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HEAVY METAL ANALYSIS IN RED OAK (*QUERCUS RUBRA*) POPULATIONS FROM A MINING REGION IN NORTHERN ONTARIO (CANADA): EFFECT OF SOIL LIMING AND ANALYSIS OF GENETIC VARIATION

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ABSTRACT

Understanding the dynamic of metals in soil and plants and population diversity in Northern Ontario is essential in determining progress toward ecosystem sustainability in reclaimed sites. The objectives of the present study were to assess the levels of metal content in soils and their accumulation in red oak plants from limed and unlimed sites. Genetic variation in red oak populations from the Northern Ontario region was also analyzed. The levels of soil acidity was lower in limed areas compared to un limed sites, an indication of the prolonged beneficial effect of liming 20 to 30 years ago on soil toxicity. The levels of total metals were very high for most elements, but the proportion of metals that were bio available and readily available to plants was very small. The enrichment factors were16.78, 4.98 and 2.94 for total arsenic, copper and nickel, respectively. The Translocation Factor (TF) values for available metals from soil to branches were high. There was more metal accumulation in leaves compared to branches. The degrees of genetic variability in red oak populations from limed and unlimed areas were compared using ISSR markers. The levels of polymorphic loci were moderate to high ranging from 44 to 65%. There were no significant differences in polymorphisms between areas that were limed and unlimed. Overall the red oak populations in stressed areas in Northern Ontario are sustainable.

Keywords: Liming, Enrichment and Translocation Factors, Red Oak (*Quercus rubra*), ISSR Markers, Northern Ontario

1. INTRODUCTION

Northern Ontario especially the Greater Sudbury region is highly known for its nickel, copper and other metal deposits. The mining, roasting and smelting of these elements have caused disastrous effects on the vegetation and overall environment (Amiro and Courtin, 1981; Gratton *et al*., 2000; Nkongolo *et al*., 2008; Vandeligt *et al*., 2011; Narendrula *et al*., 2012). The region became semi-barren to completely barren and several studies identifiedsulfur dioxide emissions and metal particulates in soil and various plant species to be the main causes of these effects. Concentrations of metal, specifically nickel and copper, have been found to be higher in areas around smelters compared to other regions (Amiro and Courtin, 1981; Narendrula *et al*., 2012; 2013). The high metal content did increase the levels of soil acidity which affects plant growth. The pHs of these soils ranged from 3.0 to 5.0 with an average of approximately 4.0. Massive restoration projects including

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liming of soils and revegetation in the Greater Sudbury Region have been implemented (Lautenbach *et al*., 1995).

Dolomitic lime which contains calcium and magnesium carbonate was applied to soils from 1980 to 1995 at different locations across Northern Ontario (Lautenbach *et al*., 1995). Specifically, the role of both calcium and magnesium together is crucial to metal toxicity as they create a competitive exclusion of metal ions from the root-hair's exchange complex, that magnesium and calcium alone is unable to perform and may affect leaf and branch tissue (Winterhalder, 1996). The process of liming aims at neutralizing soil pH and detoxifying soil from contamination. Soil liming and tree planting have proved to be visually beneficial as the once barren lands now have a prominent increase in vegetation. This is consistent with data from other regions around the world (Rodenkirchen, 1992).

Of the trees planted to date in the Northern Ontario Region of Sudbury, 95% were conifers and 5% are hardwood species because of high level of metal tolerance of conifer species (Ryan *et al*., 1986; Winterhalder, 1995; Narendrula *et al*., 2013). Red oak (*Quercus rubra*) is one of the few species found on the drier barren sites and have survived the stresses that caused the loss of the original vegetation (Winterhalder, 1996). A high level of genetic variability is one of the main prerequisites to sustainability and adaptability. Understanding the dynamic of population diversity and variation in the Red oak populations from Northern Ontario Region is essential for the ongoing monitoring of the bio-remediation program and for the assessment of the long term effects of liming on plant populations.

The objectives of the present study were to determine the effects of liming on soil and plant metal accumulation and genetic variation in red oak (*Quercus rubra*) populations.

2. MATERIALS AND METHODS

2.1. Sampling

Seven areasin Northern Ontario were selected for the present study. They included four areas consisting of paired limed and unlimed sites (Daisy Lake, Kelly Lake, Wahnapitae and Kingsway) and three reference sites (Capreol, St. Charles and Onaping Falls) (**Fig. 1**).

