American Journal of Environmental Sciences 4 (6): 608-619, 2008
ISSN 1553-345X

Iron Uptake and Translocation by Facultative and Obligate Wetland Plants

A.E. Ghaly, A. Snow, M. Kamal and S.H. Monfared
Department of Process Engineering and Applied Science, Dalhousie University
Halifax, Nova Scotia, Canada

Abstract: The effect of initial concentrations of iron in wastewater on its overall removal efficiency and accumulation in wool grass, soft rush, broad leaved cattail and soft stem bulrush plants was investigated under laboratory conditions. The total uptake and translocation of Fe in the roots, stems, leaves and flowers of each plant species were determined and the fractions of Fe removed by plant uptake and precipitation were calculated. The removal of Fe from the wastewater was influenced by the plant type, time and initial Fe concentration. The overall Fe removal efficiencies from the wastewater were 89, 74, 97 and 97%, 73, 71, 83 and 89% and 92, 44, 85 and 65% for soft stem bulrush, wool grass, soft rush and cattail in the compartments receiving tolerance (101.12 mg L^{-1}), wetland influent (7.72 mg L^{-1}) and control (1.12 mg L^{-1}) concentrations, respectively. The results showed that the concentration of Fe in the soils in the control compartments of soft stem bulrush, wool grass, soft rush and cattail decreased indicating the removal of Fe from the soil. The concentration of Fe in the soils in the wetland influent compartments of soft stem bulrush and cattail decreased and in the soils of wool grass and soft rush increased indicating the removal of Fe from the soil by the first two plants and the addition of Fe to the soil by precipitation in the other two compartments. The concentration of Fe in the soils in the tolerance compartments of soft stem bulrush, wool grass, soft rush and cattail increased indicating the precipitation of Fe from the wastewater. On a mass basis, broad-leaved cattail accumulated the greatest amount of Fe followed by soft stem bulrush, soft rush and wool grass. The root tissues accumulated significantly greater concentrations of Fe than the shoots indicating high plant availability of Fe but limited mobility once inside the plant.

Key words: Iron, wool grass, soft rush, soft stem bulrush, cattail, plant uptake

---

INTRODUCTION

Iron contamination exists in liquid waste streams from many industries including: (a) spent pickle and etch baths from plating shops and steel manufacturing[1-3] and (b) acid mine drainage, which is caused by the oxidation and hydrolysis of metal sulphides in water permeable strata or in tailings dumped on the surface[4]. Fe is also an inorganic macrocomponent in landfill leachate with a range of 3-5500 mg L^{-1}[5]. The sources of Fe contamination in landfills include: nails, metal pipes, metal containers, wood waste, concrete and soil[6].

Excess concentrations of Fe in both surface and groundwater threaten human health and the environment. Aquatic insects suffer acute toxicity at Fe concentrations of 0.320 mg L^{-1} and the lethal concentration of Fe to fish ranges from 0.3 to 10 mg L^{-1}[7]. Fe particulates in water bodies increase total suspended solids concentrations, which reduces light penetration and dissolved oxygen concentrations, damages respiratory surfaces and blankets fish spawning sites and macroinvertebrate habitats[8]. Uptake of excess quantities of iron by plants causes: damage to cellular membranes, chlorosis of leaves, reduced yield and blackening of roots[9]. Impacts of excess iron uptake in humans include: vomiting and gastrointestinal bleeding, lung inflammation, convulsions, coma, jaundice and hemosiderosis[7].

Treatment processes for the removal of iron from wastewaters include: precipitation, coagulation, membrane processes (ultrafiltration, reverse osmosis, electrolysis), ion exchange and adsorption. Capital costs and operation and maintenance commitments are high for these methods[1,2,10]. Constructed wetlands are inexpensive systems that have been used to treat many types of wastewaters contaminated with iron. Wetlands improve water quality through a variety of natural mechanisms including: sedimentation, flocculation, filtration, adsorption, chelation, precipitation, ion

---

Corresponding Author: A.E. Ghaly, Professor, Department of Process Engineering and Applied Science, Dalhousie University, Halifax, Nova Scotia, Canada Tel: (902)494-6014 Fax: (902)420-7639

exchange, bacterial mediated reactions and vegetation uptake[11,12].

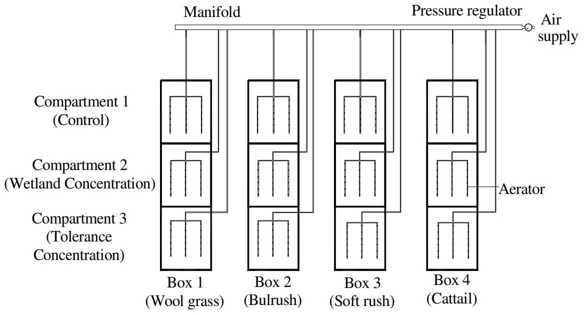
The aim of this study was to assess the performance of selective facultative and obligate wetland plants for the removal of Fe from contaminated wastewater. The specific objectives were: (a) to investigate the effect of initial Fe concentration on the overall Fe removal efficiency by obligate and facultative wetland plants, (b) to determine the total uptake and translocation of Fe in the different parts of each plant species (root, stem, leaves and flower) and (c) to determine the fractions of Fe removed by plant uptake and precipitation.

EXPERIMENTAL APPARATUS

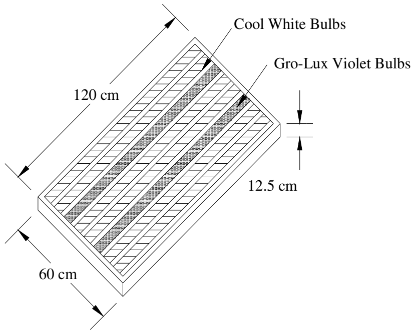
The experimental setup shown in Fig. 1 consists of holding tanks and lighting and aeration systems. Four boxes were constructed from 2.5 cm thick plywood. Each box (60×120×80 cm) was divided into three compartments (30×60×80 cm each) and each compartment contained a holding tank.

The light was provided by an artificial lighting system (625 hectolux/7200 cm^2) and was similar to the natural light required for wetland plants. Each lighting unit consisted of eight light bulbs (six 34 watts cool white fluorescent bulbs and two Gro-lux 40 watts bulbs) of 122 cm in length. The lighting system was placed on the top of each box using wooden supports in such a way that it gave a space of 140 cm clearance between the light bulbs and the water surface in the box. This space was chosen to achieve good air circulation and provide the heat and light that were required for plant growth. The lights were controlled by a timer, which was set to provide 16 hours of light per box per day and to maintain a temperature difference between the soil and the above ground part of 15°C [13].

An aeration unit was installed in the bottom of each compartment to provide oxygen for the plants. The air traveled from the main laboratory supply to a manifold with twelve outlets. Each outlet was connected to a pressure regulator (Model 129121/510, ARO, Bryan, Ohio), which was connected to an aerator located in each compartment. Each aerator consisted of a main tube (26.5 cm long) with three perforated stainless steel laterals (30 cm in length and 0.6 cm in diameter) coming off it at right angles to the main. Tygon tubing of 0.75 cm outside diameter was used to connect the main air supply, manifold and aeration unit. The pressure regulator was adjusted at 0.068 atm during the whole experimental period to give an aeration rate of 7 cm^3 min^{-1}.



