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Uptake and Translocation of Manganese by Native Tree Species in a Constructed Wetland Treating Landfill Leachates

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Abstract: A surface flow constructed wetland was used to treat stormwater runoff from surrounding watersheds which are comprised primarily of commercial properties and two former landfills. The uptake of manganese by red maple, white birch and red spruce trees growing under flooded soil conditions in the constructed wetland was compared to that of the same trees growing under well drained soil conditions in a nearby reference site. The seasonal variability of manganese and its distribution in different compartments of these trees (leaves, twigs, branches, trunk wood, trunk bark and roots) were studied. The average manganese concentrations in the aboveground compartments of red maple, white birch and red spruce trees were within the range of manganese concentrations reported in the literature for these trees. The concentrations of manganese in the aboveground compartments of red maple, white birch and red spruce trees in the reference site were significantly greater than those in the constructed wetland (with the exception of manganese concentrations in the trunk wood of red maple trees) because of the acidic soil conditions of the reference site. The percent distribution of manganese in the aboveground compartments of trees did not vary during the growing season. Higher concentrations of manganese were present in the trunk bark and either the leaves or twigs of species on both the constructed wetland and the reference site regardless of the sampling date.

Key words: Red maple, white birch, red spruce, manganese, seasonal variability, constructed wetland

INTRODUCTION

Plants have been described as solar driven pumps that can extract and concentrate metals from their environment[1]. Plants extract metals from their soil and water environments because many metals are essential nutrients including magnesium, calcium, potassium, iron, manganese, copper, zinc and molybdenum. Plants also extract metals from their environments that have not been recognized as essential nutrients including chromium, lead, cadmium, mercury and nickel[2,3]. Metals in the environment originate from natural sources such as emissions from volcanoes and forest fires and the weathering of metal enriched rocks[4] and from anthropogenic sources such as industrial activities, agricultural practices, atmospheric deposition and waste disposal operations[5-7].

The retention of metals in specific compartments and their distribution to the tissues of the plant is highly species specific and depends on metal resistance mechanisms available to the plant such as chelation of metals, compartmentalization and organic ligand exudation[8,9]. In trees, metals exceeding the metabolic needs typically are transported to the extremities such as the bark, twig tips and leaves[10].

In wetlands, flooded soils rapidly experience a decline in soil oxygen and redox potential resulting in anaerobic soil conditions. Facultative and obligate anaerobic microorganisms use carbon compounds as substrates and oxidized soil components as electron acceptors in respiration. Oxygen is the first soil component to be reduced followed by nitrate, manganese dioxide and ferric iron hydroxide[11,12]. As a result, flooded soils can have toxic concentrations of plant available nutrients such as iron and manganese compared to well drained soils[13].

The objectives of this study were: (a) to compare the uptake of manganese by native tree species growing under flooded soil conditions in a constructed wetland and those growing under well drained soil conditions in a nearby forest, (b) to evaluate the seasonal variability of manganese in these trees and (c) to determine the distribution of manganese in different compartments of these trees (leaves, twigs, branches, trunk wood, trunk bark and roots).

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MATERIALS AND METHODS

Burnside constructed wetland: A surface flow constructed wetland was established in the Burnside Industrial Park, Dartmouth, Nova Scotia, to treat stormwater runoff from the surrounding watersheds which are comprised primarily of commercial properties and two former landfills (a 5.34 ha site that operated from 1968 to 1974 and a 5.42 ha site that operated from 1976 to 1977). The aim was to protect a freshwater ecosystem that consists of a 4.6 km long brook (Wright's Brook) and two lakes (Enchanted Lake and Flat Lake). The results (Table 1) of a previously conducted environmental site assessment[14] had determined that the water entering the brook contained average iron and manganese concentrations (15.508 and 3.029 mg L^{-1}, respectively) that exceed the allowable limits established by the Canadian Water Quality Guidelines for the Protection of Aquatic Life[15,16]. The wetland consists of 9 deep open water cells that are separated by shallow internal vegetated berms and surrounded by a system of external berms with a surface area of 6300 m^2 and 2 naturally vegetated islands that are surrounded by a system of external berms with a surface area of approximately 6100 m^2. The wetland berms and cells were planted with a variety of native plant species such as *Carex crinita* (fringed sedge), *Carex lurida* (yellow green sedge), *Juncus brevicaudatus* (tweedy's rush), *Juncus effusus* (soft rush), *Scripus validus* (soft stem bulrush), *Calamagrostis canadensis* (bluejoint grass), *Alisma plantagoaquatica* (water plantain), *Pontederia cordata* (pickerelweed), *Nymphaea alba* (white waterlily) and *Potamageton natans* (pondweed). The two naturally vegetated islands consist of untamed early successional brush dominated by low shrubs such as *Comptonia peregrina* (sweet fern), *Viburnum cassinoides* (witherod) and *Spiraea alba* (meadowsweet), deciduous and evergreen trees such as *Acer rubrum* (red maple), *Betula papyrifera* (white birch) and *Picea rubens* (red spruce) and emergent macrophytes such as *Typha latifolia* (cattails).

Table 1: Heavy metal loads entering Wright's Brook[14]

Element	Average concentration (mg L^{-1})	Guidelines[15] (mg L^{-1})
Aluminium	7.720	0.005-0.100
Arsenic	0.009	0.005
Chromium	0.013	0.001-0.009
Copper	0.039	0.002-0.004
Iron	15.508	0.300
Lead	0.075	0.001-0.007
Manganese	3.029	1.000-1.500[16]
Zinc	0.158	0.030

Selection of trees and sampling locations: A survey of the vegetated islands was conducted in order to identify dominant tree species. Based on the survey, two deciduous and one evergreen tree species were selected for this study. The two deciduous species included: *Acer rubrum* (red maple) and *Betula papyrifera* (white birch) and the evergreen species was *Picea rubens* (red spruce). Samples of leaves, twigs, branches, trunk bark, trunk wood and roots from trees were collected from the two naturally vegetated islands in the constructed wetland and from a reference site. The reference site was a forested area located to the south west of the islands. It was not influenced by stormwater runoff or leachate from the former landfills and it was never flooded during the study period. According to MacDougall *et al*.[17], the soil in the reference site was porous and well drained. A total of six trees from each of red maple, white birch and red spruce were sampled (three trees from the naturally vegetated islands and three trees from the reference site). The sampling locations are shown in Fig. 1.

