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**MICRONUTRIENT CONCENTRATIONS VARY BETWEEN PEAT-MINERAL
MIX AND SUBSTRATES IN REVEGETATED SITES IN THE ALBERTA OIL SANDS**

Short title: Micronutrients in reclaimed oil sands soils

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ABSTRACT

Adequate supply of micronutrients is essential for plant growth in reclaimed sites in the Athabasca oil sands region. The objectives of this study were to determine boron, iron, manganese, copper and zinc concentrations in peat mineral mix (PMM), tailings sand (TS) and overburden (OB) materials and to assess whether lodgepole pine (*Pinus contorta*) planted on PMM over TS and white spruce (*Picea glauca*) planted on PMM over OB had low foliar micronutrient concentrations. Micronutrient concentrations determined using LiNO_3 and Mehlich-3 extractions were different between PMM and TS in the pine sites while only LiNO_3 extractable boron was different between PMM and OB in the spruce sites ($p < 0.05$). Micronutrient concentrations varied in the order of boron > iron > manganese > zinc > copper in all soil layers with concentrations ranging from 0.04 to 39.56 $\mu\text{g g}^{-1}$. The low foliar concentration of copper in pine and spruce was consistent with low LiNO_3 extractable copper in the soil in both the pine and spruce sites. We conclude that the availability of micronutrients such as copper can become a potential limitation for revegetation of white spruce but not lodgepole pine. Further studies on soil management for improving Cu availability in reclamation materials are needed for improving the growth of spruce in reclaimed soils.

Keywords: aqueous speciation, available micronutrient, deficiency, extractability, overburden, tailings sand

INTRODUCTION

Oil sands mining in northeast Alberta caused a growing anthropogenic footprint since 1967 (Gosselin et al. 2010; Schindler 2010). On-going land reclamation in this area in the past 25 years aims to achieve the equivalent land capability to that existed pre-disturbance. During initial land reclamation operations, landforms are created using either tailings sand (TS) or fine-textured overburden material (OB) as lower layers or substrates that is covered by approximately 0.2 - 0.5 m of an organic matter rich cover soil which is typically a peat-mineral mix (PMM; a mixture of salvaged peat and surface mineral soils) due to peat material being readily available in the area. Tailings sand is the by-products of the surface mining and oil sands extraction processes while OB is the geologic material lying above the economically mineable oil sands and includes Pleistocene clay deposits and Cretaceous deposits with relatively high alkalinity and salinity-sodicity (Fung and Macyk 2000). Chemical and physical constraints in some reclaimed sites such as low water and nitrogen (N) availabilities and high salinity (Duan et al. 2015a) challenge the recreation of a sustainable ecosystem. Although investigation on macronutrient status of reclaimed sites has been given significant attention in the past, the availability of micronutrients in reclaimed sites is poorly understood. A recent investigation on the foliar elemental composition of jack pine in the Athabasca oil sands region (AOSR) showed that boron (B), iron (Fe) and zinc (Zn) concentrations significantly increased with proximity to the major oil sands operations (Proemse et al. 2016), but the soil micronutrients were not studied. Although the role of B, Fe, manganese (Mn), copper (Cu) and Zn as indicators of air pollutants (Percy et al. 2013) were revealed, the influence of soil micronutrients on tree nutrition in the AOSR remains to be investigated.

Boron, Fe, Mn, Cu and Zn are essential micronutrients that play important roles in biochemical reactions related to plant growth. Although micronutrients are needed in trace amounts, deficiencies of those micronutrients in plants directly impact photosynthesis causing low dry matter production. Boron shortage affects many metabolic processes which ultimately impacts photosynthetic function (Blevins and Lukaszewski 1998) while Fe is an integral part of chlorophyll that is involved in photosynthesis (Marschner 2012). Manganese is involved in photosynthetic O₂ evolution (Hill reaction) in the chloroplast (Rutherford 1989) while Cu deficiency results in decreased photosynthetic electron transport (Mizuno et al. 1982). Zinc is essential for chlorophyll synthesis, including the activation of several enzymes and oxidation-reduction reactions (Alloway 2008). Also, micronutrients are involved in various enzymes and other physiologically active molecules associated with cell wall development, respiration, enzyme activities and N fixation (Rengel 2003; Schulin et al. 2010). Apart from the direct effect of low micronutrient availability in the soil, ratios between foliar micro- and macronutrients in tree species were found to be important for maintaining optimal leaf physiology (Sobrado 2014).

Land reclamation markedly changes soil physical, chemical and biological functions (Naeth et al. 2011; Farnden et al. 2013; Jung et al. 2014; Li et al. 2014) and available micronutrient contents in the reclaimed soil were found to be closer to or greater than that in soils under natural conditions after more than eight years after reclamation (Li et al. 2013). Development of toxic levels of available B following reclamation has been observed in some soils (Rhoades et al. 1970), whereas B deficiency is identified in soils that are highly leached (Borkakati and Takkar 2000). Plant establishment and growth in fly ash basin was limited by high pH, and the consequent deficiencies of Fe, Mn, Cu, and Zn (Haynes 2009) and the availability of these nutrients varied considerably with different land reclamation practices (Li et al. 2013). As

such, possible micronutrient limitations in re-vegetation should be studied to ensure successful land reclamation.

