Mechanisms of Manganese Removal from Wastewaters in Constructed Wetlands Comprising Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms) Grown under Different Nutrient Conditions

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ABSTRACT: This article discusses key mechanisms involved in removing 1 mg/L Mn from synthetic wastewaters in constructed wetlands comprising water hyacinth (*Eichhornia crassipes* (Mart.) Solms) grown under different nutrient levels of 1-fold (28 mg/L and 7.7 mg/L of total nitrogen and total phosphorus, respectively), 2-fold, 1/4-fold, and 1/8-fold. A mass balance was carried out to evaluate the key removal mechanisms. Phytoremediation mainly due to phytoextraction substantially contributed to manganese removal. However, chemical precipitation was absent, suggesting that manganese has a higher solubility in the given average pH (6.2 to 7.1) conditions in constructed wetlands. Bacterial mediated immobilization mechanisms also did not contribute to manganese removal. Sediments constituted a minor sink to manganese, implying that manganese has a poor adsorption potential. Constructed wetlands comprising water hyacinth are effective at removing manganese from wastewaters despite the fact that the plants are grown under higher or lower nutrient conditions. *Water Environ. Res.*, **81,** 165 (2009).

KEYWORDS: constructed wetlands, manganese, phytoremediation, wastewaters, water hyacinth (*Eichhornia crassipes* (Mart.) Solms).

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Introduction

Manganese (Mn) is a vital element to all forms of life. However, manganese is toxic to humans at very high concentrations (Gunawardhana et al., 2002; Santos-Burgoa et al., 2001); it is also toxic to plants under pH conditions less than 5 (Meagher, 2000). In Sri Lanka, contamination of groundwater and freshwater bodies with manganese and other toxic metals is a serious environmental problem in the industrial areas of Ratmalana and Moratuwa (Gunawardhana et al., 2002). This has been largely attributed to the disposal of industrial wastewaters from the textile dyeing and garment washing industries, which are predominant industries in Sri Lanka, and to open dumping of sludge produced by the wastewater treatment plants encompassing chemical precipitation (Gunawardhana et al., 2002). Furthermore, absence of a discharge standard for manganese has led to indiscriminate disposal of manganese-containing wastewaters leading to widespread pollution of surface waterbodies and groundwater (Gunawardhana et al., 2002). However, legislation currently under consideration by the Sri Lankan government would establish a discharge standard of at least 0.5 mg/L Mn for inland waters. Countries such as India have enacted a similar permissible limit for inland waters for toxic metals, including 0.5 mg/L Mn (Bhatia, 2005). In the United States, a higher standard of 1.1 mg/L Mn for inland waters has been enacted under the National Pollutant Discharge Elimination System (Ye et al., 2001a).

Presently, aeration followed by chemical precipitation are being used to remove manganese and other toxic metals such as iron (Fe) and arsenic (As) from contaminated groundwater and wastewaters in Sri Lanka. However, chemical precipitation is costly, requires intensive management and long-term maintenance, and is sometimes less efficient. Moreover, the hazardous sludge produced poses disposal problems that necessitate secure landfills. Consequently, the hazardous sludge produced is often openly dumped, resulting in recontamination of groundwater and waterways.

Constructed wetlands are effective at removing manganese, iron, and other toxic metals from polluted waters such as acid mine drainage waters (Mays and Edwards, 2001; Ye et al., 2001a, 2001b). In constructed wetlands, in situ processes such as various physicochemical processes and microbial immobilization mechanisms govern metal removal (Gavrilescu, 2004; Jayaweera et al., 2006; Jayaweera et al., 2007, 2008; Kosolapov et al., 2004; Matagi et al., 1998; Song et al., 2001). However, plant uptake or phytoremediation has not been documented to play a key role in removing metals in the case of constructed wetlands planted with emergent aquatic macrophytes such as *Typha latifolia* (Mays and Edwards, 2001; Ye et al., 2001a, 2001b). Nevertheless, recent studies reported that phytoremediation plays a key role in removing metallic pollutants in the case of floating aquatic macrophyte-based treatment systems or constructed wetlands comprising free-floating macrophytes such as water hyacinth (*Eichhornia crassipes* (Mart.) Solms) (Jayaweera et al., 2006; Jayaweera et al., 2007, 2008; Liao and Chang, 2004).

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In the present study, we attempted to investigate the key mechanisms of manganese removal from synthetic wastewaters in floating aquatic macrophyte-based treatment systems comprising water hyacinth using a mass balance analysis. Our research work also focused on the contribution of the plants grown under different nutrient levels to manganese removal mechanisms.