Sample collection: In season one, plant samples were collected when element concentrations were most stable. Element concentrations in evergreen species are most stable during the dormant season, which is typically from September through early March while element concentrations in deciduous species are most stable late in the growing season approximately two weeks before the onset of autumn coloration[18]. Therefore, plant samples were collected during the second and third weeks of September 2005. In season two, plant samples were collected in June 2006, July 2006 and August 2006. Samples of leaves were also collected in September and October 2006 upon senescence.

Samples of leaves, twigs and branches were collected with hand pruners, long handled pruners and a telescopic pruning pole. For both evergreen and deciduous species, samples of leaves or needles (whole, well formed and current year growth) and were collected from several branches representing various sides of the middle part of the live crown of each tree. Approximately 20 leaves per deciduous tree were collected and mixed to make one sample. Enough needles and twigs were collected per evergreen tree to fill an 18×15 cm plastic bag. To minimize serious injury to the trees, only one branch per tree was collected, cut into 6 inch lengths and mixed to make one sample. Wood and bark samples were collected from the trunk of each tree at breast height (1.3 m). An axe was used to peel off a sample of bark and a 25.4 cm increment borer (SUUNTO, Vantaa, Finland) with three threads and an inner bit diameter of 5 mm was used to collect a wood core. Two parallel wood cores were obtained from each sampled trunk and mixed to make one sample. Samples of roots were collected from each tree in an area between the outer branch tips and trunk. A stainless steel spade was used to expose a portion of roots around each tree and samples were collected using long handled pruners. Samples were placed in labeled resealable plastic bags and stored in a cooler (5°C). All samples were transported to the laboratory within 8 h and stored in a refrigerator at 4°C. Preparation of plant samples involved decontamination, oven drying and particle size reduction.

Decontamination of samples: According to Mills and Jones Jr.[2], when plant materials are covered with soil, dust particles or spray materials, decontamination is required. Horwitz[19] stated that the decontamination process should be performed quickly and excess washing of plant tissue, especially leaves, should be avoided to prevent leaching of minerals. However, tissue exposed to frequent rainfall such as leaves, twigs and branches need not be washed. Therefore, only root samples were washed with tap water to remove soil prior to drying.

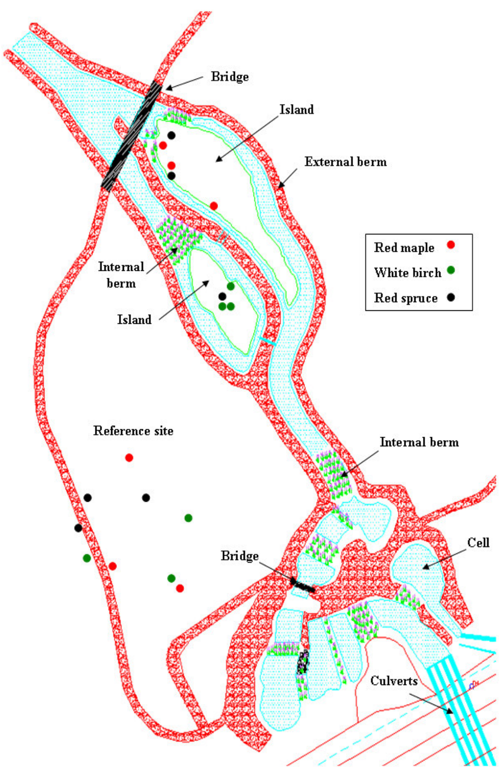

Fig. 1: Location of sampled trees

Drying of samples: Plant materials should be dried to minimize decomposition or weight loss by respiration at a temperature high enough to destroy the enzymes responsible for decomposition and sufficient for moisture removal, but below the temperature of thermal decomposition. Enzymes present in plant tissues are inactivated at temperatures above 60°C[20]. Therefore, plant tissues were placed in brown paper bags and oven dried at a temperature of 80°C for approximately 48 h in a laboratory oven (Isotemp Oven, Model # 655F, Fisher Scientific Co., Ontario, Canada).

Particle size reduction: Samples of leaves were removed from the brown paper bags and placed into resealable plastic bags and reduced in size by manually crushing the dried samples. Samples of twigs, trunk bark and trunk wood were reduced in size by hand cutting using hand pruners and then by grinding in a coffee grinder (Toastmaster, Model # 1119CAN, China). Samples of branches and roots were reduced in size by hand cutting using long handled pruners and then by grinding in a coffee grinder.

Sample analysis: Prepared samples were stored in air tight plastic containers in a refrigerator (4°C) until they were delivered to the Minerals Engineering Center at Dalhousie University, Halifax, Nova Scotia and analyzed for total manganese concentrations. A wet acid digestion procedure was performed for destruction of organic matter present in plant samples. Initially, 1.0 g of dried, ground sample was placed in a Teflon beaker and 30 mL (38 % HCl) hydrochloric acid (Cat. # A144-S212, Fisher Scientific Co., Ontario, Canada), 10 mL (70 % HNO_3) nitric acid (Cat. # A200-212, Fisher Scientific Co., Ontario, Canada), 10 mL (49 % HF) hydrofluoric acid (Cat. # A147-1LB, Fisher Scientific Co., Ontario, Canada) and 5 mL (70 % $HClO_4$) perchloric acid (Cat. # A2296-1LB, Fisher Scientific Co., Ontario, Canada) were added. Under a fume hood, the samples were heated to dryness (overnight) on a hot plate (Model # SP46925, Barnstead/Thermolyne, Dubuque, Iowa) at a temperature of 125 °C. Then, 10 mL of HCl and 30 mL of H_2O were added to dissolve the residue. Under a fume hood, the samples were heated on a hot plate at a temperature of 125°C for 30 min. The samples were filtered through Fisher # 4 filter paper (Cat. # 09-803-6A, Fisher Scientific Co., Ontario, Canada) and the filtrate was collected in a 100 mL volumetric glass flask and made up to a final volume of 100 mL with distilled-deionized water. Manganese concentrations were determined by inductively coupled plasma optical emission spectroscopy (Vista Pro, Varian Inc., Victoria, Australia) with a detection limit of 0.25 ppm for Mn.