(a) Holding Tanks



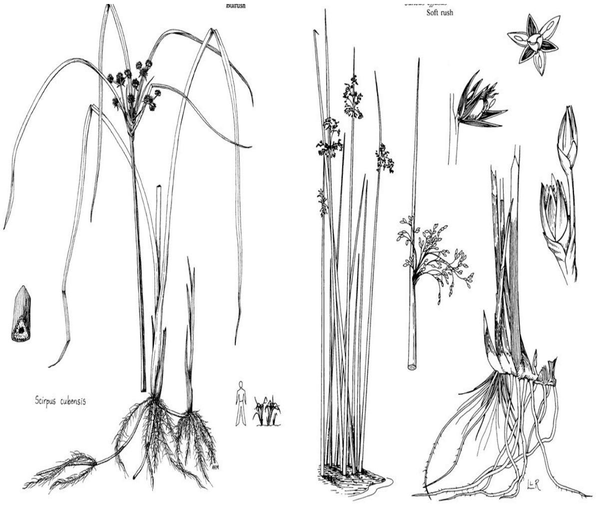
(b) Lighting System

Fig. 1: Experimental apparatus

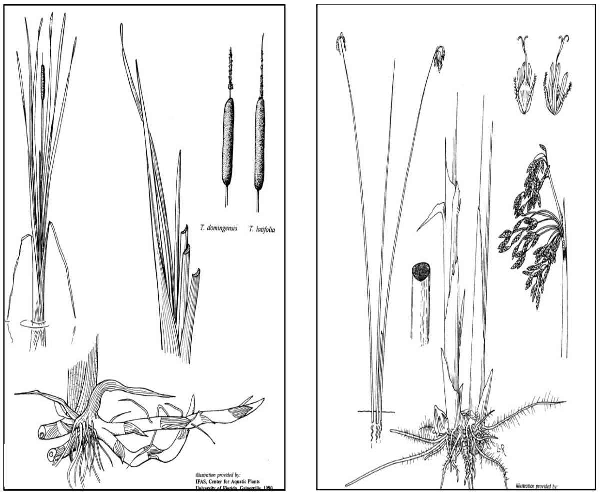
EXPERIMENTAL MATERIALS

Two facultative (wool grass and soft rush) and two obligate (broad-leaved cattail and soft stem bulrush) wetland plant species were used in the study Fig. 2. The selection of these plants was based on their dominance in the constructed wetland[14]. Both soft rush and soft stem bulrush have been listed in many references as both obligate and facultative wetland plants. These wetland plants were obtained from Environmental Concern Inc., St. Michaels, Maryland, USA.

The plants were supplied with nutrients using a fertilizer (20-20-20 Plant-Prod, Plant Products Co. Ltd., Brampton, Ontario) (817 mg of fertilizer per 1 L of water). Ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$) was used as a contaminant supply of Fe. This compound was purchased as a reagent grade chemical from Fisher Scientific, Ottawa, Ontario. Two Fe concentrations were selected to: (a) simulate concentrations in the influent of a constructed wetland[15] and (b) replicate the highest tolerance reported in the literature[16]. A control with tap water was also used. $Fe(NH_4)_2(SO_4)_2.6H_2O$ was dissolved in distilled water to achieve the appropriate contaminant level. The final concentrations of Fe used in this experiment are shown in Table 1.



(a) Wool grass (b) Soft rush



(c) Broad-leaved cattail (d) Soft stem bulrush

Fig. 2: Plant species used in the experiments

Table 1: Concentrations of Fe (mg L^{-1}) in the water

Element	Control	Wetland Influent	Tolerance Concentration
Nutrient			
Potassium	163.40	163.40	163.40
Nitrogen	163.40	163.40	163.40
Phosphorus	163.40	163.40	163.40
EDTA	008.17	008.17	008.17
Boron	000.16	000.16	000.16
Sulfur	-----	008.60	123.21
Heavy Metals			
Iron	001.12	007.72	101.12
Manganese	000.41	000.41	000.41
Copper	000.41	000.41	000.41
Zinc	000.41	000.41	000.41

EXPERIMENTAL PROCEDURE

A 10 cm layer of large gravel (1.25 cm average nominal size) was placed in each compartment to facilitate the collection of drainage water. A 35.5 cm long drainage tube, with holes in the lower 10 cm end, was placed vertically in each compartment. The drainage tube was connected to a wet vacuum pump (Bulldog 700, Shop-Vac Canada Ltd., Burlington, Ontario) to ensure complete drainage of water before introducing the next batch of contaminated water. Soil was used as a supporting media for the plants. It was placed into each compartment in layers (approximately 10 cm thick) and lightly compacted to remove excessive voids within the soil structure. One box (three compartments) was used for each plant species. About 8 plants (20-30 cm tall) were placed in each of the three compartments in each box. The start up procedure for growing wetland plants in a closed system followed that described by Mills[13]. The water level in each compartment was maintained below the root system of the plants while keeping the soil around the root system moist at all times. The plants were sprayed with the insecticide Malathion 500EC (The Solaris Group, Mississauga, Ontario) every week to control the spread of aphids in the system. The dilution rate recommended by the manufacture was followed (2.5 mL of Malathion was mixed in 1 L of water). After the startup period of 4 weeks, the experiment was run for 72 days.

The first compartment in each box was used as a control and received 30 L of tap water containing fertilizer, the second compartment received 30 L of contaminated water containing fertilizer and an Fe concentration similar to that received by a constructed wetland and the third compartment received 30 L of contaminated water containing fertilizer and an Fe concentration similar to that reported in the literature as the highest tolerance level for the four plants. The wastewater was changed every 9 days to simulate the retention time of the water in the constructed wetland[13].

SAMPLING AND ANALYSES

Water, soil and plant samples were collected from all compartments at 9 day intervals. The water samples (100 mL) were withdrawn using a 25 mL wide tip pipette and analyzed for Fe. The soil samples (50 g) were collected from the middle of each compartment and analyzed for Fe. The plant samples (root, stem, leaf and flower) were analyzed for Fe. The plant and soil samples were dried in a convection oven for 24 h at 45°C. After drying, the plant and soil samples were ground and digested with hydrochloric-nitric-hydrofluric-perchloric acids (30+10+10+5 mL g^{-1} sample) in a closed vessel at a temperature of 100°C. The Fe concentration was determined using an atomic absorption spectometer (Varion SpectrAA, Model Number: 55B, Varion, Mulgrave, Victoria, Australia).

Analysis of variance (ANOVA) was performed on the water, soil and plant data using SAS System, 5th Ed. (SAS Institute Inc., Cary, North Carolina). Duncan Multiple range tests were also performed on the data to test the differences among the levels of each factor.

RESULTS AND DISCUSSION

Wastewater Fe content: The Fe concentrations in the effluent waters and the Fe removal efficiencies for bulrush, wool grass, soft rush and cattail are shown in Table 2. The results of the analysis of variance for the Fe removal efficiencies are shown in Table 3 and 4.

