

Manganese Uptake By Facultative and Obligate Wetland Plants Under Laboratory Conditions

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Abstract: The effect of initial concentrations of manganese on the overall removal efficiency of Mn by wool grass, soft rush, broad leaved cattail and soft stem bulrush plants was investigated under laboratory conditions. The translocation of Mn in the roots, stems, leaves and flowers of each plant species was determined and the fraction of Mn removed by precipitation was calculated. The overall manganese removal efficiencies of the system during the first period were 97.66, 94.09, 98.51 and 98.44 %, 90.94, 95.47, 96.83 and 85.51 % and 92.65, 75.55, 97.56 and 75.55 % which decreased to 82.22, 89.94, 95.26 and 95.85 %, 87.78, 91.85, 90.49 and 84.16 % and 38.88, 61.61, 31.54 and 71.64 % by the end (after 72 days) of the experiment for soft stem bulrush, wool grass, soft rush and cattail in the compartments receiving tolerance concentration, landfill leachate and the control, respectively. The removal of manganese was a function of the initial Mn concentration and the higher the initial concentration the higher the removal efficiency. The results showed the addition of manganese (from wastewater) to the soil by precipitation at average rates of 2.17 and 17.19 mg/kg/day, 2.11 and 15.75 mg/kg/day, 1.71 and 15.86 mg/kg/day and 1.17 and 15.29 mg/kg/day for soft stem bulrush, wool grass, soft rush and cattail in the compartments receiving landfill leachate and tolerance concentration, respectively. The leaves of wool grass, soft rush and cattail accumulated significantly greater concentrations of manganese than the roots with translocation factors > 1 indicating high translocation of Mn from root to shoot for the control, landfill leachate and tolerance concentration, respectively.

Keywords: manganese, wool grass, soft rush, soft stem bulrush, cattail, landfill leachate, tolerance concentration

INTRODUCTION

Living plants can be compared to solar driven pumps which can extract and concentrate elements from their environment. From soil and water, all plants have the ability to accumulate heavy metals such as Mg, Fe, Mn, Zn, Cu and Mo which are essential for their growth and development^[1,2]. Certain plants also have the ability to accumulate heavy metals which have no known biological function including Cd, Cr, Pb, Co, Ag, Se and Hg^[3,4]. However, excessive accumulation of essential and nonessential heavy metals can be toxic to most plants.

The ability to both tolerate elevated levels of heavy metals and accumulate them in very high concentrations has evolved in a number of different plant species^[5]. Plants distribute metals internally in many different ways. They may localize selected metals mostly in roots and stems, or they may accumulate and

store other metals in nontoxic form for latter distribution and use. A mechanism of tolerance or accumulation in some plants apparently involves binding potentially toxic metals at cell walls of roots and leaves away from sensitive sites within the cell or storing them in a vacuolar compartment^[6,7].

Wetlands have been used to treat heavy metal contaminated wastewaters from municipal, agricultural and industrial sources. In wetlands, several processes combine to provide overall treatment including: sedimentation and filtration of solids, (b) gasification of volatile substances, (c) chemical precipitation, (d) ion exchange, and (e) plant uptake of metals^[8]. Wetland vegetation is extremely important for the nutrient transformations and transfers because it plays a key role in the cycling and temporary storage of many substances and provides habitat and energy sources to maintain a diverse microbial population in the sediments^[9,10]. It is, therefore, important to understand

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the uptake and storage of metals in various parts of wetland plants.

There are five categories of wetland plants: (a) obligate wetland plants which are found in wetlands 99 % of the time, (b) facultative wetland plants which are found in wetlands 67-99 % of the time, (c) facultative plants which are found in wetlands or non-wetlands 33-67 % of the time, (d) facultative upland plants which are found in wetlands 1-33 % of the time and (e) obligate upland plants which occur in non-wetlands >99 % of the time^[11]. The aim of this study was to assess the performance of selective facultative and obligate wetland plants for the removal of Mn from contaminated wastewater. The specific objectives were: (a) to investigate the effect of initial concentrations of manganese on the overall removal efficiency of Mn by obligate and facultative wetland plants, (b) to determine the translocation of Mn in the different parts of each plant species (root, stem, leaves and flower), and (c) to determine the fraction of Mn removed by precipitation.

EXPERIMENTAL APPARATUS

The experimental setup shown in Fig. 1 consists of holding tanks and lighting and aeration systems.

Holding Tanks: Four boxes were constructed from 2.5 cm thick plywood. Each box (60 x 120 x 80 cm) was divided into three compartments (30 x 60 x 80 cm each) and each compartment contained a holding tank.

Lighting System: The light was provided by an artificial lighting system (625 hectolux/7200 cm²) 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^[12].

Aeration System: 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

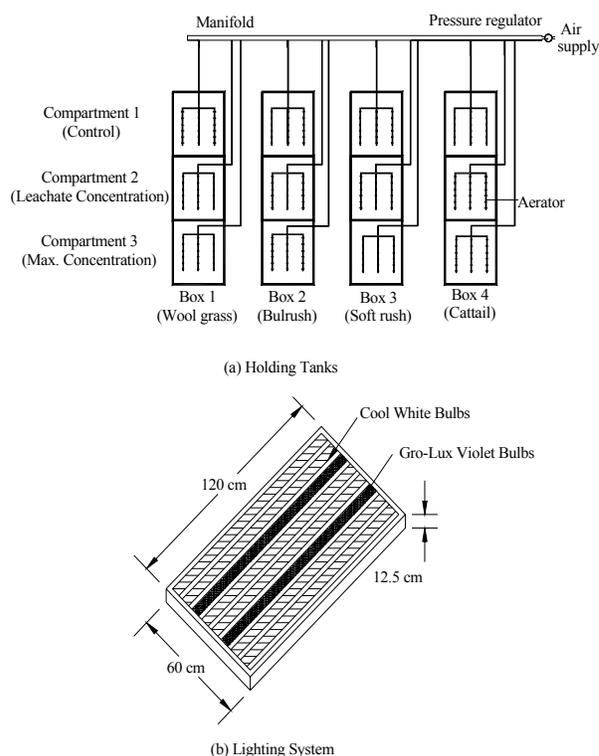
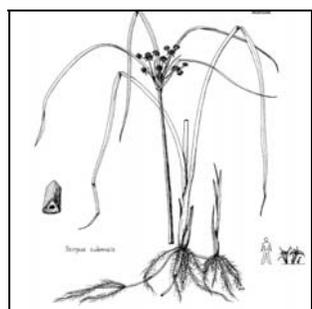


Fig. 1: Experimental apparatus

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 cm³/min.