Materials and Methods

Experimental Setup. In this study, four constructed wetlands (floating aquatic macrophyte-based plant treatment systems) comprising water hyacinth grown under different nutrient conditions were simulated in an outdoor tropical environment (i.e., on the premises of the University of Moratuwa, Sri Lanka). In this respect, batch studies were carried out only during daytime using four 590-L-capacity fiberglass tanks. Each tank was filled with 300 L of tap water (which has a temperature in the range of 28 to 33 °C and pH in the range of 6.2 to 7.1, with iron, manganese, and suspended solids levels far less than 0.3 mg/L, 0.01 mg/L, and 5 mg/L, respectively) and 30 healthy young water hyacinth plants (average height of 20 ± 2 cm). The plants were harvested from Bolgoda Lake (6° 45′ N and 79° 55′ E; near Weras Ganga) in Sri Lanka, which has been reported to contain manganese levels far less than 0.05 mg/L and total nitrogen and total phosphorus levels varying in the range of 4.6 to 39.1 mg/L and 0.136 to 1.78 mg/L, respectively (Gamage et al., 2003). The plants were transferred to the university premises in sterile plastic bags. In addition, the plants were thoroughly washed with distilled water prior to acclimatization in each setup for a period of 1 week without any added nutrients and manganese. Thereafter, nutrients of different concentrations were added to each tank. A nutrient solution (i.e., 1-fold) containing 28 mg/L total nitrogen, 7.7 mg/L total phosphorus, 29.3 mg/L K, 20 mg/L Ca (added as $CaCl_2 \cdot 7H_2O$), 18.2 mg/L Mg (added as $MgSO_4 \cdot 7H_2O$), and 23 mg/L Na with trace amounts of manganese (0.2 mg/L) and boron (0.2 mg/L) was used as the standard nutrient solution as in previous studies (Jayaweera and Kasturiarachchi, 2004; Jayaweera et al., 2006; Jayaweera et al., 2007, 2008). However, it should be noted that iron (EDTA-Fe) was not added as a trace element in order to avoid chemical precipitation of iron and subsequent coprecipitation of Mn^{2+}. The 1-fold nutrient solution was added to one of the tanks, which was labeled as 1-fold setup. Furthermore, 2-fold (56 mg/L total nitrogen and 15.4 mg/L total phosphorus), 1/4-fold (7 mg/L total nitrogen and 1.93 mg/L total phosphorus), and 1/8-fold (3.5 mg/L total nitrogen and 0.96 mg/L total phosphorus) nutrient solutions were prepared from the 1-fold nutrient solution and added to the remaining tanks labeled as 2-fold, 1/4-fold, and 1/8-fold setups, respectively. It should be noted that in this study the 2-fold and 1-fold setups were classified as nutrient-rich setups and the 1/4-fold and 1/8-fold setups were classified as nutrient-poor setups. This is because previous studies carried out in Sri Lanka revealed that water hyacinth growth is intensive (with shoot lengths greater than 35 cm and densities in the range of 80 to 90 plants/m^2) in waterbodies having total nitrogen levels exceeding 20 mg/L (Kasige et al., 2004).

At the same time nutrients were added, manganese-containing industrial wastewaters were synthetically prepared by spiking the water in each tank with analytical reagent-grade $MnCl_2 \cdot 4H_2O$ to yield a contaminant concentration of 1 mg/L Mn. It should be noted that this study targets a composite of textile-dyeing and garment-washing wastewaters, which have been reported to contain manganese levels in the range of 0.92 to 1 mg/L (Gunawardhana et al., 2002). After the addition of $MnCl_2 \cdot 4H_2O$, the water in each tank was thoroughly stirred (to enhance dissolution) using a long glass rod.

The aforementioned nutrient solutions were added every 3 weeks (to supplement the essential nutrients that are required for plant growth) without draining and refilling the tanks. Nutrient addition was carried out after harvesting the plants and sampling the wastewaters and sediments for manganese analysis. Furthermore, tap water was added to the tanks to maintain the wastewater volume at 300 L (i.e., when it was required) and the total nitrogen and total phosphorus content in the wastewaters were then analyzed prior to nutrient addition (hence, to ensure that the required total nitrogen and total phosphorus levels in the four setups are maintained). To counter water loss due to evapotranspiration at the higher ambient temperatures and, thereby, to prevent any phytotoxicity caused to the plants by the presence of a higher content of total dissolved solids (TDS) in the solutions, tap water was added as previously explained (i.e., when required) to maintain the same level (300 L) in each tank.

In addition to the aforementioned four setups, a control setup without water hyacinth was also investigated. The control was run using only tap water spiked with analytical reagent-grade $MnCl_2 \cdot 4H_2O$ to yield a contaminant concentration of 1 mg/L Mn.