The liming was previously performed through the Sudbury's Regional Land Reclamation Program using dolostone. Wahnapitae Hydro Dam site was limed in 1980, Kingsway site was limed in 1981 and Daisy Lake site was limed aerially (Lautenbach *et al*., 1995). Soil, oak leaf and branch samples were collected from each site. For each area, 10 pedons were sampled, with soil samples being collected from the surface Humus Form (LFH), as well as from the underlying mineral horizons (namely the Ae, Bm, BC and C, if present). Only the top organic layer samples were analyzed in the present study. Soil samples were air dried and stored in sealed plastic bags prior to preparation for chemical analysis. Oak leaves and branches were collected randomly from 20 individual trees and dried for further analysis.

2.2. Metal Analysis

Soil pH was measured in water and a neutral salt solution pH (0.1 M $CaCl_2$) (Carter, 1993). Total metal analysis was performed as described by (Abedin *et al*., 2012). For the estimation of total metal concentrations, a 0.5 g soil sample was treated with 10 mL of 10:1 ratio HF: HCl, heated to 110°C for 3.5 h in open 50 mL Teflon™ tube in a programmable digestion block to dry down samples, followed by addition of 7.5 mL of HCl and 7.5 mL of HNO_3 and heating to 110°C for another 4 h to dry gently. The samples were then heated to 110°C for 1 h following addition of 0.5 mL of HF, 2 mL of HCl and 10 mL of HNO_3 to reduce sample volume to 8-10 mL. On cooling, the samples are made to 50 mL with ultrapure water for subsequent analysis by plasma spectrometry.

Bio available metals were estimated by extracting 5 g of soil with 20 mL of 0.01 M $LiNO_3$ in a 50 mL centrifuge tubes in a shaker under ambient lighting conditions for 24 h at 20°C (Spiers and Abedin, 2006; Abedin *et al*., 2012). The pH ($LiNO_3$) of the suspension was measured prior to centrifugation at 3000 rpm for 20 min with filtration of the supernatant through a 0.45 µm filter into a 20 m L polyethylene tube and made to volume with deionized water. The filtrate was preserved at approximately 3°C for analysis by ICP-MS. The quality control program completed in an ISO 17025 accredited facility (Elliot Lake Research Field Station of Laurentian University) included analysis of duplicates, Certified Reference Materials (CRM's), Internal Reference Materials (IRM's), procedural and calibration blanks, with continuous calibration verification and use of internal standards (Sc, Y, Bi) to correct for any mass bias. All concentrations were calculated in mass/mass dry soil basis. The data obtained for all elements of interest in analyzed CRM soil samples were within ±12% of the certified level. Metal content in leaves and branches was determined according to the protocol described by (Nkongolo *et al*., 2008; Abedin *et al*., 2012).

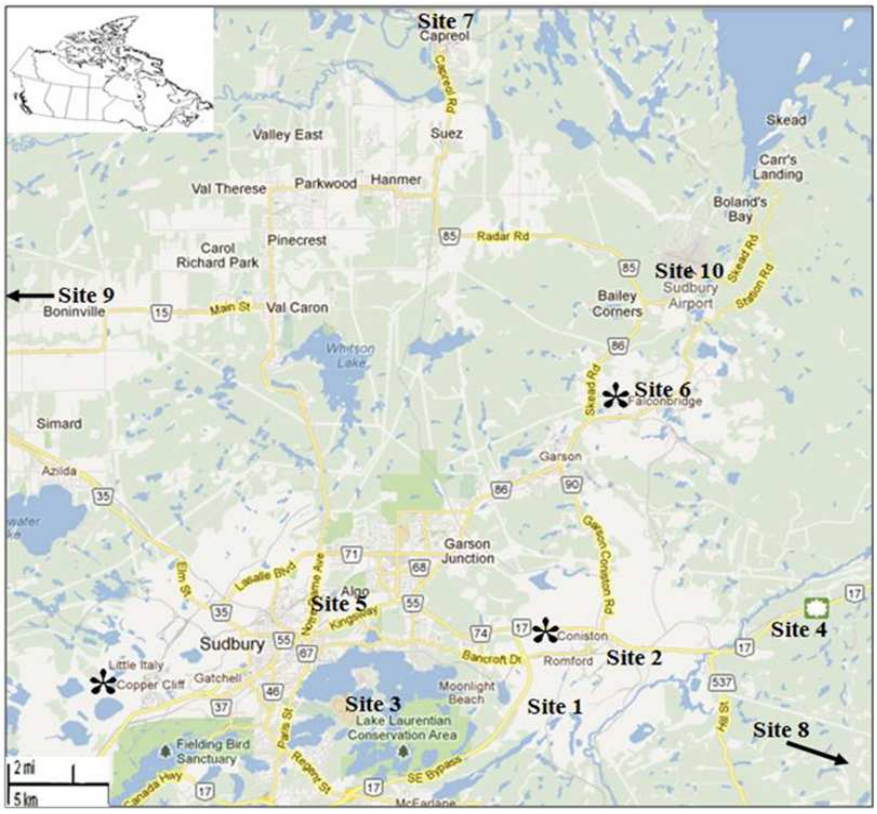

Fig. 1. Location of red oak (*Quercus rubra*) sampling sites within the greater Sudbury region in Northern Ontario. Site 1: Daisy Lake; Site 2: Wahnapitae Hydro Dam; Site 3: Laurentian; Site 4: Kukagami; Site 5: Kingsway; Site 6: Falconbridge; Site 7:

