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Research Article

Evaluation of Uranium Accumulation in Black Spruce Trees

[1]Praise Nyade, [1]Derek Wilton, [1]Henry Longerich, [2]Gary Thompson and [3]Paul McNeill
[1]Department of Earth Sciences, Memorial University, St. John's, NL, A1B 3X5, Canada
[2]College of North Atlantic, Prince Philip Drive, St John's, NL, A1C 5P7, Canada
[3]Auroral Energy Limited, St John's, NL, A1C 6H6, Canada

Abstract: This study evaluated the metal accumulation capacity of black spruce seedlings (*Picea mariana*) with a special focus on uranium and compared uptake from peat and podzol soil treated with various concentrations of uranyl nitrate hexahydrate. Bio-available indices for uranium in the substrates, accumulation factors and transfer factors for translocation of U between different plant organs were estimated. The results showed higher concentration of U in shoots with accumulation factors up to seven times greater than values determined in roots. Uranium accumulation in stems was several orders of magnitude higher than the metal content in roots and needles. Transfer from substrate to the plants was influenced by substrate-specific properties, resulting in higher uptake of U from soil than from peat. The pattern of U accumulation was consistent with that previously reported in field studies. Metal accumulation values also showed linear progression with Bio-available metal concentrations in the substrate suggesting that black spruce trees are best classified as bio-indicators of uranium content in underlying substrates rather than hyper accumulators of the metal.

Keywords: Bioaccumulation factor, bio-indicator, black spruce, transfer factor, uranium

INTRODUCTION

Multi-element analysis of vegetation has become a powerful tool for defining surficial chemical signatures of buried mineralization as well as environmental monitoring of anthropogenic inputs of pollutants (Market, 1993; Brooks *et al*., 1995; Dunn, 2007; Reid and Hill, 2010). Mineral exploration using conventional geochemical methods in glaciated and deeply weathered environments is often hampered by the significant depths of exogenous material and hence has decreased potential to detect deeply buried ore (Anand *et al*., 2007). Some plant species growing in otherwise non-prospective areas, however, have developed the ability to accumulate massive amounts of the indigenous metals in their tissues reflecting the underlying substrate chemistry without exhibiting symptoms of toxicity (Baker and Brooks, 1989). These plants highlight the buried geological features through interactions and uptake of metallic elements from many cubic meters of soil, bedrock and groundwater that surround the roots.

Coniferous trees from families *Picea*, *Pinus* and *Albies* (Dunn, 2007); deciduous plants of the family *Brassicaceae* e.g., *Arabidopsis halleri* and *Noccaea caerulescens* (Kramer, 2010); and shrubs including mountain alder, Labrador tea and aspen (Gordon, 1992; Dunn, 2007) have been perceived as metal hyper accumulators and consequently have been used as biogeochemical sampling media to detect buried ores in various reconnaissance surveys in North America and Europe (Brooks *et al*., 1995; Dunn, 2007).

Non-prospecting applications of plants, that utilize the ability of plant roots to take up and accumulate high concentrations of metallic elements from underlying substrates for environmental monitoring have gained wide acceptance (Markert, 1993; Brooks *et al*., 1995). Plants including bryophytes e.g., mosses and ferns (Kramer, 2010; Selinus, 1988, 1989) and vascular plants e.g., Labrador tea, dandelion (Gorden, 1992), coniferous trees (e.g., Norwary spruce) have been widely applied to monitor metal contamination of soils (Gordon, 1992). Specifically, *Pinuss sylvestris*, *Robinia pseudoacacia*, *Tilia cordata*, *Picea abies* and *Acer platanoides* have been variously used to monitor heavy metal (Pb, Cd, Hg, Zn, etc.) pollution from sites near point emission sources (Ozturk *et al*., 2008).

Black spruce trees have several characteristics that make them of particular interest as biogeochemical sampling media for prospecting buried ore and environmental monitoring. *Picea mariana* has broad ecological amplitude and is widely distributed across the boreal forests of North America, Europe and northern Asia. Through its use in biogeochemical studies, black spruce has gained recognition as a metal hyper accumulating plant (Dunn, 1981, 2007). Studies

Corresponding Author: Praise Nyade, Department of Earth Sciences, Memorial University, St. John's, NL, A1B 3X5, Canada

elsewhere, in particular, unpublished data from areas in Atlantic Canada have reported very low U enrichments in twigs, bark and needles collected from known mineralized areas, thus casting doubts about the metal accumulating potential of spruce trees (Dunn C.E., personal communication). Notably, biogeochemical surveys from areas in Central Labrador, returned low concentrations of metallic elements including uranium-that was essentially close to background concentrations, even in areas with known anomalous metal content in the substrate. It is these contrasting reports that necessitated this interest in investigating metal accumulation in spruce trees with a special focus on U.