Plant availability of micronutrients in the soil depends on soil pH, redox potential, texture, the quantity and quality of organic matter, mineral composition, temperature, and water regime (Panuccio et al. 2009) and therefore, soils vary widely in their ability to supply micronutrients for optimal plant growth. Due to the differences in the origin and nature of PMM, OB and TS, it is possible that micronutrient availability in reclaimed sites vary between the surface and substrate layers and may also depend on the soil reconstruction strategy (i.e., PMM over OB or PMM over TS). Associations with functional groups of natural organic matter affect bioavailability, toxicity, and mobility of micronutrients in the soil and thus organic matter-rich soils, such as peats are likely to have low available B, Mn, Cu and Zn (Alloway 2008). Humified organic matter in peat soils might form solid-phase complexes with metal ions leading to micronutrient deficiencies in plants (Andriesse 1988) suggesting possible micronutrient deficiencies in PMM. High bulk density and salinity in OB layer can lead to low available water storage capacity (Duan et al. 2015a) which might have a direct impact on macronutrient availability in OB regardless the positive relation between clay and silt fraction of soil and the micronutrient mobility (Tisdale et al. 1995). In addition, coarse-textured TS has been identified as a poor growth medium due to low cation exchange capacity and low clay and organic matter contents (Fung and Macyk 2000). Furthermore, high pH in reclaimed soils (Howat 2000) is also more likely to cause nutrient deficiencies (Zhang and Zwiazek 2016). Therefore, there is an urgent need for research on micronutrient availability and potential deficiencies in reclaimed soils to achieve successful revegetation.

This study was conducted in some reclaimed sites in the AOSR to examine the variation in microelement (B, Fe, Mn, Cu and Zn) concentrations in different reclamation materials (i.e., PMM, OB and TS) and tree species to answer the following questions: 1) what are the differences among PMM, OB and TS in terms of micronutrient concentrations determined using LiNO_3 and Mehlich-3 extractions? 2) how different soil properties in PMM, OB and TS are related to the extractable micronutrient concentrations in different soil materials? and 3) do the tree species, i.e., lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) growing in reclaimed sites experience micronutrient deficiency? Our hypotheses are that 1) the concentrations of extractable micronutrients in TS and OB is greater than that of PMM due to the low organic matter content in the substrate, due to the micronutrient complexation potential of the peat contained in PMM, and 2) based on the foliar micronutrient concentrations (critical values), tree species experience micronutrient deficiencies when they are planted on reclaimed sites with either TS or OB as the substrate. We expect that the current study on the micronutrient status of pine and spruce stands will provide new information on micronutrient management for revegetated sites in the AOSR.

MATERIALS AND METHODS

Site description

Study sites were located 22 km north of Fort McMurray (56°59 'N and 111°32 'W) in northeastern Alberta, Canada. The study area was characterized by a continental boreal climate having short and cool summers and long and cold winters. The mean annual temperature from 1971 to 2000 was 0.7 °C while the mean annual precipitation for the same period was 455.7 mm. The study area was reclaimed from an open-pit mining site to upland forests. The sites were

reconstructed using PMM as a cover soil above substrates such as TS or OB materials. Lodgepole pine was planted on PMM over TS while white spruce was planted on PMM over OB sites (Jung et al. 2014; Duan et al. 2015a). The different stands of lodgepole pine were planted between 1991 and 1996 while white spruce was planted between 1982 and 1992. Details on site characteristics and understory plant communities are given in Jung et al. (2014) and Duan et al. (2015a).

Experimental design and soil sampling

Six pine sites and six spruce sites were selected for soil sampling. At each site, a 10 x 10 m plot was set up for soil sampling. Soil samples were collected from PMM and the substrate at each site in July 2012. Soil samples were collected by depth in each site. The top 20 cm of PMM and the upper 20 cm of the substrate, measured from the PMM and substrate interface, were collected using an auger in five randomly selected locations within each plot to obtain a composite PMM or substrate sample. The thickness of the PMM layer in pine and spruce sites varied in the range of 12-30 cm and 11-48 cm, respectively (Duan et al. 2015a). When the thickness of the PMM was less than 20 cm, the entire PMM layer was sampled. All soil samples were placed in plastic bags and taken back to the laboratory. The soil samples were air-dried and used for chemical and physical analyses.