Sampling and Analysis of Manganese. Plants were cultured for a period of 15 weeks from the time of acclimatization. From each tank of water hyacinth, three plants were randomly harvested each week; the plants were then separated into aerial tissues (leaves and stems) and roots. The separate parts were oven-dried at 103 to 105 °C for 24 hours to determine the dry weights. Three 1-g samples of these oven-dried separate tissues were then ashed in a muffle furnace at 550 °C for 4 hours. The ash was transferred to a 100-mL Pyrex conical flask (Corning Incorporated Life Sciences, Lowell, Massachusetts) containing 10 to 15 mL of concentrated HCl (which was diluted to a 1:1 ratio) and the ash-concentrated HCl mixture was heated for 10 minutes using a steam bath (i.e., until the ash completely dissolved and the mixture turned pale or dull yellow in color). Thereafter, the resulting solution was cooled and then filtered (through Whatman No. 42 filter paper; Whatman plc, Kent, United Kingdom) to a 100-mL volumetric flask. The filtrate was diluted to 100 mL with distilled water prior to the analysis of manganese.

Grab samples of wastewater were also collected from each tank in triplicate at the time the plants were harvested and the samples were acidified to a pH less than 2 with concentrated HNO_3. Simultaneously, sediments including detritus produced by senescing plant tissue were also collected at the time of plant harvesting by placing three equal-size petri dishes at the bottom of each tank. The collected sediments were removed by placing an inverted petri dish on each of the three petri dishes and, after removing these petri dishes, three clean petri dishes that were previously washed with concentrated HCl and then with distilled water were placed in the tanks. All the sediment samples were first filtered then oven-dried at 70 to 80 °C for 24 hours.

All the wastewater samples were acid-digested in an autoclave at 120 °C and 1.5 psig for 30 minutes using a sample volume of 100 mL, 5 mL (5 volumes) of concentrated HCl, and 1 mL (1 volume) of concentrated HNO_3 (American Society for Testing and Materials, 1991). Similarly, in the case of sediments, acid digestion was carried out using 100 mL of distilled water (as for the wastewater samples) with 5 mL (5 volumes) of concentrated HCl and 1 mL (1 volume) of concentrated HNO_3 (American Society for Testing and Materials, 1991). The acid-digested wastewater and

sediment samples were refiltered (through Whatman No. 42 filter papers; Whatman plc) and the filtrates were topped with distilled water up to 100 mL prior to manganese analysis. All the wastewater, plant, and sediment manganese extracts were stored at a temperature less than 2 °C until analysis was performed. Quantitative analysis of manganese was carried out using a flame atomic absorption spectrophotometer (AAS) (GBC 932 Plus; GBC Scientific Equipment Pty. Ltd., Dandenong, Victoria, Australia) at a wavelength of 279.5 nm. This metal was analyzed in the AAS using an air/acetylene flame. The AAS instrument was calibrated within the linear range of analysis and a correlation coefficient of 0.98 or greater was obtained for the calibration curve. The AAS instrument was periodically checked throughout the analysis with known standards. It should be noted that the AAS instrument measured 3 readings and the average of the 3 readings were taken into consideration for the mass balance. Analytical precision was ±0.01 mg/L and the relative error for the triplicate determinations were less than 5%.

Identification of Manganese-Oxidizing Bacteria and Determination of Bacterial Density. Prior to the beginning of the study, some water hyacinth root samples were also collected using sterile containers to identify the presence of any Mn^{2+}-oxidizing bacteria. Root samples and sediment samples were also collected separately during the study period (i.e., at the time the plants were harvested) to study the variation in the density of the identified bacteria. In this respect, the dilution plate method was used to determine bacterial densities expressed as the number of colony forming units per 1 g of roots or sediments by wet weight. The dilution plate method was carried out in accordance with Bergey's Manual (Buchanan and Gibbons, 1974). Sterile agar (containing 5 g of peptone, 3 g of beef extract, and 15 g of agar dissolved in 1 L of distilled water) was used as the nutrient media in duplicate and the dilutions were prepared by dissolving 1 g of roots or sediments in 99 mL of sterile distilled water. Mixing of the root and sediment samples were carried out using a shaker (Guwina Hoffmann 221/UA.Pm1 72/35; Guwina Hoffmann, Berlin, Germany) at 200 rpm for at least 1 hour. Additionally, dilutions of 10^{-5} and 10^{-7} were used as the study progressed to avoid colony overlapping problems in the petri plates. Inoculated plates were then incubated at 28 ± 1 °C for 24 hours and the total number of colonies and different types of colonies (depending on colony morphology) were counted after the incubation period.

Bacterial differentiation was initially done by using morphological features of colonies. An identification number was given to each morphologically different colony. Pure cultures were prepared by streak plate method using nutrient agar medium. Stock cultures were prepared using nutrient agar slants for further identification processes. Finally, bacterial identification was carried out using a scanning electron microscope (Topcon ABT-32; Topcon Corporation, Tokyo, Japan), morphological features (i.e., Gram staining technique) and biochemical characteristics of cells in accordance with Bergey's Manual (Buchanan and Gibbons, 1974).

