# Uptake and Translocation of Iron by Native Tree Species In A Constructed Wetland Treating Landfill Leachates

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**Abstract:** A surface flow wetland was constructed 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. The objectives of this study were: (a) to compare the uptake of iron by red maple, white birch and red spruce trees growing under flooded soil conditions in the constructed wetland and well drained soil conditions in a nearby reference site, (b) to evaluate the seasonal variability of iron in these trees and (c) to determine the distribution of iron in different compartments of these trees (leaves, twigs, branches, trunk wood, trunk bark and roots). The average iron concentrations in the aboveground compartments of red maple, white birch and red spruce trees were within the range of iron concentrations reported in the literature for these trees. Red maple, white birch and red spruce trees in the constructed wetland had significantly greater iron concentrations in their roots than the same species in the reference site. The average iron concentrations in the leaves of red maple trees in the constructed wetland and the reference site displayed an increasing trend towards the end of the growing season while the average iron concentrations in the twigs of red maple and white birch trees in the constructed wetland and the reference site displayed maximum concentrations at the beginning of the growing season. Red maple, white birch and red spruce trees in the constructed wetland retained a major portion of their overall iron concentration in their root systems.

**Key words:** Red maple, white birch, red spruce, iron, seasonal variability and constructed wetland

## INTRODUCTION

Heavy metals are elements that have the ability to be easily molded or shaped, conduct heat and electricity and form stable cations. Air, water and soil environments may become polluted with high concentrations of heavy metals from both natural sources such as volcanoes, forest fires and mineral outcrops, and anthropogenic sources such as industrial activities, agricultural practices, atmospheric deposition and waste disposal operations[1-3]. Contamination of the environment by heavy metals can have significant impacts on both human health and ecosystems[4-6].

Current remediation methods for heavy metal contaminated environments are often expensive and physically invasive[7]. Soil remediation typically relies on physical and chemical technologies. Physical approaches isolate contaminants from the surrounding environment using capping systems and in situ vertical barriers. However, these technologies do not remove the contaminants from the soil matrix. Chemical soil remediation relies on extraction techniques such as soil washing with strong acids or solidification/stabilization with binder agents such as cement, which destroy all biological activity within the soil[8]. Wastewater remediation relies on the addition of chemical agents to the water and physical settling of metal contaminated sludge, which poses the problem of sludge disposal. Therefore, within the last fifteen years, efforts have been directed towards finding biological strategies for remediation of metal contaminated environments that are less expensive and less invasive. One such method is phytoremediation[7].

Phytoremediation is a passive technology that takes advantage of the natural processes of aquatic and terrestrial plants and microbial rhizosphere populations (bacteria and fungi) to stabilize, sequester, accumulate and/or metabolize organic and inorganic contaminants

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in polluted soils, sludges, sediments, groundwater, surface water and wastewater[9]. Plants extract metals from their soil and water environments because many metals including magnesium, calcium, potassium, iron, manganese, copper, zinc, and molybdenum are essential nutrients. Plants also extract metals from their soil and water environments that have not been recognized as essential nutrients including chromium, lead, cadmium, mercury and nickel[10,11]. Metal phytoremediation techniques that have been extensively studied include the use of hyperaccumulator plants[12-14], high biomass crop plants with chelate addition to enhance metal extraction[15,16], aquatic plants[17,18] and fast growing trees[19,20].

The use of trees to remediate heavy metal contaminated environments has several advantages: (a) trees are long lived plants with high biomass production and, therefore, have the potential to extract large concentrations of heavy metals from contaminated land and store them in woody tissues where they are less bioavailable[21], (b) trees form dense vegetative covers and have large and deep root systems, which stabilize soils and reduce mobility of contaminants[22] and (c) trees are an economic resource that can be used for the manufacture of timber products or for the production of heat, electricity and fuel[23].

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

## 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). A previously conducted environmental site assessment (Table 1) had determined that the water entering the brook contained average iron and manganese concentrations (15.508 and 3.029 mg L$^{-1}$ respectively) which exceed the allowable limits

Table 1: Heavy metal loads entering Wright's Brook

| Element | Average Concentration (mg L$^{-1}$) | Guidelines[24] (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[25] |
| Zinc | 0.158 | 0.030 |

established by the Canadian Water Quality Guidelines for the Protection of Aquatic Life[24,25]. 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).

**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
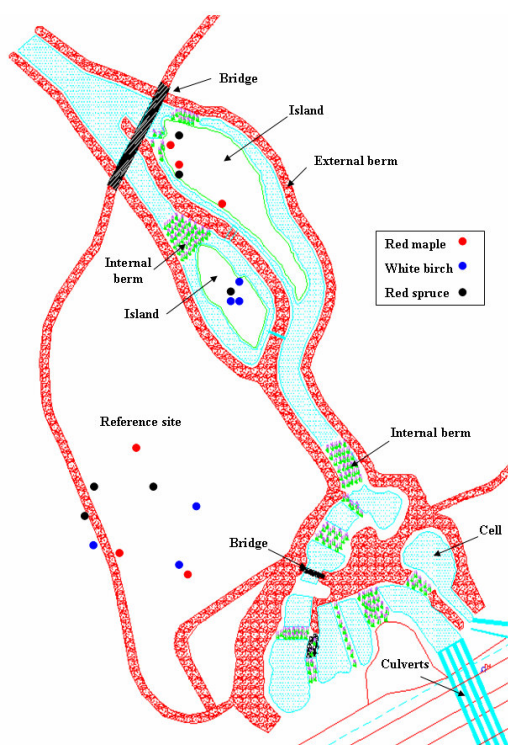
Fig. 1: Location of sampled trees

MacDougall *et al.*[26], 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[27]. 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 were whole, well formed and current year's 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 hours and stored in a refrigerator at 4°C.

**Sample preparation:** Preparation of plant samples involved decontamination, oven drying and particle size reduction.

**Decontamination:** According to Mills and Jones Jr.[10], when plant materials are covered with soil, dust particles or spray materials, decontamination is required. Horwitz[28] 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.

**Oven drying:** 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[29]. 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 the coffee grinder.

**Sample analyses:** 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 iron concentrations.

**Total iron:** 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 minutes. 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.

