DNA METHYLATION AND GENETIC VARIATION IN CANADIAN RED MAPLE (ACER RUBRUM) FROM A METAL CONTAMINATED REGION

KERSEY KALUBI[1] , MELANIE MEHES-SMITH[2],ABDEL OMRI[1] AND KABWE NKONGOLO[1]

[1]BIOLOGY DEPARTMENT AND [2]BIOMOLECULAR SCIENCES PROGRAM, LAURENTIAN UNIVERSITY, SUDBURY, ON, CANADA, P3E 2C6,

Introduction

The Greater Sudbury Region (GSR) is known for an abundance of nickel (Ni), copper (Cu) and various other metal deposits. Recent studies revealed high enrichment factors for these metals between metal-contaminated and reference sites. Red maple (*Acer rubrum*) copes with soil metal contamination by not storing Ni, Cu, and Zn in its tissues. Analysis of the effects of metals at molecular level is lacking.

Objectives

The main objective of the present study was to assess molecular changes in red maple populations growing in metal contaminated areas.

Materials and Methods

Metal Analysis

- The exchangeable cations were quantified by ICP-MS analysis of ammonium acetate (pH 7) extracts of soil samples with the total exchange capacity being estimated as the sum of the exchangeable cations.
- Cytosine and adenine methylations were measured using tandem mass spectrometry (MS/MS) coupled with LC (LC-MS/MS).
- Genetic variation was determined using inter simple sequence repeat (ISSR) markers and the distribution of methylation was established by methylation-sensitive amplified polymorphism (MSAP) analysis.
- Overall, six populations that included three metal-contaminated and three references sites were targeted

Results and Discussion

- There were significant differences in soil metal content between metal–contaminated and uncontaminated sites for the main elements (nickel, copper, zinc).
- Significant differences were also observed between these two groups for Ca^{2+}, Fe^{2+} and Mg^{2+} cation exchange capacity.
- Molecular analysis revealed difference between metal-contaminated and references sites for global DNA methylation but showed within group variation in the distribution of modification.
- MSAP analysis showed that metal contamination affects methylation of cytosine residues in CCGG motifs
- Data generated by the ISSR marker system also revealed differences in polymorphic loci patterns and Nei's gene diversity between metal-contaminated and reference sites.

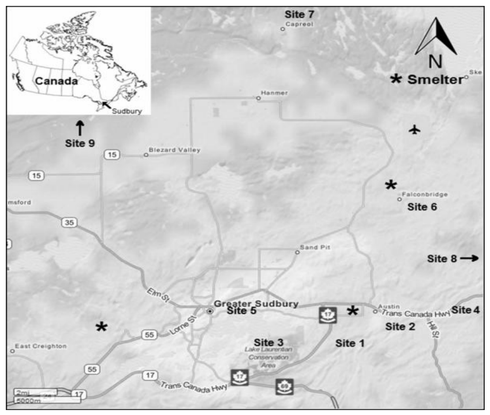

Figure 1. Location of sampling area from the Greater Sudbury region.

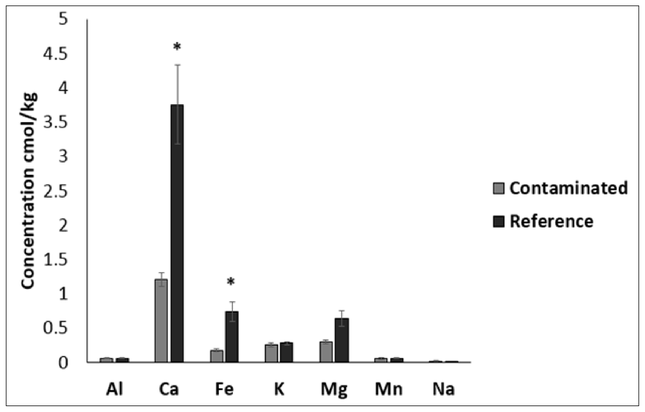

Figure 2. Cation exchange capacity of seven metals found in heavy metal contaminated sites of the Greater Sudbury Region. Means without a subscript are not significantly different based on T-test ($t \geq 0.05$). Error bars represent standard error.

