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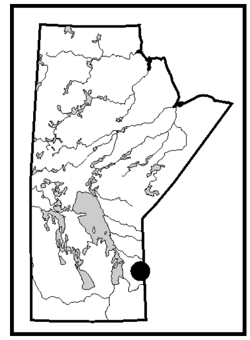
GS-26 Phytoremediation of mine tailings and bio-ore production: progress report on seed germination, plant growth and metal accumulation in seedlings planted at Central Manitoba (Au) minesite (NTS 52L13)

by S. Renault[1], C. Szczerski[1], C. Nakata[1], E. Sailerova[2] and M.A.F. Fedikow[3]



Renault, S., Szczerski, C., Nakata, C., Sailerova, E. and Fedikow, M.A.F. 2003: Phytoremediation of mine tailings and bio-ore production: progress report on seed germination, plant growth and metal accumulation in seedlings planted at Central Manitoba (Au) minesite (NTS 52L13); *in* Report of Activities 2003, Manitoba Industry, Trade and Mines, Manitoba Geological Survey, p. 200–208.

Summary

This paper summarizes the results obtained in 2002–2003 from a study initiated in 2000 to determine the potential for revegetation, phytoremediation and phytomining of gold mine tailings at Central Manitoba (Au) minesite in southeastern Manitoba. Germination rates of six grass species and two mustards species exposed to tailings in a greenhouse are presented in this progress report. Root and shoot growth was reduced in all species with a minimum decrease in altai wildrye and white mustard. A chelator was used to determine the efficiency of the mustard species to absorb copper (Cu). A new field experiment was designed in 2003 to test the benefits of peat and fertilizer. The results showed that germination and survival rates were relatively high in alfalfa, slender wheatgrass and Indian mustard when peat and fertilizer were added. Accumulation of metals in woody plants planted in 2000 and harvested after two or three growing seasons is discussed.

Introduction

The development of the mining industry in Canada has locally created considerable disturbance of our ecosystems. The re-establishment of self-sustaining ecosystems in these disturbed areas is of major concern. Reclamation is now recognized as an essential element of resource management by the industry. Research has been initiated to identify problems and develop methods to successfully reclaim disturbed areas.

In Manitoba, copper (Cu), zinc (Zn), nickel (Ni), lead (Pb), arsenic (As), cadmium (Cd) and gold (Au) can persist in the soil after the mining of sulphide and gold ores, causing environmental problems. Cleanup of such contaminated sites is often very costly. Phytoremediation, a novel technology, which uses plants to remove pollutants from the environment, is a very promising tool for the remediation of such sites. Excessive amounts of heavy metals can be toxic to most plants (Salt et al., 1995). However, some species have the ability to tolerate high levels of metals. These species could be used not only for phytoremediation and reclamation purposes but also for phytomining, a new technology that involves the use of plants to concentrate metals, followed by harvesting and metal extraction (Anderson et al., 1999). The potential use of these species in Canada may be limited by their tolerance to environmental conditions associated with the boreal forest.

Some woody plants have great potential for use in remediation processes due to their structure and physiology. A deep root system, high transpiration rate, high biomass and association with rhizospheric organisms, all these factors contribute to a higher rate of removal of metals from the soil. Woody plants live for long periods of time, therefore they have developed some systems that allow them to survive changes in the environment. Some of them are able to acclimate (induce resistance) to stress.

Heavy metal uptake is a very complex process. A variety of factors, including soil pH and interactions with metals and ions, can modify the uptake of heavy metals in plants. Recent research has shown that chelating agents facilitate metal uptake and could potentially be used to improve the performance of phytoremediation (Raskin et al., 1997).

Revegetation of mine tailings with grass species might be the first step to the successful introduction of woody species on tailings and to the determining of species suitable for phytomining purposes. Results from our study at the Central Manitoba minesite (NTS 52L13) indicated the need to add peat and fertilizer to the mine tailings material to increase the germination rate and grass seedlings survival rates. Results presented in this progress report demonstrate the effectiveness of this approach. We also report on the survival rate of and metal accumulation in woody species

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growing on untreated tailings for up to three growing seasons. The effect of adding chelating agents is also discussed.