Iron concentrations were determined by inductively coupled plasma-optical emission spectroscopy (Vista Pro, Varian Inc., Victoria, Australia) with a detection limit of 1.00 ppm for Fe.

## RESULTS AND DISCUSSION

**Uptake of iron:** Iron is an essential micronutrient that is required for plant growth. According to Decoteau[30], iron is involved in the synthesis of chlorophyll, chloroplast proteins and various enzymes. Symptoms of inadequate iron nutrition include chlorosis of young leaves and stunted root growth. Uptake of excess quantities of iron by plants can be potentially toxic because iron promotes the formation of reactive oxygen based radicals that are able to damage cellular membranes. The roots of plants exposed to excess concentrations of iron undergo blackening[31]. The iron 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 iron concentrations in the compartments of red maple, white birch and red spruce trees, respectively as affected by location.

**Leaves:** The average iron concentrations in the leaves of red maple, white birch and red spruce trees in the constructed wetland ranged from 124 to 321 mg kg$^{-1}$, from 117 to 172 mg kg$^{-1}$and from 105 to 166 mg kg$^{-1}$, respectively. The average iron concentrations in the leaves of red maple, white birch and red spruce trees in the reference site ranged from 102 to 147 mg kg$^{-1}$, from 125 to 193 mg kg$^{-1}$ and from 46 to 200 mg kg$^{-1}$, respectively.

Piczak[33], Kozlov *et al.*[34] and Heinrichs and Mayer[35] observed average iron concentrations of 128-300 mg kg$^{-1}$, 154-350 mg kg$^{-1}$and 210 mg kg$^{-1}$ in the leaves of Norway maple, mountain birch and Norway spruce trees, respectively. Reeves and Baker[36] stated that the low, normal and high concentrations of iron in dried plant leaves are 10 mg kg$^{-1}$, 60-600 mg kg$^{-1}$and 2500 mg kg$^{-1}$, respectively. The average iron concentrations in the leaves of the sampled trees fell within the normal range for iron in dried plant leaves.

The statistical analysis showed that only the concentrations of iron in the leaves of red maple trees in the constructed wetland were significantly greater than those in the leaves of red maple trees in the reference site (p-value = 0.004). Moorhead and McArthur[32] observed greater iron concentrations in the leaves of red maple trees growing in saturated soils as opposed to well drained soils and concluded that the higher concentration of iron in the leaves of red maple trees growing in saturated soils was due to the greater availability of reduced iron in saturated soil conditions.

**Twigs:** The average iron concentrations in the twigs of red maple, white birch and red spruce trees in the constructed wetland ranged from 73 to 348 mg kg$^{-1}$, from 53 to 282 mg kg$^{-1}$and from 271 to 360 mg kg$^{-1}$, respectively. The average iron concentrations in the twigs of red maple, white birch and red spruce trees in the reference site ranged from 53 to 355 mg kg$^{-1}$, from 71 to 304 mg kg$^{-1}$and from 334 to 437 mg kg$^{-1}$, respectively.

Table 2: Average Fe concentrations (mg kg$^{-1}$) in various compartments of red maple trees

| Compartment | n | Wetland | | | | | Reference | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2005 | 2006 | | | | 2005 | 2006 | | | |
| | | Sept | Jun | Jul | Aug | Sept | Sept | Jun | Jul | Aug | Sept |
| Leaves | 3 | 168 | 157 | 124 | 321 | 247 | 116 | 130 | 102 | 147 | 144 |
| | | (36) | (8) | (33) | (117) | (59) | (9) | (23) | (24) | (11) | (18) |
| Twigs | 3 | 348 | 242 | 91 | 73 | - | 355 | 164 | 61 | 53 | - |
| | | (154) | (87) | (30) | (29) | - | (275) | (29) | (17) | (8) | - |
| Branches | 3 | 73 | 139 | 31 | 63 | - | 49 | 115 | 29 | 37 | - |
| | | (29) | (158) | (8) | (19) | - | (10) | (59) | (10) | (20) | - |
| Trunk Wood | 3 | 205 | 70 | 70 | 26 | - | 362 | 80 | 30 | 14 | - |
| | | (28) | (33) | (85) | (2) | - | (130) | (25) | (11) | (3) | - |
| Trunk Bark | 3 | 221 | 91 | 126 | 168 | - | 147 | 87 | 253 | 111 | - |
| | | (111) | (65) | (22) | (33) | - | (53) | (24) | (90) | (49) | - |
| Roots | 3 | 311 | - | - | 2091 | - | 218 | - | - | 54 | - |
| | | (161) | - | - | (763) | - | (187) | - | - | (35) | - |

- Samples were not collected () Standard deviation

Table 3: Average Fe concentrations (mg kg$^{-1}$) in various compartments of white birch trees

| Compartment | n | Wetland | | | | | Reference | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2005 | 2006 | | | | 2005 | 2006 | | | |
| | | Sept | Jun | Jul | Aug | Sept | Sept | Jun | Jul | Aug | Sept |
| Leaves | 3 | 169 | 117 | 130 | 154 | 172 | 193 | 130 | 125 | 172 | 120 |
| | | (64) | (15) | (33) | (33) | (7) | (3) | (41) | (51) | (18) | (45) |
| Twigs | 3 | 282 | 176 | 83 | 53 | - | 304 | 84 | 71 | 77 | - |
| | | (121) | (35) | (12) | (8) | - | (37) | (17) | (17) | (30) | - |
| Branches | 3 | 41 | 115 | 70 | 40 | - | 49 | 77 | 79 | 45 | - |
| | | (17) | (99) | (49) | (13) | - | (12) | (56) | (46) | (18) | - |
| Trunk Wood | 3 | 257 | 120 | 68 | 68 | - | 197 | 119 | 43 | 22 | - |
| | | (217) | (76) | (83) | (46) | - | (157) | (57) | (33) | (9) | - |
| Trunk Bark | 3 | 99 | 188 | 271 | 293 | - | 39 | 244 | 159 | 117 | - |
| | | (86) | (38) | (129) | (224) | - | (10) | (140) | (34) | (59) | - |
| Roots | 3 | 336 | - | - | 845 | - | 89 | - | - | 122 | - |
| | | (190) | - | - | (494) | - | (34) | - | - | (75) | - |