2.3. Statistical Analysis

The data for the metal levels in soil and tissue samples were analyzed using SPSS 7.5™ for Windows, with all data being transformed using a $\log_{10}$ transformation to achieve a normal distribution.

Variance-ratio test was done with an assumption of data normality in the underlying population distributions of the data. ANOVA, followed by Tukey's HSD multiple comparison analysis, were performed to determine significant differences ($p<0.05$) among the sites. Data from analysis of samples from limed and no limed areas were compared using the Student-T test.

The Enrichment Factor (EF) and the Translocation Factor (TF) were determined according to the equations described by (Singh *et al*., 2010).

2.4. Molecular Analysis

Fresh *Quercus rubra* leaf samples were collected from the targeted sites in Northern Ontario based on leaf morphology. Twenty trees representing each targeted population were selected for the study. Leaf samples were wrapped in aluminum foil, immersed in liquid nitrogen and stored at-20°C until DNA extraction.

2.5. DNA Extraction

Genomic DNA was extracted from fresh frozen leaf material using the CTAB extraction protocol as described by (Nkongolo, 1999; Mehes *et al*., 2007). The protocol is a modification of (Doyle and Doyle, 1987) procedure. The modifications included the addition of 1% Poly Vinyl Pyrrolidone (PVP) and 0.2% beta mercaptanol to the Cetyl Trimethyl Ammonium Bromide (CTAB) buffer solution, two additional chloroform spins prior to the is opropanol spin and no addition of RNA se. After extraction, DNA was stored in a freezer at-20°C.

2.6. ISSR Analysis

A total of 15 ISSR primers were pre-screened for polymorphism and reproducibility of these, eight primers

were identified. These included 178 99A, 178 99B, UBC 841, UBC 825, ISSR 5, 8, 9 and 10. Five of these eight primers (178 98B, UBC 825, ISSR 5, 9 and 10) that produced strong bands were selected for ISSR analysis.

PCR amplification was carried out as described by (Mehes *et al*., 2007) in a 25 μL total volume containing a master mix of 11.4 μL distilled water, 2.5 μL $MgSO_4$, 2.1 μL 10×buffer 0.5 μL of dNTPs (equal parts dTTP, dATP, dCTP, dGTP), 0.5 μL of ISSR primer, a Taqmix of 3.475 μL distilled water, 0.4 μL 10×buffer and 0.125 μL *Taq* polymerase (Applied Biosystems) and 4 μL standardized DNA. Each primer contained a negative control of master mix and Taqmix without any DNA. All samples were covered with one drop of mineral oil to prevent evaporation and amplified with the Eppendorf Mastercycler gradient the rmocycler. The program was set to a hot start of 5 min at 95°C followed by 2 min at 85°C to which the Taqmix was added. In total, 42 cycles of 1.5 min at 95°C, 2 min at 55°C and one min of 72°C were performed. A final extension of 7 min at 72°C after which samples were removed from the the rmocycler and placed in a freezer set at s-20°C until further analysis.

Amplified DNA products were separated for analysis on a 2% agarose gel in 0.5×TBE with ethidium bromide. Then, 5 μL of 2×loading buffer were added to the PCR products and 10 μL of this solution were loaded into the wells of the gel. The gel was run at 64 V for 120 min, documented with the Bio-Rad Chemi Doc XRS system and analyzed with Image Lab Software.

The ISSR bands on each gel were scored as either present (1) or absent (0). Popgene software version 1.32 (Yeh and Boyle, 1997) was used to determine percentage of polymorphic loci, observed and effective number of alleles, Nei's gene diversity and Shannon's information index. The genetic distances were calculated using Jaccard's similarity coefficients with Free Tree Program version 1.50. A Neighbour-Joining dendogram was produced from the similarity coefficients. The method starts with a star like tree with no hierarchical structure and in a stepwise fashion finds the two operational taxonomic units that minimize the total branch length at each cycle of clustering. The unrooted tree generated by the Neighbour-Joining method is constructed under the principle of minimum evolution (Saitou and Nei, 1987).