The transfer of U from soil to plants has usually been studied in food crops/agricultural plants (Shahandeh and Hossner, 2002) and in hydroponic experiments using spiked nutrient solutions. Bioaccumulation and metal transfer factors from such studies may have limited validity for describing the behavior of U in coniferous trees. Thus, experiments using nutrient solutions as growth media give only approximate estimates of the processes occurring in the complex soil pore water under ordinary conditions and are remote from the real conditions existing in the field because soil and liquid media are absolutely different systems. Uranium commonly exists as Bio-available forms in liquid media whereas several factors affect its mobility and for that matter, its availability to biota (Shtangeev, 2008). The research described in this study investigated uranium uptake and translocation to different parts of *Picea mariana*. Substrate properties and substrate-to-plant transfer of the metal and its distribution across plant parts were examined. The goals were:

- To examine U uptake and distribution across different parts of plants grown on U enriched soil and peat
- Determine the bioaccumulation and transfer factors of U in spruce trees
- Investigate substrate impact on the uptake/ accumulation of the metal

MATERIALS AND METHODS

Reagents and materials: Acids used for sample preparation were analytical grade and included: nitric acid (70% v/v) and hydrochloric acid (38% v/v) and certified A.C.S. ammonium acetate; all supplied by Fisher Scientific (Fair Lawn, NJ, USA). Uranyl hexahydrate was supplied by BDH Chemicals Ltd., (Poole, England). A set of reference materials was included in each batch of sample preparations and analysis as a monitor of precision, accuracy, quality assurance and quality control.

The Standard Reference Materials (SRMs) comprised SRM-2710a (Montana I soil), SRM-2711a (Montana II soil), SRM-1547 (peach leaves) and SRM-1575 (pine needles) from the National Institute of Standards and Technology (NIST) (Gaithersburg, USA). Vegetative radionuclide reference materials *viz*. CLV-1 (spruce twigs) and CLV-2 (spruce needles) were obtained from the Canadian Certified Reference Material Project, CANMET Mining and Mineral Sciences Laboratories, ON, Canada. Deionized water (nano-pure water, 18 mΩ cm) was produced from a Millipore Elix-5/Mill-Q water purification system (Bedford, ON). Two-year old plants of black spruce were obtained from the plant nursery of the Forestry Division of the Department of Natural Resources, Province of Newfoundland and Labrador. Premier Horticulture Limited supplied pro-mix potting peat.

Analyses of soil and peat: Bulk podzol soil was collected from the Michelin uranium deposit, Central Mineral Belt, Labrador and was used for pot experiments in a greenhouse at the Memorial University Botanical Gardens, St. John's, NL, Canada. The soil, collected from depths of 5-15 cm was shipped to the Department of Earth Sciences, MUN where it was air dried at room temperature, sieved through a 4 mm mesh and stored at $4^{o}C$ until ready for use. Total metal content in the <250 µm fraction of soil was determined by aqua regia digestion of approximately 0.1 g samples using an ICP-MS (ELAN DRC II, PerkinElmer Sciex, Concord, ON, Canada) analysis package for a suite of 36 elements.

Mineral composition of soil was analyzed with a Mineral Liberation Analysis-Scanning Electron Microscope (MLA-SEM) using the method developed by Wilton and Winters (2012). Reagent blanks were included to ensure that solutions were not contaminated with U and other analyses from the sample preparation. Results are reported on a dry weight basis. Organic matter content of the soil was estimated by ignition at $550^{o}C$. The pH of soil and peat were determined using 1:1 slurries of substrate and deionized water. The pH meter (model 310, Orion Research Inc., FL, USA) was calibrated prior to use and has an estimated uncertainty of ±0.05 pH units. Estimates of the available U fraction in both treated and control substrates were obtained in 1 M ammonium acetate (NH_4Ac, buffered at pH 4.5) leach. Soils were analyzed for total and available U content after four weeks of treatment and stabilization following the method described above. Treated and control peat samples were ignited at 550°C and the ash dissolved in nitric acid for analysis using ICPMS.

The Tessier *et al*. (1979) method was applied to sequentially extract U sorbed onto various mineral phases in the substrates. A 0.5 g sample was sequentially subjected to a series of extractants including 1.0 M magnesium chloride for exchangeable metals; 1.0 M sodium acetate for cations bound to carbonates; 0.04 M hydroxylamine HCl in 25% acetic acid for components bound to Fe-Mn oxide phases; 0.02 M nitric acid and 5.0 M hydrogen peroxide for cations sorbed onto organic phases; and 2:1 mixture of 8 N nitric acid and 29 M hydrofluoric acid for residual components. The sum of all U fractions was compared with total U determined in each substrate by aqua regia digestion.

Plant cultivation in a greenhouse: Pots filled with 500 g (oven dried) of the <4 mm fraction of bulk soil were mounted on trays and the soil spiked with varied concentrations (100, 250 and 400 mg/kg, respectively) of uranyl nitrate hexahydrate. A second set of pots was filled with pro-mixed peat and was spiked with varied concentrations of uranyl nitrate hexahydrate. The substrates (peat and soil) were fertilized according to a fertilizer regime recommended by botanists at the MUN Botanical Gardens for black spruce seedlings and homogenized. The substrate amendments (uranium nitrate hexahydrate and soil fertilizer) were dissolved in deionized water, though the quantities used were calculated on dry weight basis. Each treatment and controls (non-amended soil and peat) were replicated ten times given a total of 100 pots.