The Fe concentration in the effluents from the soft stem bulrush compartments at the end of the 9 day retention period ranged from 1.81 to 10.80 mg L^{-1}, from 1.09 to 2.05 mg L^{-1} and from 0.05 to 0.10 mg L^{-1} for the tolerance, wetland influent and control concentrations, respectively. The Fe concentration in the effluents from the wool grass compartments at the end of the 9 day retention period ranged from 2.70 to 25.80 mg L^{-1}, from 0.86 to 2.20 mg L^{-1} and from 0.35 to 0.63 mg L^{-1} for the tolerance, wetland influent and control concentrations, respectively. The Fe concentration in the effluents from the soft rush compartments at the end of the 9 day retention period ranged from 1.26 to 2.50 mg L^{-1}, from 0.19 to 1.30 mg L^{-1} and from 0.10 to 0.18 mg L^{-1} for the tolerance, wetland influent and control concentrations, respectively. The Fe concentration in the effluents from the cattail compartment at the end of the 9 day retention period ranged from 1.26 to 2.66 mg L^{-1}, from 0.86 to 2.17 mg L^{-1} and from 0.23 to 0.54 mg L^{-1} for the tolerance, wetland influent and control concentrations, respectively.

Table 2: Fe effluent concentrations and removal efficiencies

		Bulrush		Wool grass		Soft rush		Cattail	
Time	Treatment	EC (mg L^{-1})	RE (%)	EC (mg L^{-1})	RE (%)	EC (mg L^{-1})	RE (%)	EC (mg L^{-1})	RE (%)
9	Tolerance	1.81 (0.13)	98.20	4.00 (0.26)	96.04	1.26 (0.09)	98.75	1.26 (0.08)	98.75
	Wetland	1.09 (0.08)	85.87	0.86 (0.06)	88.85	0.19 (0.01)	97.53	0.86 (0.06)	88.85
	Control	0.05 (0.003)	95.52	0.35 (0.025)	68.66	0.10 (0.006)	91.04	0.54 (0.035)	51.65
18	Tolerance	2.90 (0.17)	97.13	5.40 (0.29)	94.65	1.53 (0.09)	98.48	1.59 (0.09)	98.42
	Wetland	1.18 (0.07)	84.70	1.13 (0.07)	85.35	0.40 (0.020)	94.81	1.10 (0.06)	85.74
	Control	0.09 (0.005)	91.94	0.44 (0.026)	60.60	0.10 (0.005)	91.04	0.32 (0.017)	71.35
27	Tolerance	1.55 (0.10)	98.46	2.70 (0.16)	97.32	1.60 (0.10)	98.41	1.77 (0.10)	98.24
	Wetland	1.20 (0.08)	84.44	1.31 (0.08)	83.02	0.50 (0.03)	93.52	1.28 (0.08)	83.41
	Control	0.07 (0.004)	93.73	0.41 (0.027)	62.93	0.12 (0.007)	89.25	0.39 (0.023)	65.08
36	Tolerance	2.50 (0.13)	97.52	7.70 (0.36)	92.38	1.70 (0.09)	98.31	1.95 (0.09)	98.07
	Wetland	1.20 (0.06)	84.44	1.49 (0.08)	80.69	0.70 (0.04)	90.92	1.46 (0.07)	81.08
	Control	0.08 (0.004)	92.83	0.44 (0.022)	60.60	0.23 (0.011)	79.40	0.39 (0.018)	65.08
45	Tolerance	6.60 (0.39)	93.47	12.70 (0.75)	87.44	2.00 (0.12)	98.02	2.13 (0.13)	97.89
	Wetland	1.50 (0.09)	80.56	1.67 (0.10)	78.35	1.00 (0.06)	87.04	1.64 (0.10)	78.74
	Control	0.08 (0.005)	92.83	0.48 (0.028)	57.02	0.10 (0.006)	91.04	0.23 (0.014)	79.40
54	Tolerance	4.00 (0.20)	96.04	13.10 (0.71)	87.04	2.15 (0.11)	97.87	2.31 (0.13)	97.71
	Wetland	1.60 (0.08)	79.26	1.85 (0.09)	76.02	1.13 (0.06)	85.35	1.82 (0.10)	76.41
	Control	0.07 (0.004)	93.73	0.46 (0.023)	58.81	0.14 (0.008)	87.46	0.30 (0.016)	73.14
63	Tolerance	8.00 (0.47)	92.08	14.50 (0.85)	85.66	2.20 (0.13)	97.82	2.49 (0.15)	97.53
	Wetland	2.00 (0.12)	74.08	2.03 (0.12)	73.69	1.20 (0.07)	84.44	2.00 (0.12)	74.08
	Control	0.08 (0.005)	92.83	0.56 (0.033)	49.86	0.18 (0.011)	83.88	0.36 (0.021)	67.77
72	Tolerance	10.80 (0.51)	89.31	25.80 (1.40)	74.48	2.50 (0.12)	97.52	2.66 (.014)	97.36
	Wetland	2.05 (0.10)	73.43	2.20 (0.12)	71.49	1.30 (0.06)	83.15	2.17 (0.12)	71.88
	Control	0.10 (0.005)	91.04	0.63 (0.030)	43.59	0.17 (0.009)	84.78	0.39 (0.021)	65.08

The values are the average of 3 replicates with a coefficient of variation in the range of 0.5-2.0%

Initial Tolerance Concentration = 101.12 mg L^{-1}

Initial Wetland Influent Concentration = 7.72 mg L^{-1}

Initial Control Concentration = 1.12 mg L^{-1}

( )= standard deviation

EC= effluent concentration

RE= removal efficiency

Table 3: Analysis of variance of the Fe removal efficiency

Source	DF	SS	MS	F-Value	p-value
Total	191	34817.96	182.290		
Model	95	34571.08	363.910	141.510	0.0001
Plant type (P)	3	8688.860	2896.28	1126.25	0.0001
Time (T)	7	2143.210	306.170	119.060	0.0001
Concentration (C)	2	12989.54	6494.77	2525.55	0.0001
P×T	21	1096.960	52.2200	20.3100	0.0001
P×C	6	7711.420	1285.24	499.780	0.0001
T×C	14	810.1700	57.8700	22.5000	0.0001
P×T×C	42	1131.190	26.9300	20.4700	0.0001
Error	96	246.8700	2.57000		

$R^2 = 0.99$, CV = 1.91%

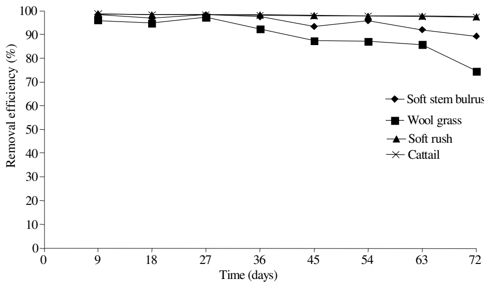
Table 4: Differences among the plant type, periods and initial Fe concentration