Measurements of soil properties

Soil texture was determined using the hydrometer method (Gee and Or 2002). Soil pH was measured in deionized water using a digital DMP-2 mV/pH meter (Thermo Fisher Scientific Inc., Waltham, MA) with soil to water ratio of 1:2 (w:v). The electrical conductivity of soil samples was measured using an AP75 portable waterproof conductivity/TDS meter (Thermo Fisher

Scientific Inc., Waltham, MA) using 30 g of air-dried soil in 60 mL deionized water after shaking for 1 h at 25 °C and filtration through Whatman No. 42 filter papers (Dellavalle 1992). Air-dried soil samples were ground with a ball mill and used to analyze for total C and N concentrations with a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Instruments, Milano, Italy) at the Lethbridge Research Centre of Agriculture and Agri-Food Canada (Duan et al. 2015a). Available N was measured by extracting soil samples with 0.5 mol L⁻¹ KCl at 1:3 soil to solution ratio (w:v), shaking at 250 rpm on a mechanical shaker for 1 h and then filtering through Whatman No. 42 filter papers. Concentrations of NH₄⁺-N and NO₃⁻-N in the extracts were analyzed using the steam distillation method using a Kjeldahl distillation system (Vapodest 20, C. Gerhardt, Königswinter, Germany) (Bremner 1996). Water extractable K was analyzed using a Perkin Elmer Optima 3000 DV inductively coupled plasma mass spectrometer (ICP-MS) (PerkinElmer Inc., Shelton, CT) after extraction with deionized water at 1:2 of soil to water ratio (w:v) and filtration.

To determine Mehlich-3 extractable B, Fe, Mn, Cu, and Zn, a 2.5 g of soil was placed in a centrifuge tube, and 25 mL of Mehlich-3 solution (0.2 mol L⁻¹ CH₃COOH, 0.25 mol L⁻¹ NH₄NO₃, 0.015 mol L⁻¹ NH₄F, 0.013 mol L⁻¹ HNO₃, 0.001 mol L⁻¹ EDTA) was added to the centrifuge tube. The mixture was then shaken for 5 min on a mechanical shaker at 120 strokes per minute, and the extract was filtered using Whatman No. 40 filter paper (Mehlich 1984). Micronutrient concentrations were measured using PerkinElmer Optima 3000 DV inductively coupled plasma mass spectrometer (ICP-MS) (PerkinElmer Inc., Shelton, CT). The LiNO₃ extraction was carried out to determine plant available micronutrient concentrations in soil samples (Abedin et al. 2012). A 2.5 g of soil was extracted using 10 mL of 0.01 mol L⁻¹ LiNO₃ solution by shaking for 24 h at 120 strokes per minute. The extractant was centrifuged at 1770 × g for 10 min and filtered through Whatman 44 filter papers and analyzed by ICP-MS for B, Fe, Mn, Cu and Zn concentrations.

Speciation of micronutrients

Soil samples (0.5 g) were extracted using 50 mL of Milli-Q water in polyethylene centrifuge tubes. The tubes were shaken on a mechanical shaker for 60 min at 40 rpm at room temperature and then centrifuged at $2767 \times g$ for 10 min. The supernatant was extracted with a $0.45 \mu\text{m}$ cellulose nitrate filter to obtain the soil extract (Fernández-Caliani et al. 2009). The soil extract was analyzed for anions (sulfate, nitrate, chloride and fluoride) using a Dionex ion chromatograph. The bicarbonate determination was done by volumetric titration with $0.02 \text{ mol L}^{-1} \text{ HCl}$. The B, Fe, Mn, Cu and Zn concentrations in the solution were analyzed by ICP-MS. Micronutrient speciation was performed using CHEAQUES Next, a Windows program for calculating chemical equilibria in aquatic systems (Verweij 2007).

Plant sampling and foliar micronutrient analysis

In October 2012, five representative trees were randomly selected in each plot with each species growing in one substrate type. One branch from the upper one-third of the live crown of each tree was collected to form a composite branch sample for each plot. Branch samples were placed in paper bags and taken back to the laboratory. The foliage from each branch was separated into current-year and 1-year-old needles. After washing twice using distilled water, current-year needles were oven-dried at 65°C for 24 h or until constant weight is achieved. Three hundred needles were randomly selected and weighed and ground to pass through a 0.15 mm sieve in preparation for nutrient analyses. Total P, K, B, Fe, Mn, Cu and Zn in needles were analyzed with the ICP-MS after digestion using concentrated HNO_3 and 30% H_2O_2 on a digestion block at 125°C

°C for 4 h (Campbell and Plank 1998) and total N in needles was determined with a Carlo Erba NA 1500 elemental analyzer.

Statistical analysis

Principle component analysis (PCA) was performed using the PROC FACTOR procedure in SAS 9.2. The first PCA was applied to the LiNO_3 and Mehlich-3 extractable micronutrient data from both pine and spruce sites. The second PCA was conducted on LiNO_3 and Mehlich-3 extracted micronutrients and soil properties. Principal components (PC) were derived from a correlation matrix and variables were transformed to z-scores prior to PCA. The final component structure was unrotated and included only PCs with eigenvalues greater than unity (Kaiser 1960). The first PCA was conducted to illustrate the similarity and/or differences among PMM, TS and OB based on extractable (i.e., both Mehlich-3 and LiNO_3) micronutrient concentrations. The purpose of the second PCA was to determine what soil properties mainly governed Mehlich-3 and LiNO_3 extractable micronutrient concentrations. Comparison of means of LiNO_3 and Mehlich-3 extracted micronutrient concentrations was performed using the Tukey's multiple comparison test. An α value of 0.05 was chosen to indicate statistical significance in all analyses. All statistical analyses were performed using the SAS software (SAS 9.2, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Differences of soil properties among reclamation materials