 - Samples were not collected () Standard deviation

Table 4: Average Fe concentrations (mg kg$^{-1}$) in various compartments of red spruce trees

| Compartment | n | Wetland | | | | Reference | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2005 | 2006 | | | 2005 | 2006 | | |
| | | Sept | Jun | Jul | Aug | Sept | Jun | Jul | Aug |
| Leaves | 3 | 166 | 132 | 105 | 158 | 99 | 200 | 46 | 76 |
| | | (66) | (30) | (51) | (86) | (26) | (168) | (8) | (12) |
| Twigs | 3 | 283 | 271 | 322 | 360 | 437 | 334 | 390 | 383 |
| | | (59) | (117) | (119) | (109) | (91) | (49) | (142) | (44) |
| Branches | 3 | 196 | 183 | 77 | 148 | 209 | 234 | 173 | 181 |
| | | (95) | (56) | (35) | (66) | (31) | (44) | (114) | (31) |
| Trunk Wood | 3 | 142 | 26 | 46 | 50 | 196 | 70 | 29 | 12 |
| | | (10) | (9) | (42) | (16) | (46) | (34) | (4) | (1) |
| Trunk Bark | 3 | 424 | 98 | 316 | 171 | 145 | 168 | 229 | 121 |
| | | (50) | (65) | (163) | (58) | (37) | (58) | (142) | (27) |
| Roots | 3 | 1063 | - | - | 851 | 139 | - | - | 81 |
| | | (984) | - | - | (452) | (87) | - | - | (26) |

- Samples were not collected () Standard deviation

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

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Red maple leaves | | | | | |
| Total | 29 | 166835 | | | |
|    Location | 1 | 43168 | 43168 | 9.77 | 0.004 |
|    Error | 28 | 123667 | 4417 | | |
| Red maple twigs | | | | | |
| Total | 23 | 556199 | | | |
|    Location | 1 | 5400 | 5400 | 0.22 | 0.647 |
|    Error | 22 | 550799 | 25036 | | |
| Red maple branches | | | | | |
| Total | 23 | 95438 | | | |
|    Location | 1 | 2128 | 2128 | 0.50 | 0.486 |
|    Error | 22 | 93310 | 4241 | | |
| Red maple trunk wood | | | | | |
| Total | 23 | 350673 | | | |
|    Location | 1 | 4817 | 4817 | 0.31 | 0.585 |
|    Error | 22 | 345857 | 15721 | | |
| Red maple trunk bark | | | | | |
| Total | 23 | 140930 | | | |
|    Location | 1 | 14 | 14 | 0.00 | 0.964 |
|    Error | 22 | 140916 | 6405 | | |
| Red maple roots | | | | | |
| Total | 11 | 9487207 | | | |
|    Location | 1 | 3404805 | 3404805 | 5.60 | 0.040 |
|    Error | 10 | 6082402 | 608240 | | |

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

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

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| White birch leaves | | | | | |
| Total | 29 | 46157 | | | |
|    Location | 1 | 1 | 1 | 0.00 | 0.982 |
|    Error | 28 | 46156 | 1648 | | |
| White birch twigs | | | | | |
| Total | 23 | 251329 | | | |
|    Location | 1 | 1233 | 1233 | 0.11 | 0.745 |
|    Error | 22 | 250097 | 11368 | | |
| White birch branches | | | | | |
| Total | 23 | 50948 | | | |
|    Location | 1 | 117 | 117 | 0.05 | 0.824 |
|    Error | 22 | 50831 | 2310 | | |
| White birch trunk wood | | | | | |
| Total | 23 | 317090 | | | |
|    Location | 1 | 6633 | 6633 | 0.47 | 0.500 |
|    Error | 22 | 310457 | 14112 | | |
| White birch trunk bark | | | | | |
| Total | 23 | 367718 | | | |
|    Location | 1 | 31901 | 31901 | 2.09 | 0.162 |
|    Error | 22 | 335817 | 15264 | | |
| White birch roots | | | | | |
| Total | 11 | 1669144 | | | |
|    Location | 1 | 704705 | 704705 | 7.31 | 0.022 |
|    Error | 10 | 964439 | 96444 | | |

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

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

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Red spruce needles | | | | | |
| Total | 23 | 143191 | | | |
|    Location | 1 | 7526 | 7526 | 1.22 | 0.281 |
|    Error | 22 | 135665 | 6167 | | |
| Red spruce twigs | | | | | |
| Total | 23 | 217948 | | | |
|    Location | 1 | 35574 | 35574 | 4.29 | 0.050 |
|    Error | 22 | 182374 | 8290 | | |
| Red spruce branches | | | | | |
| Total | 23 | 116230 | | | |
|    Location | 1 | 13920 | 13920 | 2.99 | 0.098 |
|    Error | 22 | 102310 | 4650 | | |
| Red spruce trunk wood | | | | | |
| Total | 23 | 97926 | | | |
|    Location | 1 | 693 | 693 | 0.16 | 0.696 |
|    Error | 22 | 97233 | 4420 | | |
| Red spruce trunk bark | | | | | |
| Total | 23 | 381283 | | | |
|    Location | 1 | 44462 | 44462 | 2.90 | 0.102 |
|    Error | 22 | 336821 | 15310 | | |
| Red spruce roots | | | | | |
| Total | 11 | 4585089 | | | |
|    Location | 1 | 2153921 | 2153921 | 8.86 | 0.014 |
|    Error | 10 | 2431167 | 243117 | | |

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

The average iron concentrations in the twigs of the sampled trees compare with reported values in the literature. Pastor and Bockheim[37] observed average iron concentrations of 93 and 52 mg kg$^{-1}$ in the twigs of sugar maple and trembling aspen trees. McColl[38] observed average iron concentrations of 45 mg kg$^{-1}$ in the twigs of blue gum eucalyptus trees. Brotherson and Osayande[39] observed average iron concentrations of 166.4 and 146.2 mg kg$^{-1}$ in the twigs of mountain mahogany and Utah juniper trees. Young and Guinn[40] observed iron concentrations of 58, 27 and 254 mg kg$^{-1}$ in the twigs of red maple, birch and red spruce trees respectively.