Parameter	No. of observations	Iron removal efficiency	Duncan grouping
Plant type			
Soft stem bulrush	48	89.03	A
Wool grass	48	74.32	B
Soft rush	48	91.17	C
Cattail	48	80.72	D
Periods (days)			
1 (1-8)	24	87.61	A
2 (9-17)	24	87.18	A B
3 (18-26)	24	86.57	B
4 (27-35)	24	84.36	C
5 (36-44)	24	84.25	C
6 (45-53)	24	83.08	D
7 (54-62)	24	80	E
8 (63-72)	24	77.46	F
Concentration ($mg\ L^{-1}$)			
Tolerance	64	94.98	A
Wetland influent	64	81.65	B
Control	64	75.41	C

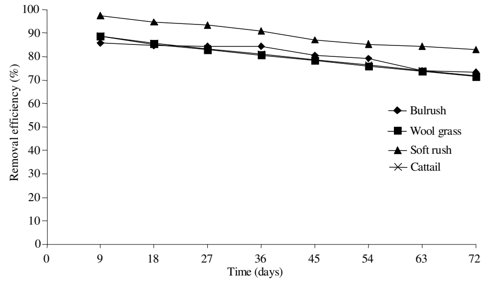
Means with the same letter are not significantly different from each other at 95% confidence level

The ANOVA results (Table 3) showed that the plant type, time and initial Fe concentration have significant effects on the Fe removal efficiency (p = 0.0001). The results also showed significant interactions (p = 0.0001) between the parameters. The results of the Duncan's Multiple Range test (Table 4) showed that the four plants used in this study were significantly different from one another in their ability to remove Fe and the three levels of Fe concentrations in the wastewater were significantly different from one another at a 95% confidence level. However, the average removal efficiencies during the periods 1 and 2, 2 and 3 and 4 and 5 were not significantly different from one another.

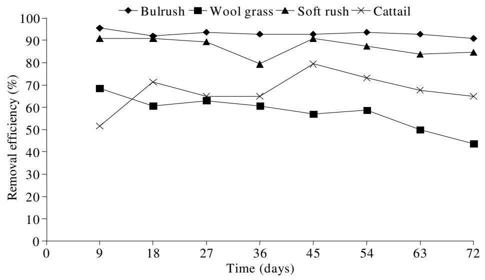
The overall Fe removal efficiencies were 98, 96, 98 and 98%, 86, 79, 98 and 89% and 96, 69, 91 and 52% during the first period which decreased to 89, 74, 97 and 97%, 73, 71, 83 and 89% and 92, 44, 85 and 65% during the last period (after 72 days) of the experiment for soft stem bulrush, wool grass, soft rush and cattail in the compartments receiving tolerance, wetland influent and control concentrations, respectively (Fig. 3). The results showed that the overall Fe removal efficiencies of the compartments receiving wetland influent concentration were lower than those receiving the tolerance concentration. This indicates that plant uptake of Fe is a function of the initial Fe concentration as reported by Kamal *et al.*[17].



(a) Tolerance concentration



(b) Wetland influent concentration,



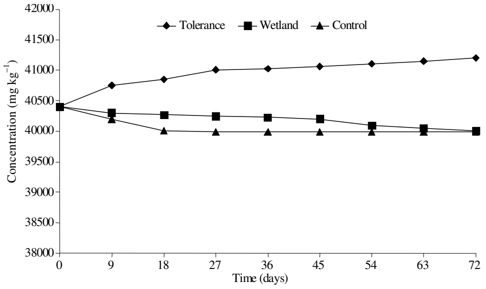
(c) Control concentration

Fig. 3: Fe removal efficiency trends for the four plants

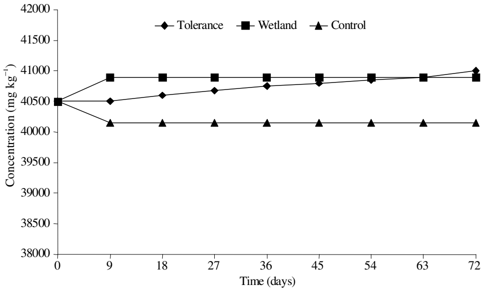
The Fe removal efficiencies obtained in this study are comparable to those reported in the literature. Lorion[18] reports Fe removal of up to 99% in treatment wetland sites. Ye *et al.*[15] reported Fe removal of up to 91% in treatment wetlands designed to treat Fe in contaminated wastewaters from coal combustion processes. Skousen *et al.*[19] reported Fe removal from acid mine drainage ranging from 28 to 99%.

It should, however, be noted that the term removed typically accounts for both plant uptake of Fe and precipitation of Fe particulates. Several reports indicated that immobilization via oxidative precipitation accounts for the majority of the reported Fe removal in treatment wetland systems[20-22]. According to Wieder[20] and Calabrese *et al.*[22], about 50 to 70% of total Fe removal is via Fe hydroxylation.

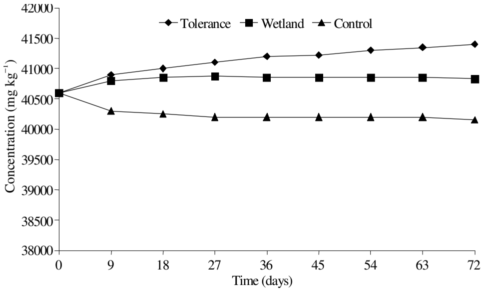
Fe concentration in soil: The changes in Fe concentration in the soil are shown in Fig. 4. The Fe concentration in the soils of soft stem bulrush decreased from 40407 to 39890 mg kg^{-1} and from 40407 to 39532 mg kg^{-1} by the end of the experiment (72 days) indicating the removal of Fe from the soil at average rates of 7.18 and 12.15 mg kg^{-1} day^{-1} for the compartments receiving control and wetland influent concentrations, respectively. It increased from 40407 to 46528 mg kg^{-1} in the compartment receiving tolerance concentration indicating the addition of Fe to the soil by precipitation at an average rate of 85.01 mg kg^{-1} day^{-1}. The concentration of Fe in the soils of wool grass decreased from 40509 to 40050 mg kg^{-1} by the end of the experiment indicating the removal of Fe from the soil at an average rate of 6.38 mg kg^{-1} day^{-1} for the control compartment and increased from 40509 to 41269 mg kg^{-1} and from 40509 to 47395 mg kg^{-1} indicating the addition of Fe to the soil by precipitation at average rates of 10.55 and 95.64 mg kg^{-1} day^{-1} in the compartments receiving wetland influent and tolerance concentrations, respectively. The concentration of Fe in the soils of soft rush decreased from 40635 to 40016 mg kg^{-1} at the end of the experiment indicating the removal of Fe from the soil at an average rate of 8.59 mg kg^{-1} day^{-1} for the control compartment and increased from 40635 to 41006 mg kg^{-1} and from 40635 to 48187 mg kg^{-1} indicating the addition of Fe to the soil by precipitation at average rates of 5.15 and 104.88 mg kg^{-1} day^{-1} in the compartments receiving wetland influent and tolerance concentrations, respectively. The concentration of Fe in the soils of cattail decreased from 40209 to 39623 mg kg^{-1} and from 40209 to 39138 mg kg^{-1} at the end of the experiment indicating the removal of Fe from the soil at average rates of 14.88 and 8.14 mg kg^{-1} day^{-1} for the compartments receiving control and wetland influent concentrations, respectively. It increased from 40209 to 45146 mg kg^{-1} in the compartment receiving tolerance concentration indicating the addition of Fe to the soil by precipitation at an average rate of 68.57 mg kg^{-1} day^{-1}.