3. RESULTS

3.1. Soil Acidity and Metal Contents

The pH values of all the sites were determined for the first top organic layer (**Table 1**). The pH in limed areas was higher compared to unlimed sites, ranging from 4.12 to 6.75. The estimated average levels of total and bio available metal concentrations in the soil samples from limed and unlimed areas are illustrated in **Table 2 and 3**. The control concentrations always showed the lowest levels for total metals analyzed.

Overall, the total magnesium was the only element measured that was significantly higher in limed compared to unlimed sites (**Table 2**). Arsenic was lower in limed compared to unlimed areas. Detectable values of metal concentrations which are bio available ranged between 0.07 mg kg^{-1} for strontium and 169.1 mg kg^{-1} for magnesium for limed sites. For unlimed areas, the level of bio-available elements ranged from 0.10 mg kg^{-1} for as to 129.2 mg kg^{-1} for K. Bio available Magnesium was as expected higher in limed compared to unlimed areas. Bio available strontium and manganese were higher in unlimed compared to limed sites (**Table 3**).

3.2. Total Metal Content in Leaves and Branches of Red Oak

Table 4 and 5 illustrate the mean total metal concentrations in leaves and branches. The total mean concentrations of cadmium and manganese in leaves were found to be significantly higher in limed compared to unlimed sites. Significant differences were also observed between limed and unlimed sites for iron, lead, magnesium and nitrogen in branches (**Table 5**).

3.3. Enrichment and Translocation Factors

The Enrichment Factor (EF) was calculated for the four elements that revealed a significant difference in concentration between metal contaminated and control sites. For total metals, the EF values were found to be 16.78, 4.98 and 2.94 for arsenic, copper and nickel, respectively. The Translocation Factors (TF) were calculated in contaminated sites from soil to branches. In general, The TFs from soil to branches were low for both total and bio available metals in reference control sites compared to contaminated sites. The TF values were very low for all total metals except for magnesium and phosphorus. The TF of bio available elements from soil to branches were very high ranging from 1.90 to 266.3 for contaminated sites (**Table 6**). There were higher metal accumulation in leaves compared to branches specifically for aluminum, copper, potassium, magnesium, sulfur and nitrogen (**Table 7**).

Table 1. The pH levels of the top organic Layer (LFH) from greater Sudbury region sites

Sampling sites	Layer 1type	pH H_2O	pH $CaCl_2$
Daisy Lake	Unlimed	4.04	3.87
	Limed	4.12	4.05
Wahnapitae hydro Dam	Unlimed	3.82	3.56
	Limed	6.75	6.34
Kingsway	Unlimed	3.87	2.35
	Limed	4.67	4.35
Capreol (control)	Unlimed	3.92	3.43
St. Charles (control)	Unlimed	3.5	3.23
Onaping Falls (control)	Unlimed	3.79	3.46

Table 2. Mean concentration of total metals elements in the limed and un imed organic surface Horizons (LFH) of soils from the Sudbury region sites (concentrations are in mg kg-1, dry weight)

	Elements [a]										
Sites	As*	C	Co	Cu	K	P	Mg	Mn	Ni	Sr	Zn
Limed	1.46±1.28	124267±26533	41±14	952±343	7066±316	571±93	2309±672	198±41	991±485	58±12	68±21
Unlimed	18.98±5.82	136583±39404	42±11	1021±356	8540±187	600±37	1548±194	242±15	1061±398	62±10	73±16

[a]Results are expressed as mean values ± standard errors; *represents significant difference between treatments based on t-test ($p \leq 0.05$); Lime and no lime sites: Daisy lake, wahnapitae hydro dam and kingsway

Table 3. Mean concentration of bio available metals elements in the limed and unlimed organic surface Horizons (LFH) of soils from the Sudbury region sites (concentrations are in mg kg^{-1}, dry weight)

Elements [a]											
Sites	As	Cd	Co	Cu	K	P	Mg*	Mn*	Ni	Sr*	Zn
Limed	0.14±0	<DL-	0.12±0	7.21±3.380	98.13±22.30	6.14±2.78	169.1±66.6	3.71±2.60	4.15±1.8	0.07±0	0.48±000
Unlimed	0.1±00	<DL-	0.31±0	12.04±3.71	129.22±24.5	6.05±2.25	39.78±5.28	13.95±3.7	6.85±2.7	0.33±0	1.48±0.80

[a]Results are expressed as mean values ± standard errors; *Represents significant difference between treatments based on t-test ($p \geq 0.05$); Limed and no limed sites: Daisy lake, wahnapitae hydro dam and kingsway; <DL indicates concentrations below detectable level

Table 4. Mean total concentration of metals in leaves of red oak (*Quercus rubra*) from the Sudbury region sites, concentrations are in mg kg-1, dry weight