The statistical analysis showed that only the concentrations of iron in the twigs of red spruce trees in the constructed wetland were significantly greater than those in the twigs of red spruce trees in the reference site (p-value = 0.050). A review of the literature revealed one study by Sailerova and Fedikow[41] who reported that the average iron concentration in the twigs of black spruce trees was not influenced by site drainage characteristics.

**Branches:** The average iron concentrations in the branches of red maple, white birch and red spruce trees in the constructed wetland ranged from 31 to 139 mg kg$^{-1}$, from 40 to 115 mg kg$^{-1}$ and from 77 to 196 mg kg$^{-1}$, respectively. The average iron concentrations in the branches of red maple, white birch and red spruce trees in the reference site ranged from 29 to 115 mg kg$^{-1}$, from 45 to 77 mg kg$^{-1}$ and from 173 to 234 mg kg$^{-1}$, respectively. There was no significant difference between the concentrations of iron in the branches of trees in the constructed wetland and the reference site.

The average concentrations of iron in the branches of the sampled trees were within the range of values reported in the literature. Morrison and Hogan[42], Pastor and Bockheim[37] and Wittwer et al.[43] observed average iron concentrations of 46-49 mg kg$^{-1}$, 32 mg kg$^{-1}$ and 92 mg kg$^{-1}$ in the branches of sugar maple, sugar maple and red pine trees, respectively. Young and Guinn[40] observed iron concentrations of 78, 50 and 66 mg kg$^{-1}$ in the branches of red maple, white birch and red spruce trees, respectively.

**Trunk wood:** The average concentrations of iron in the trunk wood of red maple, white birch and red spruce trees in the constructed wetland ranged from 26 to 205 mg kg$^{-1}$, from 68 to 257 mg kg$^{-1}$ and from 26 to 142 mg kg$^{-1}$, respectively. The average concentrations of iron in the trunk wood of red maple, white birch and red spruce trees in the reference site ranged from 14 to 362 mg kg$^{-1}$, from 22 to 97 mg kg$^{-1}$ and from 12 to 196 mg kg$^{-1}$, respectively. There was no significant difference between the concentrations of iron in the trunk wood of trees in the constructed wetland and the reference site.

Miller[44] stated that elements such as iron are typically present in dry wood at concentrations of <100 mg kg$^{-1}$. Esch *et al.*[45] observed average iron concentrations of 8.8, 12 and 64 mg kg$^{-1}$ in the trunk wood of birch, oak and beech trees. Morrison and Hogan[42], Pastor and Bockheim[37] and Schmitt *et al.*[46] observed average iron concentrations of 19 mg kg$^{-1}$, 9.3 mg kg$^{-1}$ and 12 mg kg$^{-1}$ in the trunk wood of sugar maple, sugar maple and white birch trees, respectively. However, iron concentrations greater than 100 mg kg$^{-1}$ have also been reported in the literature. Heinrichs and Mayer[35] observed average iron concentrations of 270 and 180 mg kg$^{-1}$ in the trunk wood of European beech and Norway spruce trees.
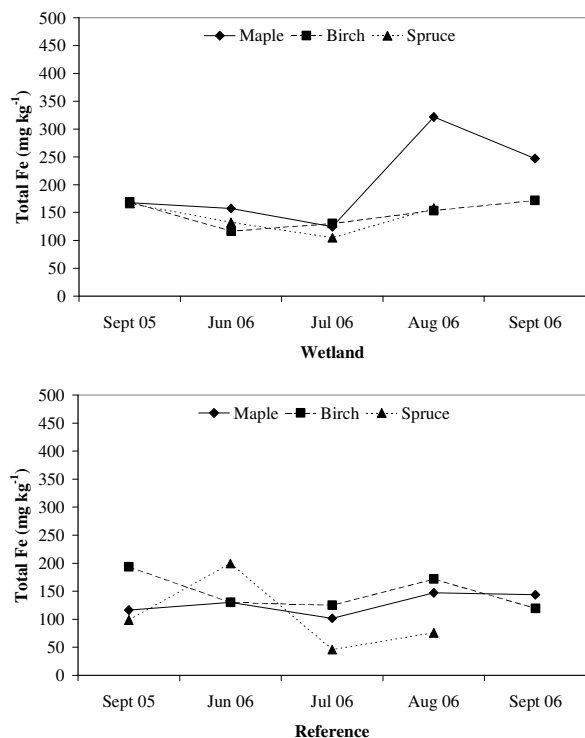


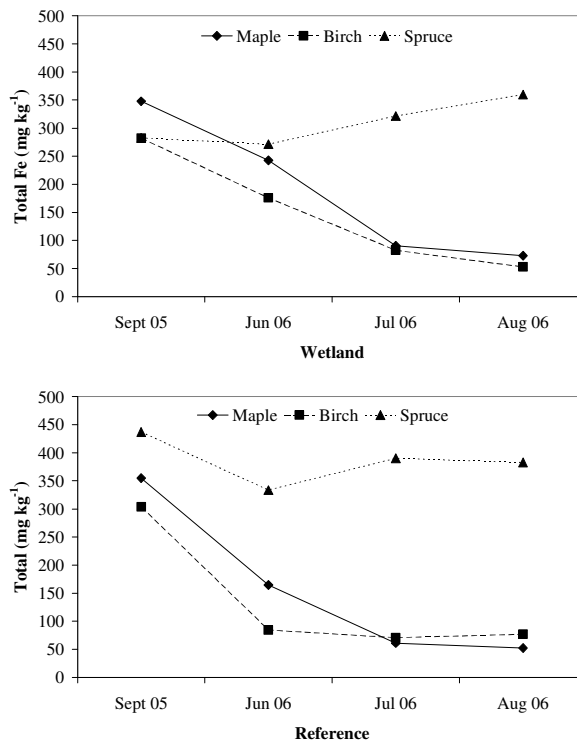Fig. 3: Seasonal variations of Fe in twigs