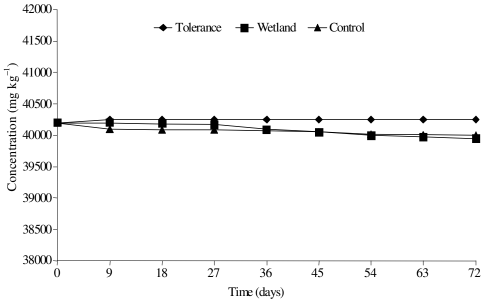
(a) Soft stem bulrush



(b) Wool grass



(c)): Soft rus



(d) Cattai

Fig. 4: Fe concentrations in the soil

These results indicated that a portion of the Fe removed from the wastewater was by the precipitation mechanism and that the rate of precipitation was affected by the initial Fe concentration in the wastewater and the plant type. Wiessner *et al.*[23] conducted experiments to determine the effectiveness of various small scale constructed wetland systems for the removal of Fe from acid mine drainage under field conditions and showed that 92.1% of the Fe input to the system was associated with the soil. Eckhardt *et al.*[24] operated a constructed wetland system for 410 days for the treatment of landfill leachates and found that the concentrations of Fe in the sediment increased from 578 to 2720 mg kg^{-1} demonstrating that the sediment was the main sink for Fe removal. Collins *et al.*[25] examined the element concentrations in the sediments of a constructed wetland that was receiving metal contaminated effluent from a coal pile runoff basin and found that Fe increased in the sediments from below detection limits to 317 μg g^{-1}.

Plant uptake of Fe: The average total accumulation of Fe was 1229, 9740 and 17327 mg for soft stem bulrush, 837, 1381 and 2281 mg for wool grass, 1721, 3348 and 4862 mg for soft rush and 4308, 12488 and 24282 mg for broad-leaved cattail in the compartments receiving the control, wetland influent and tolerance concentrations, respectively. The ANOVA analysis (Table 5) showed that the plant type and Fe concentration have significant effects on the plant uptake (p = 0.0001). The analysis also showed significant interactions (p = 0.0001) between the parameters. The results of Duncan's Multiple Range test (Table 6) showed that the four plants used in this study were significantly different from one another in their ability to accumulate Fe and the three levels of Fe concentrations in the wastewater were significantly different from one another at a 95% confidence level.

Table 5: Analysis of variance of the Fe accumulation in plant tissues

Source	DF	SS	MS	F-Value	P
Total	23	1344437517	58453805		
Model	11	1340933352	121903032	417.5	0.0001
Plant type (P)	2	442051159	221025579	756.9	0.0001
Concentration (C)	3	615293689	205097896	702.4	0.0001
P×C	6	283588502	47264750	161.9	0.0001
Error	12	3504166	292014		

$R^2 = 0.99$, CV = 7.46%

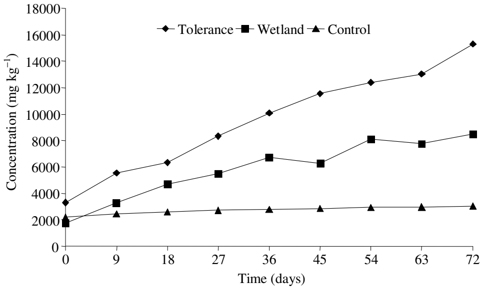
Table 6: Differences among the plant type and initial Fe concentration

Parameter	No. of observations	Total iron in plant tissues	Duncan grouping
Plant type			
Soft stem bulrush	6	9791.3	A
Wool grass	6	1526.2	B
Soft rush	6	3421	C
Cattail	6	14226.2	D
Iron concentration (mg L^{-1})			
Tolerance	8	12612.3	A
Landfill leachate	8	7003.2	B
Control	8	2107.9	C

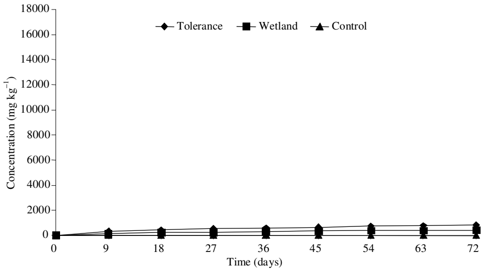
Means with the same letter are not significantly different from each other at 95% confidence level.

Sanchez *et al.*[26] reported Fe concentrations of 2370, 86 and 515 mg kg^{-1} in aquatic moss (*Brachythecium rivulare*), soft rush (*Juncus effusus*) and board leaf cattail (*Typha latifolia*) obtained from sites near an old lead-zinc mine, respectively. The authors stated that the concentration of Fe in plant tissues was plant specific and was not related to the Fe concentration in the sediments. Samecka-Cymerman and Kempers[27] collected plant samples from impacted sites around a former open cut brown coal mine. At one site, the Fe concentration in the water was 9 mg L^{-1} and the Fe concentrations in the leaves of *Phyragmites australis, Potamogeton natans* and *Iris pseudoacorus* were 612, 2100 and 1360 mg kg^{-1}, respectively. At a second site, the Fe concentration in the water was 130 mg L^{-1} and the Fe concentrations in the leaves of *Phragmites austrlis, Juncus bulbosus, Phalaris arundinacea* and *Carex remota* were 841, 974, 995 and 2150 mg kg^{-1} for, respectively. The authors stated that the accumulation of Fe in plant tissues was affected by the Fe concentration in the water.

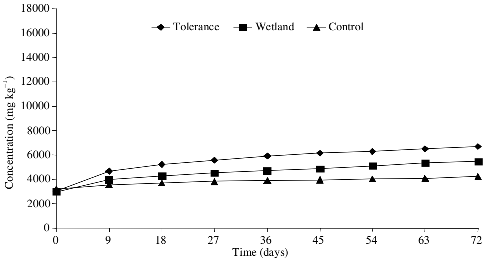
Distribution of iron in plant tissues: The concentrations of Fe in various plant tissues are shown in Figs. 5-8. The results indicated that all of the four plant species had the ability of absorbing Fe through the root system and translocating it into the various parts of the plant. The concentrations of Fe in the roots, leaves and flowers of soft stem bulrush increased by the end of the experiment (72 days) from the initial values of 2423, 13 and 3869 mg kg^{-1} to 3062, 18 and 4222 mg kg^{-1}, to 8482, 415 an 5459 mg kg^{-1} and to 15 290, 813 and 6695 mg kg^{-1} in the compartments receiving the control, wetland influent and tolerance concentrations, respectively. The concentrations of Fe in the roots, stems, leaves and flowers of wool grass increased by the end of the experiment from the initial values of 1533, 26, 102 and 28 mg kg^{-1} to 4220, 88, 181 and 105 mg kg^{-1}, to 6372, 166, 232 and 260 mg kg^{-1} and to 9620, 260, 252 and 415 mg kg^{-1} in the compartments receiving the control, wetland influent and tolerance concentrations, respectively. The Fe concentrations in the roots, leaves and flowers of soft rush increased by the end of the experiment from the initial values of 2479 and 69 mg kg^{-1} (no flowers) to 3840, 130 and 596 mg kg^{-1}, to 5242, 145 and 845 mg kg^{-1} and to 6432, 160 and 1094 mg kg^{-1} in the compartments receiving the control, wetland influent and tolerance concentrations, respectively. The Fe concentrations in the roots and leaves of cattail increased by the end of the experiment from the initial values of 288 and 113 mg kg^{-1} to 2680 and