Elements[a]																		
Sites	Al	As	C	Ca	Cd*	Co	Cu	Fe	K	P	Pb	Mg	S	Mn*	Ni	N	Sr	Zn
Limed	1385	0.25	469750	6647	0.080	0.460	17.6	92.90	12435	2431	<DL	2367	2148	677	27	24182	9.40	2600
	±111	±0.3	±14020	±560	±0.05	±0.46	±3.8	±12.5	±2708	±521	-	±619	±522	±279	±6	±6372	±3.0	±3.3
Unlimed	1401	0.97	470000	7282	0.230	0.150	13.6	1010	11690	2149	<DL	2434	2172	1195	24	25541	9.60	2700
	±103	±0.7	±13839	±567	±0.04	±0.15	±1.9	±100	±2076	±498	-	±269	±401	±237	±4	±4451	±1.5	±3.3

[a]Results are expressed as mean values ± standard errors; *Represents significant difference between treatments based on t-test ($p \leq 0.05$); Lime and no lime sites: Daisy lake, wahnapitae hydro dam and kingsway; <DL indicates concentrations below detectable level

Table 5. Mean total concentration of metals in branches of red oak (*Quercus rubra*) from the Sudbury region sites, concentrations are in mg kg-1, dry weight

	Elements [a]																	
Sites	Al	As	C	Ca	Cd	Co	Cu	Fe*	K	P	Pb*	Mg*	Mn	S	Ni	N*	Sr	Zn
Limed	811	0.99	490750	5457	0.29	0.82	33.5	269	6630	1342	3.35	1098	282	962	25	10430	15.5	220
	±146	±0.58	±5662	±904	±0.14	±0.32	±14	±192	±1833	±363	±2.0	±517	±126	±208	±9	±2760	±6.4	±1.3
Unlimed	942	0.99	497000	6451	0.56	0.52	15.2	98	5655	1265	0.36	888	524	735	19	9044	17.9	1800
	±164	±0.41	±3216	±648	±0.10	±0.21	±3	±40.6	±836	±166	±0.36	±91.4	±153	±134	±5	±1937	±2.7	±1.7

[a]Results are expressed as mean values ± standard errors; *Represents significant difference between treatments based on t-test ($p \leq 0.05$); Lime and no lime sites: Daisy lake, wahnapitae hydro dam and kingsway

Table 6. The translocation factors for total and bio available metals from soil to branches in metal-contaminated sites

Elements										
Translocation factor	As	Co	Cu	K	P	Mg	Mn	Ni	Sr	Zn
Total metal from Soil to branches	0.01	0.02	0.04	0.89	2.69	0.62	1.32	0.05	0.38	0.35
Bio available metal from soil to branches	1.90	4.82	4.14	94.20	266.32	8.45	41.08	7.51	138.46	32.2

Table 7. Mean concentration of total metals in leaves and branches of red oak (*Quercus rubra*) from the Sudbury region sites, concentrations are in mg kg-1, dry weight

Elements[a]															
Type	Al	As	Ca	Cd	Co	Cu	Fe	K *	P	Pb *	Mg*	Mn	Ni	Sr	Zn
Leaves	1190	0.60	6718	0.200	0.20	14.2	87.80	10967	2138	0.020	2568	9320	220	9.40	2700
	±152	±0.4	±421	±0.03	±0.2	±1.7	±10.3	±1377	±326	±0 .02	±295	±168	±3.5	±1.2	±1.90
Branches	7620	0.80	6587	0.500	0.50	19.7	139.1	53280	1123	1.300	1071	4040	190	20.3	21000
	±131	±0.3	±553	±0.10	±0.2	±5.0	±63.0	±7460	±157	±0 .70	±198	±970	±4.1	±3.2	±1.20

Table 8. The nucleotide sequences of ISSR primers used to amplify DNA from red oak samples

ISSR Primer Identification	Nucleotide sequence(5' → 3')		GC content (%) fragment size range (bp)
HB 15	$(GTG)_3GC$	72	270-2200
HB 13	$(GAG)_3GC$	72	170-1360
ISSR 17898A	$(CA)_6AG$	50	270-1450
ISSR 17898B	$(CA)_6GT$	50	210-1300
ISSR UBC 841	GAAG $(GA)_6YC$	50	250-1100
ISSR UBC 829	$(TG)_7C$	53	200-4000
ISSR UBC 827	$(AC)_8G$	60	400-1000
ISSR UBC825	$(AC)_8T$	53	650-1000
SC ISSR 10	$(CTT)_5(CCT)_6CT$	51	
SC ISSR 9	$(GATC)_3GC$	57	305-9000
SC ISSR 8	$(AGAT)_4GY$	27	
SC ISSR 7	$(AGG)_5GY$	65	
SC ISSR 6	$(TTG)_5CB$	35	
SC ISSR 5	$(ACG)_4$	67	310-1500
SC ISSR 4	$(CGT)_4C$	69	

