake could be influenced by the amount of metal present in the soil (Heggo and Angle, 1990).

The practicality of applying the amendments was an important component of research to develop potential solutions or programs for the revegetation of Flin Flon/Creighton and/or other smelter-impacted landscapes. The WB amendment was too light-weight for application in the field study. The WB did not stay in place on site and therefore is not a practical choice, alone, for *in situ* revegetation in that type of landscape. There may be potential to mix WB, and/or other amendments with a tackifying agent to help hold them in place. The challenge with doing this and why it was not explored for this study was because of the variable terrain in Flin Flon/Creighton. The terrain makes it hard to get a vehicle into the remote locations in order to apply the mixture on a large scale. The EMF amendment required slightly more work in application as it needed to be placed in the tree seedling planting hole. Furthermore, research should be conducted to determine the long term effect of planting trees and vegetation in the rock outcrop soil pockets.

The small area of these soil pockets could potentially have long-term detrimental effects on the survival of the vegetation planted. It is probable that the root growth of the tree species will become restricted by the amount of soil present in the soil pockets and will have reduced access to essential nutrients in the small areas. The pre-treatment of areas to allow the amendments to act on the soil prior to planting the tree plugs may also create a more conducive environment to tree survival initially when planted. Winterhalder (2000) observed in Sudbury, ON that the effects of the dolomitic limestone on the soil took a few years to increase the soil

pH. Further research could therefore be conducted to find the effects of applying soil amendments and leaving them for a period of time before planting the tree seedlings and understory species to allow the amendments to act upon the soil properties and potentially increase the survival rate of the vegetative species.

The variation present throughout the Flin Flon/Creighton area may be one of the major complicating factors in an *in situ* remediation project. Some areas had overall more survival and growth than others indicating that one treatment may not be effective across the entire landscape. For the amendments examined in this project, it was found that compost had the greatest overall positive contributions including increased understory survival and growth and the ability to increase the pH and base saturation of the soils.

Recommendations based on this study for Flin Flon/Creighton would be to establish a secondary *in situ* trial that focuses on trembling aspen seedlings and tufted hairgrass in combination with compost, dolomitic limestone and fertilizer. Different techniques for applying compost could be experimented with, for examples, mixing the amendment into the soil or applying the amendment prior to planting or seeding the area. Allowing an *in situ* project to remain in place for a longer period of time would also help determine if there will be complications with the amount of soil present in the rock outcrop areas.

Other remediation techniques such as transplanting soil containing propagule banks from healthy forest systems within the area to assist in natural recovery of the site may be an option. Research has been successfully conducted on the use of donor soils for many other mine impacted sites but only recently has research began to look at boreal forest communities (Holmes, 2001; Mackenzie and Naeth, 2010). This method transports and deposits soil on the landscape that has not been smelter-impacted. This increases the amount of soil present and the number of propagules present in the area. A few considerations with this type of revegetation project would be access to equipment that can maneuver through the landscape and the associated cost of this type of project.