Objectives

The long-term goal of this study is to define limiting factors for phytoremediation of mine tailings and other sites contaminated with heavy metals. The main objectives are to 1) establish the scientific basis for the remediation of mine tailings and extraction of heavy metals by phytomining techniques and 2) gain practical experience in the routine remediation of mine tailings and, possibly, in the extraction of heavy metals. Parameters guiding the study are 1) that the species selected for the study will include plants native to the surrounding environment and 2) that the suitability of the selected species for the phytomining of base metals and gold will be tested in terms of the quality and costs of bio-ore production and economic effectiveness.

Geological setting of the Central Manitoba (Au) deposit

Tailings associated with the Central Manitoba (Au) deposit were selected for initial phytoremediation and phytomining studies. A description of the geological setting of this deposit is detailed in Renault et al. (2000, 2001).

Geochemical characterization of tailings and description of experimental sites

Three experimental sites were chosen based on their proximity to the tailings edge and vegetation cover, exposure to sun and wind and drainage conditions (Renault et al., 2000, 2002). In 2003, a fourth site (site 4) was selected in the middle of the tailings on the east side of the minesite. At sites 1 and 2, pH values typically range from 3.5 to 4 in oxidized zones and 6.5 to 7 in non-oxidized zones. At site 3, pH values ranged from 5 to 7. The pH of the fourth site varied from 7.5 to 8.

Results

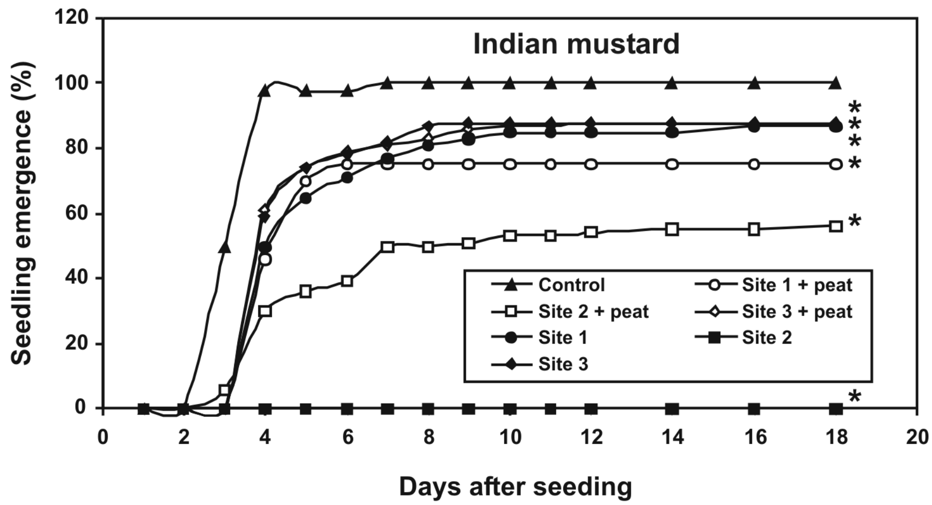
Greenhouse study

Germination experiments conducted in 2001 and 2002 were repeated in 2003 to provide sufficient data for statistical analysis. Seeds of the following species were planted in the greenhouse: Indian mustard (*Brassica juncea*), white mustard (*Sinapis alba*), slender wheatgrass (*Agropyron trachycaulum*), altai wildrye (*Elymus angustus*), reed canary grass (*Phalaris arundinacea*), creeping foxtail (*Alopecurus arundinaceus*), streambank wheatgrass (*Agropyron riparium*) and tall fescue (*Festuca alatior*). The experimental design is described in Renault et al. (2002).

White mustard planted on tailings from sites 1 and 3, with and without peat amendment, had high germination rates while Indian mustard showed a reduction in the germination rate (Fig. GS-26-1) suggesting that Indian mustard was more affected by the tailings than white mustard. Although Indian mustard and white mustard germinated on tailings from sites 1 and 3, they were not able to survive for three months at the sites without peat (Renault et al., 2002). The addition of peat to the tailings increased germination rates particularly at site 2 (Fig. GS-26-1, -2). The germination rates of grasses, including creeping foxtail, reed canary grass, streambank wheatgrass, altai wildrye, slender wheatgrass and tall fescue, were significantly reduced by the tailings (Fig. GS-26-2). Altai wildrye was the only species able to germinate on tailings from site 2 (Fig. GS-26-2). In addition, the germination rate of altai wildrye seeds was higher for plants grown on tailings from site 1 and 3 than for those grown on control substrate (Fig. GS-26-2).