Pots were watered with deionized water (18 mΩ cm @ 25°C) to 70-80% field moisture capacity and left for 28 days to attain equilibrium. The two-year-old seedlings of *Picea mariana* were transplanted from potting compost into pots containing U-enriched substrates and controls and were periodically watered by adding water to the trays. Watering of the potted seedlings via trays was aimed at minimizing possible contamination of stems and needles by U-enriched soil particles through splashing during aerial watering. Tray watering also eliminated leaching of metals from the surface to deeper layers. Another set of samples was comprised of seedlings cultivated in non-amended soils and peat and was treated once a month with a dose of 250 mg/kg uranyl nitrate solution. Plants were grown for 16 weeks in the greenhouse with a natural day light/night regime in the summer of 2011. At harvest the aerial portions of the plants were removed just above substrate (soil/peat) level using a pair of Teflon coated shears, while roots were carefully extracted from soil and washed copiously with tap water, which was followed by deionized water.

Plant analyses: Plant parts (roots, stem and needles) were washed with deionized water after harvest. Roots, stems and needles were oven dried, pulverized in a cap mill and accurately weighted samples of approximately 1.0 g were ignited at 450°C following which the ash was dissolved in HNO_3 for ICPMS analysis.

Statistical analyses and quality control: Data were analyzed and presented as mean and standard deviation (X±S.D.) of ten replicates using SPSS statistical package (version 20 for mac). Multiple comparisons of means were determined by the Turkey test. Student's t-tests were used to detect significant differences between mean U concentrations in substrates and plant parts. The precision of analytical procedures was expressed as Relative Standard Deviation (RSD) calculated as the standard deviation divided by the mean. The RSD values for uranium in all samples ranged from 3-10%. The recovery rates for U in the standard reference materials were within 90±10%. Table 1 shows uranium recovery in standard reference materials.

Table 1: Uranium recovery in standard reference materials

Standard reference materials	Measured value (µg/g)	Certified value (µg/g)
NIST SRM 1547	0.012	0.015*
NIST SRM 1575	0.017	0.020
NIST SRM 2711a	2.660	3.010
NIST SRM 2710a	8.800	9.110
CLV-1	81	86.800
CLV-2	3.200	3.600

*: Recommended value

Biological Accumulation Factor (BAF) was calculated as the ratio of metal content in shoots to the available metal concentration in soil (Negri and Hinchman, 2000; Kidd *et al*., 2007); The Translocation Factor (TF) was defined as the ratio of the metal content (mg/kg) in plant stem to that in the roots (Sheppard and Evenden, 1988; Ramaswami *et al*., 2001; Kidd *et al*., 2007; Al-Qahtani, 2012) and the Bioavailability Index (BI) was described as ratio of available metal concentration (mg/kg) to the total metal concentration (mg/kg) in soil (Kidd *et al*., 2007). All concentrations were computed on a dry weight basis.

RESULTS AND DISCUSSION

Podzol soil collected for the study was largely sandy, coarse grained and light brown in color. Mineral Liberation Analyzer-Scanning Electron Microscope (MLA-SEM) analysis of the <250 µm fraction showed that the dominant mineral composition of the soil was albite (68%), quartz (17%), low Fe muscovite (6%) and small amounts of hornblende. The pH ranges determined in soil and peat were 4.2-4.6 and 3.9-4.3, respectively. It is anticipated that the acidic pH of both substrates promoted solubilization and increased mobility of uranyl ions in substrate pore water as well as speciation of U(VI) organic species (such as fulvic and humic acids) in peat. The pH of treated substrates did not change significantly over the period of stabilization indicating that substrate amendments had no significant effect on pH. The organic matter content of the soil ranged from 4.6-4.8%. The soil content of essential plant nutrients including, S, P, Ca and B were lower than mean concentrations reported for podzols (Kabata-Pendias and Pendias, 2001).

Table 2: Sequential chemical extraction of U from control and amended substrates

	Soil treatment (mg/kg)				Peat treatment (mg/kg)			
Mineral phase	Control	100	250	400	Control	100	250	400
Exchangeable phase	0.06	8.8	16.5	23	0.48	16.30	37	41
Carbonate phase	3.90	11	20	26.6	0.02	9.02	18	16
Fe-Mn oxide phase	34	38	50.3	67	<DL	3.90	9	7
Organic phase	9	9	16.2	24.3	5.70	36	59	81
Residual phase	19	26.1	47	78	6	4.80	5	3

<DL: Below limit of detection

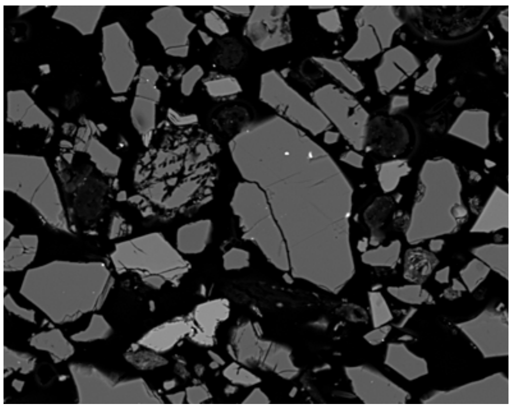

Fig. 1: Si_uraninite particle (white) in albite host grains (grey) on untreated soil

Table 2 illustrates the level of U fractionation in various mineral phases from the <250 µm fraction of non-treated (control) and amended soil. MLA-SEM analysis of non-amended soil using Back Scattered Electron (BSE) image gray scale underscored the occurrence of uranium largely as uraninite particles encapsulated by albite and quartz crystals (Fig. 1). The sequestration of U within the dominant minerals makes it less available for uptake by biota. Moisture contents of soil and peat were 5.1 and 18%, respectively.