(a) Roots

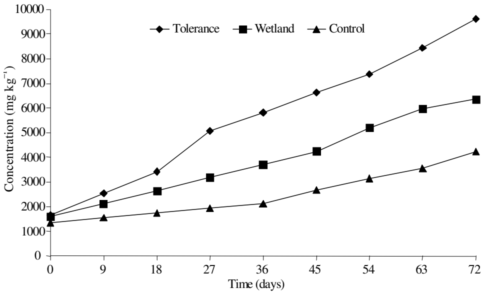


(b) Leaves

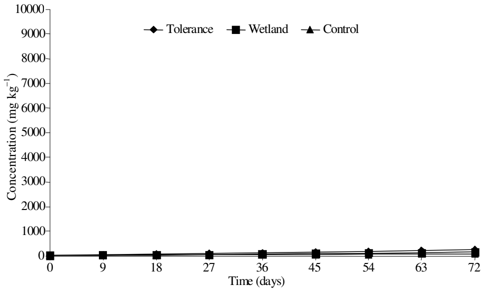


(c) Flowers

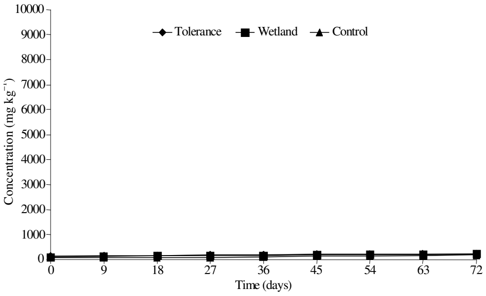
Fig. 5: Fe concentrations in the various parts of soft stem bulrush



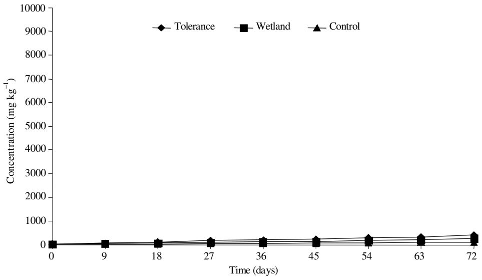
(a) Roots



(b) Stem



(c) Leaves

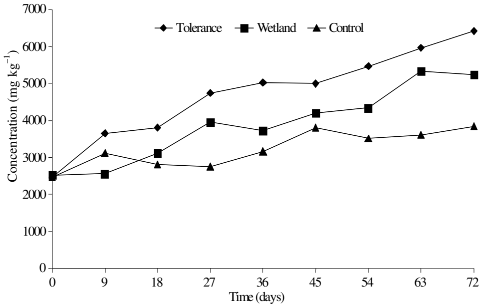


(d) Flowers

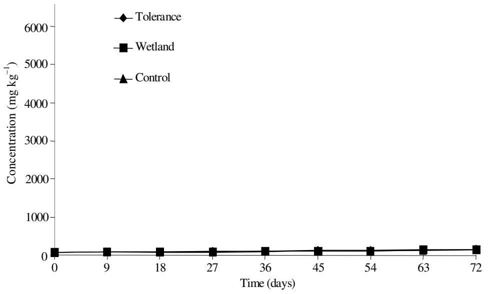
Fig. 6: Fe concentrations in the various parts of wool grass

820 mg kg^{-1}, to 7145 and 2980 mg kg^{-1} and to 14171 and 5788 mg kg^{-1} in the compartments receiving the control, wetland influent and tolerance concentrations, respectively.

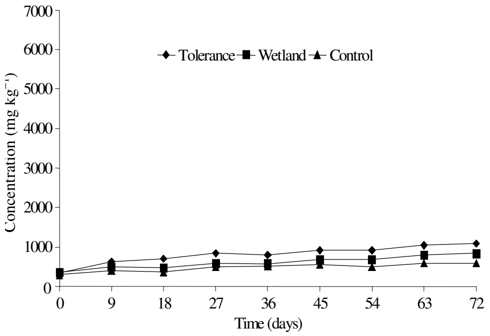
The results also indicated that the highest amount of Fe accumulated in the total plant tissues was in cattail with concentrations of 19194 and 9847 mg kg^{-1} and the lowest was in soft rush with concentrations of 4808 and 3281 mg kg^{-1} in the compartments receiving tolerance and wetland influent concentrations, respectively. The highest amount of Fe accumulated in the root tissues was in cattail with concentrations of 13501 and 7019 mg kg^{-1} and the lowest was in soft rush with concentrations of 3964 and 2728 mg kg^{-1} in compartments receiving the tolerance and wetland influent concentrations, respectively. The highest amount of Fe accumulated in the leaf tissues was in cattail with concentrations of 5693 and 2828 mg kg^{-1} and the lowest was in soft rush with concentrations of 95 and 73 mg kg^{-1} in compartments receiving the tolerance and wetland influent concentrations, respectively. The highest amount of Fe accumulated in



(a) Roots



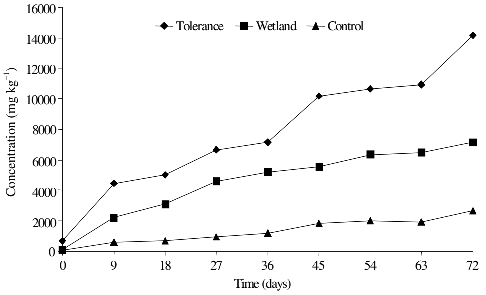
(b) Leaves



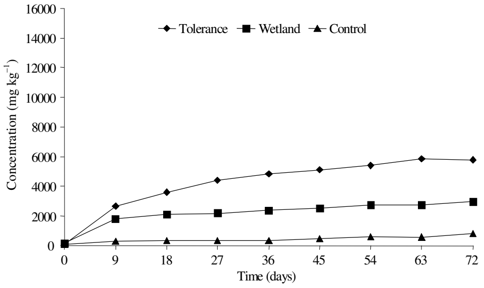
(c) Flowers

Fig. 7: Fe concentrations in the various parts of soft rush

the flower tissues was in soft stem bulrush with concentrations of 3639 and 2494 mg kg^{-1} and the lowest was in wool grass with concentrations of 379 and 231 mg kg^{-1} in compartments receiving the tolerance and wetland influent concentrations, respectively. It is clear from the results obtained from the present study that the four plants used were superior accumulators of Fe. However, plant species differ widely in their ability to accumulate and translocate heavy metals. The root tissues accumulated significantly greater concentrations of Fe than shoots as shown in Table 7 indicating high plant availability of the substrate metals as well as its limited mobility once inside the plant. The translocation factor (concentration of Fe in aboveground tissues divided by that in belowground tissues) has been used as an indicator of the mobility of the element in plant tissues.