In a separate experiment, the following species were used to study the effects of tailings on plant growth: Indian mustard, white mustard, reed canary grass, altai wildrye and tall fescue. Shoot heights, shoot and root fresh weights were determined after seven weeks of treatment. The results are shown in Table GS-26-1. The presence of tailings reduced the shoot height of all the species and reduced both root and shoot fresh weights with the exception of Indian mustard (Table GS-26-1). Although this species was more affected at the germination stage by the tailings than the other selected species, the plant seems more resistant to tailings at the early growth stage. White mustard and altai wildrye showed less growth inhibition than tall fescue and reed canary grass (Table GS-26-1). Overall, root growth was more reduced than shoot growth (Table GS-26-1) resulting in a decrease in the root to shoot ratio. This will further affect plant growth by limiting the amount of water and nutrient absorbed by the plant roots. These results suggest that the addition of peat to the tailings does not seem to be sufficient to enable successful revegetation; nutrients are required to improve plant growth.

To test the efficiency of white mustard and Indian mustard to absorb Cu, a chelator, ethylenediaminetetraacetic acid (EDTA), was used. Chelators are used to enhance the plant's ability to absorb Cu from the tailings. Thirty-five-day-old



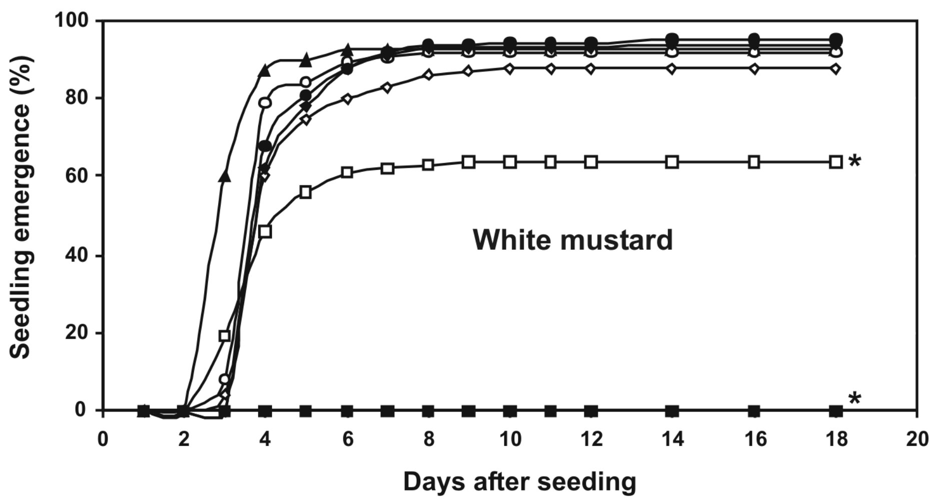


Figure GS-26-1: Germination (seedling emergence) of Indian mustard and white mustard planted on tailings with and without peat amendment (significant difference from the control [P=0.05]).*

seedlings growing on tailings from site 3 amended with peat were treated with EDTA (0.5 to 2 mM) for 21 days. The presence of EDTA increased the accumulation of Cu in both the shoots and roots of seedlings. A 2 mM concentration increased the accumulation by a factor of two in roots and a factor of approximately three in shoots. However, the total amount of Cu absorbed by these seedlings (38 µg/plant) remained relatively low as it represented less than 0.01% of the total dry plant biomass. For a successful phytoremediation, the accumulated metal should account for 0.1% of the total dry biomass (Baker and Brooks, 1989). These results can be explained at least in part by the pH of the tailings. In this experiment the pH was around 6.5, at this pH the availability of Cu is reduced, this could therefore explain the low accumulation of Cu in plants even when EDTA was added. This suggests that in tailings areas of neutral or high pH the Cu uptake will be reduced and therefore Cu toxicity is not likely to be an issue. However, the problem is that the pH of the tailings changes over time depending on environmental conditions.

Field study

In 2003, a new experiment was set up on site 4. The top 10 to 15 cm of the tailings was mixed using a rotor tiller. Seeds of the following species were planted in June: Indian mustard (*Brassica juncea*), alfalfa (*Medicago sativa*), meadow fescue (*Festuca pratensis*) and slender wheatgrass (*Agropyron trachycaulum*). Seeds were covered with a layer of peat (3.0 ± 0.5 cm). Seedlings were fertilized (20:20:20 / N:P:K) twice and watered four times over a two-month period (June–August).