Statistical analysis: The data was analyzed statistically using a one-way analysis of variance. The statistical analyses were performed using SPSS (SPSS Inc., SPSS 14.0.1, Chicago, IL) and differences were considered significant at a p-value = 0.05 (95% confidence interval).

RESULTS AND DISCUSSION

Uptake of manganese: Manganese is an essential micronutrient in plant nutrition and has several functions. Manganese is involved in photosynthesis, it is a constituent of some respiratory enzymes and enzymes responsible for protein synthesis and it functions in the formation of chlorophyll[13,21]. Symptoms of manganese deficiency include interveinal chlorosis of young leaves and brown speckling and bronzing with abscission of developing leaves. Uptake of excess concentrations of manganese by plants can result in deformed leaves, chlorotic areas, dead spots and stunted growth[13]. The manganese concentrations in the different compartments of each tree are shown in Tables 2-4. Tables 5-7 display the analysis of variance for the total manganese concentrations in the compartments of red maple, white birch and red spruce trees, respectively as affected by location.

Leaves: The average manganese concentrations in the leaves of red maple, white birch and red spruce trees in the constructed wetland ranged from 116 to 405 mg kg^{-1}, from 252 to 426 mg kg^{-1} and from 104 to 168 mg kg^{-1}, respectively. The average concentrations of manganese in the leaves of red maple, white birch and red spruce trees in the reference site ranged from 223 to 480 mg kg^{-1}, from 553 to 852 mg kg^{-1} and from 351 to 920 mg kg^{-1}.

The manganese concentrations in the leaves of trees examined in this study are within the range reported in the literature. Piczak[22] observed average manganese concentrations of 41.3-700 and 106-409 mg kg^{-1} in the leaves of Norway maple and weeping birch trees. Mankovska[23] and Elowson and Rytter[24] observed average manganese concentrations of 1025 and 450 mg kg^{-1} in the needles of Norway spruce and grey alder trees. Young and Guinn[25] observed average manganese concentrations of 765, 315 and 1400 mg kg^{-1} in the leaves of red maple, white birch and red spruce trees, respectively.

The low, normal and high concentrations of manganese in dried plant leaves are 5, 20-400 and 2000 mg kg^{-1}, respectively[26]. The average manganese concentrations in the leaves of the sampled trees fall within or above the normal range for manganese in dried plant leaves.

The statistical analysis showed that the concentrations of manganese in the leaves of red maple, white birch and red spruce trees in the reference site were significantly greater than those in the leaves of trees in the constructed wetland (p-values = 0.056, 0.001 and 0.000). High levels of manganese in the leaves and needles of trees have been associated with acidic soils[27-31]. Lin *et al*.[28] observed manganese concentrations in the range of 552-896 mg kg^{-1} in the needles of balsam fir trees that were growing in soil with an average pH of 3.75. Kolb and McCormick[29] observed average manganese concentrations of 2459 and 2452 mg kg^{-1} in the leaves of sugar maple trees that were growing in soil with an average pH < 4.1. Kazda and Zvacek[30] observed average manganese concentrations of 704±389 mg kg^{-1} in the needles of Norway spruce trees that were growing in soil with an average pH <4.0.

Twigs: The average manganese concentrations in the twigs of red maple, white birch and red spruce trees in the constructed wetland ranged from 162 to 178 mg kg^{-1}, from 104 to 121 mg kg^{-1} and from 77 to 93 mg kg^{-1}, respectively. The average manganese concentrations in the twigs of red maple, white birch and red spruce trees in the reference site ranged from 273 to 313 mg kg^{-1}, from 159 to 225 mg kg^{-1} and from 436 to 590 mg kg^{-1}, respectively.

The average manganese concentrations in the twigs of the sampled trees agree with reported values in the literature. McColl[32] observed average manganese concentrations of 250 mg kg^{-1} in the twigs of blue gum eucalyptus trees. Brotherson and Osayande[33] observed average manganese concentrations of 12.0 and 13.1 mg kg^{-1} in the twigs of mountain mahogany and Utah juniper trees. Young and Guinn[25] observed average manganese concentrations of 475, 129 and 580 mg kg^{-1} in the twigs of red maple, white birch and red spruce trees, respectively.

The statistical analysis showed that the concentrations of manganese in the twigs of red maple, white birch and red spruce trees in the reference site were significantly greater than those in the twigs of trees in the constructed wetland (p-values = 0.001, 0.000 and 0.000). A review of the literature revealed one study by Sailerova and Fedikow[10] who reported that the average manganese concentrations in the twigs of black spruce trees growing on well drained sites was significantly greater than those in trees growing on poorly drained sites.

Branches: The average manganese concentrations in the branches of red maple, white birch and red spruce trees in the constructed wetland ranged from 78 to 117 mg kg^{-1}, from 69 to 85 mg kg^{-1} and from 59 to 115 mg kg^{-1}, respectively. The average manganese concentrations in the branches of red maple, white birch and red spruce trees in

Table 2: Average Mn concentrations (mg kg^{-1}) in red maple trees

	Wetland					Reference Site				
Compartment	2005	2006				2005	2006			
	Sept	Jun	Jul	Aug	Sept	Sept	Jun	Jul	Aug	Sept
Leaves	329	116	167	274	405	420	223	334	480	557
	(228)	(86)	(88)	(175)	(321)	(197)	(88)	(158)	(236)	(198)
Twigs	162	176	172	178	-	273	313	280	281	-
	(38)	(54)	(54)	(115)	-	(95)	(48)	(98)	(96)	-
Branches	91	86	78	117	-	138	143	101	152	-
	(24)	(12)	(22)	(46)	-	(12)	(50)	(22)	(36)	-
Trunk wood	32	47	25	37	-	46	43	52	39	-
	(5)	(23)	(6)	(6)	-	(6)	(17)	(18)	(12)	-
Trunk bark	244	223	233	214	-	388	386	358	393	-
	(38)	(39)	(61)	(70)	-	(89)	(79)	(76)	(123)	-
Roots	98	-	-	128	-	133	-	-	105	-
	(32)	-	-	(57)	-	(77)	-	-	(57)	-