Variation of Manganese in Different Components of the Constructed Wetlands. A detailed mass balance analysis was carried out to investigate the key mechanisms of manganese removal. In this respect, the mass balance analysis was conducted using a Microsoft Excel 2003 spreadsheet (Microsoft Corporation, Redmond, Washington) that incorporated continuity equations. After completion of the mass balance analysis, the amount of manganese partitioned into each component of the constructed wetlands at any given time period was expressed as a percentage.

Results and Discussion

Results of the mass balance analysis revealed that manganese removal was largely governed by phytoremediation, with all four setups exhibiting the highest manganese removal efficiencies during the period of maximum phytoremediation (Figures 1 and 2). Moreover, the four setups reported the lowest manganese levels (<0.2 mg/L) in the wastewater during the period of maximum phytoremediation (Figure 3). In contrast, the control setup showed a loss of 1 to 7.2% of manganese (data not shown), and this manganese removal may have been attributed to adsorption to the tank walls.

Analysis of the aerial tissues and roots of water hyacinth showed that most of the phytoremediated manganese was localized in the aerial tissues, suggesting that phytoextraction was the key mechanism of phytoremediation (Figure 4). However, other reports have shown that water hyacinth accumulates higher levels of manganese in roots than in aerial tissues (Abou-Shanab et al., 2007; Mehra et al., 1998; Mishra et al., 2008; Rodriguez et al., 1998; Soltan and Rashed, 2003; Vesk et al., 1999). According to work by Abou-Shanab et al. (2007), Rodriguez et al. (1998), Soltan and Rashed (2003), and Vesk et al. (1999), plaques of iron oxyhydroxides formed due to radial oxygen loss under low redox potential (Eh) conditions and even due to oxygen evolution as a result of root-associated microbial metabolism have been reported to adsorb or coprecipitate other metals, including manganese. Nevertheless, in this study iron was present at levels far less than 0.3 mg/L; hence, any possibilities of iron plaque formation on the roots were unlikely for significant immobilization of the manganese to occur in the roots. Therefore, based on our results and a review of work by Abou-Shanab et al. (2007), Rodriguez et al. (1998), Soltan and Rashed (2003), and Vesk et al. (1999), it appears that under conditions where metal-to-metal interactions are minimal, manganese in the form of Mn^{2+} (which has a high solubility under the given average pH conditions in the range of 6 to 7) could be readily assimilated by the plant roots and then translocated to the aerial tissues.

Results of this study also revealed that the manganese levels accumulated in the plant biomass were substantially higher than the manganese levels accumulated by water hyacinth growing in Bolgoda Lake, where manganese concentrations in water hyacinth were in the range of 90 to 100 mg/kg dry weight (unpublished data). Furthermore, manganese levels accumulated in the plant biomass were substantially higher (especially during the period of maximum phytoremediation) than the manganese levels accumulated by water hyacinth growing in other freshwater bodies (Figures 1 and 4). For example, Abou-Shanab et al. (2007) revealed that water hyacinth growing in Lake Mariout, southwest of Alexandria, Egypt (where the manganese concentrations in the lake were around 1.8 mg/L), contained 112 mg/kg Mn dry weight. According to Tiwari et al. (2007), manganese concentrations in water hyacinth harvested from Shahpura Lake, Bhopal, India (where the manganese levels in the lake were in the range of 0.02 to 0.09 mg/L), were variable in the range of 409.1 to 929.3 mg/kg dry weight. Studies carried out by Mehra et al. (1998) documented that the manganese content in water hyacinth rooted along the riverbanks of the River Yamuna in Delhi, India, was around 580 to 591 mg/kg dry weight. However, recent studies carried out by Mehra et al. (2000) revealed that the manganese content in water hyacinth occurring along the riverbanks of the River Yamuna was in the range of 200 to 800 mg/kg dry weight (except at a few locations where the plant biomass

