Evaluation of nickel toxicity in *Deschampsia cespitosa*: analysis of morphological changes and DNA methylation

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Introduction

The Greater Sudbury Region has, for the last 130 years, been a location known for its mineral deposits. The extraction procedure used on these deposits has left the area's soils metal-contaminated and difficult for plants to colonize. One plant which thrived in the region is *D. cespitosa,* a species known to be an excluder, restricting metal accumulation to their roots with limited translocation to aerial tissue. The potential for phytoextraction is therefore high in *D. cespitosa.* The exclusion mechanism is unknown and there is limited information on the regulation of genes associated with metal transport and tolerance.

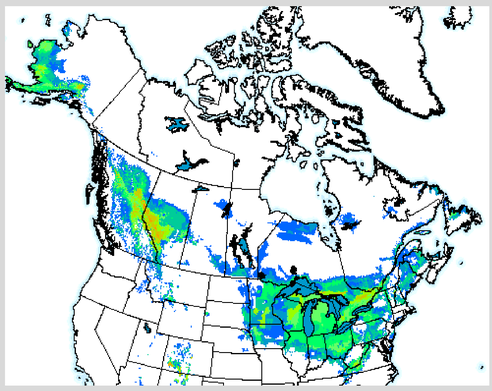

Figure
1a: Tufted hairgrass (*D. cespitosa)*
1b: Geographical distribution of Tufted hairgrass

Objectives

- To assess the toxicity of nickel and copper in *D. cespitosa.*
- To determine metal regulation levels in *D. cespitosa* contaminated with different nickel concentrations.
- To determine metal regulation levels in *D. cespitosa* contaminated with different copper concentrations.

Materials and Methods

Field experiment
- three metal-contaminated sites and three reference sites were selected.
- Leaf and root samples were collected from ten specimens per site, flash frozen with liquid nitrogen, and stored at -20^{0}C until RNA extraction.

Growth chamber assays
- Seeds were harvested from two metal-contaminated sites, and were germinated in a Petawawa box.
- Seedlings were grown in a growth chamber until an average height of 20 cm.
- Nickel treatment included three doses of nickel nitrate: 5.6 mg/kg, 1,600 mg/kg and 4,800 mg/kg.
- Copper treatment included three doses of copper sulfate: 9.16 mg/kg, 1,312mg/kg, 3,936 mg/kg.
- Water was used as a control (0 mg/kg).
- The experiment was a completely randomized block with seven to ten replications.
- Damage rating (based on a scale of 1 to 10) and root and leaf biomass were recorded to determine the effect of nickel on plant aspect and growth.
- Analyses of variance were performed using SPSS version 20 to determine significant differences between treatments.

Gene Regulation
- Expression of genes associated with Ni and Cu in other plant species will be assessed in *D. cespitosa* exposed to different metal concentrations using Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR).

Results and Discussion

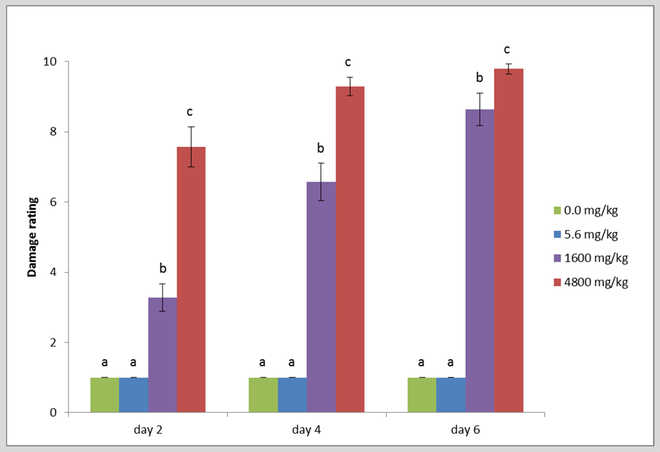

Figure 2a: Damage rating (based on a scale of 1 to 10) for *D. cespitosa*, treated with three different doses of nickel (5.6 mg/kg, 1,600 mg/kg and 4,800 mg/kg). Water was used as a control (0 mg/kg).