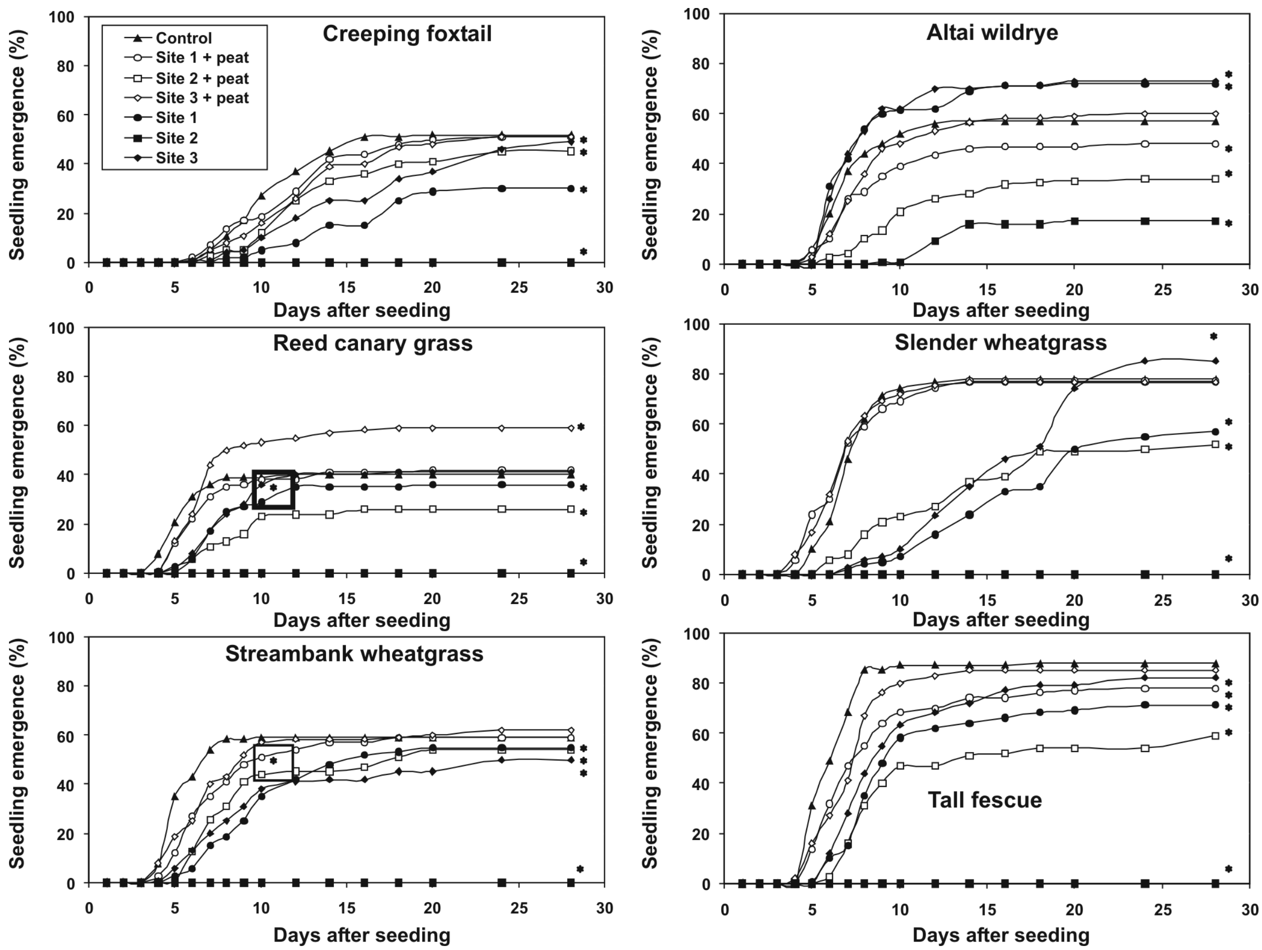


Figure GS-26-2: Germination (seedling emergence) of creeping foxtail, reed canary grass, streambank wheatgrass, altai wildrye, slender wheatgrass and tall fescue planted on tailings with and without peat amendment (significant difference from the control [P=0.05]).*

The highest germination rate occurred in Indian mustard (86.5%); unfortunately, the plants were severely damaged by insects. Alfalfa (Fig. GS-26-3a) and slender wheatgrass (Fig. GS-26-3b) had relatively high germination rates and survival rates (over 60%) while meadow fescue had lower germination (52%) and survival (20%) rates. However, the fescue plants that survived had a relatively high biomass (Fig. GS-26-3c).