- Samples were not collected, () Standard deviation

Table 3: Average Mn concentrations (mg kg^{-1}) in white birch trees

	Wetland					Reference				
Compartment	2005	2006				2005	2006			
	Sept	Jun	Jul	Aug	Sept	Sept	Jun	Jul	Aug	Sept
Leaves	273	252	316	352	426	852	553	694	695	540
	(44)	(131)	(101)	(154)	(189)	(586)	(229)	(348)	(281)	(48)
Twigs	105	104	109	121	-	177	159	225	218	-
	(35)	(38)	(15)	(31)	-	(82)	(7)	(67)	(54)	-
Branches	69	83	85	84	-	163	164	170	196	-
	(16)	(14)	(22)	(19)	-	(59)	(36)	(47)	(15)	-
Trunk wood	34	35	46	24	-	77	72	65	64	-
	(8)	(1)	(29)	(6)	-	(24)	(15)	(18)	(15)	-
Trunk bark	1033	1054	1087	985	-	1374	1178	1332	1158	-
	(411)	(278)	(122)	(113)	-	(383)	(147)	(194)	(262)	-
Roots	118	-	-	262	-	90	-	-	254	-
	(34)	-	-	(154)	-	(50)	-	-	(161)	-

- Samples were not collected, () Standard deviation

Table 4: Average Mn concentrations (mg kg^{-1}) in red spruce trees

	Wetland				Reference			
Compartment	2005	2006			2005	2006		
	Sept	Jun	Jul	Aug	Sept	Jun	Jul	Aug
Leaves	168	104	143	165	887	351	464	920
	(54)	(51)	(56)	(64)	(392)	(302)	(112)	(299)
Twigs	77	81	88	93	590	436	485	565
	(12)	(13)	(13)	(14)	(328)	(196)	(203)	(208)
Branches	115	78	59	79	382	319	380	366
	(34)	(18)	(11)	(9)	(149)	(148)	(163)	(152)
Trunk Wood	52	38	39	37	186	151	149	137
	(6)	(3)	(11)	(3)	(24)	(54)	(59)	(49)
Trunk Bark	419	323	310	321	917	731	813	742
	(53)	(21)	(43)	(90)	(258)	(109)	(191)	(159)
Roots	98	-	-	80	156	-	-	349
	(27)	-	-	(35)	(140)	-	-	(82)

- Samples were not collected, () Standard deviation the reference site ranged from 101 to 152 mg kg^{-1}, from 163 to 196 mg kg^{-1} and from 319 to 382 mg kg^{-1},respectively. These values are comparable to data reported in the literature. Morrison and Hogan[34], Schmitt *et al*.[35] and Wittwer *et al*.[36] observed average manganese

Table 5: One-way analysis of variance for the total Mn concentrations in the compartments of red maple trees as affected by location

Source	DF	SS	MS	F	P
Red maple leaves					
Total	29	1258581			
Location	1	156819	156819	3.99	0.056
Error	28	1101763	39349		
Red maple twigs					
Total	23	183686			
Location	1	78891	78891	16.56	0.001
Error	22	104795	4763		
Red maple branches					
Total	23	32599			
Location	1	9923	9923	9.63	0.005
Error	22	22676	1031		
Red maple trunk wood					
Total	23	4419			
Location	1	523	523	2.95	0.100
Error	22	3897	177		
Red maple trunk bark					
Total	23	237000			
Location	1	139843	139843	31.67	0.000
Error	22	97157	4416		
Red maple roots					
Total	11	29478			
Location	1	108	108	0.04	0.852
Error	10	29370	2937		

Differences are considered significant at a p-value = 0.05 (95% confidence interval)

concentrations of 264-411, 543 and 304 mg kg^{-1} in the branches of sugar maple, white birch and red pine trees, respectively. Young and Guinn[25] observed average manganese concentrations of 830, 273 and 1076 mg kg^{-1} in the branches of red maple, white birch and red spruce trees, respectively.

The statistical analysis showed that the concentrations of manganese in the branches of red maple, white birch and red spruce trees in the reference site were significantly greater than those in the branches of trees in the constructed wetland (p-values = 0.005, 0.000 and 0.000). A review of the literature revealed no specific studies on the accumulation of manganese in the branches of trees growing on well drained acidic soils compared to saturated soils.

Trunk wood: The average manganese concentrations in the trunk wood of red maple, white birch and red spruce trees in the constructed wetland ranged from 25 to 47 mg kg^{-1}, from 24 to 46 mg kg^{-1} and from 37 to 52 mg kg^{-1}, respectively. The average manganese concentrations in the trunk wood of red maple, white birch and red spruce trees in the reference site ranged from 39 to 52 mg kg^{-1}, from 64 to 77 mg kg^{-1} and from 137 to 186 mg kg^{-1}, respectively.

Table 6: One-way analysis of variance for the total Mn concentrations in the compartments of white birch trees as affected by location

Source	DF	SS	MS	F	P
White birch leaves					
Total	29	2508391			
Location	1	882711	882711	15.20	0.001
Error	28	1625680	58060		
White birch twigs					
Total	23	88742			
Location	1	43350	43350	21.01	0.000
Error	22	45392	2063		
White birch branches					
Total	23	71761			
Location	1	51894	51894	57.46	0.000
Error	22	19867	903		
White birch trunk wood					
Total	23	12974			
Location	1	7350	7350	28.75	0.000
Error	22	5624	256		
White birch trunk bark					
Total	23	1511798			
Location	1	292604	292604	5.28	0.031
Error	22	1219194	55418		
White birch roots					
Total	11	178319			
Location	1	972	972	0.05	0.820
Error	10	177347	17735		

Differences are considered significant at a p-value = 0.05 (95% confidence interval)

Table 7: One-way analysis of variance for the total Mn concentrations in the compartments of red spruce trees as affected by location

Source	DF	SS	MS	F	P
Red spruce needles					
Total	23	3049082			
Location	1	1562130	1562130	23.11	0.000
Error	22	1486951	67589		
Red spruce twigs					
Total	23	1638807			
Location	1	1129702	1129702	48.82	0.000
Error	22	509105	23141		
Red spruce branches					
Total	23	3049082			
Location	1	1562130	1562130	23.11	0.000
Error	22	1486951	67589		
Red spruce trunk wood					
Total	23	101705			
Location	1	78204	78204	73.21	0.000
Error	22	23500	1068		
Red spruce trunk bark					
Total	23	1651574			
Location	1	1255838	1255838	69.82	0.000
Error	22	395736	17988		
Red spruce roots					
Total	11	192625			
Location	1	80033	80033	7.11	0.024
Error	10	112591	11259		

Differences are considered significant at a p-value = 0.05 (95% confidence interval

Saarela *et al*.[37] observed average manganese concentrations of 32-83 mg kg^{-1} in the trunk wood of

Scots pine trees. Basham and Cowling[38] observed average manganese concentrations of 16 and 62 mg kg^{-1} in the trunk wood of weeping birch and European spruce trees. Young and Guinn[25] observed average manganese concentrations of 72, 34 and 144 mg kg^{-1} in the trunk wood of red maple, white birch and red spruce trees, respectively. Miller[39] stated that elements such as manganese are typically present in dry wood at concentrations of < 100 mg kg^{-1}. The average manganese concentrations in the trunk wood of the sampled tree species are all less than 100 mg kg^{-1} with the exception of the trunk wood from red spruce trees in the reference site.