EXPERIMENTAL PROCEDURES

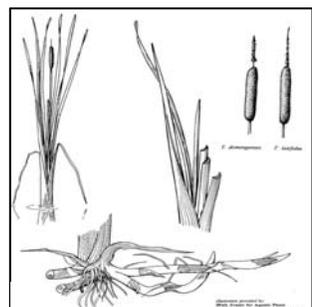
Wetland Plants: Two facultative (wool grass and soft rush) and two obligate (broad-leaved cattail and soft stem bulrush) wetland plant species were used in the study (Fig. 2). The selection of these plants was based on their dominance in the constructed wetland^[13]. Both soft rush and soft stem bulrush 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.



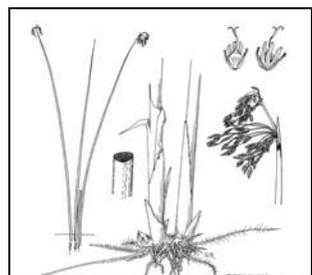
(a) Wool grass



(b) Soft rush



(c) Broad-leaved cattail



(d) Soft stem bulrush

Fig. 2: Plant species used in the experiments

Contaminant Preparation: The plants were supplied with nutrients using a fertilizer (20-20-20 Plant-Prod, Plant Products Co. Ltd., Brampton,, Ontario) (817 mg daily basis.

of fertilizer per 1 L of water). Manganous sulfate ($MnSO_4 \cdot H_2O$) was used as a contaminant supply of manganese. This compound was purchased as a reagent grade chemical from Fisher Scientific, Ottawa, Ontario. Manganese concentrations were selected to: (a) simulate concentrations in the influent of a constructed wetland^[14] and (b) replicate the highest tolerance reported in the literature^[9]. $MnSO_4 \cdot H_2O$ was dissolved in distilled water to achieve the appropriate contaminant level. The final concentrations of manganese used in this experiment are presented in Table 1.

Experimental Protocol : 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^[12]. 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 a Mn concentration similar to that received by a constructed wetland and the third compartment received 30 L of contaminated water containing fertilizer and a Mn 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]. The insecticide was sprayed every week and the growth of individual plants was observed on a

Table 1: Concentrations of manganese (mg/L) in the water

Element	Control	Landfill Leachate	Tolerance Concentration
<i>Nutrient</i>			
Potassium	163.40	163.40	163.40
Nitrogen	163.40	163.40	163.40
Phosphorus	163.40	163.40	163.40
EDTA	8.17	8.17	8.17
Boron	0.16	0.16	0.16
Sulfur	-----	8.60	123.21
<i>Heavy Metals</i>			
Iron	0.82	0.82	0.82
Manganese	0.41	2.21	15.41
Copper	0.41	0.41	0.41
Zinc	0.41	0.41	0.41

MnSO₄.H₂O contributed sulfur in addition to manganese.

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 Mn. The soil samples (50 g) were collected from the middle of each compartment and analyzed for Mn. The plant samples (root, stem, leaf, and flower) were analyzed for Mn. The plant and soil samples were dried in a convection oven for 24 hours at 45 °C. After drying, the plant and soil samples were ground and digested with hydrochloric-nitric-hydrofluoric-perchloric acids (30+10+10+5 mL/g sample) in a closed vessel at a temperature of 100 °C. The Mn concentration was determined using an atomic absorption spectrometer (Varion SpectrAA, Model Number: 55B, Varion, Mulgrave, Victoria, Australia).

Statistical Analysis: 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 DISCUSSIONS

Water: The manganese concentrations in the effluent waters and the manganese removal efficiencies for bulrush, wool grass, soft rush and cattail are shown in Table 2. The results of the analysis of variance performed on the manganese removal efficiencies are shown in Tables 3 and 4.

The manganese concentration in the effluent from the soft stem bulrush compartment at the end of the 9 day retention period ranged from 0.20 to 0.30 mg/L, from 0.36 to 2.74 mg/L and from 0.03 to 0.25 mg/L for landfill leachate, tolerance concentration and control, respectively. The concentration of manganese in the effluent from the wool grass compartment at the end of the 9 day retention period ranged from 0.10 to 0.18 mg/L, from 0.91 to 1.55 mg/L and from 0.10 to 0.36 mg/L for the landfill leachate, tolerance concentration and control, respectively. The manganese concentration in the effluent from the soft rush compartment at the end of the 9 day retention period ranged from 0.07 to 0.21 mg/L, from 0.23 to 0.73 mg/L and from 0.02 to 0.28 mg/L for the landfill leachate, tolerance concentration and control, respectively. The manganese concentration in the effluent from the cattail compartment at the end of the 9 day retention period ranged from 0.13 to 0.35 mg/L, from 0.24 to 0.64 mg/L and from 0.10 to 0.13 mg/L for the landfill leachate, tolerance concentration and control, respectively.

The ANOVA results showed that the plant type, time and Mn initial concentration have significant effects on the Mn removal efficiency (P = 0.0001). The results also showed significant interactions (P = 0.0001) between these parameters. The results of Duncan's Multiple Range test showed that the four plants used in this study were significantly different from one another in their ability to remove manganese and the three levels of initial manganese concentrations in the wastewater were significantly different from one another at a 95% confidence level. However, the average removal efficiency during the periods 4, 5 and 6 were not significantly different from one another at a 95% confidence level.

The overall manganese removal efficiencies of the system during the first period were 97.66, 94.09, 98.50 and 98.44 % and 90.94, 95.47, 96.83 and 85.51 % which decreased to 82.22, 89.94, 95.26 and 95.84 % and 87.77, 91.85, 90.49 and 84.15 % by the end (after 72 days) of the experiment for soft stem bulrush, wool grass, soft rush and cattail in the compartments receiving tolerance concentration and landfill leachate, respectively (Figure 4). The results showed that the compartments receiving landfill leachate had overall manganese removal efficiencies lower than those of the compartments receiving the tolerance concentration. This indicates that the removal of manganese was a function of the initial concentration and the higher the initial concentration the higher the removal efficiency. Kamal et al.^[15] reported similar results for Fe and Hg.