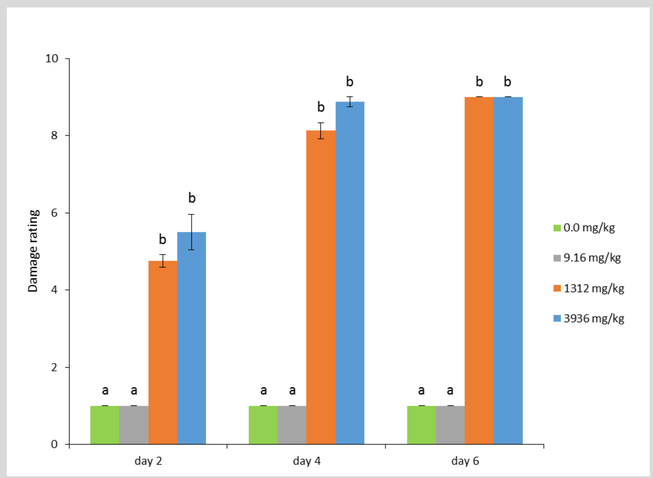

Figure 2b: Damage rating (based on a scale of 1 to 10) for *D. cespitosa*, treated with three different doses of copper (9.16 mg/kg, 1,312 mg/kg and 3,936 mg/kg). Water was used as a control (0 mg/kg).

Table 2a: mean height difference and biomasses for nickel controlled experiment.

	Growth rate (cm)	Day 7 Biomass (g)	
Treatment	7 days	leaf	root
0.0 mg/kg	8.34ab	3.70a	3.34a
	±1.12	±0.31	±0.38
5.6 mg/kg	11.42a	2.76a	2.71ab
	±0.67	±0.31	±0.31
1600 mg/kg	4.50bc	0.67b	1.37bc
	±0.58	±0.13	±0.44
4800 mg/kg	4.00c	1.00b	0.84c
	±0.46	±0.17	±0.17

Table 2b: mean height difference and biomasses for copper controlled experiment.

	Growth rate (cm)	Day 7 Biomass (g)	
Treatment	7 days	leaf	root
0.0 mg/kg	4.38a	6.18a	5.04a
	±1.78	±0.58	±0.43
9.16 mg/kg	3.88a	5.72a	5.54a
	±1.16	±0.45	±0.53
1312 mg/kg	0.25b	1.79b	2.23b
	±1.41	±2.81	±2.88
3936 mg/kg	0.75b	2.81c	2.88b
	±1.19	±0.28	±0.22

Results and Discussion

Table 1a: Candidate genes for nickel tolerance in *D. cespitosa*.

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

Table 1b: Candidate genes for copper tolerance in *D. cespitosa*.

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

Nickel assays
- No damage to plants was observed during the seven days of treatment with 5.6 mg/kg dose.
- Significant differences for damage rating, root and leaf biomass were observed between the 1,600 mg/kg dose (representing the total Ni level found in contaminated Greater Sudbury soil) and the control (0.0mg/kg).
- The 4,800 mg/kg treatment was extremely toxic as all the plants were dying (damage rating of 8 on a scale of 1 to 10) within 48 hours after the treatment.

Copper controlled experiment
- No damage to plants was observed during the seven days of treatment with the 9.16 mg/kg dose.
- Significant differences for damage rating, root and leaf biomass were observed between the 1,312 mg/kg dose (representing the total Cu level found in contaminated Greater Sudbury soil) and the control (0.0 mg/kg).
- The 3,936 mg/kg treatment was extremely toxic as all the plants were dying (damage rating of 8 on a scale of 1 to 10) within 48 hours after the treatment.
- Overall, Cu was more toxic than Ni in the assays.

Future directions

The regulation of tolerance genes associated with Ni and Cu tolerance is being assessed using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Future studies will include transcriptome analysis and the identification of novel genes.

Acknowledgements

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