(a) Roots



(b) Leaves

Fig. 8: Fe concentrations in the various parts of cattail

The results obtained from this study are consistent with pervious studies[28-30]. Outridge and Noller[28] reported that the concentrations of heavy metals in the root tissues of freshwater macrophytes from polluted areas were usually found to contain higher concentrations of most metals compared to the aboveground parts. The exclusion of metals from aboveground tissues has been suggested as a metal tolerant strategy by *T. latifolia*[31]. The elevated metal concentrations in the underground tissues and low translocation to the aboveground tissues in the wetland plants examined might also suggest that they are capable of rather well balanced uptake and translocation of Fe under heavily iron-polluted conditions. Gries and Garbe[32] indicate that a well-balanced and largely independent ion budget is typical of many grass-line species and is supposed to partly create their wide ecological amplitude. In the view of toxicology, this could be a desirable property, as Fe would not be passed into the food chain via herbivores and thus avoid potential risk to the environment.

Sources of bioavailable Fe: The bioavailable Fe in the current system was made of two portions: (a) the Fe in

Table 7: Fe uptake and distribution in plant tissues

	Total uptake* (mg)	Iron distribution Root (mg)	Stem (mg)	Leaves (mg)	Flowers (mg)	Translocation factor+
Soft stem bulrush						
Tolerance	17327.2 (916.20)	16760.0 (1185.1)	NA	480.0 (33.9)	87.2 (6.17)	0.033
Wetland influent	9740.0 (536.60)	9440.0 (667.5)	NA	240.0 (16.9)	60.0 (4.24)	0.031
Control	1229 (71.61)	3368.2 (234.5)	NA	10.4 (1.3)	50.6 (2.31)	0.018
Wool grass						
Tolerance	2280.8 (18.70)	2229.6 (157.7)	12.8 (0.91)	28.8 (2.1)	8.8 (0.62)	0.016
Wetland influent	1380.8 (81.79)	1337.6 (94.6)	7.2 (0.51)	29.6 (2.1)	4.8 (0.34)	0.026
Control	836.8 (49.30)	886.2 (52.1)	4.4 (0.21)	23.5 (1.9)	2.0 (0.14)	0.033
Soft rush						
Tolerance	4794.4 (179.09)	4756.8 (336.3)	NA	7.2 (0.51)	29.6 (2.09)	0.007
Wetland influent	3348.0 (189.64)	3273.6 (231.5)	NA	58.4 (4.12)	16.0 (1.13)	0.022
Control	1720.8 (100.06)	2649.6 (141.6)	NA	5.2 (0.12)	11.3 (0.56)	0.006
Cattail						
Tolerance	24282.4 (1322.26)	18361.6 (1298.4)	NA	5920.8 (418.6)	NA	0.320
Wetland influent	12128.0 (685.97)	9545.6 (674.9)	NA	2941.6 (208.0)	NA	0.310
Control	4308.4 (254.91)	3580.5 (250.6)	NA	809.4 (60.1)	NA	0.226

The values are the average of 8 replicates with a coefficient of variation in the range of 1-5%. *: Total iron accumulation after 72 days
+: Translocation factor (concentration of iron in the aboveground tissues divided by that in belowground tissues), NA: Not applicable

Table 8: Sources of Fe assimilated by the plants

Iron concentration	Plant	Accumulation in plant (mg)	Removal from water (mg)	(%)	Removal from soil (mg)	(%)
Control						
	Soft stem bulrush	1229	177	14.4	1052	85.6
	Wool grass	836.8	81.1	9.69	755.7	90.31
	Soft rush	1720.8	159	9.24	1561.8	90.76
	Cattail	4308.4	108.3	2.50	4200.7	97.5
Wetland influent						
	Soft stem bulrush	9740	1497	15.370	8243	84.63
	Wool grass	1380.8	1475.9	106.88	95.1	-6.88
	Soft rush	3348.3	1659	49.550	1689.3	50.45
	Cattail	12488	1482	11.870	11006	88.13
Tolerance						
	Soft stem bulrush	17327.2	23124	133.45	5796.8	-33.45
	Wool grass	2281.6	21619.2	947.55	19409.6	-847.55
	Soft rush	4862.3	23817	489.82	18954	-389.82
	Cattail	24282	23783	97.940	498	2.05

The values are the average of 3 replicates with a coefficient of variation in the range of 0.6-2.0%

the water from fertilization and pollutants and (b) the Fe that already existed in the soil. Schulte[33] stated that the bioavailable portion of Fe in soil ranges from 5-10% of the total Fe content in the soil.

Therefore, the total plant uptake of Fe may include that in the wastewater plus some of the bioavailable Fe in the soil. The results showed that the Fe concentration in the plant tissues is directly proportional to the initial Fe concentration in the wastewater. Due to the high affinity of the four plants for Fe, the plants in the compartments receiving the control and wetland influent utilized some of the bioavailable Fe found in the soil as shown Table 8.

CONCLUSIONS

The overall Fe removal efficiencies from the wastewater were 89, 74, 97 and 97%, 73, 71, 83 and 89% and 92, 44, 85 and 65% for soft stem bulrush, wool grass, soft rush and cattail in the compartments receiving tolerance, wetland influent and control concentrations, respectively. The removal of Fe from the wastewater was influenced by the plant type, time and initial Fe concentration. The results showed that the concentration of Fe in the soils in the control compartments of soft stem bulrush, wool grass, soft rush and cattail decreased indicating the removal of Fe from the soil at average rates of 7.18, 6.38, 8.59 and

14.88 mg kg^{-1} day^{-1}, respectively. The concentration of Fe in the soils in the wetland influent compartments of soft stem bulrush and cattail decreased indicating removal of Fe from the soil at average rates of 12.15 and 8.14 mg kg^{-1} day^{-1}, respectively. It however increased in the soils of wool grass and soft rush indicating addition of Fe to the soil by precipitation from the wastewater at average rates of 10.55 and 5.15 mg kg^{-1} day^{-1}. The concentration of Fe in the soils in the tolerance compartments of soft stem bulrush, wool grass, soft rush and cattail increased indicating the addition of Fe to the soil by precipitation from the wastewater at average rates of 85.01, 95.64, 104.88 and 68.57 mg kg^{-1} day^{-1}, respectively. The results showed that the plant type and initial Fe concentration have significant effects on the accumulation of Fe in plants. On a mass basis, broad-leaved cattail accumulated the greatest amount of Fe followed by soft stem bulrush, soft rush and wool grass. The root tissues accumulated significantly greater concentrations of Fe than the shoots indicating high plant availability of Fe but limited mobility once inside the plant.

ACKNOWLEDGMENTS

This research was funded by the ELJB Foundation of Montreal.