Table 2: Manganese effluent concentration and removal efficiency

Time	Treatment	Bulrush		Wool grass		Soft rush		Cattail	
		EC (mg/L)	RE (%)	EC (mg/L)	RE (%)	EC (mg/L)	RE (%)	EC (mg/L)	RE (%)
9	Tolerance	0.36 (0.03)	97.66	0.91 (0.06)	94.09	0.23 (0.02)	98.51	0.24 (0.02)	98.44
	Leachate	0.20 (0.01)	90.94	0.10 (0.01)	95.47	0.07 (.005)	96.83	0.32 (0.02)	85.51
	Control	0.03 (0.002)	92.65	0.10 (.002)	75.55	0.10 (0.001)	97.56	0.10 (0.007)	75.55
18	Tolerance	0.36 (0.02)	97.66	1.10 (0.07)	92.86	0.33 (0.02)	97.86	0.55 (0.04)	96.43
	Leachate	0.21 (0.01)	90.49	0.10 (0.01)	95.47	0.12 (0.01)	94.56	0.28 (0.02)	97.32
	Control	0.05 (0.003)	87.78	0.18 (.012)	55.99	0.02 (0.001)	95.11	0.10 (0.006)	75.55
27	Tolerance	0.63 (0.03)	95.91	1.37 (0.08)	91.11	0.43 (0.03)	97.21	0.55 (0.03)	96.43
	Leachate	0.30 (0.02)	86.41	0.10 (0.01)	95.47	0.14 (0.01)	93.66	0.13 (0.01)	94.11
	Control	0.08 (0.005)	80.44	0.24 (.014)	41.32	0.04 (0.002)	90.22	0.11 (0.007)	72.37
36	Tolerance	0.80 (0.06)	94.81	1.37 (0.10)	91.11	0.63 (0.05)	95.91	0.73 (0.06)	95.26
	Leachate	0.29 (0.02)	86.87	0.14 (0.01)	93.66	0.21 (0.01)	90.49	0.18 (0.01)	91.85
	Control	0.13 (0.010)	68.22	0.30 (.024)	26.65	0.08 (0.006)	80.44	0.13 (0.010)	67.97
45	Tolerance	0.70 (0.05)	95.45	1.01 (0.07)	93.45	0.65 (0.05)	95.78	0.46 (0.03)	97.01
	Leachate	0.26 (0.02)	88.23	0.16 (0.01)	92.75	0.20 (0.02)	90.49	0.13 (0.01)	94.11
	Control	0.15 (0.010)	63.32	0.36 (.025)	11.98	0.11 (0.007)	73.11	0.13 (0.095)	97.23
54	Tolerance	2.19 (0.11)	85.78	1.28 (0.06)	91.69	0.73 (0.04)	95.26	0.55 (0.03)	96.43
	Leachate	0.30 (0.02)	86.41	0.15 (0.01)	93.21	0.21 (0.01)	90.49	0.28 (0.01)	87.32
	Control	0.20 (0.010)	51.10	0.18 (.009)	55.01	0.15 (0.007)	63.33	0.11 (0.005)	73.35
63	Tolerance	2.58 (0.17)	83.25	1.10 (0.07)	92.86	0.63 (0.04)	95.91	0.55 (0.04)	96.43
	Leachate	0.26 (0.02)	88.23	0.16 (0.01)	92.76	0.20 (0.01)	90.49	0.13 (0.01)	94.12
	Control	0.20 (0.013)	51.10	0.17 (.011)	58.43	0.24 (0.015)	41.32	0.12 (0.007)	71.64
72	Tolerance	2.74 (0.16)	82.22	1.55 (0.09)	89.94	0.73 (0.04)	95.26	0.64 (0.04)	95.85
	Leachate	0.27 (0.02)	87.78	0.18 (0.01)	91.85	0.21 (0.01)	90.49	0.35 (0.02)	84.16
	Control	0.25 (0.015)	38.88	0.16 (.009)	61.61	0.28 (0.017)	31.54	0.12 (0.007)	71.64

The values are the average of 3 replicates with a coefficient of variation in the ranges of 1.0 – 6.0 %.

Initial tolerance concentration = 15.41 mg/L.

Initial landfill leachate concentration = 2.21 mg/L.

Initial control concentration = 0.41 mg/L.

() standard deviation

EC effluent concentration

RE removal efficiency

The manganese removal efficiencies obtained in this study are comparable to those reported in the literature. Groudeva et al.^[16] conducted a study to determine the ability of a constructed wetland planted (*Typha latifolia*, *Typha angustifolia*, *Phragmites communis*, *Scirpus lacustris* and *Juncus* spp.) to treat waters contaminated with crude oil and toxic heavy metals and reported initial manganese concentrations in the water of 0.64 - 3.74 mg/L and effluent manganese concentrations of <0.5 mg/L. Mitchell and Karathanasis^[17] conducted a study to examine metal removal efficiencies from a metal chloride enriched wastewater by simulated constructed wetlands planted

with soft stem bulrush and cattail and found that the average retention of manganese was 24 % over a 12 week period. Brodie^[18] evaluated manganese removal efficiencies of aerobic constructed wetlands planted with cattail, wool grass, sedge and rush treating drainage from coal mining and processing facilities and coal fired power plants and reported that manganese removal efficiencies were in the range of 60 - 96 %.

Ye et al.^[9] stated that phytoremediation mechanisms prove relatively insignificant, accounting for approximately 1 – 4 % of manganese removal in wetlands. Previous studies^[19-23] showed that constructed wetlands are less successful in removing manganese.

Skousen et al.^[24] reported that oxidized manganese does not readily precipitate unless the pH of water is at least 7.0, with precipitation most commonly occurring in waters with pHs greater than 10.0. In addition, manganese oxidation is sensitive to the presence of Fe²⁺ which can prevent or even reverse manganese oxidation^[24]. In the current study, the pH of the wastewaters was not acidic, hence removal of Mn via precipitation took place in the system. The soil analysis results showed increased Mn concentrations. BCMELP^[25] reported that the most effective removal mechanism for manganese in treatment wetland systems is settling, as 90 to 95% of total waterborne manganese binds to particulate matter.

