

# Genetic analysis of *Betula papyrifera* (Marsh) populations from a mining reclaimed region: expression of Ni and Cu tolerance genes

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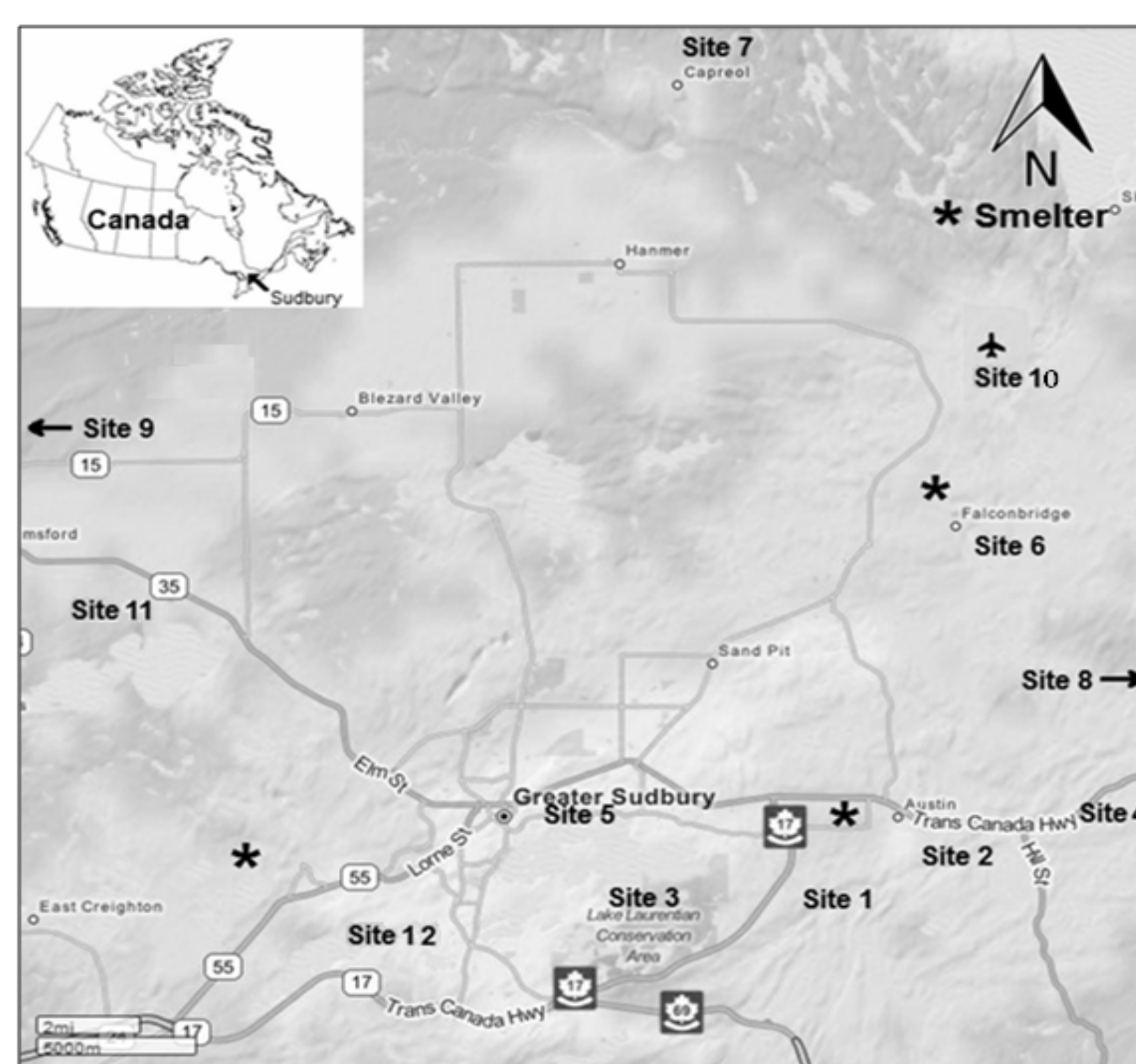
## Abstract

White birch (*Betula papyrifera*) is an open pollinate species that is dominant in Northern Ontario after land reclamation. In fact, this species represents 65% of all trees in the region. The main objective of the present study is to assess the expression of Ni and Cu tolerance genes in white birch. We found that *B. papyrifera* is a Ni and Zn accumulator with a translocation factor of 6.4 and 81, respectively and an indicator of Cu and Pb. Several genes involved in Ni and Cu tolerance from different plant species are being examined using qRT-PCR. Other candidate genes will be identified in *B. papyrifera* by transcriptome analysis. The molecular data will be examined within the context of heavy metal accumulations in soil and plants. Preliminary results of RT-PCR are described.