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7. APPENDICES

APPENDIX A

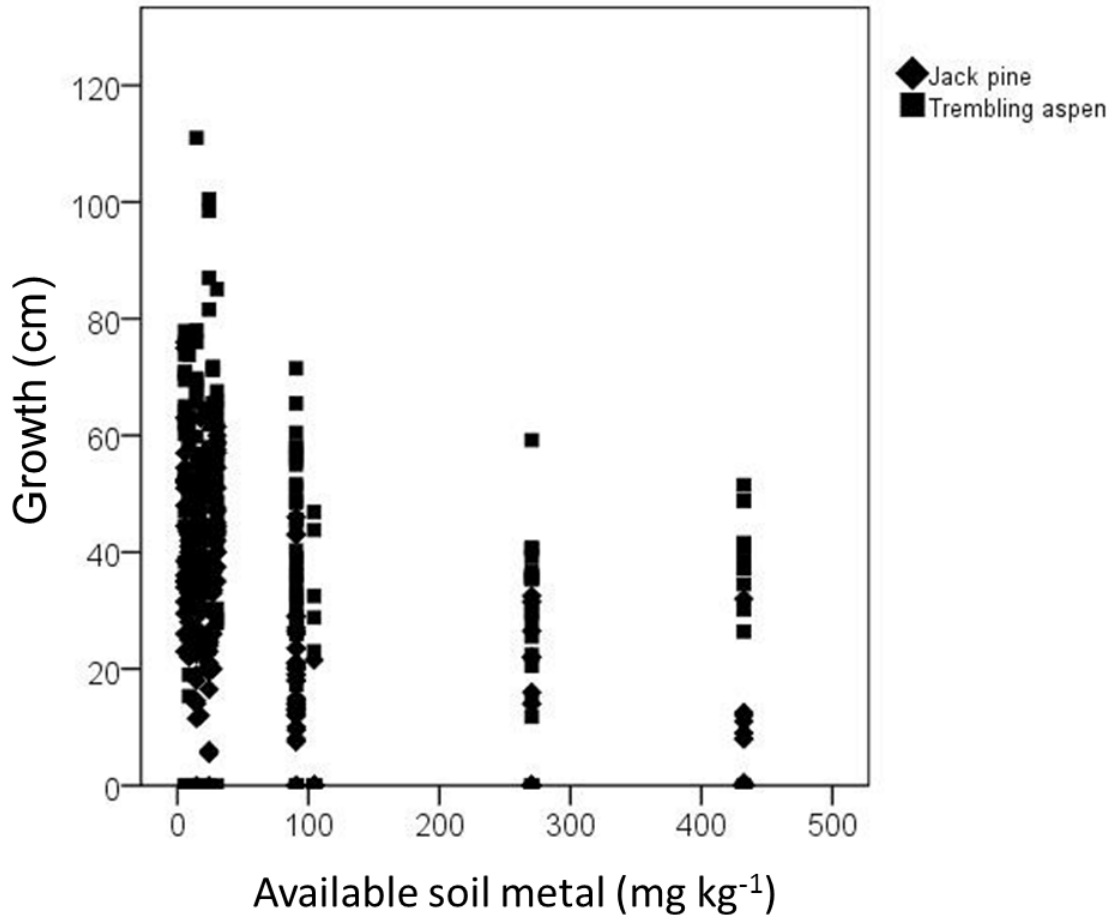


Figure A.1. Growth (change in height from time of planting to harvest) (cm) of trembling aspen and jack pine and the available soil metal concentration (averaged Al, Cd, Cu, Ni, Zn).

APPENDIX B

Table B.1 Pearson product moment correlations between root, shoot or total plant biomass, metal concentrations in shoots and roots for trembling aspen and soil chemical properties including soil extractable metal concentrations in the field trial experiment at Flin Flon/Creighton. Values are correlation coefficients 'r'.

	Soil Cd	Soil Cu	Soil Zn	Soil Al	Soil TC	Soil NO ₃ ⁻	Soil NH ₄ ⁺	CECe	Soil S
Total Biomass	- 0.27*	-0.47 **	-0.25	- 0.40**	-0.31*	-0.31*	-0.14	-0.20	-0.33*
Root Biomass	-0.26 *	- 0.49 **	- 0.27 *	-0.42 **	-0.35**	-0.31*	-0.18	-0.27*	-0.32*
Shoot Biomass	- 0.26*	0.43**	-0.23	-0.36**	-0.27*	-0.28*	-0.11	-0.16	0.31*
Root Cd	0.31*	0.08	0.35**	-0.26*	0.61**	0.61**	0.60**	0.31*	0.02
Root Cu	0.75*	0.86**	0.59**	0.09	0.26*	0.58**	-0.16	-0.21	0.79**
Root Zn	0.79**	0.87**	0.57**	0.05	0.15	0.54**	-0.28*	-0.32*	0.83**
Root Al	0.15	0.36**	-0.02	0.36**	0.28 *	-0.15	-0.46**	0.05	0.41**
Root Ni	0.57**	0.60**	0.38**	-0.10	0	0.30*	-0.35**	-0.21	0.60**
Shoot Cd	-0.14	-0.21	-0.18	-0.01	-0.08	-0.14	-0.03	0.25	-0.03
Shoot Cu	0.50**	0.66**	0.38**	0.24	0.41**	0.58**	0.22	0.19	0.53**
Shoot Zn	0.51**	0.33*	0.43**	-0.16	0.08	-0.28*	-0.14	-0.36*	0.30*
Shoot Al	-0.26*	-0.20	-0.33**	0.05	-0.13	-0.20	0.02	0.54**	-0.08
Shoot Ni	-0.13	-0.13	0.07	-0.24	-0.26*	0.26*	-0.30*	-0.39**	-0.19