Table 3: Analysis of variance of the manganese removal efficiency

Source	DF	SS	MS	F-Value	P
Total	191	67075.38	351.18		
Model	95	66831.56	703.49	276.99	0.0001
Plant Type (P)	3	2552.19	850.73	334.97	0.0001
Time (T)	7	4719.77	674.25	265.48	0.0001
Concentration (C)	2	36853.10	18426.55	7255.27	0.0001
P x T	21	5011.22	238.63	93.96	0.0001
P x C	6	4693.16	782.19	307.98	0.0001
T x C	14	5121.54	365.82	144.04	0.0001
P x T x C	42	7880.57	187.63	73.88	0.0001
Error	96	243.82	2.54		

R² = 0.99
CV = 1.91%

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

Parameter	Number of Observations	Mn Removal Efficiency	Duncan Grouping
Plant Type			
Soft stem bulrush	48	81.36	A
Wool grass	48	77.07	B
Soft rush	48	86.19	C
Cattail	48	85.45	D
Periods (days)			
1 (1-8)	24	91.14	A
2 (9-17)	24	88.42	B
3 (18-26)	24	85.65	C
4 (27-35)	24	80.94	D
5 (36-44)	24	79.29	E F
6 (45-53)	24	80.07	D E
7 (54-62)	24	78.83	F
8 (63-72)	24	75.79	G
Concentration (mg/L)			
Tolerance	64	93.93	A
Landfill	64	90.61	B
Leachate	64	63.02	C
Control			

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

Soil: The change in manganese concentrations in the soils are shown in Figure 5. The concentration of

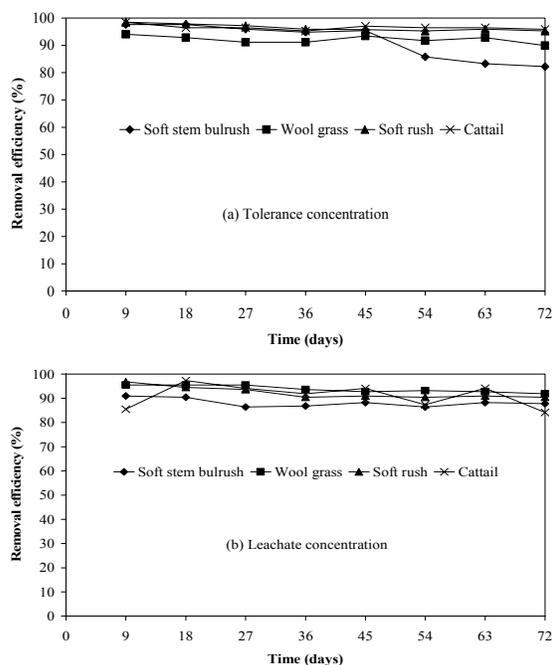


Fig. 4: Manganese removal efficiency trends for the four plants

manganese in the soils of soft stem bulrush, wool grass, soft rush and cattail increased to 639 and 1721 mg/kg, to 645 and 1627 mg/kg, to 635 and 1654 mg/kg and to 586 and 1603 mg/kg by the end of the experiment (72 days) in the compartments receiving the landfill leachate and tolerance concentration, respectively. These results showed the addition of manganese (from wastewater) to the soil by precipitation at average rates of 2.17 and 17.19 mg/kg/day, 2.11 and 15.75 mg/kg/day, 1.71 and 15.86 mg/kg/day and 1.17 and 15.29 mg/kg/day for soft stem bulrush, wool grass, soft rush and cattail in the compartments receiving landfill leachate and tolerance concentration, respectively.

A possible reason for an increase in manganese concentrations in the soil could be sedimentation of manganese precipitates and manganese bound to particulate matter. Eckhardt et al.^[26] conducted a study to determine the ability of a 2 cell, vegetated surface flow wetland to treat leachate from a landfill in New York and found that the concentrations of manganese in the sediment of the first and second cells increased over time and were 307 and 298 mg/kg after two years, respectively. Ye et al.^[9] conducted a study to determine the ability of a 4 cell, vegetated surface flow wetland to treat leachate from an electrical power station in