Soil pH was alkaline in both PMM and substrate in most sites except for some TS samples (Table 1) and was about two units greater than the soil pH in native boreal ecosystems (Howat 2000). Soil pH was not significantly different between the PMM and the substrate in each site

type (pine vs spruce sites). Higher pH in PMM than in natural peat materials was attributed to the clay-rich, calcium-absorbing mineral soil in the PMM and the windblown deposits from nearby exposed tailings (Rowland 2008). Also, saline-sodic overburden and tailings sand with a high sodium concentration can have Na translocate to the PMM by the capillary action (Jorenush and Sepashkah 2003) and form sodium bicarbonate due to the reaction with water resulting in a significant increase in soil pH when hydrolyzed (Marschner 2012). However, EC in the pine and spruce sites were less than the critical values of 4 dS m^{-1} for causing salt stress in trees growing in the AOSR (Howat 2000), but high EC was the unique characteristic that differentiated spruce sites from pine sites based on the substrate type. The EC in PMM and OB was 2-fold and 13-fold greater than the PMM and TS, respectively.

Peat-mineral mix in pine and spruce sites had greater N, P, K and total carbon content than the TS or OB substrate in each site type as expected (Table 1). The high total carbon (C) content and the low bulk density in PMM in both sites are indicative of the higher organic matter content in PMM as compared to the substrate in each site. Soil organic carbon content in PMM is highly correlated with microbial respiration rate, enzyme activities, cation exchange capacity and bulk density (Kong et al., 1980). Conversely, low carbon content in OB and TS caused a significant decrease in phenoloxidase activities and increased the dissimilarities of microbial communities between natural and reclaimed sites (Dimitriu et al. 2010).

The low cation exchange capacity, the absence of clay and the extremely low total C content in TS together were reflective of the poor nutrient status in TS as a substrate compared to OB (Table 1). Such undesirable soil chemical properties of TS can adversely affect plant growth (Fung and Macyk 2000). High bulk density in TS was caused by the sandy texture of TS, while soil compaction by heavy equipment or highly compacted reconstructed materials caused high

bulk density in OB with high clay content. Water storage is also influenced to a large extent by soil organic carbon content in PMM (Duan et al. 2015a). Increasing organic matter content increases the amount of water held at field capacity more than that held at the permanent wilting point, thereby increasing the water holding capacity in the soil (Hudson 1994). Unlike PMM, low water holding capacity and water availability in the TS were attributed to the high macroporosity (Jung et al. 2014) that can affect nutrient availability.

Distribution of micronutrient concentrations varies among PMM, TS and OB

There was no significant relationship between micronutrient concentrations from LiNO_3 and Mehlich-3 extractions for most of the elements analyzed ($p = 0.221$), except for B in PMM in spruce sites. The independence of the micronutrient concentrations measured by the two extractants indicates that they are not extracting micronutrients from the same pools in PMM, TS, and OB. The greater B concentration measured by LiNO_3 than by Mehlich-3 indicates that the former is a strong extractant for B in reclamation materials due to possible exchange reactions between nitrate for H_2BO_3^- . Although Mehlich-3 is a versatile extractant for extraction of multi-elements, the superior extractability of B by LiNO_3 over Mehlich-3 in reclamation material has not been reported elsewhere to the best of our knowledge. The greater extractability of cationic micronutrients by Mehlich-3 is expected since it is a weak acid that is thought to remove a number of cations in the exchangeable fraction, soil solution and a part of the adsorbed or complexed forms (Cancela et al. 2002). Specifically, Fe, Mn, Cu and Zn are extracted by ammonium and the EDTA in the Mehlich-3 extractant. On the other hand, LiNO_3 is a neutral electrolyte that does not change soil pH during extraction and provides a realistic estimate of phytoavailable trace element concentrations under natural pH conditions as compared with other more aggressive extractants (Abedin et al. 2012).

The extractability of all five micronutrients by each extractant was in the same order for samples from PMM and substrates in pine and spruce sites: $\text{Fe} > \text{Mn} > \text{B} > \text{Zn} > \text{Cu}$ (Table 2). A similar order of LiNO_3 extractable Fe, Mn, Zn and Cu was also found in soils collected from a reclaimed mining area (Li et al. 2013). There are significant variations in LiNO_3 and Mehlich-3 extractable micronutrient concentrations in the PMM and in both substrates (Table 2). Mehlich-3 extractable concentrations of Fe, Mn, Cu and Zn were greater than LiNO_3 extractable micronutrient concentrations in pine and spruce sites. The PCA of the LiNO_3 and Mehlich-3 extractable micronutrient concentrations of all soil samples provides a clear differentiation of PMM and the substrate in each of the pine and spruce sites (Figure 1). The first axis in the PCA accounted for 44% of the variation in the data set and differentiated PMM and TS of pine sites. The second axis in the PCA explained 20% of the variation in the data set and differentiated PMM and OB of spruce sites. A greater difference was found between PMM and the substrate in pine sites than in spruce sites, since most of the PMM and OB samples showed a positive correlation with PC1 while TS samples were negatively correlated with PC1 differentiating TS largely from PMM in pine sites. Such results are expected due to the contrasting differences in both physical and chemical properties between PMM and TS in comparison with PMM and OB (Table 1).