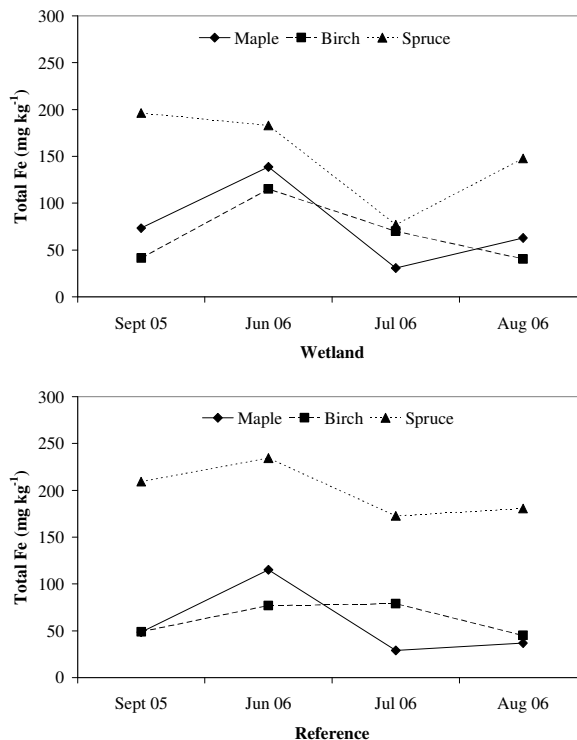Fig. 2: Seasonal variations of Fe in leaves



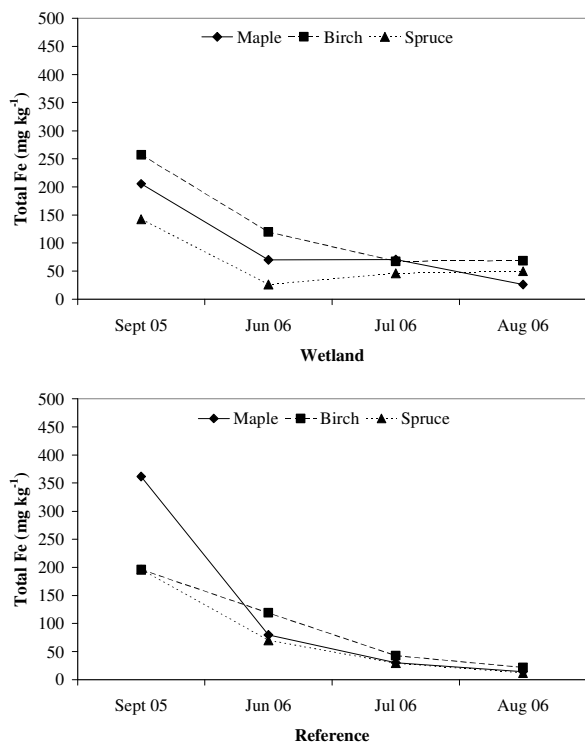Fig. 4: Seasonal variations of Fe in branches

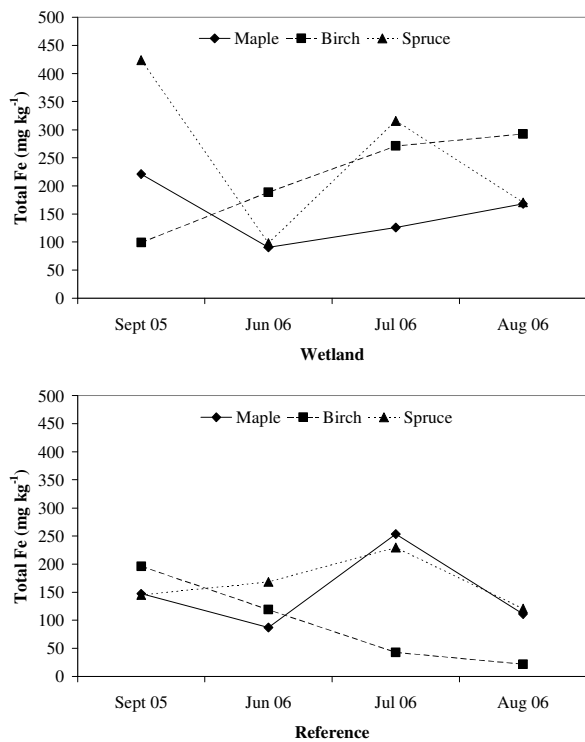Fig. 5:    Seasonal variations of Fe in trunk wood



Fig. 6: Seasonal variations of Fe in trunk bark

**Roots:** The average concentrations of iron in the roots of red maple, white birch and red spruce trees in the constructed wetland during the second sampling period were 2091 mg kg$^{-1}$, 845 mg kg$^{-1}$ and 851 mg kg$^{-1}$, respectively. The average concentrations of iron in the roots of red maple, white birch and red spruce trees in the reference site during the second sampling period were 54 mg kg$^{-1}$, 122 mg kg$^{-1}$ and 81 mg kg$^{-1}$, respectively.

Vogt et al.[48] observed average iron concentrations of 150 and 110 mg kg$^{-1}$ in the roots of hemlock and fir trees. Pastor and Bockheim[37] observed average iron concentrations of 170 and 120 mg kg$^{-1}$ in the roots of sugar maple and quaking aspen trees. Rodriguez-Barrueco[49] observed average iron concentrations of 1685-5110 mg kg$^{-1}$ in the roots of European alder trees. Young and Guinn[40] observed average iron concentrations of 120, 308 and 293 mg kg$^{-1}$ in the roots of red spruce, white birch and red maple trees, respectively.

The statistical analysis showed that the concentrations of iron in the roots of red maple, white birch and red spruce trees in the constructed wetland were significantly greater than the concentrations of iron in roots of species in the reference site (p-values = 0.040, 0.022 and 0.014). Iron becomes more soluble under anaerobic soil conditions and its concentration in the roots of plants has been reported to increase with flooding[50,51]. Shrive and McBride[52] observed a reddish brown plaque on the root system of red maple seedlings after flooding with municipal solid waste landfill leachate. Levan and Riha[53] reported that black spruce trees grown under flooded and non-flooded conditions had iron concentrations of 2240 and 193 mg kg$^{-1}$ in their roots, respectively.