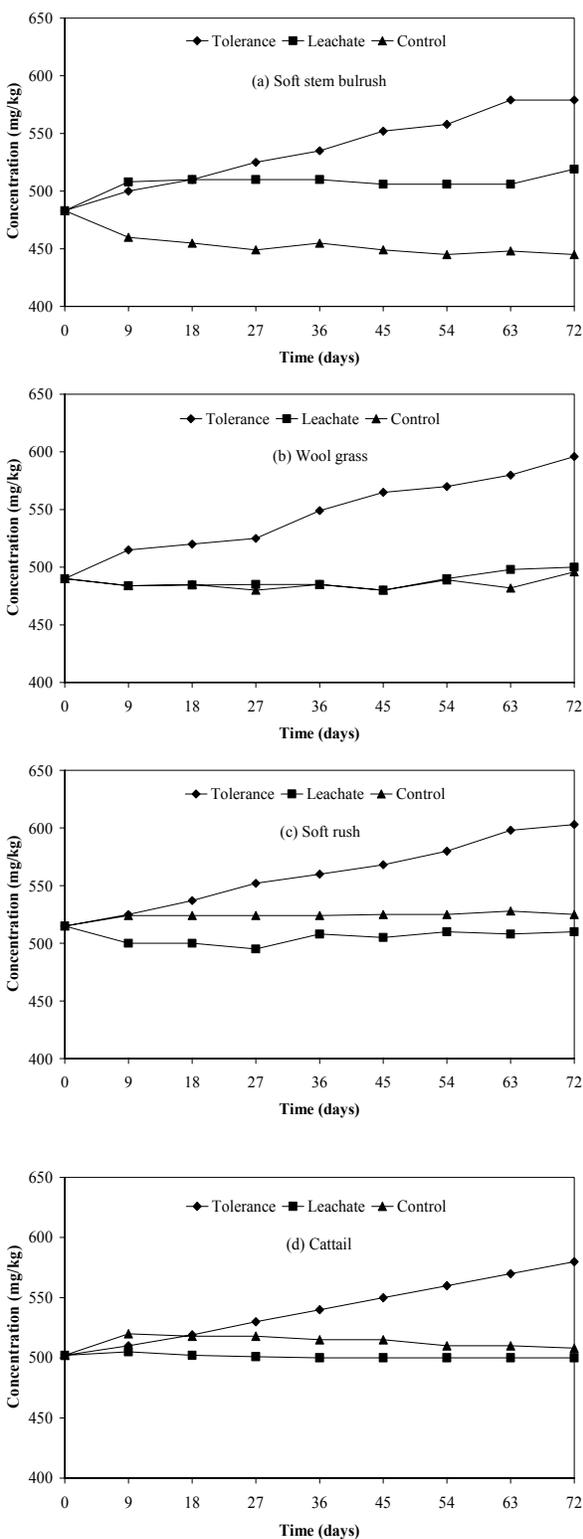


Fig. 5: Manganese concentrations in the soil

Pennsylvania and found that the average manganese concentrations increased over the two year study period and were 3310, 1350, 830 and 872 mg/kg in the uppermost sediments of cells 1 – 4, respectively. Ye et al.^[27] conducted a study to determine the ability of a 10 year old, 2 cell, vegetated surface flow wetland to treat leachate from an electrical utility in Alabama and found that average concentrations of manganese in the sediment from the two cells were in the range of 200 to 400 mg/kg.

A possible reason for an increase in manganese concentrations in the soil could be sedimentation of manganese precipitates and manganese bound to particulate matter. Eckhardt et al.^[26] conducted a study to determine the ability of a 2 cell, vegetated surface flow wetland to treat leachate from a landfill in New York and found that the concentrations of manganese in the sediment of the first and second cells increased over time and were 307 and 298 mg/kg after two years, respectively. Ye et al.^[9] conducted a study to determine the ability of a 4 cell, vegetated surface flow wetland to treat leachate from an electrical power station in Pennsylvania and found that the average manganese concentrations increased over the two year study period and were 3310, 1350, 830 and 872 mg/kg in the uppermost sediments of cells 1 – 4, respectively. Ye et al.^[27] conducted a study to determine the ability of a 10 year old, 2 cell, vegetated surface flow wetland to treat leachate from an electrical utility in Alabama and found that average concentrations of manganese in the sediment from the two cells were in the range of 200 to 400 mg/kg.

The concentration of manganese in the soil of the control compartment of soft rush increased to 530 mg/kg indicating addition of manganese to the soil at an average rate of 0.25 mg/kg/day while the concentration of manganese in the soil of the control compartments of soft stem bulrush, wool grass and cattail decreased to 454, 481 and 476 mg/kg indicating removal of manganese from the soil at average rates of 0.70, 0.17 and 0.36 mg/kg/day, respectively.

A possible reason for a decrease in the manganese concentrations in the soil of the control compartments for soft stem bulrush, wool grass and cattail could be uptake of the bioavailable fraction of manganese from the soil. The bioavailable manganese in the system is made of two portions: (a) the manganese in the water from fertilizers and reagents and (b) the manganese that naturally existed in the soil. Therefore, the total plant uptake of manganese may include that in water plus some of the bioavailable Mn in the soil. Due to the high affinity of plants for manganese, some plants in the

compartments with low Mn concentrations (control and landfill leachate) utilized the bioavailable portion of Mn in the soil.

Plants: The concentrations of manganese in plant tissues of the four plants are shown in Figures 6 - 9. The initial concentrations of manganese in the roots, leaves and flowers of soft stem bulrush were 62 mg/kg, 84 mg/kg and 239 mg/kg which increased by the end of the experiment (72 days) to 140, 148 and 156 mg/kg, to 150, 210 and 268 mg/kg, and to 419, 599 and 788 mg/kg in the compartments receiving the control, landfill leachate and tolerance concentration, respectively. The initial concentrations of manganese in the roots, stems, leaves and flowers of wool grass were 39 mg/kg, 28 mg/kg, 46 mg/kg and 20 mg/kg which increased by the end of the experiment (72 days) to 63, 81 and 90 mg/kg, to 103, 196 and 289 mg/kg, to 148, 170 and 191 mg/kg, and 56, 204 and 351 mg/kg in the compartments receiving the control, landfill leachate and tolerance concentration, respectively. The initial concentrations of manganese in the roots, leaves and flowers of soft rush were 65 mg/kg, 81 mg/kg, and 275 mg/kg which increased by the end of the experiment (72 days) to 97, 157 and 217 mg/kg, to 171, 242 and 313 mg/kg, and to 421, 446 and 471 mg/kg in the compartments receiving the control, wetland influent and tolerance concentration, respectively. The results showed that the manganese concentration in the plant tissues is directly proportional to the initial manganese concentration in the wastewater (Table 5). Marschner^[1] stated that the Mn content of plants is not only affected by plant characteristics, but also by the pool of available Mn which is highly controlled by soil properties. Samecka-Cymerman and Kempers^[28] collected plant samples from 9 different impacted sites around a former open cut brown coal mine and found that the manganese concentration in the leaves of the various plant samples (*Phragmites australis*, *Potamogeton natans*, *Iris pseudoacorus*, *Juncus effusus*, *Drepanocladus aduncus*, *Juncus bulbosus*, *Phalaris arundinacea*, *Carex remota* and *Calamagrostis epigeios*) increased with the increase of manganese concentration in the water. Kabata-Pendias and Pendias^[29] suggested that because Mn seems to be easily taken up by plants when it occurs in soluble