The statistical analysis showed that the concentrations of manganese in the trunk wood of white birch and red spruce trees in the reference site were significantly greater than those in the trunk wood of trees in the constructed wetland (p-values = 0.000 and 0.000). A review of the literature revealed no specific studies on the accumulation of manganese in the trunk wood of trees growing on well drained acidic soils compared to saturated soils.

Trunk bark: The average manganese concentrations in the trunk bark of red maple, white birch and red spruce trees in the constructed wetland ranged from 214 to 244 mg kg^{-1}, from 985 to 1087 mg kg^{-1} and from 310 to 419 mg kg^{-1}, respectively. The average manganese concentrations in the trunk bark of red maple, white birch and red spruce trees in the reference site ranged from 358 to 393 mg kg^{-1}, from 1158 to 1374 mg kg^{-1} and 731 to 917 mg kg^{-1}, respectively.

The average manganese concentrations in the trunk bark of the sampled species were within the range of values reported in other studies. Morrison and Hogan[34] observed average manganese concentrations of 683 mg kg^{-1} in the trunk bark of sugar maple trees. Heinrichs and Mayer[40] observed average manganese concentrations of 1600 and 355 mg kg^{-1} in the trunk wood of European beech and Norway spruce trees. Young and Guinn[25] observed average manganese concentrations of 668, 275 and 612 mg kg^{-1} in the trunk bark of red maple, white birch and red spruce trees, respectively.

The statistical analysis showed that the concentrations of manganese in the trunk bark of red maple, white birch and red spruce trees in the reference site were significantly greater than those in the trunk bark of trees in the constructed wetland (p-values = 0.000, 0.031 and 0.000). A review of the literature revealed no specific studies on the accumulation of manganese in the trunk bark of trees growing on well drained acidic soils compared to saturated soils.

Roots: The average manganese concentrations in the roots of red maple, white birch and red spruce trees in the constructed wetland ranged from 98 to 128 mg kg^{-1}, from 118 to 262 mg kg^{-1} and from 80 to 98 mg kg^{-1}, respectively. The average manganese concentration in the roots of red maple, white birch and red spruce trees in the reference site ranged from 105 to 133 mg kg^{-1}, from 90 to 254 mg kg^{-1} and from 156 to 349 mg kg^{-1}, respectively.

Vogt *et al.*[41] observed average manganese concentrations of 580 and 850 mg kg^{-1} in the roots of hemlock and fir trees. Rodriguez-Barrueco[42] observed average manganese concentrations of 137-223 mg kg^{-1} in the roots of European alder trees. Turner *et al.*[43] observed average manganese concentrations of 142 mg kg^{-1} in the roots of red alder trees. Young and Guinn[25] observed average manganese concentrations of 657, 122 and 892 mg kg^{-1} in the roots of red maple, white birch and red spruce trees, respectively.

The statistical analysis showed that only the concentrations of manganese in the roots of red spruce trees in the reference site were significantly greater than those in the roots of trees in the constructed wetland (p-value = 0.024). A review of the literature revealed no specific studies on the accumulation of manganese in the roots of trees growing on well drained acidic soils compared to saturated soils.

Seasonal variations in total manganese: The seasonal variations in the average total manganese concentrations in the various compartments of the different tree species are shown in Figs. 2-6. Seasonal changes in the uptake of manganese may be evident when plant samples are collected over an extended period of time and could be attributed to several factors including: (a) developmental changes during the growth of the plant, (b) weather conditions that affect the evapotranspiration rate of the plant, (c) shunting of metals to plant tissues such as leaves and (d) seasonal changes in the availability of metals in the soil[10,44]. Tables 8-12 display the analysis of variance for the total manganese concentrations in the leaves, twigs, branches, trunk wood, trunk bark and roots of trees in the constructed wetland and the reference site as affected by the sampling date.

Leaves: The average manganese concentrations in the leaves of red maple trees in the constructed wetland and the reference site increased throughout the growing season from 116±86 and 223±88 mg kg^{-1} in June 2006 to 405±321 and 557±198 mg kg^{-1} in September 2006. However, these increases were not significantly affected by the sampling date (p-values = 0.436 and 0.271). The average manganese concentrations in the leaves of white birch trees in the constructed wetland and the reference site and red spruce trees in the constructed wetland were not significantly affected by the sampling date. The average manganese concentration in the needles of red spruce trees in the reference site was affected by the sampling date at a 90% confidence interval with a minimum value of 351±302 mg kg^{-1} in June 2006.