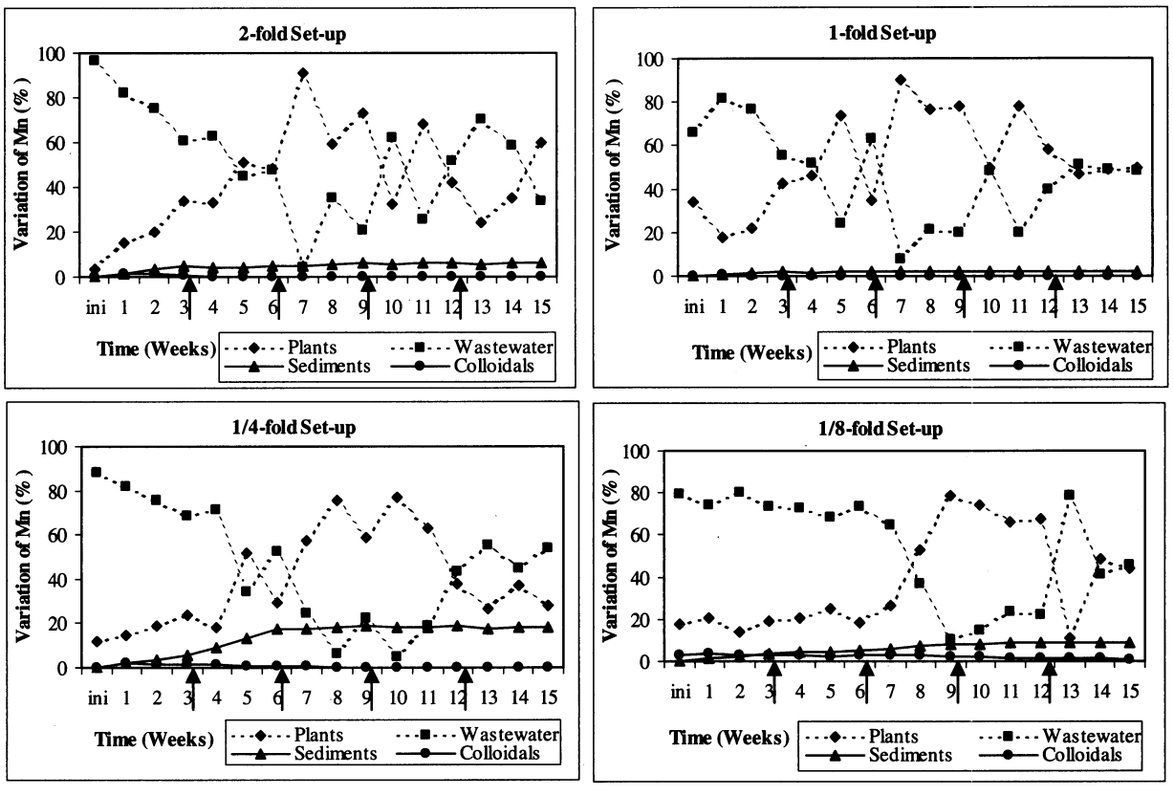

Figure 1—Variation of manganese in constructed wetlands. Note that "ini" denotes the initial week of nutrient addition with $MnCl_2 \cdot 4H_2O$ and the manganese levels remaining in the wastewater during this period present the levels detected approximately 6 hours after the addition of manganese. The arrows denote the addition of nutrients.

manganese levels had exceeded 1000 mg/kg Mn dry weight). The only exception is that water hyacinth occurring in the Nile River in Egypt were found to contain higher manganese concentrations (manganese levels were in the range of 1925 to 2275 mg/kg dry weight) than the concentrations accumulated by the plants in the present study (except for the plants cultured in the 1/8-fold setup during the period of the 8th to 11th weeks) (Soltan and Rashed, 2003; Figure 4).

Recent studies documented that the daily net growth rate of water hyacinth is at a maximum when the plants have attained 5 to 6 weeks of age and removal of nutrients maximized during the period of maximum growth (Jayaweera and Kasturiarachchi, 2004). Because manganese is also an essential metal to plants, it was expected that water hyacinth would maximize phytoremediation of manganese during the period of maximum growth. Nevertheless, our results showed that water hyacinth maximized manganese removal after the period of maximum growth (Figure 1). One plausible explanation is that plant maturity may have played an important role in manganese removal. This is because plants with higher maturity stages are known to have higher capabilities for absorption as they have a larger biomass that could be associated with a large number of active absorption sites (Choo et al., 2006). Furthermore, an increase in metal accumulation in matured plant tissue has been reported due to increased permeability and metabolic activities associated with increasing age (Choo et al., 2006).

Water hyacinth cultured in the nutrient-rich 2-fold and 1-fold setups showed higher phytoremediation efficiencies of 91 and 90%, respectively, during the 7th week, with a plant biomass accumulation of approximately 1363 mg/kg Mn dry weight and 1198 mg/kg Mn dry weight in the 2-fold and 1-fold setups, respectively (Figures 1 and 4). However, the plants cultured in the lowest nutrient-containing 1/8-fold setup showed higher plant biomass accumulations toward the latter stages of the study (Figure 4). This may have been attributed to the fact that the mechanisms involved in root uptake of essential elements as manganese were activated during highly nutrient-starved conditions (Kumar et al., 1995).

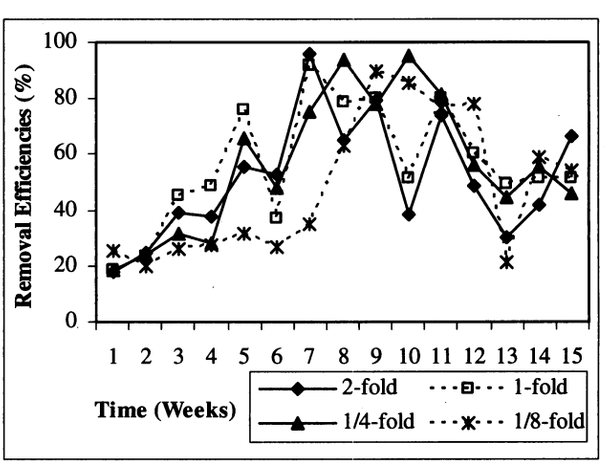

Figure 2—Variation of manganese removal efficiencies from wastewaters.