Acknowledgements

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Results

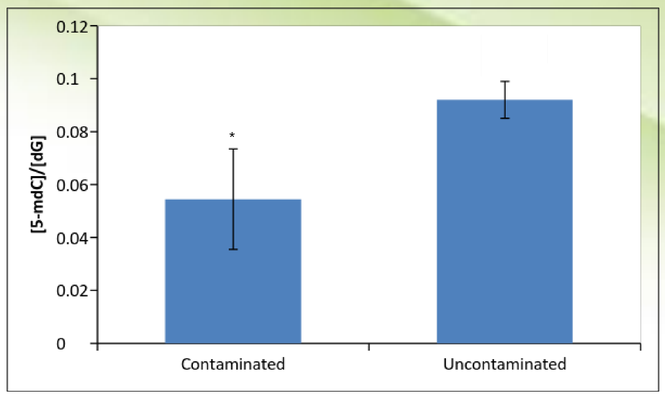

Figure 3. Level of cytosine methylation in metal-contaminated and reference sites

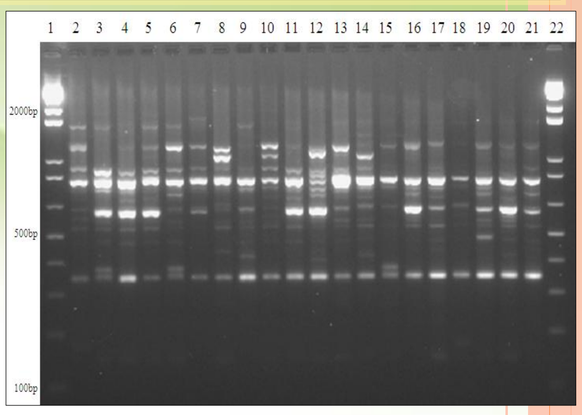

Figure 4: ISSR amplification of red maple samples with primer 17898B. Lane 1 and 22 contain 1Kb+ ladder and lanes 2 to 21 contains samples from site 5 (Kingsway).

Table 1. The nucleotide sequence of ISSR primers that amplified DNA from red maple (*Acer rubrum*) populations samples.

ISSR Primer	Nucleotide sequence (5' → 3')
HB 15	GTGGTGGTGGC
HB 13	GAGGAGGAGGC
17898A	CACACACACACAAG
17898B	CACACACACACAGT
UBC 841	GAAGGAGAGAGAGAGAYC
UBC 829	TGTGTGTGTGTGTGC
UBC 827	ACACACACACACACACG
UBC825	ACACACACACACACACT
SC ISSR 10	CTTCTTCTTCTTCTTCCTCCTCCTCCTCCTCCTCT
SC ISSR 9	GATCGATCGATCGC
SC ISSR 8	AGATAGATAGATAGATAGATGY
SC ISSR 7	AGGAGGAGGAGGAGGGY
SC ISSR 6	TTGTTGTTGTTGTTGCB
SC ISSR 5	ACGACGACGACG
SC ISSR 4	CGTCGTCGTCGTC

Possible nucleotides for base B are C, G or T and for base Y are C or T. Primers in bold were used for ISSR analysis.

Table 2. Genetic Variability parameters of *Acer rubrum* based on ISSR data

Sites	Polymorphism	h	I	Na	Ne
Daisy lake	63.64	0.1356	0.2218	1.6364	1.2082
Dam	63.64	0.1292	0.2149	1.6364	1.1913
Kingsway	70.45	0.1665	0.2671	1.7045	1.2621
Falconbridge	57.95	0.1443	0.2302	1.5795	1.2264
Capreol	70.45	0.1682	0.2675	1.7045	1.2712
St.Charles	75.00	0.1825	0.2911	1.7500	1.2893
Means	**66.855**	**0.1544**	**0.2488**	**1.6685**	**1.2414**

Conclusions

- The genetic variation were high for both contaminated and reference sites
- Preliminary data show that DNA methylation could be associated with metal contamination

References

1. He, X.J., T. Chen, and J.-K Zhu. 2011. Regulation and function of DNA methylation in plants and animals. Cell Research 21: 442-465.
2. Kalubi, K., Mehes-Smith, M., & Omri, A. 2015. Molecular analysis of red maple (Acer rubrum) populations from a reclaimed mining region in Northern Ontario (Canada):soil metal accumulation and translocation in plants Ecotoxicology. 24:636–647

LaurentianUniversity UniversitéLaurentienne