Differences between the sum of uranium fractions from the sequential extraction regime and concentrations determined by total digestion (aqua regia) of substrate were remarkable. For instance, while the sum of U fractions in the control soil was 88 mg/kg, the total uranium content determined via aqua regia digestion was 153 mg/kg. This indicates incomplete liberation of U adsorbed to crystalline mineral and/or organic phases in soil by the extractants. Results of sequential extraction (Table 2) revealed that uranium was largely sequestered onto Fe-Mn oxide and residual phases. The fraction of uranium adsorbed to exchangeable phases was <0.08% of total U determined in soils. On the contrary, the organic phase was the dominant geochemical phase in peat where U interacts with polar organic functional groups through cation exchange and complexation reactions with humic and fulvic acids in peat (Stevenson, 1994). Mechanisms of acidification in peat have been recognized as: cation exchange, dissociation of organic acids and sulphate reduction (Clymo, 1964; Stevenson, 1994).

The sequential chemical extraction data also highlighted a general increase in the exchangeable (Bio-available) fraction of U with increasing substrate concentration of uranyl nitrate (Table 2). This could be due to relatively high acidity of peat. An exhaustion of the cation adsorption and/or exchangeable sites in peat could also account for the relatively high exchangeable fraction. Uranium concentrations in exchangeable fraction of non-amended peat (control) was determined to be below the detection limit, however, concentrations of 1.4, 5.5 and 18.03 mg/kg were respectively determined in peat treated with doses 100, 250 and 400 mg/kg uranyl nitrate hexahydrate. Uranyl nitrates are generally soluble at acidic pH and dissociate to form free mobile uranyl (UO_2^{2+}) species which are readily available for incorporation into biota (Langmuir, 1978; Ebbs *et al*., 1998).

It has long been established that the total concentration of a metal in a substrate is a poor indicator of its bioavailability (Hamon and McLaughlin, 2003; Kidd *et al*., 2007). Uranium exists in several different chemical forms in substrates and it is important to recognize that the most readily available forms are the soluble forms transported as uranyl ions (UO_2^{2+}) under acidic condition or hexavalent carbonate complexes (Negri and Hinchman, 2000) at alkaline pH of substrate pore water. An example is shown in Table 3 and 4 where substantial differences were reported for total U content and available concentrations in soil and peat. Consequently, plant metal bioavailability is a function of the metal availability index determined as the ratio of available metal concentration (mg/kg) in a substrate to total metal content (mg/kg) of substrate. Metal availability indices computed in this study (Table 3 and 4) suggest greater solubility and mobility of uranyl species in peat than in soil.

It might be expected that uptake and accumulation of uranium would be greatest in parts of black spruce grown in pots containing treated peat since higher availability indices of metals in growth media are known to correlate with their bioavailability to plants. On the contrary, U uptake and accumulation was

Table 3: Total U content in treated soils and bioavailability indices for U after 4 weeks of stabilization

Treatment (mg/kg)	Mean (mg/kg)	Max. (mg/kg)	Min. (mg/kg)	Bio-available (mg/kg)	Bioavailability index
Control	153	161	115	3.070	0.02
100	288	310	263	10.70	0.04
250	330	427	357	16.50	0.05
400	513	548	501	26	0.05
Determined after sixteen weeks of plant growth					
250/month	449	482	423	14.03	0.03

Max.: Maximum; Min.: Minimum

Table 4: Total U content in treated peat and bioavaibility indices for U after 4 weeks of stabilization

Treatment (mg/kg)	Mean (mg/kg)	Max. (mg/kg)	Min. (mg/kg)	Bio-available conc. (mg/kg)	Bioavailability index
Control	6	12	2.4	0	<DL
100	115	132	111	24	0.21
250	252	263	245	37	0.15
400	422	451	392	41	0.10
Determined after sixteen weeks of plant growth					
250/month	371	407	339	15.1	0.04

<DL: Below limit of detection; Max.: Maximum; Min.: Minimum

less in seedlings grown on U-enriched peat than those grown in soils treated with uranium. The statistically significant differences ($p<0.01$) between U content in plants grown on U treated peat and those grown on spiked soil suggests that uranium uptake in spruce trees is not entirely a function of metal availability indices. Other biotic and abiotic factors may discriminate against root development and consequently metal uptake. Specifically, mechanisms associated with the development of root rhizosphere and mycorrhyzae can be pH sensitive, hence, their development may be inhibited by extreme pH conditions in peat (Shtangeeva, 2008). In addition, protons and phytosiderophores released by the root are known to greatly influence the microenvironment and significantly impact cation uptake rates of plant roots (Laurie and Manthey, 1994).