Even though the extractability of micronutrients was similar in PMM, TS and OB, the available fraction of the total extractable pool (i.e., LiNO_3 extractable micronutrient concentration expressed as a percent of the Mehlich-3 extractable micronutrient concentration) varied depending on the micronutrient and the soil layer. For instance, only 0.1 to 5.8% of Mehlich-3 extractable Fe was available whereas 2.5 to 62% of the extractable Zn was available (Table 2), indicating the different ability of PMM, TS and OB to retain micronutrients. This emphasizes the importance of exploiting micronutrients in reclamation materials using different extractants since Mn, Cu and Zn

are also heavy metals with a potential environmental risk when present at high concentrations. Copper and Zn were the least extractable micronutrients in both site types. Copper has been identified as the least mobile micronutrient among the studied micronutrients in forest soils (Mortvedt et al. 1991) since Cu reside mostly in unavailable forms and has lower mobility and bioavailability than Zn (Hickey and Kittrick 1984). The Mehlich-3 extractable Cu concentration was greater in pine and spruce sites than the Cu concentration of $0.19 \mu\text{g g}^{-1}$ found in natural forest soils (Entry et al. 1987) indicating high amounts of Cu in reclaimed soils. The large difference between LiNO_3 and Mehlich-3 extractable Fe and Mn concentrations in pine and spruce sites indicated that both elements resided mainly in a pool that is not readily available. Water-soluble and exchangeable Fe are plant-available forms of Fe, but in organic soils 50% of the Fe was found in the organic and the sulfide fractions (Hoffman and Fletcher 1978).

Our hypothesis of substrates having greater micronutrient concentrations than PMM is valid only for the pine sites where LiNO_3 extractable micronutrient concentrations were significantly greater in TS than in PMM for most of the micronutrients studied. In pine sites, LiNO_3 extractable Fe, Mn and B concentrations were significantly greater in TS than in PMM (Table 2) likely due to low organic matter and relatively low pH (Table 1) despite the reported negative impact of TS on plant growth (Fung and Macyk 2000). Such findings suggest the potential of the TS substrate to provide micronutrients for tree growth in reclaimed lands in the AOSR. The LiNO_3 extractable B concentration in TS and OB samples were significantly greater than that in the PMM in each site type with the lowest LiNO_3 extractable B found in pine sites (Table 2). Soil moisture status is the main environmental factor affecting the availability of B to plants, and that relates to the lowest LiNO_3 extractable B in PMM in pine sites where water limitation has been identified (Duan et al.

2015). Moreover, relatively high pH in PMM could lead to a greater absorption of B (Barrow 1989) in PMM than the substrates in both sites.

Soil properties governing LiNO_3 and Mehlich-3 extractable micronutrient concentrations vary depending on the site type.

A PCA was used to derive some relationships between soil properties and micronutrient concentrations in PMM in both sites due to the high Mehlich-3 extractable micronutrient concentration in PMM. However, some significant correlations between LiNO_3 extractable micronutrient concentrations and soil properties of pine and spruce sites were found for PMM but not for TS and OB (Supplementary Table S1). For pine sites, PC1 and PC2 explained 39% and 30% of the variance in the data set, respectively, indicating that both PCs are equally important (Table 3). In general, soil reaction seems to be the most important factor affecting LiNO_3 extractable micronutrient concentrations (except Fe) in pine sites while Mehlich-3 extractable pool (except Zn) were related to soil physical conditions associated with total C and bulk density. In spruce sites, PC1 accounted for 63% of the variation in the data set and exemplified that soil attributes such as total C content, pH, EC and bulk density influenced both LiNO_3 and Mehlich-3 extractable cations in spruce sites.

The above results showed that micronutrient concentrations in different pools largely depend on more than one soil factor, and that further studies on the behavior of these elements in reclamation materials are warranted. Although soil physical and chemical properties were comparable in PMM in two site types, the underlying mechanisms for micronutrient extractability varied, partly due to the effect of the vegetation type as shown by specific relationships between soil and foliar nutrients in the pine and spruce sites (Supplementary Table S2). This calls for site-

specific fertilizer studies even though the reclamation material is of the same origin. The interrelationships between micro- and macronutrients under low water availability and high salinity (Duan et al. 2015a) might further aggravate the effect of low available macronutrients on tree growth. The Mehlich-3 extractable Cu in PMM showed a significantly negative relationship with available N in both site types while available P and water extractable K were also negatively related to the Mehlich-3 Cu in PMM of spruce sites (Supplementary Table S2).