Table 9. Genetic variability parameters of red oak (*Quercus rubra*) populations based on ISSR data

Populations (Distances from smelters)	P (%)	Na	Ne	h	I
Daisy lake limed (<5 km)	51.77	1.52	1.21	0.13	0.20
Daisy lake unlimed (<5 km)	52.48	1.52	1.20	0.13	0.20
Wahnapitae hydro dam limed (<5 km)	51.77	1.52	1.20	0.13	0.21
Wahnapitae hydro dam unlimed (<5 km)	54.61	1.55	1.21	0.13	0.21
Kingsway limed (5-15 km)	54.61	1.55	1.18	0.12	0.19
Kingsway unlimed (5-15 km)	58.87	1.59	1.22	0.14	0.23
St. Charles (control>15 km)	43.97	1.44	1.21	0.13	0.20
Onaping falls (control>15 km)	51.06	1.51	1.22	0.13	0.21
Mean	46.01	1.54	1.22	0.14	0.22

Genetic diversity descriptive statistics P: Percentage of polymorphic loci; Na: Observed number of alleles; Ne: Expected number of alleles; h: Gene diversity (Nei, 1973); I: shannon's information index

3.4. ISSR Analysis of Red Oak Populations from Targeted Sites

3.4.1. Genetic Diversity

Table 8 describes the main characteristics of ISSR primers screened in the present study. **Figure 2** depicts amplified products of genomic DNA from red oak maple samples using the ISSR primer 5. The percentage of polymorphic loci (%), the observed Number of alleles (Na), the effective Number of alleles (Ne), Nei's gene diversity (h) and Shannon's Information index (I) were estimated and they are described in **Table 9**.

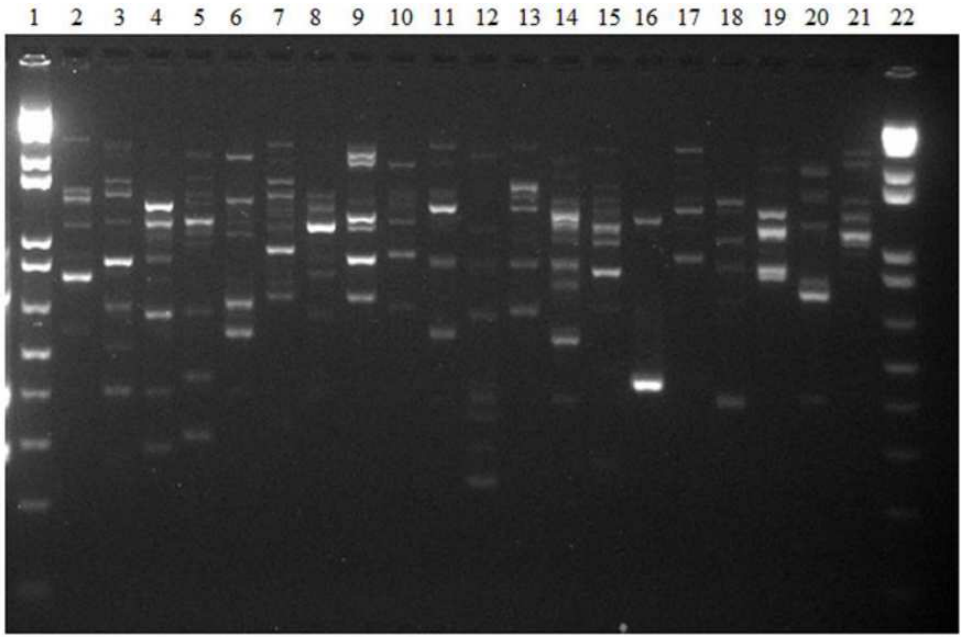

Fig. 2. ISSR amplification of red oak samples with primer ISSR 5. Lanes 1 and 22 contain 1 kb+ ladder; Lanes 2-21 contain red oak

The mean values for Na, Ne, h and I were 1.54, 1.22, 0.14 and 0.22. The level of inter-population polymorphism was 97.87%. The total gene diversity (HT) and the mean gene diversity between populations (HS) were 0.21 and 0.14, respectively. The population differentiation (GST) value was 0.34 and the estimated gene flow (Nm) was 0.98. The levels of polymorphism per primer were 61, 73, 66, 56 and 48% for 178 98 B, UBC 825, ISSR 10, ISSR 9 and ISSR 5 primers, respectively.