Visual symptoms and plant growth: Growth of seedlings of *Picea mariana* was normal at all substrate U concentration. No visible signs of metal toxicity were observed even in pots where the total U concentration was >500 mg/kg. A trend of higher biomass production was observed with increasing substrate content of uranyl nitrate hexahydrate. An increase attributed to concomitant increase in nitrate content of the substrate due to increasing concentrations of the U spike (uranyl nitrate hexahydrate). Slight chlorosis was observed on needles of plants grown in control pots (unspiked growth media), but this did not affect plant growth. The highest biomass growth was observed in plant grown on substrates treated with 400 mg/kg single dose followed by those grown on media treated on a monthly basis with 250 mg/kg uranyl nitrate hexahydrate. It is instructive to note that this pattern was observed in plants grown in both peat and soils and underscores the high tolerance of *Picea mariana* for U.

Uranium accumulation in plant parts: The content of U and plant nutrients, in particularly, Ca, P and Ni in

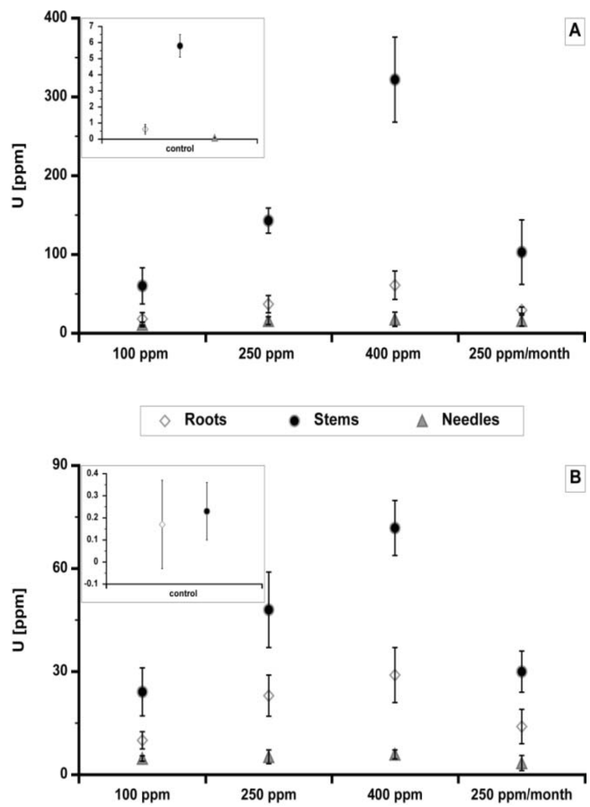

Fig. 2: (a) U in parts of spruce trees after 16 weeks of growth in soil treated with varied doses of uranyl nitrate hexahydrate, (b) U content in parts of spruce trees after 16 weeks of growth in peat spiked with varied concentrations of uranyl nitrate hexahydrate

the black spruce seedlings after 16 weeks growth in modified growth media (peat and soil) clearly showed preferential accumulation in the stem and followed the order stem>roots>needles. For instance, maximum U accumulation values of 336, 78 and **7** mg/kg, respectively were determined in stem, roots and needles respectively of plants grown in soils spiked with 400 mg/kg and maximum values of 112, 34 and 2.14 mg/kg, respectively detected in stem, roots and needles of plants grown in peat spiked with 400 mg/kg U solution (Fig. 2a and 2b).

The metal content in stems also increased progressively with increasing substrate concentration. In Particular, U contents in stems of plants grown in soils treated with 100, 250 and 400 mg/kg, respectively were 10, 24 and 50 folds greater than concentrations determined in stems of control plants. A similar pattern of accumulation was observed in roots and stems of plants grown in pots containing treated peat. This observation is consistent with findings of Dunn (1981, 2007) who reported that U preferentially accumulates in younger twigs of black spruce trees. Analysis of variance indicated significant differences ($p<0.05$) between mean U concentrations in stems and roots of plants grown on treated media and those grown in the control group. Accumulation of the metal in stems and roots of plants grown on spiked substrates was several orders of magnitude greater than U content in parts of plants grown in control media.

As mentioned previously, results indicated substantial differences in the magnitude of metal accumulation between parts of plants grown on U spiked peat and those grown in pots containing treated soils. Ramaswani *et al*. (2001), observed that substrate type can greatly affect the sorption and desorption of metals. In a comparative study of radionuclide uptake and translocation in plants grown in two different soil types (a sandy-loam soil and a soil rich in organic matter) and a hydroponic medium, Ramaswami *et al*. (2001) reported that the efficiency of uranium uptake decreased sharply from hydroponic to sandy and especially, organic soil, indicating that soil organic matter sequestered U, rendering it unavailable for plant uptake. Similarly, Sheppard and Evenden (1988) reported diverse concentrations of U in stems and leaves of sunflowers grown in several different types of soils contaminated with U. Their study revealed a higher accumulation in organs of plants grown in calcareous soils than acidic soils. Variability of U uptake in response to substrate heterogeneity is largely attributed to substrate specific properties that influence U chemistry.