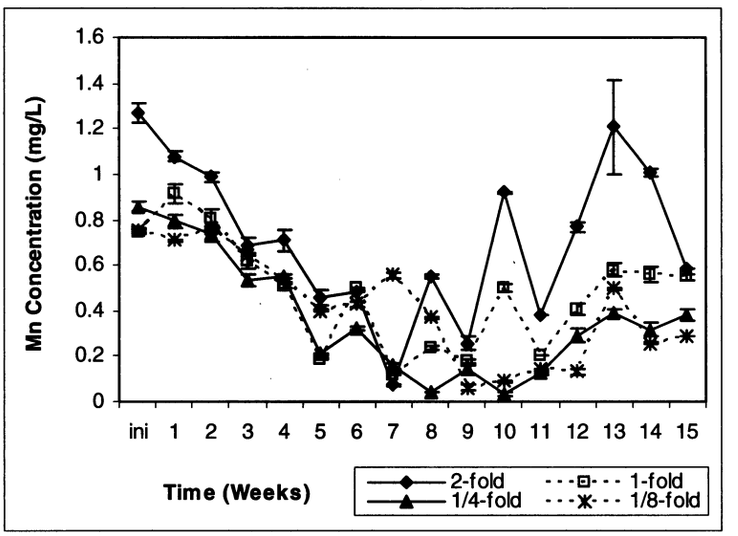

Figure 3—Variation of manganese levels in constructed wetlands. Vertical bars denote standard errors (n = 3).

According to reports by Liao and Chang (2004), Wang et al. (2002), and Zhu et al. (1999), from the standpoint of phytoremediation, a good phytoremediator is regarded as a plant that has an ability to accumulate more than 5000 mg/kg dry weight of a given element and to exhibit a bioconcentration factor (BCF) (i.e., the ratio of the metal concentration in the plant tissues at harvest to the initial metal concentration in the external environment) of 1000 or more. In this study, water hyacinth did not accumulate manganese greater than 5000 mg/kg dry weight. Therefore, only the BCF was considered to evaluate the effectiveness of the plants as a phytoremediator. Hence, in light of the results presented in Figure 4 (which also shows the BCF by considering the total manganese levels accumulated in plant tissue at harvest and the initial manganese levels), the plants met the criteria during the period of maximum phytoremediation, thereby further justifying that the plants played a crucial role in the removal of manganese. In the case of the 1/8-fold setup, the BCF was greater than 2000 during the period of the 8th to 11th weeks (Figure 4).

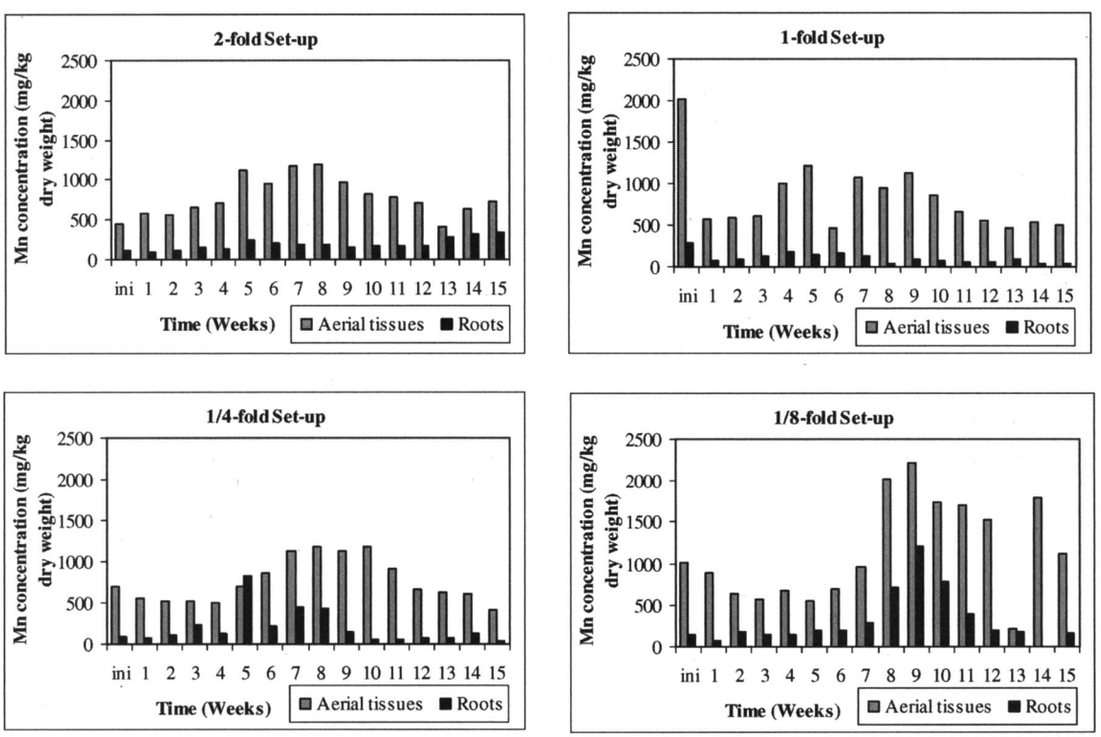

Figure 4—Total concentration of manganese (in mg/kg dry weight) in water hyacinth biomass during different stages of plant growth.