## Background

Since 1883, the Greater Sudbury Region located in Northern Ontario has been renowned for its sulfur-rich deposits in nickel, copper and iron. This led to the rapid boom of the mining industry and the establishment of many mining companies within the region, such as VALE and Xstrata. The Greater Sudbury Region is also known as one of Canada's most ecologically disturbed areas. Over 100 million tonnes of sulfur dioxide and tens of thousands tonnes of cobalt, copper, nickel and iron have been released from roast pits and smelters (Freedman and Hutchinson, 1980). This caused localized heavy metal pollution and acidification of surrounding ecosystems (Winterhalder, 1995). In the last 30 years, emission control and the Sudbury Re-greening Program contributed significantly to the re-establishment of vegetation in contaminated sites.

White birch is one of the dominant tree species of the Boreal Forest in Northern Ontario. It is a pioneer species and rapidly colonizes open areas. Little is known about heavy metal stress in this species even though it plays such a key role in forest sustainability. Studies have shown that birch is fairly resistant to heavy metal contamination (Kirkey *et al.*, 2012). We have shown that genetic variation in white birch growing in the Greater Sudbury Region is low to moderate. This suggests that the targeted populations are sustainable (Theriault *et al.*, 2014; Theriault *et al.*, 2013).



**Figure 1.0:** Location of studied white birch populations in the Greater Sudbury Region. Site 1: Daisy Lake; Site 2: Dam; Site 3: Laurentian; Site 4: Kukagami; Site 5: Kingsway; Site 6: Falconbridge; Site 7: Capreol (control); Site 8: St. Charles (control); Site 9: Onaping Falls (control); Site 10: Airport; Site 11: Azilda; Site 12: Kelly Lake.

## Methods

### Sampling

White birch leaves and roots were sampled from six locations throughout the Greater Sudbury Region, three from heavy metal contaminated sites and 3 controls (**Figure 1.0**). Secondary roots were collected and washed. The collected samples were flash frozen and stored at -80°C for the RNA extraction.

### RNA extraction

The total RNA was extracted from white birch leaves and roots using Chang *et al.*, 1993 protocol with modifications. The RNA was quantified via fluorometry and treated with DNase.

### qRT-PCR

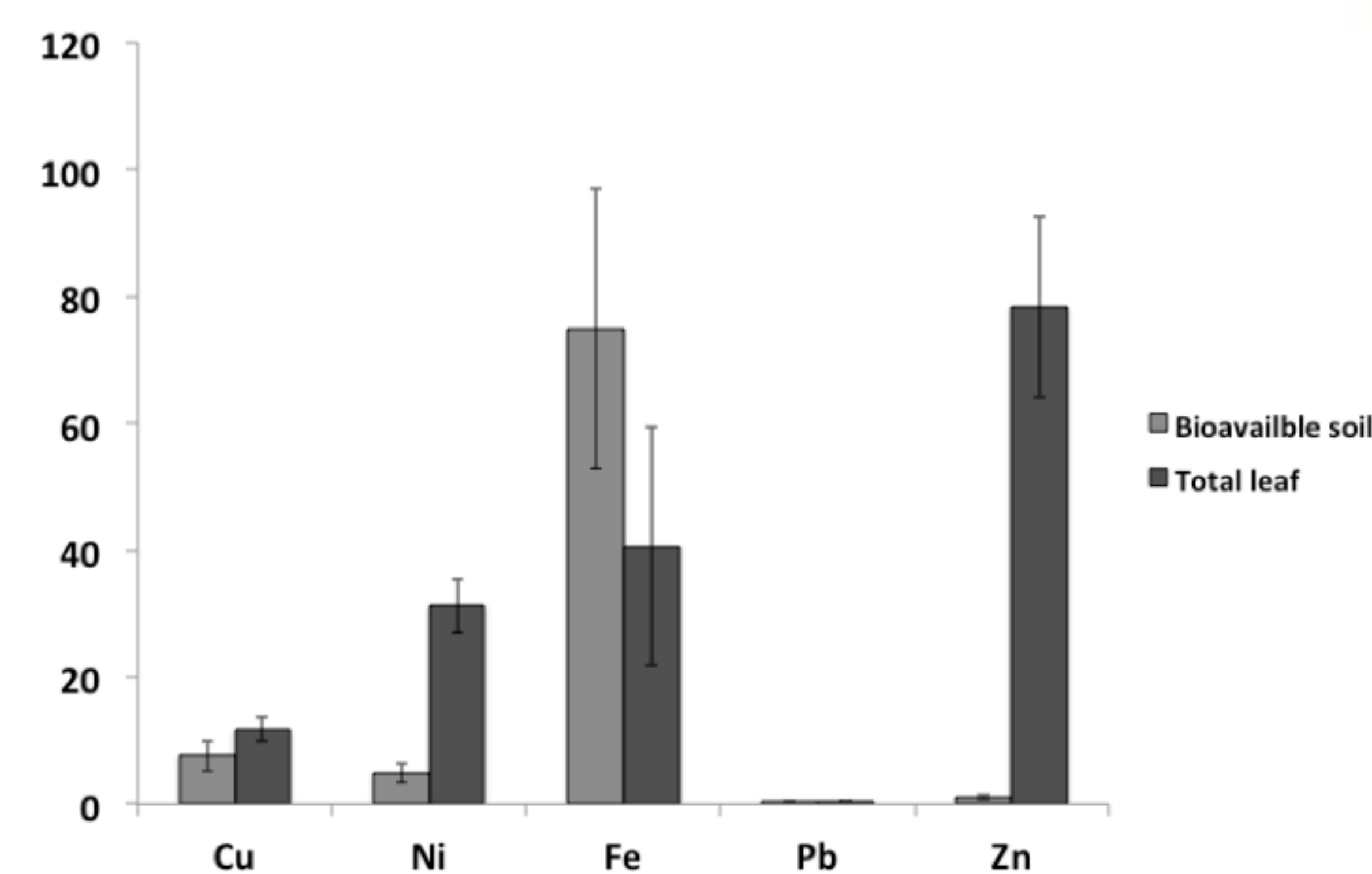
Eleven genes involved in nickel or copper resistance in other species were selected for this study (**Table 2.0**). Primers were designed using the genome of a close relative, dwarf birch (Wang *et al.*, 2012). The cDNA was generated using the High-Capacity cDNA Reverse Transcription Kit from Life Technologies. To confirm that the candidate genes are expressed in white birch, regular RT-PCR was performed on selected samples.