**Seasonal variations in total iron:** The seasonal variations in the average total iron concentrations in the various compartments of the different tree species are shown in Fig. 2-6. When plant samples are collected over a prolonged period of time, seasonal changes in the uptake of metals such as iron may be evident[41]. These changes could be attributed to: (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[54]. Tables 8-13 display the analysis of variance for the total iron concentrations in the leaves,

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

| Source | DF | Wetland | | | | Reference | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | F | P | SS | MS | F | P |
| Red maple leaves | | | | | | | | | |
| Total | 14 | 116133.6 | | | | 7532.9 | | | |
| Date | 4 | 76614.9 | 19153.7 | 4.847 | 0.020 | 4318.9 | 1079.7 | 3.359 | 0.055 |
| Error | 10 | 39518.7 | 3951.8 | | | 3214.0 | 321.4 | | |
| White birch leaves | | | | | | | | | |
| Total | 14 | 19904.4 | | | | 26251.7 | | | |
| Date | 4 | 6939.1 | 1734.8 | 1.338 | 0.322 | 12849.1 | 3212.3 | 2.397 | 0.120 |
| Error | 10 | 12965.3 | 1296.5 | | | 13402.7 | 1340.3 | | |
| Red spruce needles | | | | | | | | | |
| Total | 11 | 37164.9 | | | | 98500.0 | | | |
| Date | 3 | 6901.6 | 2300.5 | 0.608 | 0.628 | 40090.0 | 13363.3 | 1.830 | 0.220 |
| Error | 8 | 30263.3 | 3782.9 | | | 58410.0 | 7301.3 | | |

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

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

| Source | DF | Wetland | | | | Reference | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | F | P | SS | MS | F | P |
| Red maple twigs | | | | | | | | | |
| Total | 11 | 219712.7 | | | | 331086.7 | | | |
| Date | 3 | 153662.0 | 51220.7 | 6.204 | 0.018 | 177864.7 | 59288.2 | 3.096 | 0.089 |
| Error | 8 | 66050.7 | 8256. | | | 153222.0 | 19152.8 | | |
| White birch twigs | | | | | | | | | |
| Total | 11 | 128944.7 | | | | 121152.0 | | | |
| Date | 3 | 96502.0 | 32167.3 | 7.932 | 0.009 | 115414.7 | 38471.6 | 53.644 | 0.000 |
| Error | 8 | 32442.7 | 4055.3 | | | 5737.3 | 717.2 | | |
| Red spruce twigs | | | | | | | | | |
| Total | 11 | 100592.9 | | | | 81780.9 | | | |
| Date | 3 | 14620.9 | 4873.6 | 0.454 | 0.722 | 16108.9 | 5369.6 | 0.654 | 0.603 |
| Error | 8 | 85972.0 | 10746.5 | | | 65672.0 | 8209.0 | | |

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

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

| Source | DF | Wetland | | | | Reference | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | F | P | SS | MS | F | P |
| Red maple branches | | | | | | | | | |
| Total | 11 | 71152.9 | | | | 22156.9 | | | |
| Date | 3 | 18624.9 | 6208.3 | 0.946 | 0.463 | 13908.9 | 4636.3 | 4.497 | 0.040 |
| Error | 8 | 52528.0 | 6566.0 | | | 8248.0 | 1031.0 | | |
| White birch branches | | | | | | | | | |
| Total | 11 | 36545.7 | | | | 14284.9 | | | |
| Date | 3 | 11091.7 | 3697.2 | 1.162 | 0.382 | 2948.3 | 982.8 | 0.694 | 0.581 |
| Error | 8 | 25454.0 | 3181.8 | | | 11336.7 | 1417.1 | | |
| Red spruce branches | | | | | | | | | |
| Total | 11 | 61106.0 | | | | 41203.7 | | | |
| Date | 3 | 25698.7 | 8566.2 | 1.935 | 0.202 | 7191.0 | 2397.0 | 0.564 | 0.654 |
| Error | 8 | 35407.3 | 4425.9 | | | 34012.7 | 4251.6 | | |

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

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

| Source | DF | Wetland | | | | Reference | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | F | P | SS | MS | F | P |
| Red maple trunk wood | | | | | | | | | |
| Total | 11 | 72604.0 | | | | 273252.7 | | | |
| Date | 3 | 54406.7 | 18135.5 | 7.973 | 0.009 | 238075.3 | 79358.4 | 18.048 | 0.001 |
| Error | 8 | 18197.3 | 2274.7 | | | 35177.3 | 4397.2 | | |
| White birch trunk wood | | | | | | | | | |
| Total | 11 | 195750.9 | | | | 114705.7 | | | |
| Date | 3 | 71748.9 | 23916.3 | 1.543 | 0.277 | 56680.3 | 18893.4 | 2.605 | 0.124 |
| Error | 8 | 124002.0 | 15500.3 | | | 58025.3 | 7253.2 | | |
| Red spruce trunk wood | | | | | | | | | |
| Total | 11 | 28526.9 | | | | 68705.7 | | | |
| Date | 3 | 24248.3 | 8082.8 | 15.113 | 0.001 | 62121.0 | 20707.0 | 25.158 | 0.000 |
| Error | 8 | 4278.7 | 534.8 | | | 6584.7 | 823.1 | | |

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

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

| Source | DF | Wetland | | | | Reference | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | F | P | SS | MS | F | P |
| Red maple trunk bark | | | | | | | | | |
| Total | 11 | 64778.7 | | | | 76137.7 | | | |
| Date | 3 | 28566.7 | 9522.2 | 2.104 | 0.178 | 48321.0 | 16107.0 | 4.632 | 0.037 |
| Error | 8 | 36212.0 | 4526.5 | | | 27816.7 | 3477.1 | | |
| White birch trunk bark | | | | | | | | | |
| Total | 11 | 221056.7 | | | | 114760.3 | | | |
| Date | 3 | 69785.3 | 23261.3 | 1.230 | 0.360 | 65960.9 | 21986.9 | 3.604 | 0.065 |
| Error | 8 | 151271.3 | 18908.9 | | | 48799.3 | 6099.9 | | |
| Red spruce trunk bark | | | | | | | | | |
| Total | 11 | 265816.0 | | | | 71004.9 | | | |
| Date | 3 | 192504.7 | 64168.2 | 7.002 | 0.013 | 19664.9 | 6554.9 | 1.021 | 0.433 |
| Error | 8 | 4278.7 | 9163.9 | | | 51340.0 | 6417.5 | | |