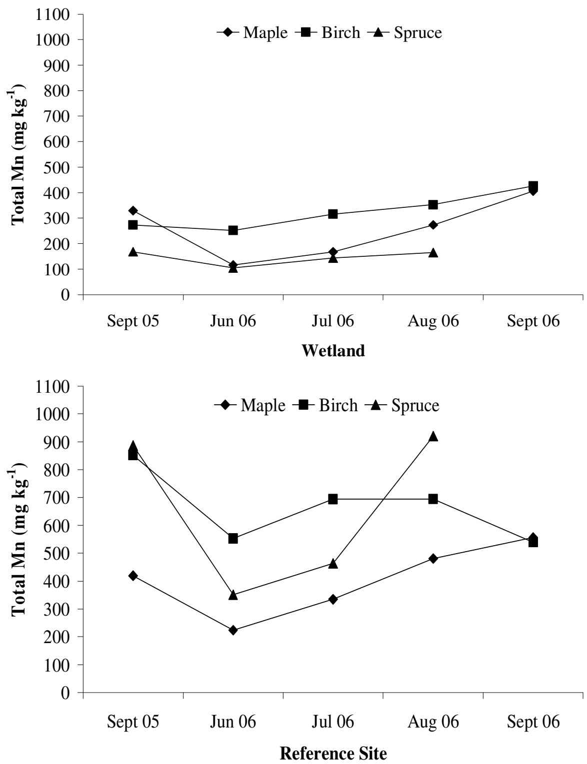

Fig. 2: Seasonal variability of Mn in the leaves of trees

Piczak *et al*.[22] observed an increase in the concentration of manganese in the leaves of Norway maple trees during the growing season and reported that the average manganese concentration in the leaves of maple