Elemental analysis of plant tissues

In September 2002, tamarack (*Larix laricina*), jack pine (*Pinus banksiana*) and yellow willow (*Salix lutea*) seedlings planted in the field in 2000 and white spruce (*Picea glauca*) and red-osier dogwood (*Cornus stolonifera*) planted in 2001were harvested, washed carefully and freeze-dried prior to elemental analysis. Lichen (*Stereocolum sp.*), willow, white spruce and tamarack growing naturally on tailings were also used for elemental analysis. The results of the analysis (Table GS-26-2) showed that tamarack and willow naturally growing on site 1 had a relatively high level of boron (B) (over 100 ppm in shoots) compared to what is normally found in plants (10 to 20 ppm). Similar results were found in 2002 for tamarack and willow seedlings planted on site 1. At this concentration toxicity can occur (Dudley, 1994). Boron can therefore be a concern for plant survival on the tailings. The level of copper was elevated in roots of dogwood, tamarack and jack pine (Table GS-26-2). Further work will be necessary to determine if the high values reflect only adsorption of copper on root surface or uptake by the plants. Shoots of jack pine, white spruce, tamarack and dogwood seedlings planted on site 1 also showed relatively high concentration of copper compared to the seedlings naturally growing on site 1. These results suggest that plants growing on sites may have developed mechanisms to limit the uptake of copper to be able to survive on tailings. On the other hand, white spruce and tamarack growing on site 1 had high levels of Manganese (Mn) while willow had high levels of zinc (Zn) and cadmium (Cd). Lichens accumulated relatively high levels of aluminium (Al), titanium (Ti), iron (Fe), arsenic (As) and lead (Pb) (Table

Table GS-26-1: Shoot height, root and shoot fresh weights of mustard and grass seedlings planted on tailings amended with peat and on control substrate (sand with peat, no tailings).

	Control substrate (peat with sand)	Site 1 (with peat)	Site 3 (with peat)
Plant species		**Shoot height (cm)**	
Indian mustard	68 ± 3	46 ± 5*	37 ± 5*
White mustard	69 ± 3	19 ± 5*	10 ± 1*
Tall fescue	39 ± 4	20 ± 1*	17 ± 1*
Altai wildrye	48 ± 6	32 ± 4*	22± 2*
Reed canary grass	40 ± 4	14 ± 2*	16 ± 2*
		Root fresh weight (g)	
Indian mustard	0.19 ± 0.02	0.20 ± 0.04	0.17 ± 0.03
White mustard	0.26 ± 0.04	0.09 ± 0.03*	0.04 ± 0.01*
Tall fescue	0.48 ± 0.1	0.05 ± 0.01*	0.04 ± 0.01*
Altai wildrye	0.54 ± 0.04	0.18 ± 0.01*	0.13 ± 0.01*
Reed canary grass	1.51 ± 0.11	0.06 ± 0.03*	0.04 ± 0.01*
		Shoot fresh weight (g)	
Indian mustard	2.26 ± 0.23	2.93 ± 0.50	2.25 ± 0.39
White mustard	2.37 ± 0.37	0.99 ± 0.52*	0.45 ± 0.12*
Tall fescue	0.48 ± 0.09	0.11 ± 0.01*	0.07 ± 0.02*
Altai wildrye	0.64 ± 0.10	0.29 ± 0.03*	0.16 ± 0.02*
Reed canary grass	0.95 ± 0.11	0.12 ± 0.05*	0.10 ± 0.02*

Figure GS-26-3a: Alfalfa on site 4, amended with peat, two months after seeding.