The levels of genetic variation in all populations were moderate to high. In fact the percentage of polymorphic loci varied between 43.97% (St. Charles) and 64.54% (Capreol). The observed number of alleles ranged from 1.44 (St. Charles) to 1.65 (Capreol) with a mean of 1.54. The expected number of alleles ranged from 1.18 (Kingsway, limed) to 1.28 (Capreol) with a mean of 1.22. Nei's gene diversity, h, ranged from 0.12 (Kingsway, limed) to 0.17 (Capreol) with a mean of 0.14. Shannon's information index revealed a range between 0.19 (Kingsway, limed) and 0.27 (Capreol) with a mean of 0.22. The mean polymorphic indices were determined for limed and unlimed area. No significant differences for the level of polymorphism was observed between the two groups. The mean levels of polymorphism were 53 and 55% for limed and unlimed populations, respectively.

4. DISCUSSION

4.1. Long Term Effects of Liming on Soil Toxicity

Several studies have been conducted on the fitness and viability of the introduced plant species (Backor and Fahselt, 2004; Vandeligt *et al*., 2011; Narendrula *et al*., 2013). The present study is the first that assesses the sustainability of a hardwood species in stressed areas in Northern Ontario.

Monitoring the long term consequences of metal contamination as well as the results of land remediation is important to determine ecological stability. The levels of total metal concentrations in the organic-rich upper soil Horizon (LFH) were consistent with previous studies and confirm that the metal particulates found in soil are dominantly due to an airborne cause (Amiro and Courtin, 1981; Gratton *et al*., 2000; Nkongolo *et al*., 2008; Vandeligt *et al*., 2011; Narendrula *et al*., 2012; 2013).

The bio available metal level in soil analyzed in the present study was determined to be a critical aspect of phytoxicity. Although total amounts of metal content in soil of certain sites were high, the percentage of bio available metal relative to total metal was much lower. This indicates that the metal amount readily available to

plants is small and therefore the toxicity of such contaminated soils might be minimal.

The most profound effect of liming is the high level of pH in limed sites compared to unlimed areas. Soil pH is an important factor in regulating metal and nutrient content. If it is too low, the availability of nutrients to plants may be disrupted as seen in the barren lands of the Sudbury region (Rodenkirchen, 1992; Winterhalder, 1996). Increasing soil pH will also affect the rate of organic matter mineralization. The pH values found in this present study shows that the liming of soils 20 to 30 years ago continues to produce long term advantages. High pH values are also reflected on the metal content as there were significantly lower bio available concentrations of aluminum, iron and strontium in limed areas. It should be pointed that although smelters are still currently in use, pH values were not as low as values recorded prior to liming. For example, extremes pH values were once recorded to have been slightly over 2 (Winterhalder, 1996). The current improvement in soil toxicity even in unlimed sites compared to past records are likely the results of the abatement policies.

Overall, the application of dolomitic limes over 20 years ago continue to have an impact on total concentration of metals of interest for Northern Ontario that include total Cu, Ni, Co, Zn and Cd The levels of these metals in limed sites were lower than unlimed areas. Total arsenic concentrations were significantly higher in limed areas compared to reference unlimed areas. The amount of bio available strontium was significantly lower in limed sites compared to unlimed sites. A measurable decrease in bio available amounts of Co, Cu, K, Mg, Ni and Zn was observed in limed compared to unlimed sites. The amounts of bio available Ca and Mg is measurably higher in limed areas which reflects the addition of calcitic and dolomitic lime. The neutralizing power of the limestone was expected to halt eventually leading to a deterioration of the plant community. However, as the present study illustrated, this is not the case. The lasting effect of liming for over 30 years may be the result of soil detoxification requiring roots to penetrate further into the soil and cycling calcium and magnesium from lower horizons to the surface layer. This phenomenon is referred to as a cation pump (Jordan, 1987).

Total cadmium and manganese were significantly different in leaf samples from limed compared to unlimed areas. Iron, lead, magnesium and nitrogen were significantly different in branch samples of limed compared to unlimed areas.

4.2. Enrichment and Translocation Factors

The Enrichment Factor (EF) was calculated to establish the degree of soil contamination and heavy metal accumulation. It is the ratio between the concentration of metals in contaminated soil and the concentration of metals in reference sites. Values greater than 1 indicate environmental pollution (Singh *et al*., 2010). The EF for the present study was determined for the three elements which contained a significant difference between contaminated and reference sites. These elements included As, Cu and Ni. Their respective EF values were 16.78, 4.98 and 2.94. EF for arsenic, copper and nickel are far above the value of contamination resulting in high availability and distribution of metals in soil. The high EF values of copper and nickel are attributed to the mining and smelting activities which occur in Sudbury. These values may be an indication that there may ultimately be an increase in metal accumulation in plants located on reference sites (Gupta *et al*., 2008).