Micronutrient speciation modeling

There were some differences in speciation among micronutrients, but speciation of a particular micronutrient did not vary significantly between pine and spruce sites regardless of the material (PMM or substrate) being compared (Figure 2). Such similarity in aqueous speciation of micronutrients is likely due to the similar soil pH between PMM and substrates. A greater number of aqueous species was found for Cu than for Mn and Zn in soil extracts (Figure 2). Species containing hydroxyl group comprised a major fraction of dissolved Fe, Cu, and Zn. Other anions such as nitrate, phosphate, sulfate, and chloride were not candidates for forming ion pairs or complexes with Fe, Mn, Cu and Zn as is reflected in the low concentration (i.e., < 1%) of these anions in the soil extract (data not shown). Subtle amounts of free ions of micronutrients were present in the soil extract showing the restricted mobility of free ions in the soil (Figure 2). Hence, cation exchange may not be the primary pathway of replenishing the available pool of micronutrients in soils.

The aqueous speciation of Cu and Zn was similar as both existed mainly in the hydrolyzed form in the soil extract. Both precipitation and adsorption of Zn and Cu increase as the soil pH increases, causing low availability of Zn and Cu as compared to Fe and Mn (Mortvedt et al.

1991). Moreover, both Cu^{2+} and Zn^{2+} form strong complexes with functional groups associated with the humic acid component of peat (Dissanayake and Weerasooriya, 1981). In the neutral pH range, about 70% of Cu was hydrolyzed but precipitation of Cu(II)OH_2 may not be the reason for the low LiNO_3 extractable Cu in PMM since Cu may precipitate at high pH range and high solution concentration of Cu^{2+} (Harter 1983). However, when compared with Cu, precipitation of Zn(OH)_2 is a possibility due to much greater LiNO_3 extractable Zn concentration (Table 2). The general order of affinity of the cationic micronutrient with organic matter was reported as $\text{Cu} > \text{Fe} > \text{Mn} > \text{Zn}$ (Park et al. 2011) confirming the high affinity of Cu to be retained by the organic fraction due to the formation of Cu complexes in the soil (Agbenin 2010) and inner-sphere complexation of Cu(II) (Karlsson et al. 2006). The greater proportion of complexes and ion pairs of Cu than those of Mn and Zn in the soil extract indicates the high potential of Cu to react with organic matter in reclaimed soils (Figure 2). Adsorption reactions between Cu^{2+} and humic fractions of Sphagnum peat moss (Gardea-Torresdey et al. 1996) lower the free Cu^{2+} ion concentration seven orders of magnitude below the total Cu^{2+} concentration (Benedetti and Van Riemsdijk 1995).

Speciation of Fe in soils was determined as FeIII(OH)_4^- (76%) and $\text{FeIII(OH)}_{3(\text{aq})}$ (24%) while B was present as free H_2BO_3^- (aq) and $\text{H(H}_2\text{BO}_3)_{(\text{aq})}$. The presence of hydrolysis species of Fe(III) indicated that soil pH was the main factor determining Fe species in soil solution (Lindsay and Schwab 1982). In pine sites, 92% of B was present as free H_2BO_3^- (aq) in PMM while only $\text{H(H}_2\text{BO}_3)_{(\text{aq})}$ was present in TS. In spruce sites, 99% of B was present as $\text{H(H}_2\text{BO}_3)_{(\text{aq})}$ in PMM, while 90% of B was present as free HBO_3^- (aq) in OB. High Mehlich-3 extractable B in TS and OB (Table 2) is supported by the presence of $\text{H(H}_2\text{BO}_3)_{(\text{aq})}$ in two substrates. Relatively low B in PMM compared to substrates could be due to the fraction of B associated with various soil

components including adsorbed and occluded B in mineral phase which is not readily available in the soil solution (Kot 2000; Kot et al. 2012).

Foliar micronutrient concentrations and the ratio of foliar macro- to micronutrients

The foliar analysis provides an index of the amount of nutrients taken up by the trees and this method is considered to be most useful for identifying severely deficient nutrients (Carter 1992) and also as an indicator of soil nutrient availability (Brockley 2001). In both species, the foliar concentration of micronutrients in current year's foliage varied in the order of $\text{Fe} > \text{Mn} > \text{B} > \text{Zn} > \text{Cu}$ (Figure 3). Nutrient deficiencies in pine and spruce were determined using the critical micronutrient concentrations reported for *Pinus* and *Picea* species as shown in Figure 3 (Beaton et al. 1965; Ingestad 1958; Stoate 1950). The foliar Cu concentration in spruce ranged from 1.7 to $6.9 \mu\text{g g}^{-1}$ indicating that Cu was deficient in four of six sites studied. Therefore, out of the five micronutrients, only Cu was identified to be low in spruce trees, consistent with the low availability of Cu in PMM and OB that could lead to reduced tree growth (Mizuno et al. 1982). This partially supports the second hypothesis. However, symptoms of Cu deficiency are less specific than that of other micronutrients (Reuther and Labanauskas 1966) and might not be revealed unless foliar samples were monitored. Both pine and spruce had sufficient B, Fe, Mn and Zn and also Cu in pine regardless of the varying extractability of these nutrients in PMM and substrate in each site type.