GS-26-2). Tamarack roots accumulated more cobalt (Co), nickel (Ni) and silver (Ag) than the other species with the exception of dogwood roots which accumulated a higher amount of Co. Vanadium (V), chromium (Cr) and sodium (Na) were below detection level in all species. The levels of macronutrients such as magnesium (Mg) and potassium (K) were generally lower than the normal range, confirming that mineral deficiency is likely to be a limiting factor for plant growth in the tailings. Levels of calcium (Ca), strontium (Sr) and barium (Ba) were within the normal range for plants. The level of Au in jack pine and tamarack roots was significantly higher than in the other plant species.

Future work

Further work will focus on enhancing the growth rate of the selected species to determine the potential of these species for revegetation, phytoremediation and phytomining. The focus of the next field season will be the determination

Figure GS-26-3b: Slender wheat-grass on site 4, amended with peat, two months after seeding.

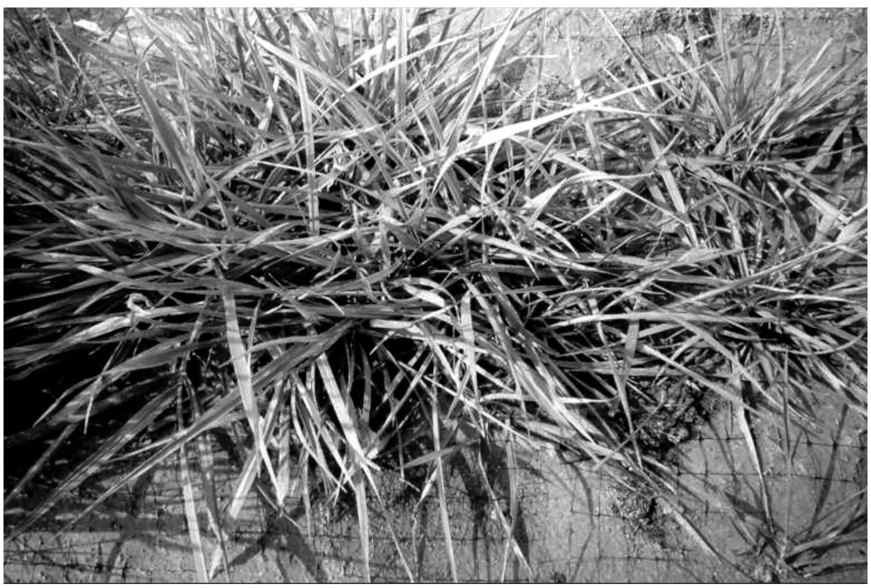


Figure GS-26-3c: Meadow fescue on site 4, amended with peat, two months after seeding.

of the best combination of peat and fertilizer to increase the long-term survival rate and growth of plant species on tailings. In 2003, woody plants grown for three summers on tailings were harvested and will be analyzed for element accumulation to complete this study. Additional samples from lichens, willow, white spruce and tamarack growing naturally on the site were collected to increase the sample size to provide sufficient data for statistical analysis.

Economic considerations

This research has direct economic benefits to Canada. The exploitation of natural resources has considerably disturbed our ecosystems, many of them have never been reclaimed. The development of methods of revegetation, reclamation, remediation and potential recovery of metals (phytomining) have obvious benefits for the mining and forestry industries and for the future environment of Canada. The research will lead to the development of techniques that will enable the mining industry to continue operations with reduced impact on the environment. This new technology would also create new markets for the plant nursery industry.

Acknowledgments

The authors would like to thank S. Green for greenhouse and fieldwork assistance.

Table GS-26-2: Elemental analysis of lichen, plant shoots and roots harvested from the field (sites 1 and 3). All values are in ppm, unless otherwise noted.