Table 1—Predominant bacterial types occurring in the roots of water hyacinth harvested from Bolgoda Lake, Sri Lanka. Note that densities are expressed as colony forming units per gram of roots by wet weight.

	Setup			
Bacterial type	**2-fold**	**1-fold**	**1/4-fold**	**1/8-fold**
Initial week (before treatment)				
Azotobacter	10^4–10^5	10^4–10^5	1–3 × 10^4	1–2 × 10^4
Clostridium	10^4–10^5	10^3–10^4	10^3–10^4	1–3 × 10^4
Enterobacter	10^4–10^5	1 × 10^4	1 × 10^4	10^3–10^4
Pseudomonas	10^3–10^5	1 × 10^4	10^3–10^4	1–2 × 10^4
Staphylococcus	1 × 10^4	10^4–10^5	1 × 10^4	1–2 × 10^4
15^{th} week				
Enterobacter	1.1–1.2 × 10^3	10^3–10^4	1.1–2 × 10^4	1.7–1.8 × 10^3
Pseudomonas	10^3–10^4	10^3–10^4	1.4–1.5 × 10^3	1.3 × 10^3
Staphylococcus	10^3–10^4	10^3–10^4	1.1 × 10^3	1 × 10^3

It was evident that the plants cultured in the four setups were tolerant of the manganese levels used in the present study as the plants did not show any signs of phytotoxicity such as chlorosis of leaves and the appearance of necrotic spots on mature leaves. However, the plants did not exhibit any continuous removal of manganese throughout the study period. This is because the plants exhibited signs of active effluxing of manganese, possibly as a mechanism of avoiding phytotoxicity from excessive manganese accumulations (Figure 1) as has been observed in other studies for metals such as iron, aluminium (Al), cadmium (Cd), and zinc (Zn) (Jayaweera et al., 2006; Jayaweera et al., 2007, 2008).

In this study, abiotic oxidation of Mn^{2+} to Mn^{4+} and subsequent chemical precipitation in the form of MnO_2 and $Mn(OH)_4$ did not contribute to manganese removal though formation of manganese-containing colloidals (precipitates or filterable manganese) and, later, manganese-containing sediment accumulation was evident (Figure 1). In fact, the accumulation of manganese-containing sediments seemed to be not substantial (Figure 1), although dissolved oxygen levels were sufficiently high in the range of 7.25 to 7.5 mg/L at the time of commencement of the study (data not shown). In general, approximately 0.15 to 0.30 mg/L of dissolved oxygen is required to oxidize 1 mg/L of Mn^{2+} either partially to Mn^{3+} or completely to Mn^{4+} (Johnson and Younger, 2005). The key explanation to account for the absence of chemical precipitation of manganese is that the kinetics for manganese oxidation are slow (i.e., the activation energy required for Mn^{2+} oxidation is high) and manganese has a higher solubility, hence requiring higher pH conditions in the range of 8 to 8.6 for chemical precipitation to occur (Batty and Younger, 2007; Batty et al., 2002; Hallberg and Johnson, 2005; Johnson and Younger, 2005; Sheoran and Sheoran, 2006; Town and Filella, 2002; Ye et al., 2001a, 2001b). In other words, chemical precipitation of manganese does not readily occur in constructed wetlands comprising water hyacinth stands, where the average pH is in the range of 6 to 7.

Moreover, this study showed that there was no bacterial mediated oxidation and subsequent precipitation of MnO_2 because our preliminary bacterial identification test results did not reveal the presence of any Mn^{2+}-oxidizing bacteria such as *Leptothrix*, *Sphaerotilus*, *Metallogenium*, and *Crenothrix* sp. in the water hyacinth roots in moderately high or high densities (Table 1). Additionally, there was no evidence to manifest that dissimilatory SO_4^{2-} reduction mediated by sulfate-reducing bacteria (SRB) governed manganese removal as the study progressed. This is because any generation of H_2S gas was undetected (olfactory detection) and the pH in any of the setups did not increase significantly, thereby manifesting that HCO_3^- alkalinity generation was insignificant (data not shown). Furthermore, the redox potential in the wastewater column in any of the setups did not decrease to low values in the range of −200 to −100 mV as the study progressed (data not shown), although drastic depletions in the dissolved oxygen levels were noticed in the nutrient-rich 2-fold and 1-fold setups (data not shown) due to rapid vegetative reproduction of the plants, thereby intensely covering the tanks. However, according to Hallberg and Johnson (2005), manganese does not readily form an insoluble sulfide phase, although other studies have shown that Mn^{2+} ions could form MnS (Cabrera et al., 2006; Kosolapov et al., 2004; La force et al., 2002).