As shown in a previous study, application of a complete fertilizer (i.e., including B, Fe, Mn, Cu, and Zn) in the same study sites did not affect foliar micronutrient concentrations in both species (Duan et al. 2015b). Such findings are expected for B, Fe, Mn and Zn since both pine and spruce had sufficient foliar concentrations of these micronutrients. The lack of response to Cu

application suggests possible retention of applied Cu in reconstructed soils, similar to that reported in James and Barrow (1981) and McBride (1981). Even though strong correlations between shoot tissue and LiNO_3 extractable concentrations of trace elements have been reported (Abedin et al. 2012), such relationships were not found in the present study. Such discrepancy between our study and Abedin et al. (2012) may reflect the differences between results from a field study and a greenhouse study.

Foliar analysis information can also be used to monitor foliar nutrient balance (Brockley 2001). Since nutrient ratios have shown to be promising in identifying nutrient deficient sites (Hockman and Allen 1990; Valentine and Allen 1990), nutrient ratios may be especially important if one or both of the elements are near deficiency levels (Marschner 2012). Although pine and spruce were grown over differently reconstructed sites, the ratios of macronutrient:micronutrient were comparable for most of the nutrients studied (Figure 4). Therefore, pine and spruce exhibited the capability to maintain leaf nutrient balances while growing on soils with different levels of macro- and micronutrient availabilities. The ratios between N, P, K and Cu were high as expected. Regardless the N limitation in spruce sites (Duan et al. 2015a), ratios between foliar N and micronutrients were comparable to that in pine sites except for Cu. However, N/Cu, N/B and N/Fe were considerably low in both pine and spruce compared the upper threshold values reported for these two species (Brockley and Simpson 2004) which might have an effect on tree growth. For instance, N metabolism is abnormal in the leaves from a copper-deficient tree and abnormal amounts of complex N compounds were formed at the expense of the carbohydrate reserves (Gilbert et al. 1946). Moreover, fertilizer induced Cu deficiency (Amponsah et al. 2005) may further aggravate the low Cu level in spruce during fertilizer application to overcome low N

availability in spruce sites (Duan et al. 2015). An improper N-Cu balance might also cause a disorder known as "ammoniation" by heavy application of N fertilizers (Camp et al. 1939).

Implications for Land Reclamation

Our results demonstrated low Cu availability in PMM in spruce sites and that adds to the previous findings on the soil nutrient limitations of PMM (e.g., Hemstock et al. 2010; Duan et al. 2015), indicating the necessity for enhancing Cu availability in reclaimed soils. The frequent occurrence of Cu-deficiency in peat soils reclaimed for agriculture has led to the term "reclamation disease" for this condition (Welch et al. 1991). Since Cu reacts with inorganic and organic compounds within the soil (James and Barrow 1981; McBride 1981), monitoring the beneficial effects on tree form and height development as well as changes in Cu availability in the soil upon fertilizer application is needed.

The time since reclamation exerts a large influence on the content and distribution of available micronutrients in the soil (Li et al. 2013). Although available B, Fe, Mn and Zn concentrations in pine and spruce sites are at sufficiency levels, further monitoring of the availability of those micronutrients in reclaimed sites is worthwhile because weathering, decomposition of organic matter and changes in soil water regime can alter the solubility and availability of micronutrients. Research on the distribution of micronutrients in the surface soil layer during land reclamation showed that Cu and Zn move slowly and gradually upwards as water evaporates (Li et al. 2013). This phenomenon can be particularly important for pine sites where high evapotranspiration demand is a possibility (Duan et al. 2015a). Hence, the availability of Cu and Zn may be further reduced in pine sites as compared to other micronutrients. The large pool of Mehlich-3 extractable Fe in PMM in both sites is indicative of the potential of Fe being

released to the soil over the course of time due to the decomposition of organic matter and mineral weathering that could lead to high available Fe in PMM. Available Fe in soil increased and exceeded the available Fe in natural soils as the time since reclamation increased (Li et al. 2013). The increased Fe availability may negatively affect the nutrient balance in vegetation over time

A slightly acidic soil pH and a proper balance with other nutrients will help maintain the optimum Cu level in spruce sites. Soil pH between 5 to 6.5 has been identified to be optimal for surface materials for revegetation in boreal forests, particularly for planting coniferous trees, in northern Alberta (Macyk et al. 1993). Soil management to reduce pH in PMM should be taken into consideration when planning reclamation practices to avoid nutrient limitations. Further research on soil reaction mechanisms that control the solubility and mobility of micronutrients in PMM, TS and OB will improve our understanding of the availability of nutrients in reclaimed soils.

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Table 1. Properties (means with SD in parenthesis) of soils collected from lodgepole pine and white spruce sites selected for this study in the Athabasca oil sands region, Alberta (Duan et al. 2015a).