Samples	Pre-ash wt (g)	Ash wt (g)	Ash (%)	B	Na (%)	Mg (%)	Al	K (%)	Ca (%)	Ti	V	Cr	Mn
Lichen[1]	1	0.076	7.6	BDL	BDL	0.05 ± 0.01	775 ± 307	0.2 ± 0.0	1.2 ± 0.8	27.7 ± 6.4	BDL	BDL	19 ± 6
Willow[2]													
Shoot - site 1	1	0.08	8	182 ± 30	BDL	0.08 ± 0.01	79 ± 5	0.4 ± 0.1	2.6 ± 0.1	4.7 ± 0.3	BDL	BDL	55 ± 3
White spruce[2]													
Shoot - site 1	1	0.032	3.2	27 ± 2	BDL	0.05 ± 0.00	57 ± 10	0.3 ± 0.0	0.7 ± 0.1	4.0 ± 0.6	BDL	BDL	196 ± 15
Tamarack[2]													
Shoot - site 1	0.994	0.041	4.1	110 ± 19	BDL	0.04 ± 0.00	80 ± 9	0.5 ± 0.1	0.6 ± 0.1	8.5 ± 0.6	BDL	BDL	159 ± 13
White spruce[3]													
Shoot - site 1	1	0.035	3.5	33 ± 3	BDL	0.09 ± 0.00	174 ± 2	0.4 ± 0.1	0.5 ± 0.1	6.9 ± 0.8	BDL	BDL	96 ± 45
Root - site 1	0.944	0.039	4.1	16 ± 1	BDL	0.05 ± 0.00	213 ± 44	0.1 ± 0.0	0.9 ± 0.2	10.3 ± 3.6	BDL	BDL	27 ± 13
Dogwood[3]													
Shoot - site 1	1	0.026	2.6	34 ± 16	BDL	0.05 ± 0.02	47 ± 10	0.2 ± 0.1	0.7 ± 0.1	3.5 ± 0.7	BDL	BDL	19 ± 10
Root - site 1	1	0.097	9.7	36 ± 10	BDL	0.08 ± 0.02	341 ± 168	0.3 ± 0.1	0.7 ± 0.1	6.5 ± 1.2	BDL	BDL	107 ± 13
Willow[4]													
Shoot - site 3	0.78	0.047	6	26 ± 2	BDL	0.08 ± 0.01	202 ± 72	0.3 ± 0.0	1.8 ± 0.1	6.2 ± 1.6	BDL	BDL	74 ± 14
Root - site 3	0.856	0.036	4.2	12 ± 1	BDL	0.06 ± 0.01	79 ± 15	0.3 ± 0.0	1.0 ± 0.2	3.8 ± 1.3	BDL	BDL	37 ± 3
Tamarack[4]													
Shoot - site 1	0.944	0.052	5.5	49 ± 17	BDL	0.07 ± 0.01	315 ± 18	0.3 ± 0.0	0.7 ± 0.1	8.5 ± 0.6	BDL	BDL	111 ± 35
Root - site 1	1	0.194	19.4	30 ± 9	BDL	0.07 ± 0.02	505 ± 160	0.2 ± 0.1	2.5 ± 0.4	9.6 ± 2.7	BDL	BDL	73 ± 32
Jack pine[4]													
Shoot - site 1	1	0.03	3	28 ± 9	BDL	0.09 ± 0.01	216 ± 23	0.4 ± 0.0	0.5 ± 0.1	7.6 ± 1.2	BDL	BDL	24 ± 5
Root - site 1	0.715	0.021	3	22 ± 6	BDL	0.08 ± 0.01	175 ± 38	0.4 ± 0.1	0.7 ± 0.1	5.8 ± 0.8	BDL	BDL	12 ± 3
Shoot - site 3	1	0.032	3.2	10 ± 1	BDL	0.07 ± 0.01	283 ± 101	0.2 ± 0.0	0.5 ± 0.1	13.0 ± 4.8	BDL	BDL	22 ± 5
Root - site 3	0.98	0.045	4.6	8 ± 1	BDL	0.07 ± 0.01	386 ± 135	0.3 ± 0.0	0.8 ± 0.1	14.7 ± 3.1	BDL	BDL	44 ± 11
Normal range in plants				10 - 20	0.02 - 0.5	0.2 - 0.3	10 - 200	0.5 - 1	0.5 - 1	0.5 - 5	0.5	1.5 - 2	10 - 200

Table GS-26-2: Elemental analysis of lichen, plant shoots and roots harvested from the field (sites 1 and 3). All values are in ppm, unless otherwise noted. (continued)