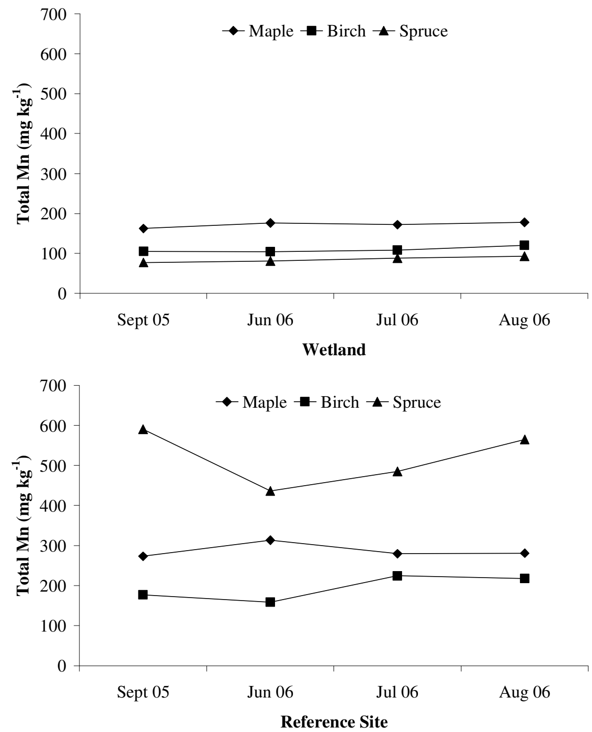

Fig. 3: Seasonal variability of Mn in the twigs of trees

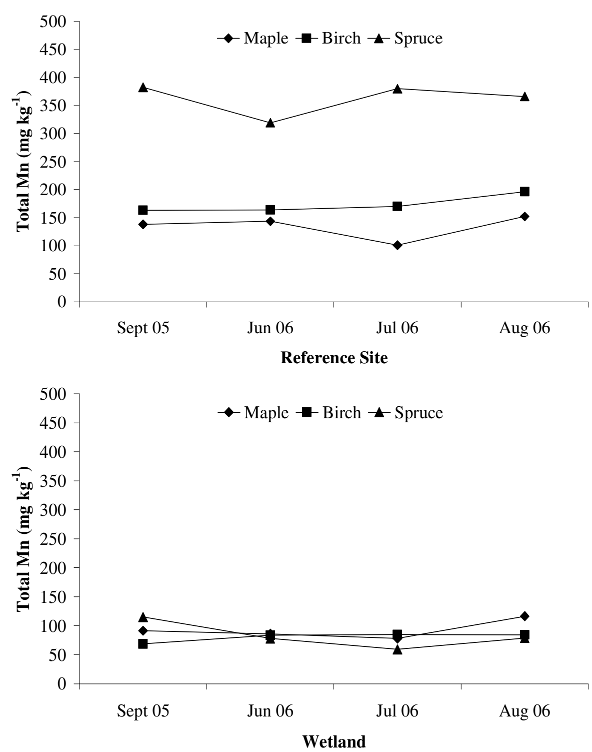

Fig. 4: Seasonal variability of Mn in the branches of trees

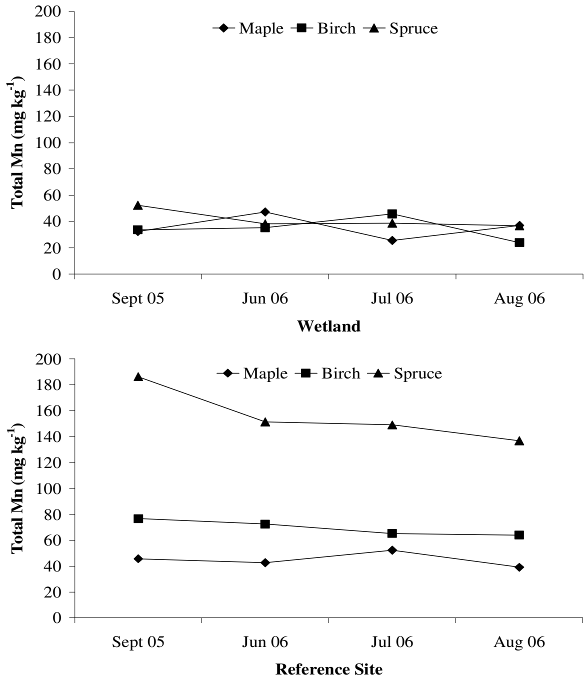

Fig. 5: Seasonal variability of Mn in the trunk wood of trees

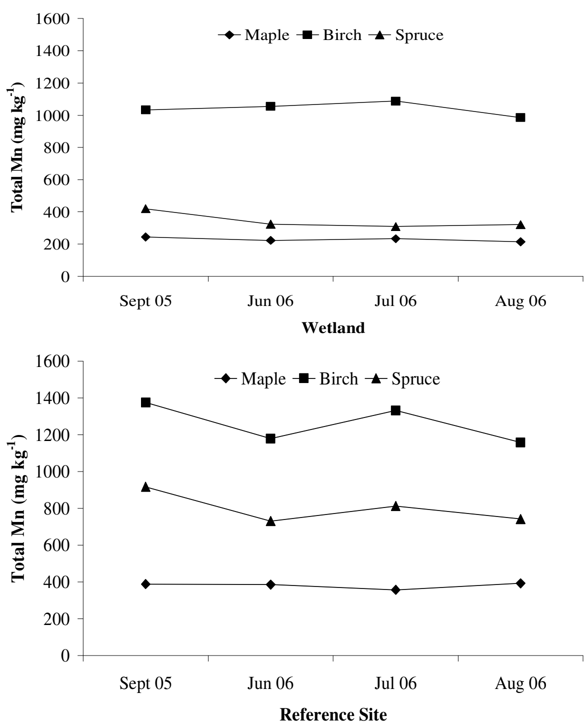

Fig. 6: Seasonal variability of Mn in the trunk bark of trees

trees from three sampling sites increased from 41.3 to 70.4 mg kg^{-1}, from 364 to 700 mg kg^{-1} and from 118 to 187 mg kg^{-1}. Oleksyn *et al.*[45] observed an accumulation of manganese in the leaves of silver birch trees during the growing season. They reported an increased average manganese concentration in the leaves from 237 mg kg^{-1} during leaf formation to 421 mg kg^{-1} during leaf senescence. Moorhead and McArthur[46] observed an accumulation of manganese in the leaves of red maple trees during the growing season. Rodriguez-Barrueco *et al.*[42] observed an accumulation of manganese in the leaves of European alder trees prior to leaf fall and reported an increased average manganese concentration in the leaves from 269 to 1014 mg kg^{-1}. Lea *et al.*[47] observed a steady increase in the concentration of manganese in the leaves of sugar maple trees during the growing season and reported an increased average manganese concentration in the leaves from 820 mg kg^{-1} in June to 2130 mg kg^{-1} in October.

Twigs: The average manganese concentrations in the twigs of red maple, white birch and red spruce trees in the constructed wetland and the reference site were not significantly affected by the sampling date. A review of the literature revealed no specific studies on the seasonal variability of manganese in the twigs of trees.

Branches: The average manganese concentrations in the branches of red maple and white birch trees in the constructed wetland and the reference site and red spruce trees in the reference site were not significantly affected by the sampling date. The average manganese concentration in the branches of red spruce trees in the constructed wetland was significantly affected by the sampling date at a 90% confidence interval (p-value = 0.055) with a maximum concentration of 115±34 mg kg^{-1} in September 2005. A review of the literature revealed no specific studies on the seasonal variability of manganese in the branches of trees.

Trunk wood: The average manganese concentrations in the trunk wood of red maple and white birch trees in the constructed wetland and the reference site and red spruce trees in the reference site were not significantly affected by the sampling date. The average manganese concentrations in the trunk wood of red spruce trees in the constructed wetland were significantly affected by the sampling date at a 90% confidence interval (p-value = 0.064) with a maximum concentration of 52±6 in September 2005.

Table 8: One-way analysis of variance for the total Mn concentrations in the leaves of trees in the constructed wetland and the reference site as affected by date

		Wetland				Reference			
Source	DF	SS	MS	F	P	SS	MS	F	P
Red maple leaves									
Total	14	568062.9				533699.7			
Date	4	166244.3	41561.1	1.034	0.436	201045.7	50261.4	1.511	0.271
Error	10	401818.7	40181.9			332654.0	33265.4		
White birch leaves									
Total	14	235457.3				1390223			
Date	4	57428.7	14357.2	0.806	0.548	194848.9	48712.2	0.408	0.799
Error	10	178028.7	17802.9			1195374.0	119537.4		
Red spruce leaves									
Total	11	33186.3				1453765.0			
Date	3	7724.9	2574.9	0.809	0.524	759747.7	253249.2	2.919	0.100
Error	8	25461.3	3182.7			694017.3	86752.2		

Differences are considered significant at a p-value = 0.05 (95% confidence interval)

Table 9: One-way analysis of variance for the total Mn concentrations in the twigs of trees in the constructed wetland and the reference site as affected by date

		Wetland				Reference			
Source	DF	SS	MS	F	P	SS	MS	F	P
Red maple twigs									
Total	11	41626.9				63168.3			
Date	3	424.9	141.6	0.028	0.993	2860.3	953.4	0.126	0.942
Error	8	41202.0	5150.3			60308.0	7538.5		
White birch twigs									
Total	11	8114.9				37276.9			
Date	3	488.3	162.8	0.171	0.913	9039.6	3013.2	0.854	0.503
Error	8	7626.7	953.3			28237.3	3529.7		
Red spruce twigs									
Total	11	1786.0				507318.9			
Date	3	466.0	155.3	0.941	0.465	45724.9	15241.6	0.264	0.849
Error	8	1320.0	165.0			461594.0	57699.3		

Differences are considered significant at a p-value = 0.05 (95% confidence interval)

Table 10: One-way analysis of variance for the total Mn concentrations in the branches of trees in the constructed wetland and the reference site as affected by date

		Wetland				Reference			
Source	DF	SS	MS	F	P	SS	MS	F	P
Red maple branches									
Total	11	9250.9				13424.9			
Date	3	2540.9	846.9	1.010	0.437	4557.6	1519.2	1.371	0.320
Error	8	6710.0	838.7			8867.3	1108.4		
White birch branches									
Total	11	3116.7				16750.7			
Date	3	547.3	182.4	0.568	0.651	2202.0	734.0	0.404	0.755
Error	8	2569.3	321.2			14548.7	1818.6		
Red spruce branches									
Total	11	8228.3				195784.3			
Date	3	4882.9	1627.6	3.892	0.055	7798.9	2599.6	0.111	0.951
Error	8	3345.3	418.2			187985.3	23498.2		

Differences are considered significant at a p-value = 0.05 (95% confidence interval)

Table 11: One-way analysis of variance for the total Mn concentrations in the trunk wood of trees in the constructed wetland and the reference site as affected by date

		Wetland				Reference			
Source	DF	SS	MS	F	P	SS	MS	F	P
Red maple trunk wood									
Total	11	2055.0				1841.7			
Date	3	767.0	255.7	1.588	0.267	272.3	90.8	0.463	0.716
Error	8	1288.0	161.0			1569.3	196.2		
White birch trunk wood									
Total	11	2662.9				2960.9			
Date	3	730.3	243.4	1.008	0.438	338.3	112.8	0.344	0.795
Error	8	1932.7	241.6			2622.7	327.8		
Red spruce trunk wood									
Total	11	800.7				22699.7			
Date	3	461.3	153.8	3.625	0.064	4093.7	1364.6	0.587	0.641
Error	8	339.3	42.4			18606.0	2325.8		