REFERENCES

1. Park, D., D.S. Lee, J.M. Park, H.D. Chun, S.K. Park, I. Jitsuhara, O. Miki and T. Kato, 2005. Metal recovery from electroplating wastewater using acidophilic iron oxidizing bacteria: Pilot-Scale feasibility test. Ind. Eng. Chem. Res., 44: 1854-1859.
2. Kadirvelu, K., K. Thamaraiselvi and C. Namasivayam, 2001. Removal of heavy metals from industrial wastewaters by adsorption onto activated carbon prepared from an agricultural solid waste. Bioresour. Technol., 76: 63-65.
3. Ho, G.E., P.J. Murphy, N. Platell and J.E. Wajon, 1984. Iron removal from TiO_2-Plant acidic wastewater. J. Environ. Eng., 110 (4): 828-846.
4. Gray, N.F., 1998. Acid mine drainage composition and the implications for its impact on lotic systems. Water Res., 32 (7): 2122-2134.
5. Christensen, T.H., P. Kjeldsen, P.L. Bjerg, D.L. Jensen, J.B. Christensen, A. Baun, H.J. Albrechtsen and G. Heron, 2001. Biogeochemistry of landfill leachate plumes. Appl. Geochem., 16 (7-8): 659-718.
6. Weber, W.J., Y.C. Jang, T.G. Townsend and S. Laux, 2002. Leachate from land disposed residential construction waste. J. Environ. Eng., 128 (3): 237-245.
7. Moore, J.W., 1991. Inorganic Contaminants of Surface Water. Springer-Verlag, New York.
8. Cripps, S. and M. Kumar, 2003. Environmental and other impacts of aquaculture. In: Lucas, J.S. and P.C. Southgate, (Eds.). Aquaculture: Farming Aquatic Animals and Plants. Blackwell Publishing, Oxford, England. pp:74-99.
9. Schmidt, W., 1999. Review mechanisms and regulation of reduction-based iron uptake in plants. New Phytol., 141 (1): 1-26.
10. Matin, A., M.A. Awan and M.M. Aslam, 2003. A cost effective treatment method for the removal of iron from water and wastewater. Elect. J. Environ. Agric. Food Chem., 2 (5): 558-562.
11. Mitchell, G.F., C.L. Hunt and Y. Su, 2002. Mitigating highway runoff constituents via a wetland. Transport. Res. Record, 1808: 127-133.
12. Dunbabin, J.S. and K.H. Bowmer, 1992. Potential use of constructed wetlands for treatment of industrial wastewaters containing metals. Sci. Total Environ., 111 (2-3): 151-168.
13. Mills, J., 2003. Greenhouse Manager. Department of Biology. Dalhousie University, Halifax, Nova Scotia. (Personal Communication).
14. Galbrand, C., 2004. Naturalized treatment wetlands for contaminant removal: A case study of the Burnside engineering wetland for treatment of landfill leachate. Master of Environmental Studies. Dalhousie University, Halifax, Nova Scotia.
15. Ghaly, A.E. and R. Cote, 2001. Engineered Wetland Technology for Treatment of Industrial Park Contaminants, Technical Report, Dalhousie University, Halifax, Nova Scotia.
16. Ye, Z.H., S.N. Whiting, Z.Q. Lin, C.M. Lytle, J.H. Qian and N. Terry, 2001a. Removal and distribution of iron, manganese, cobalt and nickel within a Pennsylvania constructed wetland treating coal combustion by-product leachate. J. Environ. Qual., 30:1464-1473.
17. Kamal, M., A.E. Ghaly, N. Mahmoud and R. Cote, 2004. Phytoaccumulation of heavy metals by aquatic plants. Environ. Int., 8: 1029-1039.
18. Lorion, R., 2001. Constructed Wetlands: Passive Systems for Wastewater Treatment: Technology Status Report. http://www.clu-in.org/download/remed/constructed_wetlands.pdf
19. Skousen, J.A., K. Sexstone, J. Garbutt and J. Sencindiver. 1994. Acid mine drainage treatment with wetlands and anoxic limestone drains. In: Kent, D.M. (Ed.). Applied Wetland Science and Technology. Lewis Publishing, Boca Raton, FL. pp: 263-281.

20. Wieder, R.K., 1988. Determining the capacity for metal retention in man-made wetlands constructed for treatment of coalmine drainage. Mine Drainage and Surface Mine Reclamation. United States Bureau of Mines, Pittsburgh, PA. pp: 375-381.
21. Perry, A. and R.L.P. Kleinmann, 1991. The use of constructed wetlands in the treatment of acid mine drainage. Natural Resour. Forum, 15 (3): 178-184.
22. Calabrese, J.P., A.J. Sexstone, D.K. Bhumbla, G.K. Bissonnette and J.C. Sencindiver, 1991. Application of constructed cattail wetlands for the removal of iron from acid mine drainage. Proceedings, 2nd International Conference on the Abatement of Acidic Drainage, Montreal, Canada, 16-18.
23. Wiessner, A., P. Kuschk, S. Buddhawong, U. Stottmeister, J. Mattusch and M. Kastner, 2006. Effectiveness of various small-scale constructed wetland designs for the removal of iron and zinc from acid mine drainage under field conditions. Eng. Life Sci., 6 (6): 584-592.
24. Eckhardt, D.A. V., J.M. Surface and J.H. Peverly, 1999. A constructed wetland system for treatment of landfill leachate, Monroe County. In: Mulamoottil, G., E.A. McBean and F. Rovers (Eds.). Constructed Wetlands for the Treatment of Landfill Leachates. CRC Press, Boca Raton, Florida, New York.
25. Collins, B.S., R.R. Sharitz and D.P. Coughlin, 2005. Elemental composition of native wetland plants in constructed mesocosm treatment wetlands. Bioresour. Technol., 96: 937-948.
26. Sanchez, J., M.C. Vaquero and I. Legorburu, 1994. Metal pollution from old lead-zinc mine works: Biota and sediment from Oiartzun Valle. Environ. Technol., 15:1069-1076.
27. Samecka-Cymerman, A. and A.J. Kempers, 2001. Bioindication of heavy metals with aquatic macrophytes: The case of a stream polluted with power plant sewages in Poland. J. Toxicol. Environ. Health-Part A, 62 (1): 57-67.
28. Outridge, P.M. and B.N. Noller, 1991. Accumulation of toxic trace elements by freshwater vascular plants. Rev. Environ. Contamination Toxicol., 121: 1-63.
29. Fitzgerald, E.J., J.M. Caffrey, S.T. Nesaratnam and P. McLoughlin, 2003. Copper and lead concentrations in salt marsh plants on the Suir Estuary, Ireland. Environ. Pollut., 123: 67-74.
30. Cardwell, A.J., D.W. Hawker and M. Greenway, 2002. Metal accumulation in aquatic macrophytes from Southeast Queensland, Australia. Chemosphere, 48: 653-663.
31. Taylor, G.J. and A.A. Crowder, 1983. Uptake and accumulation of heavy metals by Typha latifolia in wetlands of Sudbury, Ontario region. Can. J. Bot., 61: 63-73.
32. Gries, C. and D. Garbe, 1989. Biomass and nitrogen, phosphorus and heavy metal content of *Phragmites australis* during the third year growing season in a root zone wastewater treatment. Archiv für Hydrobiologie, 117: 97-105.
33. Schulte, E.E., 2004. Understanding Plant Nutrients: Soil and Applied Iron. Cooperative Extension Publications. University of Wisconsin. http://cecommerce.uwex.edu/pdfs/A3554.PDF34.