Plant species	Soil layer	Soil property								
		Clay (g kg ⁻¹)	pH	Electrical conductivity (dS m ⁻¹)	Bulk density (Mg m ⁻³)	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	Inorganic N (mg kg ⁻¹)	Available P (mg kg ⁻¹)	Water extractable K (mg kg ⁻¹)
Pine	PMM	235(48)	7.80(0.07)	0.98(0.21)	0.95(0.21)	75.77(1.05)	2.57(1.05)	4.16(2.11)	1.56(1.05)	7.22(2.26)
	TS	< DL ^a	6.68(1.04)	0.22(0.15)	1.37(0.17)	1.87(0.96)	0.02(0.01)	1.96(0.43)	0.41(0.10)	2.39(0.91)
Spruce	PMM	127(18)	7.69(0.43)	2.27(1.23)	0.89(0.33)	86.15(73.66)	3.61(3.46)	3.41(2.54)	0.80(0.47)	19.25(6.94)
	OB	280(43)	7.60(0.19)	3.03(2.17)	1.28(0.15)	40.27(4.57)	0.46(0.11)	1.93(0.52)	0.49(0.17)	11.42(5.19)

Note: Data in table are means of nine replicates

^a DL stands for detection limit.

Table 2. Micronutrient concentrations in soils collected from lodgepole pine and white spruce sites.

Extraction method	Tree species	Soil layer ^a	B	Fe	Mn	Cu	Zn
Micronutrient concentration ($\mu\text{g g}^{-1}$)							
LiNO ₃	Pine	PMM	17.28b ^b	0.30b	0.12b	< DL ^c	0.17a
		TS	33.88a	6.67a	0.30a	< DL	0.05b
	Spruce	PMM	22.42b	14.07	0.54	< DL	0.07
		OB	39.56a	10.51	0.32	0.04	0.11
Mehlich-3	Pine	PMM	2.11a	289.24a	14.06a	1.36a	3.01a
		TS	0.41b	54.96b	3.80b	0.33b	0.80b
	Spruce	PMM	2.93	240.56	14.24	1.70	4.43
		OB	3.30	221.81	33.55	1.50	4.31

Note: Data in table are means of six replicates per treatment.

^a PMM, TS, and OB stand for peat-mineral soil mix, tailings sand and overburden, respectively.

^b Different letters indicate significant differences in micronutrient concentration between PMM and substrate of each site type at $p < 0.05$ (i.e., between PMM and TS in pine sites and between PMM and OB in spruce sites).

^cDL stands for detection limit.

Table 3. Principal components extracted from a correlation matrix based on soil properties and micronutrients in pine and spruce sites.

Variable	Variable	Pine		Spruce	
		PC1	PC2	PC1	PC2
Soil properties	Total C	-0.75^a	-0.53	-0.97	0.13
	Clay	-0.56	0.35	0.39	-0.68
	pH	0.51	0.65	0.91	-0.20
	Electrical conductivity	-0.02	0.95	0.54	0.52
	Bulk density	0.97	0.15	0.93	-0.16
Mehlich-3 extractable micronutrients	B	-0.98	0.13	-1.00	-0.02
	Fe	-0.93	0.15	-0.82	-0.34
	Mn	0.85	-0.09	0.75	0.24
	Cu	0.68	-0.02	0.89	0.30
	Zn	-0.26	0.65	-0.81	0.35
LiNO ₃ extractable micronutrients	B	0.13	-0.92	0.93	-0.13
	Fe	0.51	0.45	-0.95	0.17
	Mn	-0.19	0.78	-0.95	0.16
	Zn	0.16	-0.62	0.23	0.87
Eigenvalue		5.49	4.24	9.52	2.77
% Variance		39.22	39.22	63.50	63.51
Cumulative explanation		30.22	69.54	18.50	82.01

^a Values in the table are the correlation of each variable with each principal component. Bold values indicate the strongest associations.

Figure 1. Principal component analysis of the LiNO_3 and Mehlich-3 extractable micronutrient concentrations in soils collected from lodgepole pine and white spruce sites selected for this study in the Athabasca oil sands region, Alberta. PMM, TS, and OB stand for peat-mineral soil mix, tailings sand and overburden, respectively.

Figure 2. Results of Mn, Zn and Cu speciation simulation for solutions extracted from soils in lodgepole pine and white spruce sites selected for this study in the Athabasca oil sands region, Alberta. PMM, TS, and OB stand for peat-mineral soil mix, tailings sand and overburden, respectively.

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70 Figure 3. Foliar micronutrient concentrations (means $\pm$ SD) in lodgepole pine and white spruce.
71 The value above each column is the critical concentration of a particular micronutrient.
72 Concentrations below the critical concentration indicate a deficiency in respective tree species
73 (Beaton et al. 1965; Ingestad 1958; Stoate 1950).

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Figure 4. Ratios between foliar concentration of nitrogen, phosphorus and potassium and the studied micronutrients (B, Fe, Mn, Cu and Zn) in lodgepole pine and white spruce.