## Results

**Table 1.0:** Total and bioavailable concentration of heavy metals in contaminated sites from the Greater Sudbury Region.

Metal (mg/kg)	Cu	Ni	Fe	Pb	Zn
Total	991	1192	29200	736	82
Std. Error	±242	±336	±3453	±141	±13
Bioavailable	7.53	4.89	74.92	0.23	0.97
Std. Error	±2.44	±1.57	±22.00	±0.07	±0.26

Sites: Daisy Lake, Dam, Laurentian, Kingsway, Falconbridge, Kukagami, Azilda and Airport



**Figure 2.0** Bioavailable and total concentration of Cu, Ni, Fe, Pb and Zn in soil and in white birch leaves from contaminated sites (Daisy lake, Dam, Laurentian, Kingsway, Falconbridge, Kukagami, Azilda and Airport). Significant differences were found among bioavailable and leaf heavy metal concentration ( $t \leq 0.05$ ).

**Table 2.0** Candidate genes for Cu and Ni resistance in white birch

Metal	Gene	Species	Reference
Cu	Copper-transporting ATPase (RAN1)	<i>Arabidopsis thaliana</i>	Kobayashi <i>et al.</i> , 2008
	Multi-drug resistance-associated protein (MRP4)	<i>Betula pendula</i>	Keinänen <i>et al.</i> , 2007
	Copper transporter protein (COPT1)	<i>Arabidopsis thaliana</i>	Sancenón <i>et al.</i> , 2004
	Metallothionein (MT2b)	<i>Arabidopsis thaliana</i>	Guo <i>et al.</i> , 2008

Table 2.0 (continued)

Ni	Serine acetyltransferase (SAT)	<i>Thlaspi goesingense</i>	Freeman <i>et al.</i> , 2004
	Glutathione reductase (GR)	<i>Thlaspi goesingense</i>	Freeman <i>et al.</i> , 2004
	Nicotianamine synthase (NAS3)	<i>Noccaea caerulescens</i> ; <i>Thlaspi caerulescens</i>	Visioli <i>et al.</i> , 2014; Mari <i>et al.</i> , 2006
	Metal transporter Nramp3	<i>Noccaea caerulescens</i>	Visioli <i>et al.</i> , 2014
	Metal transporter Nramp4	<i>Noccaea caerulescens</i>	Visioli <i>et al.</i> , 2014
	1-aminocyclopropane-1-carboxylic acid deaminase (ACC)	<i>Brassica napus</i>	Stearns <i>et al.</i> , 2005
	High affinity nickel transporter family protein (AT2G16800)	<i>Arabidopsis thaliana</i>	

**Table 3.0** Expression of candidate genes in white birch

Gene	Primers (5'-3')	Amplicon size	Expression
Glutathione reductase (GR)	AGCGGTATTGACGAATTCTGGGGT TGGCCAGGGATAGGGGGACG	169bp	Root/Leaf
High affinity nickel transporter family protein (AT2G16800)	GAGCTCTCTGGGGGTGTGGC TGCCGGCACGACCATCATCA	335bp	Root/Leaf
Metal transporter Nramp3	GTTTTGCCTCTCTGGGCTGGR GTTGGGACCAATCTTCTTGACTGT	307bp	Root
Multi-drug resistance-associated protein (MRP4)	GCTTGATCCTCTGGCTTCTACTTTG CCACTTCTGTGGACCAACAAC	380bp	Root

## Discussion

- Bioavailable metals are much lower than total metals in the soil.
- White birch accumulate nickel and zinc in their leaves.
- Four genes involved in Ni or Cu tolerance were found to be expressed in white birch growing in the Greater Sudbury Region.

## Future Directions

- Perform the transcriptome analysis on white birch sampled from contaminated and control areas
- Conduct pot experiments with controlled levels of Ni and Cu
- Measure expression levels of tolerance genes from wild white birch populations and pot experiments

## Acknowledgements

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