No significant differences were observed between the uptake of essential plant nutrients in cultivars grown on control media and those grown on U-enriched substrates. However, treatments of the soils significantly increased the uptake of Fe, Cu and Al with increasing soil concentration of uranyl nitrate. As explained by Shtangeeva (2008), uptake mechanisms in plants are selective, thus plants preferentially acquire some elements over others. Elsewhere the presence of elevated nutrient levels in substrates were reported to correlated with higher uptake of U, as a consequence of a higher metabolism (Negri and Hinchman, 2000).

Concentrations of U determined in spruce needles were markedly low. Mean concentrations in the young needles grown in control soil were 0.08 and ranged up to 9.8 mg/kg, respectively when grown in soil spiked with 400 mg/kg of uranyl nitrate solution. Generally, concentrations determined in the needles were well within the ranges previously reported in field samples. For example, mean U concentrations of 0.069 and 0.37 mg/kg were, respectively reported for young and old black spruce in background samples collected from a bog habitat located near the Key Lake uranium mill in northern Saskatchewan (Thomas, 2000). It must, however, be emphasized that increases in needle contents of the metal were not always significant for some plants, nevertheless, mean concentrations exhibited a trend of rising concentration with increasing substrate content due to excessively high concentrations (outliers due to contamination) determined in needles from two plants. Dunn (2007) reported a mean U concentration of 1.64 ppm in matured needles collected in a regional biogeochemical survey at the McClean Lake area of northern Saskatchewan, Canada. Conversely studies undertaken by Nash and Ward (1977), in the vicinity of Midnite U mine in Washington demonstrated high concentrations of U (up to 200 ppm) in needles of coniferous trees.

The metal content of roots, stems and needles of spruce seedlings grown in pots containing substrates treated with one-time-dose of 250 mg/kg uranyl nitrate hexahydrate were consistently higher than those grown in non-treated pots but were later spiked on monthly with 50 mL of 250 mg/kg U solution (Fig. 2a and 2b). The low uptake of U from the monthly spiked substrates could be attributed to the considerable heterogeneity in the distribution of uranyl cations along the substrate profile. Thus, migration of available forms of the metal could be constrained by the level to which water containing dissolved solute (uranyl ions) rises by capillary fringe and consequently, leads to less availability of mobile species in upper layers of media treated on per month basis. Processes including diffusion, advective flow and capillary rise were identified as mechanisms responsible for vertical migration of elements and for creation of surface soil anomalies in reviews authored by Cameron *et al*. (2004) and Aspandiar *et al*. (2004). They however established that upward migration of dissolved species by these mechanisms could be slow.

In general, mean U contents of roots and stems exhibited linear correlations with Bio-available concentrations (exchangeable fraction) of uranium in the both substrates (Table 3 and 4). For instance, mean exchangeable fraction of U increased from the 0.06 mg/kg in non-amended soil (control) to 23 mg/kg in soils treated with 400 mg/kg dose of uranyl nitrate (Table 2) with corresponding increases in root, stem and needles concentration of the metal. Similarly, U contents in organs of plants grown on amended peat showed a linear progression with available metal content in the growth media. The high metal uptake and accumulation in the plant organs is, however, not unexpected given the low pH and high solubility of uranyl nitrate in acidic pore water (Ebbs *et al*., 1998; Langmuir, 1978). The correlation presented in Fig. 2a is congruent with the correlation pattern observed for uranium accumulation in roots and stems of the plants grown in pots containing treated peat. The toxicity levels of the metal in black spruce trees are uncertain, especially since concentrations in excess of 2000 mg/kg have been reported in ash of spruce twigs sampled from areas adjacent to the Rabbit Lake open pit operation in northern Saskatchewan, Canada (Dunn, 2007).

Non-essential elements, uptake by plants are controlled by their quest for micronutrients and their capacity to absorb and eliminate toxic elements. The quantities of metals taken up by plants according to Markert (1993) vary for different plant species and are dependent on the tolerance and adaptation of plant tissues to high metal concentrations. According to the Markert (1993) classification of plants as excluders, bio-indicators and accumulators and depicted in Fig. 2b, black spruce trees from this study best qualify as indicators of available metal content in the underlying substrates (Fig. 3a). The pattern of accumulation observed for Ni, Co, Cu, Cr and Pb or Zn fell short of Brooks *et al*. (1995) definition for accumulator plants hence spruce trees from this study and results of other field studies undertaken in Labrador, Canada (not published) do not qualify classified as metal hyper accumulators.

Uptake of U from the terrestrial environment by plants is a complicated function of various interrelated biotic and abiotic factors (substrate controls). As mentioned, plant properties have huge influence on U uptake and may vary between cultivars of the same species (Hamon and McLaughlin, 2003) (Fig. 3b). More importantly, factors including root architecture, rhizosphere chemistry, expression and affinity of root surface transporter proteins for metals, internal compartmentalization of metals and xylem loading of metals significantly regulate speciation of metal cations in rhizosphere environment, cation absorption and translocation and accumulation of elements may operate differently within individual plants (Hamon and McLaughlin, 2003; Smical *et al*., 2008).