Differences are considered significant at a p-value = 0.05 (95% confidence interval)

Table 12: One-way analysis of variance for the total Mn concentrations in the trunk bark of trees in the constructed wetland and the reference site as affected by date

		Wetland				Reference			
Source	DF	SS	MS	F	P	SS	MS	F	P
Red maple trunk bark									
Total	11	24814.9				72342.3			
Date	3	1492.3	497.4	0.171	0.913	2296.9	765.6	0.087	0.965
Error	8	23322.7	2915.3			70045.3	8755.7		
White birch trunk bark									
Total	11	564128.7				655065.0			
Date	3	16384.7	5461.6	0.080	0.969	106395.0	35465.0	0.517	0.682
Error	8	547744.0	68468.0			548670.0	68583.8		
Red spruce trunk bark									
Total	11	49610.3				346126.3			
Date	3	23464.9	7821.6	2.393	0.144	65800.9	21933.6	0.626	0.618
Error	8	26145.3	3268.2			280325.3	35040.7		

Differences are considered significant at a p-value = 0.05 (95 % confidence interval)

A review of the literature revealed one study on the seasonal variability of manganese in the trunk wood of trees. Laureysens *et al*.[48] observed that the average manganese concentration in the wood of poplar clones did not significantly increase or decrease during the growing season.

Trunk bark: The average manganese concentrations in the trunk bark of red maple, white birch and red spruce trees were not significantly affected by the sampling date. Laureysens *et al*.[48] also observed that the average manganese concentration in the bark of poplar clones did not significantly increase or decrease during the growing season.

Roots: The average manganese concentrations in the roots of red maple, white birch and red spruce trees were not significantly affected by the sampling date. However, a review of the literature revealed no specific studies on the seasonal variability of manganese in the roots of trees.

Manganese distribution within trees: Figure 7 displays the percent distribution of manganese in the above and belowground compartments of wetland and reference tree species. Higher concentrations of manganese were present in the trunk bark and either the leaves or twigs of species on both the constructed wetland and the reference site regardless of the sampling date.

Wittwer *et al*.[36] and Zottl[49] observed higher concentrations of manganese in the leaves and trunk bark of Norway spruce trees in Germany and of red pine trees in New York, respectively. Zottl[49] noted that high concentrations of manganese in the trunk bark are typical for conifer trees growing on acid soils. Higher concentrations of manganese in the leaves and trunk bark of mature white birch trees were also observed by Schmitt *et al*.[35] and Young and Guinn[25].

Manganese was present in higher concentrations in the trunk bark of species compared to the trunk wood. In the constructed wetland, the average concentrations of manganese in the trunk bark of trees were 6.4-30.0 fold

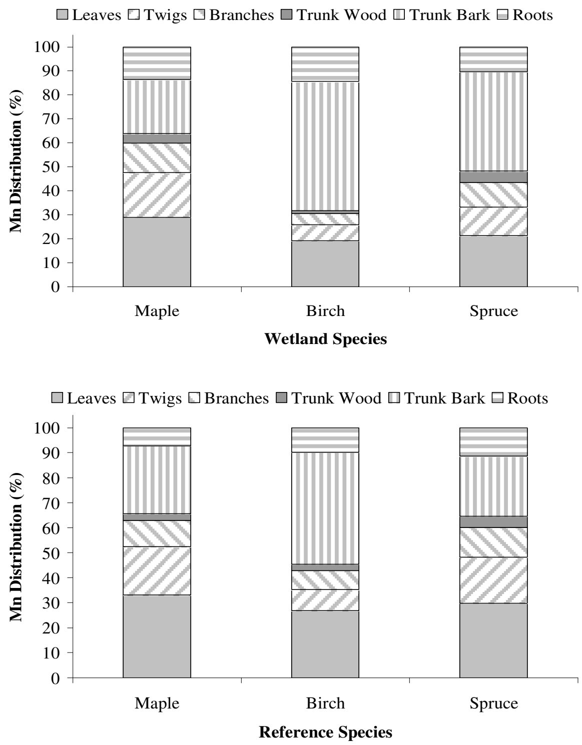

Fig. 7: Mn distribution (%)

higher than the trunk wood concentrations. In the reference site, the average concentrations of manganese in the trunk bark were 5.1-18.1 fold higher than the trunk wood concentrations. Vogt *et al*.[41] observed a 3.8 fold and a 2.6 fold increase in the concentrations of manganese in the trunk bark of fir and hemlock trees, respectively compared to the trunk wood concentrations. Morrison and Hogan[34], Schmitt *et al*.[35], Wittwer *et al*.[36] and Zottl[49] observed a 9.9 fold, 5.6 fold, 2.6 fold and a 6.6 fold increase in the concentrations of manganese in the trunk bark compared to the trunk wood concentrations of sugar maple, Norway spruce, white birch and red pine trees, respectively. Young and Guinn[25] observed a 4.3 fold, 8.1 fold and a 9.3 fold increase in the concentrations of manganese in the trunk bark of red spruce, white birch and red maple trees, respectively compared to the trunk wood concentrations. According to Heinrichs and Mayer[40], high concentrations of manganese in the surface compartments of biomass, such as the leaves and bark, compared to the wood are caused by either selective uptake and storage of manganese in these compartments or deposition and fixation of atmospheric substances.

CONCLUSIONS

The average manganese concentrations in the aboveground compartments of red maple, white birch and red spruce trees were within the range of manganese concentrations reported in the literature for these trees. The concentrations of manganese in the aboveground compartments of red maple, white birch and red spruce trees in the reference site were significantly greater than those in the constructed wetland (with the exception of manganese concentrations in the trunk wood of red maple trees) because of the acidic soil conditions of the reference site. The percent distribution of manganese in the aboveground compartments of trees did not vary during the growing season. Higher concentrations of manganese were present in the trunk bark and either the leaves or twigs of species on both the constructed wetland and the reference site regardless of the sampling date. Manganese was present in higher concentrations in the trunk bark of species compared to the trunk wood. In the constructed wetland, the average concentrations of manganese in the trunk bark of trees were 6.4-30.0 fold higher than the trunk wood concentrations while in the reference site, the average concentrations of manganese in the trunk bark were 5.1-18.1 fold higher than the trunk wood concentrations.

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