Samples	Fe (%)	Co	Ni	Cu	Zn	As	Sr	Ag	Cd	Ba	Au (ppb)	Pb
Lichen[1]	0.29 ± 0.10	0.6 ± 0.2	BDL	355 ± 109	81 ± 23	8 ± 4	9 ± 5	0.5 ± 0.3	2.3 ± 0.3	9.3 ± 2.0	10 ± 4	25 ± 8
Willow[2]												
Shoot - site 1	0.03 ± 0.01	3.3 ± 0.1	BDL	36 ± 5	287 ± 28	BDL	22 ± 0.8	BDL	6.9 ± 0.2	10 ± 2	9 ± 1	0.6 ± 0.1
White spruce[2]												
Shoot - site 1	0.01 ± 0.00	0.3 ± 0.1	BDL	21 ± 4	52 ± 2	BDL	23 ± 1	BDL	0.1 ± 0.0	47 ± 3	5 ± 2	0.2 ± 0.0
Tamarack[2]												
Shoot - site 1	0.02 ± 0.00	0.5 ± 0.1	BDL	34 ± 11	19 ± 3	BDL	50 ± 2	BDL	0.2 ± 0.0	8.5 ± 1.2	4 ± 1	0.5 ± 0.1
White spruce[3]												
Shoot - site 1	0.07 ± 0.01	3.8 ± 2.4	BDL	273 ± 109	35 ± 4	BDL	27 ± 5	0.1 ± 0.1	0.2 ± 0.1	16.4 ± 1.2	67 ± 39	0.9 ± 0.1
Root - site 1	0.04 ± 0.01	10.8 ± 6.6	7 ± 7	4.2 ± 1.7	62 ± 26	BDL	17 ± 1	0.7 ± 0.4	1.8 ± 1.0	7 ± 1	55 ± 11	0.9 ± 0.1
Dogwood[3]												
Shoot - site 1	0.02 ± 0.01	1.3 ± 0.7	BDL	269 ± 111	13 ± 1	BDL	13 ± 2	BDL	0.1 ± 0.1	2.6 ± 2.6	9 ± 3	0.3 ± 0.1
Root - site 1	0.04 ± 0.01	19.4 ± 9.3	8 ± 7	4,879 ± 3,013	82 ± 10	BDL	10 ± 2	1.6 ± 0.4	1.6 ± 0.4	1.7 ± 2.4	54 ± 21	2.1 ± 0.8
Willow[4]												
Shoot - site 3	0.06 ± 0.02	0.9 ± 0.2	BDL	82 ± 26	120 ± 12	BDL	9 ± 1	BDL	3 ± 1	BDL	13 ± 10	1.2 ± 0.4
Root - site 3	0.02 ± 0.01	0.5 ± 0.1	BDL	220 ± 68	60 ± 10	BDL	6 ± 1	BDL	1.9 ± 0.4	BDL	32 ± 14	1.0 ± 0.4
Tamarack[4]												
Shoot - site 1	0.12 ± 0.01	2.0 ± 0.2	3 ± 3	331 ± 32	14 ± 1	BDL	49 ± 9	0.3 ± 0.1	0.5 ± 0.1	6.1 ± 0.4	73 ± 31	1.3 ± 0.1
Root - site 1	0.15 ± 0.06	15.5 ± 2.0	19 ± 5	1,907 ± 326	50 ± 1	BDL	21 ± 3	11.2 ± 10.3	1.8 ± 0.1	3.0 ± 3.0	184 ± 68	2.1 ± 0.5
Jack pine[4]												
Shoot - site 1	0.08 ± 0.01	1.0 ± 0.1	BDL	271 ± 45	17.5 ± 3.3	BDL	4 ± 1	BDL	0.26 ± 0.08	BDL	26 ± 4	0.9 ± 0.1
Root - site 1	0.04 ± 0.01	2.0 ± 0.3	BDL	1,098 ± 322	14.7 ± 3.7	BDL	6 ± 1	1.0 ± 0.5	0.33 ± 0.07	BDL	172 ± 120	0.9 ± 0.2
Shoot - site 3	0.08 ± 0.03	0.8 ± 0.2	BDL	105 ± 22	20 ± 2	BDL	3 ± 0	BDL	0.25 ± 0.04	BDL	5 ± 3	2.2 ± 0.2
Root - site 3	0.10 ± 0.03	2.2 ± 0.5	BDL	1,361 ± 383	22 ± 5	BDL	4 ± 1	BDL	0.44 ± 0.08	BDL	25 ± 4	3.7 ± 0.7
Range in plants	0.01 - 0.02	0.5 - 3	1	6 - 40	25 - 100	1 - 2	50	0.2 - 2	0.1 - 1	10 - 100	NA	1 - 10

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