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

Table 13: One-way analysis of variance for the total Fe concentrations in the roots of trees in the constructed wetland and the reference site as affected by date

| Source | DF | Wetland | | | | Reference | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | F | P | SS | MS | F | P |
| Red maple roots | | | | | | | | | |
| Total | 5 | 5969527 | | | | 112874.8 | | | |
| Date | 1 | 4754380 | 4754380.2 | 15.650 | 0.017 | 40837.5 | 40837.5 | 2.268 | 0.207 |
| Error | 4 | 1215147 | 303786.7 | | | 72037.3 | 18009.3 | | |
| White birch roots | | | | | | | | | |
| Total | 5 | 949257.3 | | | | 15181.3 | | | |
| Date | 1 | 388112.7 | 388112.7 | 2.767 | 0.172 | 1600.7 | 1600.7 | 0.471 | 0.530 |
| Error | 4 | 561144.7 | 140286.2 | | | 13580.7 | 3395.2 | | |
| Red spruce roots | | | | | | | | | |
| Total | 5 | 2409665 | | | | 21502.0 | | | |
| Date | 1 | 67416.0 | 67416.0 | 0.115 | 0.751 | 4930.7 | 4930.7 | 1.190 | 0.337 |
| Error | 4 | 2342249 | 585562.3 | | | 16571.3 | 4142.8 | | |

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

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 iron concentrations in the leaves of red maple trees in the constructed wetland were significantly affected by the sampling date (p-value = 0.020) and displayed an increasing trend towards the end of the growing season with a maximum iron concentration of 321±117 mg kg$^{-1}$ in August 2006. A similar pattern was observed for red maple trees in the reference site at a confidence interval of 90% (p-value = 0.055) where the average concentration of iron in the leaves reached a minimum value of 102±24 mg kg$^{-1}$ in July 2006. The average iron concentrations in the leaves of white birch and red spruce trees in the constructed wetland and the reference site were not affected by the sampling date.

Mertens et al.[54] and Laureysens et al.[55] stated that the accumulation of iron in the leaves of deciduous trees, such as red maple trees in the present study, prior to leaf senescence was an excretion mechanism of excess metals by the trees. The accumulation of iron in the leaves of Norway maple and white birch trees in urban areas of Poland during the growing season was observed by Piczak et al.[33] who reported increased concentrations of iron in the leaves of maple and birch trees from 196 and 160 mg kg$^{-1}$ in June to 300 and 245 mg kg$^{-1}$ in October, respectively. Rodriguez-Barrueco et al.[49] observed an accumulation of iron in the leaves of European alder trees in Spain prior to leaf senescence and reported an increased average concentration of iron in the leaves from 314 mg kg$^{-1}$ in April to 404 mg kg$^{-1}$ in November. Lea et al.[56] noted an accumulation of iron in the leaves of yellow birch trees in New York during the growing season and reported an increased average concentration of iron in the leaves from 67.60±24.13 mg kg$^{-1}$ in June to 106.91±38.53 mg kg$^{-1}$ in October.

**Twigs:** The average iron concentrations in the twigs of red maple trees in the constructed wetland and the reference site were significantly affected by the sampling date (p-values = 0.018 and 0.089) at a 95% and a 90% confidence interval, respectively, with the highest iron concentrations in September 2005 of 348±154 mg kg$^{-1}$ and 355±275 mg kg$^{-1}$ and in June 2006 of 242±87 mg kg$^{-1}$ and 164±29 mg kg$^{-1}$. A similar pattern was observed for white birch trees in the constructed wetland and the reference site where the average iron concentration in the twigs

reached maximum concentrations of 282±121 mg kg$^{-1}$ and 76±35 mg kg$^{-1}$ in September 2005 and June 2006 and 304±37 mg kg$^{-1}$ in September 2005. The average iron concentrations in the twigs of red spruce trees in the constructed wetland and the reference site were not significantly affected by the sampling date.

The maximum concentrations of iron in the twigs in June 2006 could be attributed to a high metal uptake compared to growth rate at the beginning of the growing season. This was followed by a period of active growth, which diluted the iron concentrations[54]. Ferm and Markkola[57] observed a maximum iron concentration during the growing season in the twigs of 1 year old and 15 year old downy birch trees of approximately 150 mg kg$^{-1}$ in the months of June and July.

**Branches:** The average iron concentration in the branches of red maple trees in the constructed wetland was not significantly affected by the sampling date. The average iron concentration in the branches of red maple trees in the reference site was significantly affected by the sampling date (p-value = 0.040) and displayed a maximum value in June 2006 of 115±59 mg kg$^{-1}$. The average concentrations of iron in the branches of 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 iron in tree branches.

**Trunk wood:** The average concentrations of iron in the trunk wood of red maple and red spruce trees in the constructed wetland were significantly affected by the sampling date with maximum concentrations of 205±28 mg kg$^{-1}$ and 142±10 mg kg$^{-1}$ in September 2005. A similar pattern was observed for red maple and red spruce trees in the reference site where the average concentration of iron in the trunk wood reached maximum values of 362±130 and 196±46 mg kg$^{-1}$ in September 2005. The average concentrations of iron in the trunk wood of white birch trees in the constructed wetland and the reference site were not significantly affected by the sampling date.

A review of the literature revealed one study by Laureysens et al.[55] who reported that the average iron concentration in the wood of poplar clones did not significantly increase or decrease during the growing season. In the present study, if the data from September 2005 is removed from the statistical analysis, then the concentrations of iron in the trunk wood of red maple, white birch and red spruce trees were not significantly affected by the sampling date.

**Trunk bark:** The average iron concentrations in the trunk bark of red maple and white birch trees in the constructed wetland were not significantly affected by the sampling date. The average iron concentrations in the trunk bark of red maple and white birch trees in the reference site were significantly affected by the sampling date at a 95 % and a 90 % confidence interval (p-values = 0.037 and 0.065), respectively with maximum concentrations of 147±43 mg kg$^{-1}$ and 253±90 mg kg$^{-1}$ in September 2005 and July 2006 for maple and 244±140 mg kg$^{-1}$ and 159±34 mg kg$^{-1}$ in June 2006 and July 2006 for birch. The average iron concentration in the trunk bark of red spruce trees in the constructed wetland was significantly affected by the sampling date (p-value = 0.013) with maximum values of 424±50 mg kg$^{-1}$ and 316±163 mg kg$^{-1}$ in September 2005 and July 2006. The average iron concentration in the trunk bark of red spruce trees in the reference site was not significantly affected by the sampling date. A review of the literature revealed no specific studies on the seasonal variability of iron in the trunk bark of trees.