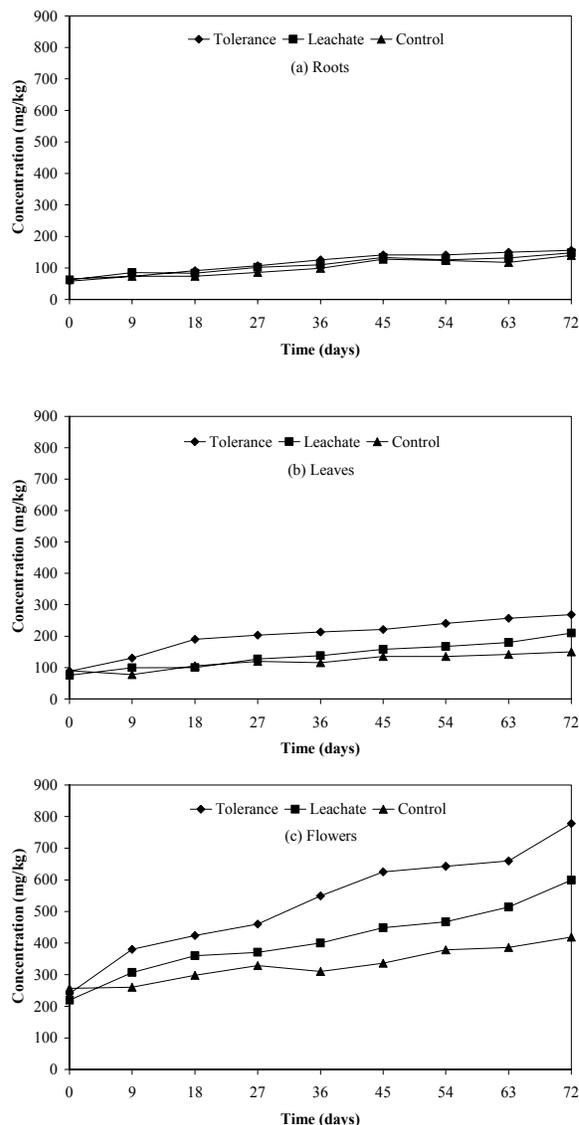


Fig. 6: Manganese concentrations in the various parts of the soft stem bulrush

forms in soils, the Mn content of plants should be a direct function of the soluble Mn pool in soils.

The highest amount of manganese accumulated in the total plant tissues was in soft stem bulrush with concentrations of 1202, 957 and 709 mg/kg and the lowest was in wool grass with concentrations of 921, 649 and 370 mg/kg for the compartments receiving tolerance concentration, landfill leachate and control, respectively. The highest amount of manganese accumulated in the root tissues was in soft rush with concentrations of 217, 157 and 97 mg/kg and the lowest