Rhizosphere chemistry greatly influences the pH of ambient soil solution through proton (H^+) /hydroxide (OH^-) release/uptake at root surfaces (Sylvia *et al*., 2005) which produce pH variations of up 2.5 pH units in the rhizosphere environment when compared to the bulk soil solution (Romheld *et al*., 1984). Organic chemicals such as mucilage, exudates and lysates released from the roots into the immediate vicinity of rhizosphere have the potential of increasing

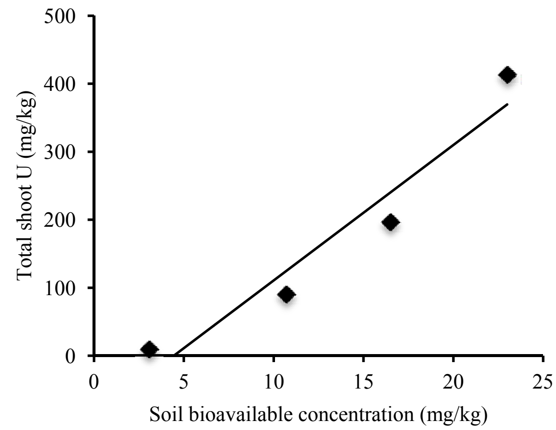

Fig. 3a: Relationship between bio-available concentration of U (mg/kg) in soil and total U content in shoots of black spruce seedlings

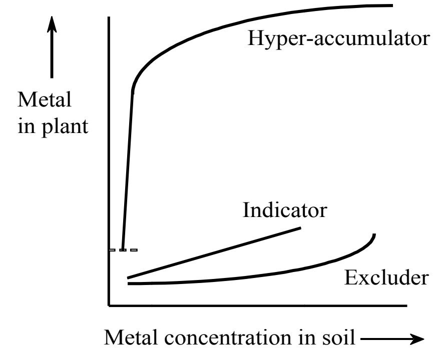

Fig. 3b: Characteristic relationships between increasing substrate metal concentration and plant metal concentration for different categories of plant species (Markert, 1993; Hamon and McLaughlin, 2003)

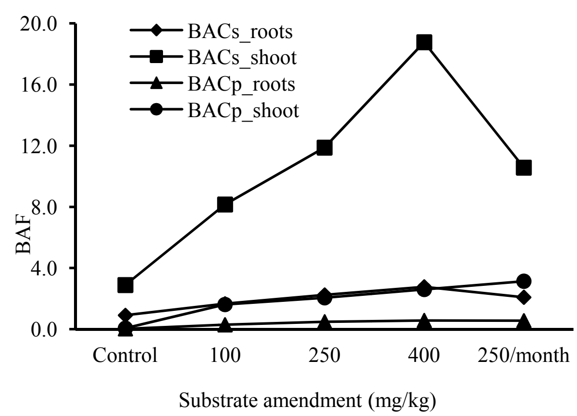

Fig. 4: A plot of bioaccumulation factors of U in roots and shoots of black spruce seedlings grown substrates spiked with varied concentrations of U solutions

Table 5: Bioaccumulation and transfer factors for uranium uptake in spruce seedlings grown on substrates treated with varied concentration of uranium nitrate

Substrate Treatment (mg/kg)	Soil				Peat			
	BAF (roots)	BAF (shoots)	TF (stem)	TF (needles)	BAF (roots)	BAF (shoot)	TF (stem)	TF (needles)
Control	0.91	2.89	2.14	0.01	0.03	0.07	1.35	0.00
100	1.66	8.15	3.29	0.19	0.29	1.62	2.41	0.20
250	2.24	11.88	3.86	0.11	0.49	2.06	2.09	0.11
400	2.77	18.76	5.28	0.09	0.57	2.60	2.48	0.08
250/month	2.09	10.57	3.52	0.16	0.56	3.14	2.14	0.11

microbiological and biochemical activity with resultant changes in redox conditions of cations in the vicinity of the roots. Colonization of root surface by micorrhizal fungi due to a pH altering effect of rhizosphere chemistry greatly increases the absorbing surface area of plant roots and improves metal uptake from substrate pore water (Sylvia *et al*., 2005; Kabata-Pendias and Pendias, 2001; Chunilall *et al*., 2005).

Bioaccumulation and transfer factors: In Table 5 bioaccumulation and transfer factors for U in organs of spruce plants grown in control media and in pots treated with varied doses of uranyl nitrate are presented. The calculated BAF values showed linear association with U content in the substrates (Fig. 4). Results in Table 5 also demonstrated great disparities in the magnitude of BAF values for U accumulation in aboveground shoots and plant roots. Accumulation of the metal in stems was several orders of magnitude greater than quantities determined in roots. This observation, however, contradicted results by Ramaswami *et al*. (2001) and Shtangeeva (2008) who reported higher BAF for U accumulation in roots of herbs, including, sunflower, Indian mustard, spring vetch, bush bean, hairy vetch and juniper, rye grass and wheat grown in U contaminated soils and U-enriched soils, respectively. Furthermore, needle to stem transfer ratios of U clearly showed marginal accumulation in the needles. This study corroborated findings of a previous study where U accumulation was determined in needles of spruce trees sampled from areas of known uranium mineralization in the Athabasca region of Saskatchewan (Dunn, 1981, 2007). Dunn (1981) suggested that the low enrichment in needles could be explained by barrier mechanisms that potentially inhibit the transport of the metal along the vascular system to the needles. In addition, Sheppard and Evenden (1988) and Shahandeh and Hossner (2002) attributed a low accumulation of U in aerial parts of plants to limited mobility along plant vascular systems and tissues caused by the tendency of U to absorb on cell wall materials.