**Roots:** The average iron concentrations in the roots of red maple trees in the constructed wetland in August 2006 were significantly greater than in September 2005 (p-value = 0.017) with a maximum concentration of 2091±763 mg kg$^{-1}$. The average iron concentration in the roots of red maple trees in the reference site, white birch and red spruce trees in the constructed wetland and the reference site were not affected by the sampling date.

A review of the literature revealed no specific studies on the seasonal variability of iron in the roots of trees. However, significant differences in the concentration of iron in the roots of red maple in August 2006 could be attributed to exposure of the roots to flooded soil conditions for a longer period of time.

**Iron distribution within trees:** Fig. 7 displays the percent distribution of iron in the above and belowground compartments of wetland and reference tree species in August 2006. It is clear that red maple, white birch and red spruce trees in the constructed wetland retain a major portion of their overall iron concentration in their root systems. Under flooded soil conditions, some species have the ability to transfer oxygen from their aboveground compartments to their roots creating a thin oxidized layer around the surface of their roots[58]. At a pH greater than 3.5 with oxygen present, soluble and bioavailable ferrous iron is oxidized to insoluble ferric iron which cannot be readily taken up by plants and may accumulate on the outside

surface of the roots[59,60]. Consequently, the concentration of iron in the roots is quite high when compared to aboveground plant parts. As a result, the trees that were exposed to flooded soil conditions were able to exclude high concentrations of iron in their aboveground tissues.
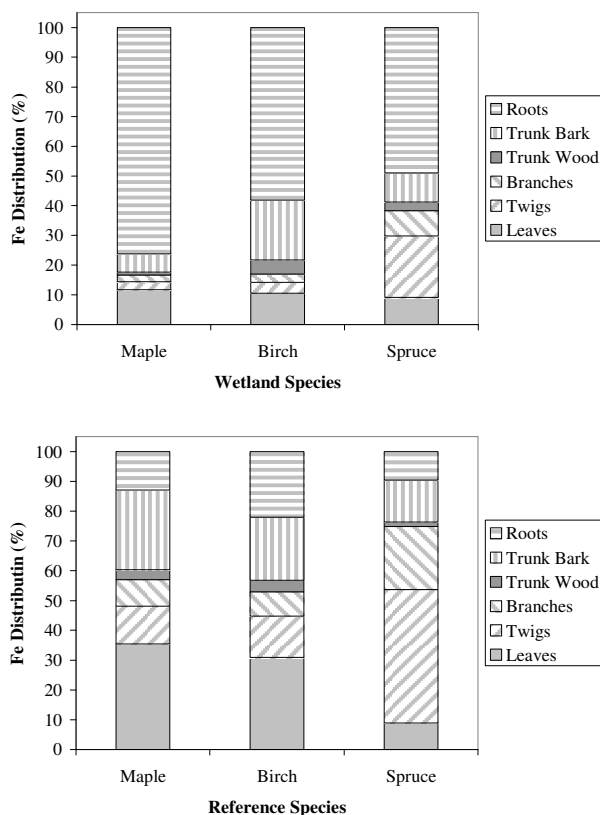


Fig. 7: Fe distribution (%)

In aboveground compartments, iron was present in higher concentrations in the leaves and trunk bark of red maple and white birch trees in the constructed wetland and the reference site in August 2006. Higher concentrations of iron in the leaves and trunk bark of mature white birch trees were also observed by Schmitt *et al.*[46] and Young and Guinn[40]. Morrison and Hogan[42] and Pastor and Bockheim[37] observed higher concentrations of iron in the leaves and trunk bark of sugar maple trees in Ontario and Northern Wisconsin, respectively.

Iron was present in higher concentrations in the trunk bark of species compared to the trunk wood. In the constructed wetland, the average concentrations of iron in the trunk bark of trees were 1.6-3.8 fold higher than the trunk wood concentrations. In the reference site, the average concentrations of iron in the trunk bark

were 1.2-2.2 fold higher than the trunk wood concentrations. Morrison and Hogan[42], Schmitt *et al.*[46] and Wittwer et al.[43] observed a 2.8 fold, 3.3 fold and a 9.2 fold increase in the concentrations of iron in the trunk bark of sugar maple, white birch and red pine trees, respectively compared to the trunk wood concentrations. Young and Guinn[40] observed a 5.9 fold, 5.5 fold and a 5.0 fold increase in the concentrations of iron in the trunk bark of red spruce, white birch and red maple trees, respectively compared to the trunk wood concentrations.

## CONCLUSIONS

The average iron concentrations in the aboveground compartments of red maple, white birch and red spruce trees were within the range of iron concentrations reported in the literature for these trees regardless of the sampling location. The concentrations of iron in the roots of red maple, white birch and red spruce trees in the constructed wetland were significantly greater than those in the roots of the same species in the reference site because of the flooded soil conditions in the constructed wetland. The sampling date had a significant impact on the concentration of iron in the compartments of different tree species. The average iron concentrations in the leaves of red maple trees in the constructed wetland and the reference site displayed an increasing trend towards the end of the growing season, which could be attributed to an excretion mechanism of excess metals by the trees. The average iron concentrations in the twigs of red maple and white birch trees in the constructed wetland and the reference site displayed maximum concentrations at the beginning of the growing season, which could be attributed to a high metal uptake compared to growth rate. Red maple, white birch and red spruce trees in the constructed wetland retained a major portion of their overall iron concentration in their root systems. In aboveground compartments, higher concentrations of iron were present in the leaves and trunk bark of species on both the constructed wetland and the reference site. Iron was present in higher concentrations in the trunk bark of species compared to the trunk wood. In the constructed wetland, the average concentrations of iron in the trunk bark of trees were 1.6-3.8 fold higher than the trunk wood concentrations while in the reference site, the average concentrations of iron in the trunk bark were 1.2-2.2 fold higher than the trunk wood concentrations.

## ACKNOWLEDGEMENTS

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