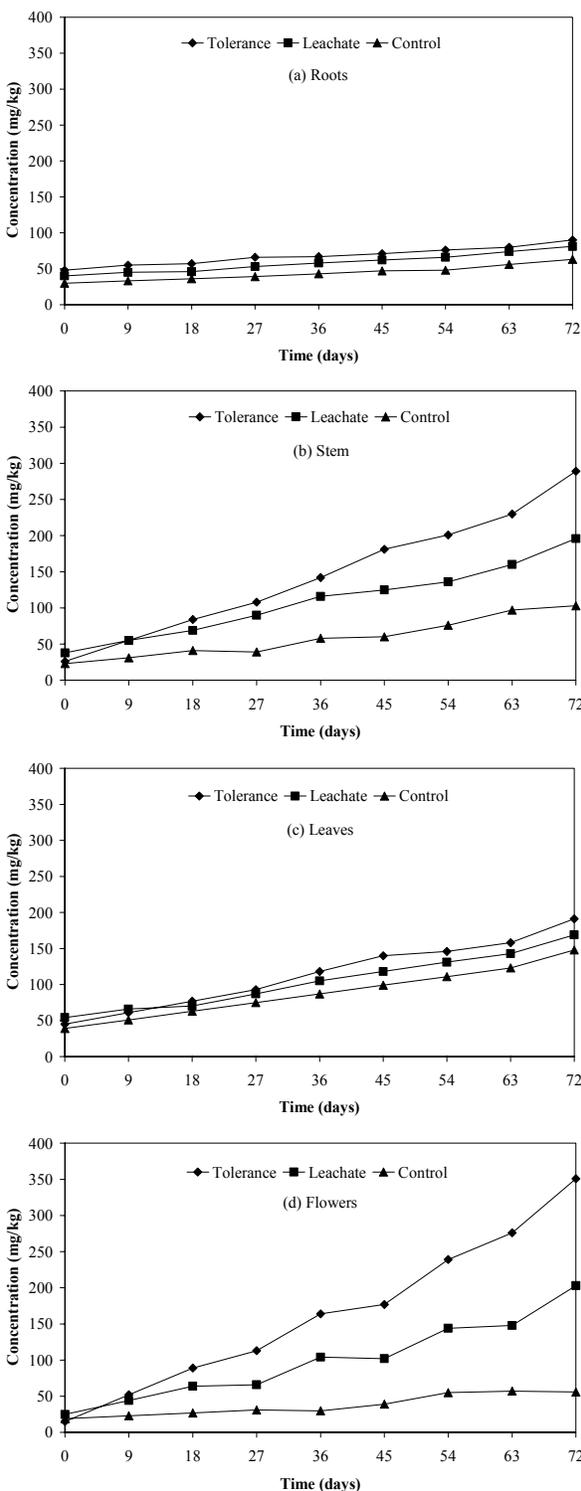


Fig. 7: Manganese concentrations in the various parts of the wool grass

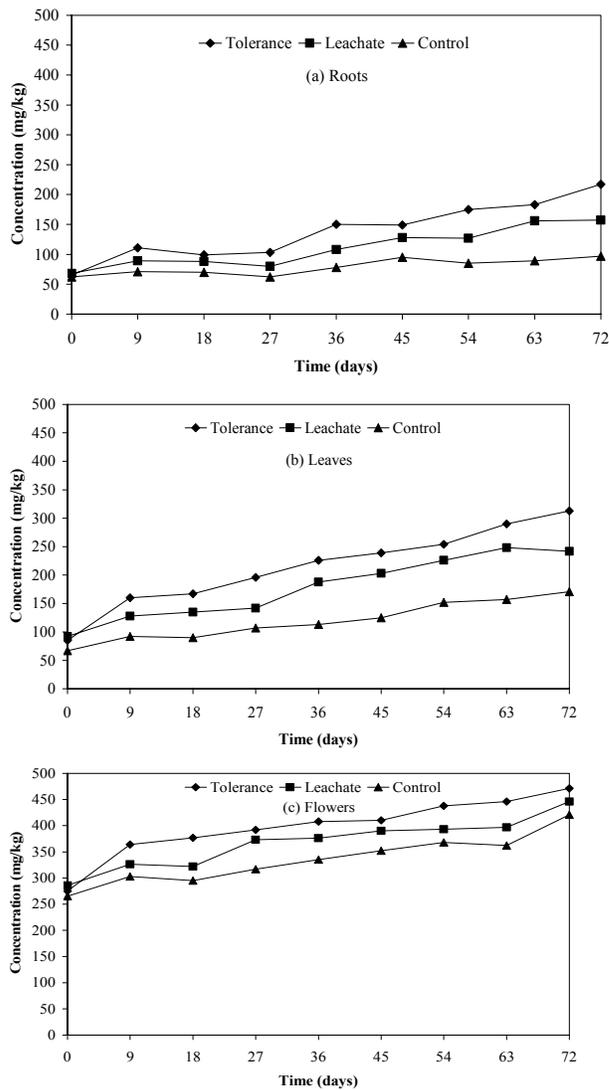


Fig. 8: Manganese concentration in the various parts of the soft rush

was in wool grass with concentrations of 90, 81 and 63 mg/kg for compartments receiving the tolerance concentration, landfill leachate and control, respectively. The highest amount of manganese accumulated in the leaves was in cattail with concentrations of 783, 757 and 730 mg/kg and the lowest was in wool grass with concentrations of 191, 169 and 148 mg/kg in compartments receiving the tolerance concentration, landfill leachate and control, respectively. The highest amount of manganese accumulated in the flowers was in soft stem bulrush with concentrations of 778, 599 and 419 mg/kg and the lowest was in wool grass with concentrations of 351,

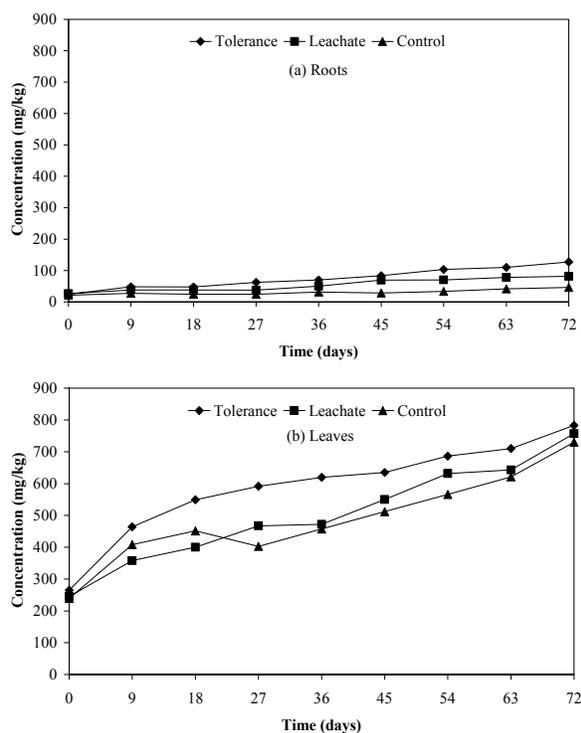


Fig. 9: Mn concentrations in the various parts of cattail

203 and 56 mg/kg in compartments receiving the tolerance concentration, landfill leachate and control, respectively.

Kabata-Pendias and Pendias^[29] reported that the Mn concentration fluctuates greatly within the plant parts and within the vegetative period. Resh^[30] stated that immobile elements such as Mn cannot be translocated from their original site of deposition to the actively growing region of the plant and a deficiency of an immobile element appears on young leaves on the upper portion of the plant. Page et al.^[31] demonstrated that ⁵⁴Mn remained in the oldest leaves of white lupin and was not transported to the younger leaves. Nable and Loneragan^[32] examined the mobility of manganese from old leaves during vegetative growth by following manganese redistribution in parts of subterranean clover plants. In plants transferred from nutrient solutions with 1 μM Mn²⁺ to solutions without Mn²⁺, there was no net loss of manganese from old leaves, although plants developed severe manganese deficiency symptoms in young leaves.

The ANOVA results (Table 6) showed that the plant type and the initial manganese concentration have significant effects on the plant uptake (P = 0.0001). There were significant interactions (P = 0.0001) between the parameters. The results of Duncan's Multiple Range test (Table 7) showed that the four plants used in this study (soft stem bulrush, wool grass, soft rush and cattail) were significantly different from one another in their ability to accumulate manganese and the three levels of the initial manganese concentrations were significantly different from one another at a 95% confidence level.

Table 5: Sources of manganese assimilated by the plants

Treatment	Plant	Total Uptake ⁺		From Water		From Soil	
		(mg/kg)	(mg) ⁺⁺	(mg)	(%)	(mg/kg)	(%)
Control	Bulrush	709	144.8 (10.24)	65.4 (4.62)	45.17	79.4 (5.61)	54.83
	Wool grass	370	40.8 (2.62)	47.4 (3.35)	117.32	-6.6 (0.47)	-17.32
	Soft rush	689	131.4 (9.29)	70.2 (4.96)	53.42	61.2 (4.33)	46.58
	Cattail	776	544.6 (32.09)	70.5 (4.99)	12.95	474.1 (33.52)	87.05
Leachate	Bulrush	957	210.4 (14.88)	468.0 (33.09)	222.43	-257.6 (18.21)	-122.43
	Wool grass	649	52.5 (4.12)	497.5 (35.17)	947.61	-445.0 (31.47)	-847.61
	Soft rush	845	290.8 (16.49)	489.0 (34.57)	209.6	-255.8 (18.09)	-109.64
	Cattail	838	607.2 (35.78)	476.2 (33.67)	78.42	131.0 (9.26)	21.57
Tolerance	Bulrush	1202	258.4 (18.27)	3387.0 (239.49)	1310.8	-3128.6 (221.23)	-1210.8
	Wool grass	921	81.5 (4.09)	3326.0 (240.94)	4081	-3338.0 (236.03)	-4802.9
	Soft rush	1001	372.6 (26.34)	3567.0 (252.22)	957.32	-3194.4 (225.87)	857.32
	Cattail	910	678.8 (43.63)	3570.0 (252.43)	525.92	-2891.2 (204.44)	-425.92

The values are the average of 3 replicates with a coefficient of variation in the range of 1.5-6.7%