The statistically significant differences ($p<0.01$) between BAF and TF values of plants grown in U enriched soils and those grown on U enriched peat (Table 5) agree with observations of Cummins (1994) that bioaccumulation and bioconcentration factors for a metal in a particular species are greatly affected by substrate properties and environmental conditions. As anticipated, plants grown in soils treated with a single dose of 250 ppm uranyl nitrate consistently recorded higher BAF and TF values than their counterparts grown on soils treated with monthly dose of 50 mL of 250 ppm uranyl nitrate solution. Conversely, BAF and TF values determined in plants grown on peat treated with 250 mg/kg uranyl nitrate on a monthly basis were significantly greater than those determined in plant grown on peat treated with single dose of 250 ppm U solution (Table 5).

In spite of the considerable interest in the evaluation of U uptake and bioaccumulation factors in plants, insufficient data exist relating uptake to the fraction of U that is actually available to plants (Negri and Hinchman, 2000). In other words, U accumulation ratios in most studies were computed using total U content in the substrates and may in effect underestimate the accumulation factors or otherwise not correctly reflect the uptake of mobile species. The lack of agreement in the literature on the computation of the metal accumulation ratios in plants and the obvious substrate controls on metal availability make it difficult to compare metal accumulation ratios from different studies. There are no published bioaccumulation ratios for U accumulation in black spruce trees.

Figure 4 demonstrates the relationship between bioaccumulation of U in roots and shoots of black spruce plants and the metal content in the substrates. Plants grown on U-enriched soils showed a clear association between the magnitude of accumulation and substrate content (available fraction) of the metal. The translocation of the metal to needles was however marginal (transfer ratios ≤ 0.2) and exhibited no clear correlation with substrate contents (Table 5). The statistically significant differences between stem to root transfer ratios (TF_{stem}) and needle to stem transfer ratios ($TF_{needles}$) of U corroborates the presence of a possible restriction on internal translocation of the metal ions to the needles.

CONCLUSION

Although U is not known to be essential or beneficial to plants, many species will absorb and incorporate it into their biomass along with other metals. Results from this study shows that black spruce plants are capable of accumulating anomalous quantities of metals in their tissues which reflect the substrate content. However, the level of accumulation is greatly dependent on the degree of metal availability in the substrate. The magnitude of metal accumulation in parts of black spruce plants followed the order stem>roots>needles. The trend of U accumulation was comparable for plants grown in U enriched peat and soil although the magnitude of metal accumulation differed markedly. Uranium Accumulation Factors (BAF) were higher in shoots compared to values determined in roots. The transfer of the metal to needles was relatively slow in plants grown in soil and peat with TF$\leq$0.2. Based on the determined BAF and TF values and the magnitude of accumulation of U black spruce can best be described as a bio-indicator of the metal since the U content in the plant parts examined increased linearly with increasing available concentration in substrates. Consequently, the magnitude of U uptake is a function of available metal content in the substrate. It is evident from the experimental data that substrate controls have a significant impact on U uptake by the black spruce trees. More importantly metal availability in the substrate appears to be the dominant factor responsible for U uptake and accumulation in organs of black spruce.

Implications for mineral exploration and environmental monitoring: Exploration challenges have changed over the past decade and future discoveries of ore deposits are expected to shift to regions overlain by transported overburden. Exploration geologists consider transported overburden to be an impediment to mineral prospecting, however evidence now exists that metal accumulating plants growing on exogenous cover have the potential of defining signatures of buried mineralization. Results from this study indicate that black spruce stems are capable of providing variably focused expression of concealed uranium mineralization in areas covered by exogenous material. The linearity of the quantities of U accumulated in parts of spruce trees with the available concentrations in substrates provided great contrast between background (control) and areas of elevated uranium concentration. This property coupled with an extensive lateral root system makes black spruce an excellent sampling medium for expressing mineralization haloes in areas where access to mineralized bedrock is limited by extensive cover material. Compared with mineral prospecting methods such as drilling and soil geochemical surveys, which are arduous to undertake and very expensive, plant sampling and analysis is fast, relatively inexpensive and has minimal to no environmental impact. As a bio-indicator, the black spruce plant has great potential for use as tool for environmental monitoring. Use of plants provides a direct expression of pollutant impact on the environment that is not readily obtainable by other environmental monitoring techniques. It is apparent that U uptake by spruce trees is substrate-dependent and greatly influenced by available metal pools in the underlying substrate.

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