Translocation of metals from soil or roots to above ground tissues is a crucial physiological process in an effective utilization of plant to remediate polluted sites (Zacchini *et al*., 2009; Galfati *et al*., 2011; Majid *et al*., 2012). In the present study, the Translocation Factor (TF) was calculated as the ratio of the concentration of total metal in plant tissues and the concentration of total and bio available metals in corresponding soil. It is used to determine relative translocation of metals from the soil to branches. The TF for total metals were relatively low for the majority of elements. But the TF values based on bio available metals in soils were high for both contaminated and uncontaminated sites.

The accumulation of metals in leaves was greater than in branches indicating a higher mobility of elements within the plant tissues itself. Singh *et al*. (2010) estimated the translocation factor based on total metal in soil only. This approach is not accurate because it underestimates the translocation by considering a large proportion of total metals that are in forms that are not readily available to plants. The assessment of translocation factors based on total metals in roots or bio available elements in soil such as described in the present study should provide reliable information on metal movement from soil to aerial plant tissues.

The analysis of plant tissues was important in determining the level of metal uptake and the mobility of elements from the soil. There was also a significant difference in total potassium, lead, magnesium, manganese, sulfur and nitrogen between leaves and

branches. The levels of these metals were higher in leaves compared to branches with the exception of lead.

The amount of total metal concentrations found in the tissues is much lower than the total amount in soil, but significantly higher than the bio available metals in soil. This indicates that Red oak plants are able to accumulate metals in their tissues. This is consistent with (Leavitt *et al*., 1979) who reported accumulations of Ag, Cd, Cu and Zn metals in red oaks tissues and exclusion of Pb in leaves and twig. Based on the result of the present study, red oak might play a role in phytoextraction of metals from the Sudbury soils. Considering that this species represents up to 10% of all tree species in the targeted sites, it can contribute to phytostabilisation (a component of phytoremediation) of restored forests, since it is able to grow and survive on heavily contaminated soil.

4.3. ISSR Analysis

Determining plant sustainability is a critical process in monitoring land restoration. A decline in genetic variability may stunt the species gene pool and therefore decreases its chances of survival in environmental stresses. In the present study, northern red oak samples from 13 populations were analyzed using ISSR markers to determine the level of genetic variability.

Several molecular markers have been used to determine genetic variability in different taxa. The Inter-Simple Sequence Repeat (ISSR) system is a very useful and efficient tool used in assessing genetic variation of plant populations. It combines the advantages of SSRs and Amplified Fragment Length Polymorphism (AFLP) to the universality of Random Polymorphic DNA (RAPD) (Reddy *et al*., 2002).

In the present study, the level of polymorphic loci ranged from 43.97 to 64.54%. This indicates a moderateto high levelsof genetic variability. No significant differences in polymorphisms were observed between populations fromlimed and unlimed areas. This is consistent with previous data on conifer species (Vandeligt *et al*., 2011; Dobrzeniecka *et al*., 2011; Narendrula *et al*., 2013). This lack of significant differences for genetic variability among populations suggests that the level of metals in plant tissue is too low to play an important role in genotypic selection that can affect the allelic frequencies within the targeted population.

Nei's gene diversity (h) was also determined. The mean value of h for red oak populations analyzed was low (0.14) indicating that allelic frequencies were quite similar for the targeted red oak populations. Shannon's Information index (I) values (mean of 0.22) indicates an uneven distribution of alleles in the populations.

5. CONCLUSION

The results of the present study revealed that the highest concentrations of metal and nutrients were observed in the topmost organic layer, LFH. The pH levels of limed areas were higher than unlimed sites, an indication of the prolonged beneficial effect of liming 20 to 30 years ago on soil toxicity. The proportion of total metals that was bio available and readily available to plants was very small. The concentrations of metal in plant tissue were found to be higher than the bio available metals in soil. The Enrichment Factor (EF) varied between 2.94 and 16.78 indicating environmental pollution. The Translocation Factors (TF) from soil to branches were low for most total elements. But the TF values for bio available metals from soil to branches were high. There was more metal accumulation in leaves compared to branches. The ISSR analysis revealed that the genetic variability within red oak populations in the Greater Sudbury Region (GSR) was moderate to high ranging from 43.97-64.54%.

5.1. Future Research Directions, Limitations and Implication

Knowledge of coping mechanisms of red oak to soil metal accumulation is essential to our understanding of the metal tolerance strategy for different hardwood species. The low levels of TF suggest that red oak is likely not the species of choice for bioremediation.

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