+ Total iron accumulation after 72 days

() Standard deviation

++ Calculated from plant weight

Table 6: Analysis of variance of the manganese accumulation in plant tissues

Source	DF	SS	MS	F-Value	P
Total	23	1192725.75	51857.64		
Model	11	118676.12	107887.38	217.05	0.0001
Plant Type (P)	2	74287.28	37143.64	74.73	0.0001
Concentration (C)	3	1086547.82	362182.61	728.66	0.0001
P x C	6	25926.03	4321.00	8.69	0.0008
Error	12	5964.75			
R ²	= 0.99				
CV	= 7.65%				

Table 7: Differences among the plant type and initial manganese concentration

Parameter	Number of Observations	Total Mn in Plant Tissues	Duncan Grouping
Plant Type			
Soft stem bulrush	6	214.78	A
Wool grass	6	56.82	B
Soft rush	6	258.06	C
Cattail	6	636.48	D
Mn Concentration (mg/L)			
Tolerance	8	361.16	A
Wetland Influent	8	288.45	B
Control	8	224.99	C

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

Table 8: Manganese uptake and distribution in plant tissues

	Total Uptake ^s (mg)	Manganese Distribution				Translocation Factor ⁺
		Roots (mg)	Stem (mg)	Leaves (mg)	Flowers (mg)	
Soft stem bulrush						
Tolerance	258.4 (18.27)	137.6 (9.73)	NA	108.0 (7.64)	12.9 (0.91)	0.87
Landfill Leachate	210 (14.88)	120.8 (8.54)	NA	80.8 (5.71)	9.0 (0.64)	0.74
Control	144.8 (10.24)	119.0 (5.4)	NA	58.5 (3.89)	6.5 (0.23)	0.54
Wool grass						
Tolerance	81.5 (4.09)	19.5 (0.17)	14.7 (1.04)	35.0 (2.47)	8.0 (0.57)	2.95
Landfill Leachate	52.5 (4.12)	11.4 (0.81)	8.8 (0.62)	27.8 (1.96)	4.2 (0.31)	3.57
Control	40.8 (2.62)	9.5 (1.20)	4.9 (0.53)	25.16 (2.12)	1.2 (0.12)	3.29
Soft rush						
Tolerance	372.6 (26.34)	182.4 (12.89)	NA	182.4 (12.0)	7.6 (0.53)	1.04
Landfill Leachate	290.8 (16.49)	107.0 (7.55)	NA	120.0 (8.49)	64.0 (4.52)	1.72
Control	131.4 (9.29)	46.3 (4.28)	NA	92.5 (7.89)	6.3 (0.31)	2.13
Cattail						
Tolerance	678.8 (43.63)	140.1 (9.91)	NA	538.7 (38.09)	NA	3.85
Landfill Leachate	607.2 (35.78)	74.8 (5.28)	NA	532.5 (37.65)	NA	7.11
Control	544.6 (32.09)	46.0 (1.02)	NA	503.7 (10.23)	NA	10.95

The values are the average of 8 replicates with a coefficient of variation in the range of 2-6%

* Total iron accumulation after 72 days

+ Translocation factor: concentration of iron in the above ground tissues divided by below ground tissues.

NA Not applicable

Peltier, et al.^[33] collected common reeds (*Phragmites*) grown along the edge of the waterline of a metal-contaminated wetland having manganese concentration of 0.96 mg/L and found that the concentrations of manganese were 1027, 138 and 46 mg/kg in roots, stem and leaves, respectively. Sanchez et al.^[34] sampled aquatic moss (*Brachythecium rivulare*), soft rush (*Juncus effusus*), and board leaf cattail (*Typha latifolia*) from different sites near an old lead-zinc mine and

found the manganese concentrations in the leaves were 1850, 345 and 1950 mg/kg for moss, soft rush and board leaf cattail, respectively. It appears from the results obtained from the present study that the four plants used were somewhat inferior accumulators of manganese compared to the plants used in other studies.

The leaves accumulated significantly greater concentrations of manganese than the roots (Table 8), indicating high plant availability of the substrate metal.

Generally, Mn is known to be taken up and translocated within plants rapidly. Manganese is reported to occur in plant fluids and extracts mainly as free cationic forms and is likely to be transported as Mn^{2+} but redistribution of Mn in the phloem is very limited and may depend on the plant species and developmental stage^[31]. However, its complexing compounds with organic molecules of 1000 to 5000 molecular weights have been found in phloem exudates^[1]. Van Goor and Wiersma^[35] reported a much lower Mn concentration in phloem exudates than in leaf tissues and concluded that a slight transport of Mn through the phloem vessels is responsible for the low concentration of Mn in fruits, seeds, and storage roots.

CONCLUSIONS

The overall manganese removal efficiencies of the system during the first period were 97.66, 94.09, 98.51 and 98.44 % and 90.94, 95.47, 96.83 and 85.51 % and 92.65, 75.55, 97.56 and 75.55 % which decreased to 82.22, 89.94, 95.26 and 95.85 % and 87.78, 91.85, 90.49 and 84.16 % and 38.88, 61.61, 31.54 and 71.64 % by the end (after 72 days) of the experiment for soft stem bulrush, wool grass, soft rush and cattail in the compartments receiving tolerance concentration landfill leachate and the control, respectively. The removal of manganese was a function of the initial Mn concentration and the higher the initial concentration the higher the removal efficiency. The results showed the addition of manganese (from wastewater) to the soil by precipitation at average rates of 2.17 and 17.19 mg/kg/day, 2.11 and 15.75 mg/kg/day, 1.71 and 15.86 mg/kg/day and 1.17 and 15.29 mg/kg/day for soft stem bulrush, wool grass, soft rush and cattail in the compartments receiving landfill leachate and tolerance concentration, respectively. The concentration of manganese in the soil of the control compartment of soft rush increased at an average rate of 0.25 mg/kg/day while the concentration of manganese in the soil of the control compartments of soft stem bulrush, wool grass and cattail decreased at average rates of 0.70, 0.17 and 0.36 mg/kg/day, respectively. The highest amount of manganese accumulated in the total plant tissues was in soft stem bulrush with concentrations of 1202, 957 and 709 mg/kg and the lowest was in wool grass with concentrations of 921, 649 and 370 mg/kg for the compartments receiving tolerance concentration, landfill leachate and control, respectively.

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