Therefore, it seems that portions of manganese that were immobilized in the form of manganese-containing colloidals (which later settled as sediments under calm and quiescent conditions) may have been a consequence of the formation of rhodochrosite ($MnCO_3$). This reaction is known to occur when carbonates (produced as a byproduct of the respiration of bacterial biofilms present in the water hyacinth roots) react with Mn^{2+} (Green et al., 2003; Hallberg and Johnson, 2005; Hansel et al., 2001; La force et al., 2002; Sheoran and Sheoran, 2006). However, formation of $MnCO_3$ was not substantial considering the pattern of manganese-containing sediment accumulation (Figure 1). Therefore, it seems that the partial pressure of CO_2 was not sufficiently high during the study period (Green et al., 2003; and Sheoran and Sheoran, 2006), suggesting that bacterial biofilm activity was not high during daytime to result in high levels of CO_2. This is evidenced by the fact that the bacterial biofilms did not show any significant exponential growth throughout the study as shown in Table 1 (except for the fact that the *Azotobacter* sp. showed a complete elimination in the roots and sediments from the 1st week and *Clostridium* sp. also showed a complete elimination in the roots from the 2nd week). The exact mechanisms pertaining to this insignificant growth of bacterial biofilms are not known. However, there may have been a possibility that the bacterial biofilms (except for *Azotobacter* sp.), including *Clostridium* strains (which survived in the sediments), showed some resistance to manganese stress. Possible mechanisms of resistance may have been binding of manganese to the anionic polymeric substances (EPS) that encase the biofilms (hence retarding their diffusion within the biofilms) as well as to the cells at the biofilm–bulk liquid interface and exclusion by the cell surface

proteins (Teitzel and Parsek, 2003). It is known that the EPS matrix (comprising polysaccharides, proteins, and nucleic acids) contain negatively charged phosphate, sulfate, and carboxylic acid groups that could bind heavy metals (Teitzel and Parsek, 2003).

In the case of *Clostridium* sp., it appears that the manganese levels used in this study did not have any direct toxic effects because *Clostridium* sp. survived in the sediments with time (data not shown). Therefore, the presence of *Clostridium* sp. in the water hyacinth roots up to the period of the 1st week (data not shown) manifested that certain *Clostridium* strains are aerotolerant (Karnholz et al., 2002; Rolfe et al., 1978), although *Clostridium* sp. are generally known to be obligate anaerobes. Nevertheless, the complete elimination of *Clostridium* sp. in the water hyacinth roots after the 1st week of the study (data not shown) manifested that they were unable to survive the aerobic conditions (which were present in the first few weeks of the study) for longer periods. However, their presence in the sediments manifested that the bottom of the tanks became more anaerobic than the upper layers of the wastewater column as the study progressed. This was attributed to the rapid vegetative reproduction of the plants, thereby covering the entire setups (hence restricting atmospheric oxygen diffusion at the air–wastewater interface) and subsequently providing favorable conditions for the survival of the obligate metal-tolerant strains of *Clostridium*.

Furthermore, the insignificant or low accumulation of manganese-containing sediments suggests that Mn^{2+} ions having a low adsorption potential poorly complexed with sediments including detritus (which were produced by senescing plant tissue, particularly the roots, during the latter stages of water hyacinth growth) (Figure 1). Insignificant complexation of manganese with organic matter has also been confirmed in other studies (Town and Filella, 2002; Ye et al., 2001a, 2001b).

Conclusions

The present study revealed that batch-type constructed wetlands comprising water hyacinth plants could effectively remediate wastewaters having manganese levels in the range of 0.92 to 1 mg/L. It was also concluded that phytoremediation by water hyacinth (largely through the process of phytoextraction) is a significant contributor to manganese removal, regardless of whether the plants are grown under higher or lower nutrient conditions. However, abiotic oxidation of manganese with subsequent chemical precipitation and sedimentation did not contribute to manganese removal, implying that the average pH conditions in constructed wetlands do not provide favorable conditions for chemical precipitation reactions. Additionally, there was no evidence of bacterial mediated mechanisms of manganese immobilization, especially precipitation and SRB mediated reactions. Adsorption to sediments constituted a minor sink to manganese.

Based on the results of this study, it appears that complete harvesting of plants grown under higher nutrient levels is necessary after a period of approximately 7 weeks. Additionally, complete harvesting of plants grown under lower nutrient levels is required after a period of 9 to 10 weeks to maximize the phytoremediation potential of the plants and to simultaneously avoid possible recycling of accumulated manganese through active effluxing.

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