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

Table B.2. Pearson product moment correlations between root, shoot or total plant biomass, metal concentrations in shoots and roots for jack pine and soil chemical properties including soil extractable metal concentrations in the field trial experiment at Flin Flon/Creighton. Values are correlation coefficients 'r'

	Soil Cd	Soil Cu	Soil Zn	Soil Al	Soil TC	Soil NO ₃ ⁻	Soil NH ₄ ⁺	CECe	Soil S
Total Biomass	-0.47**	-0.54**	-0.54**	-0.24	-0.28*	-0.40**	-0.01	0.37**	-0.21
Root Biomass	-0.49**	-0.58**	-0.55**	-0.29*	-0.28*	-0.40**	0.01	0.37**	-0.26*
Shoot Biomass	-0.46**	-0.53**	-0.53**	-0.22	-0.27*	-0.42**	-0.20	0.36*	-0.2
Root Cd	0.25*	0.26*	0.21	0.15	0.34**	0.32*	0.26*	0.18	0.28*
Root Cu	0.70**	0.81**	0.50**	0.09	0.16	0.51**	-0.23	-0.23	0.77**
Root Zn	0.48**	0.49**	0.33*	-0.03	0.06	0.37**	-0.22	-0.19	0.44**
Root Al	0	0.61**	0.31*	0.02	0.02	0.34**	-0.27*	-0.18	0.63**
Root Ni	0.71**	0.71**	0.48**	-0.02	0.13	0.48**	-0.22	-0.19	0.72**
Shoot Cd	0.48**	0.53**	0.36**	0.01	0.13	0.36**	-0.13	-0.15	0.51**
Shoot Cu	0.71**	0.83**	0.72**	0.14	0.54**	0.69**	0.14	-0.09	0.61**
Shoot Zn	0.44**	0.49**	0.31*	0.03	0.08	0.31*	-0.16	-0.17	0.47**
Shoot Al	0.35**	0.43**	0.21	0.05	0.06	0.25*	-0.14	-0.06	0.44**
Shoot Ni	0.42**	0.44**	0.32*	0.03	0.01	0.28*	-0.23	-0.32*	0.35**

*, **, *** Significant at the 0.05 and 0.01 probability levels, respectively

Table B.3. Pearson product moment correlations between total plant biomass, biomass metal concentrations and soil properties (available metals, total carbon, NO_3^- , NH_4^+ , CECe, and soil available S) for tufted hairgrass in the field trial experiment at Flin Flon/Creighton. Values are correlation coefficients 'r'.

		Soil Cd	Soil Cu	Soil Zn	Soil Al	Soil TC	Soil NO_3^-	Soil NH_4^+	CECe	Soil S
Total Biomass		-0.31*	-0.32*	-0.28*	-0.27*	-0.31*	-0.41**	-0.23*	-0.17	-0.11
Biomass	Cd	0.13	0.08	0.37**	-0.17	0.41**	0.24	0.25*	0.08	-0.07
Biomass	Cu	0.17	0.13	0.42**	-0.14	0.48**	0.28*	0.32*	0.11	-0.02
Biomass	Zn	0.12	0.07	0.34**	-0.2	0.36**	0.16	0.21	0.02	-0.09
Biomass	Al	-0.19	-0.19	-0.18	-0.15	-0.08	-0.16	-0.08	0.06	-0.08
Biomass	Ni	-0.14	-0.24	-0.02	-0.40**	-0.16	-0.23	-0.16	-0.21	-0.17

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

APPENDIX C

C.1. Photographs of various sites in Flin Flon/Creighton at the time of planting (a) and the time of harvest (b).



Site 1



Site 2



Site 4



Site 5



Site 6



Site 7



Site 8



Site 9



Site 11



Site 12