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SUSTAINABLE DEVELOPMENT OF WATER RESOURCES, WATER SUPPLY AND ENVIRONMENTAL SANITATION

Removal Mechanisms and Efficiencies of Selected Heavy Metals in Constructed Wetlands

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*The removal efficiencies and mechanisms of Pb, Cr, Cd and Zn removal were investigated in batch type constructed wetlands comprising water hyacinth (*Eichhornia crassipes* (Mart.) Solms) using a mass balance analysis. This study was conducted for 15 weeks in four 590 l capacity tanks containing 3.1, 5, 0.2 and 1.1 mg/l of Pb, Cr, Cd and Zn respectively. Removal efficiencies from the wastewaters were in the range of 92.1-99.6%, 31.3-100%, 46.3-94.4% and 25.1-94.8% for Pb, Cr, Cd and Zn, respectively. The results showed that water hyacinth roots play a crucial role in the removal of Pb and Cr by means of precipitation, though phytoremediation was insignificant. However, phytoremediation through rhizofiltration seemed to govern the removal of Cd and Zn. We conclude that constructed wetlands comprising water hyacinth is a promising low cost option for removing the above heavy metals within the ranges investigated.*

Introduction

HEAVY metal pollution of soils and freshwater environments is becoming a potential environmental problem in the industrial areas of developing countries due to the disposal of untreated or partially treated industrial wastewaters from various anthropogenic sources such as metal finishing and galvanizing industries (Gunawardhana et al., 2002). At present in developing countries chemical precipitation is being widely practiced to treat heavy metal containing wastewaters. However, these techniques are costly, need continued supervision and sometimes pose operational problems. In the past decades, therefore research efforts have been directed towards the use of constructed wetlands comprising aquatic plants as an alternative low cost means of treating heavy metal containing wastewaters. This is because most wetland plants have a unique ability to take up or phytoremediate heavy metals (Matagi et al., 1998; Zhu et al., 1999). Of particular interest is the tropical free-floating freshwater weed water hyacinth (*Eichhornia crassipes* (Mart.) Solms) due to its exceptional ability to take up heavy metals (Gopal, 1987; Vesik and Allaway, 1997; Chua, 1998; Lytle et al., 1998; Vesik et al., 1999; Zhu et al., 1999; Cordes et al., 2000; Wang et al., 2002; Ingole and Bhole, 2003; Soltan and Rashed, 2003; Jayaweera et al., 2005). Besides phytoremediation in situ processes such as various physicochemical processes and microbial immobilization mechanisms are known to govern heavy metal removal (Kadlec and Knight, 1996; Matagi et al., 1998; Gavrilescu, 2004; Kosolapov et al., 2004). However, the heavy metal removal mechanisms seem to be complex (Matagi et al., 1998). In this paper we attempt to discuss the removal efficiencies and the key mechanisms involved in the treatment of metal finishing wastewaters containing Pb, Cr, Cd or Zn in batch type constructed wetlands (floating

aquatic macrophyte-based treatment systems) comprising water hyacinth stands.

Materials and methods**Experimental set-up**

In this study four batch type constructed wetlands comprising water hyacinths (floating aquatic macrophyte-based treatment systems) were simulated in an open area by using four 590 l capacity fiberglass tanks filled with 300 l of fresh water and 20 healthy young water hyacinth plants (average height of 20 ± 2 cm) collected from various nearby canals infested with this weed. These plants were first acclimatized for a period of one week (i.e., without any nutrients and heavy metals) prior to the startup batch study which was carried out for a period of 15 weeks. A nutrient solution containing 56 and 15.4 mg/l of total nitrogen (TN) and total phosphorus (TP), respectively with 29.3 K mg/l, 18.2 Mg mg/l (as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 20 Ca mg/l (as $\text{CaCl}_2 \cdot 7\text{H}_2\text{O}$), 2.5 Fe mg/l (as EDTA-Fe), 0.4 B mg/l (as H_3BO_3) and 0.4 Mn mg/l ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$) was added to each tank based on the recipes used in other studies (Jayaweera and Kasturiarachchi, 2004; Jayaweera et al., 2005). Simultaneously the water in one of the tanks was spiked with analytical reagent (A.R.) grade $\text{Pb}(\text{NO}_3)_2$ to yield a concentration of 3.1 Pb mg/l and the remainders were individually spiked respectively with A.R. grade $\text{K}_2\text{Cr}_2\text{O}_7 \cdot 5\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ or $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ to yield a concentration of 5 Cr mg/l, 0.2 Cd mg/l and 1.1 Zn mg/l, respectively. The Pb and Zn concentrations were selected for the study based on the results reported in the study carried out by Gunawardhana et al. (2002) whereas the Cr concentration was selected based on previous studies on Cr toxicity limits for water hyacinth (Gopal, 1987; Ingole and Bhole, 2003) and the Cd concentration was selected in

view of the fact that lower concentrations have been reported to result in higher bioconcentration factors (BCF) for Cd in water hyacinth (Wang et al., 2002). After the addition of heavy metal salts water in all tanks were thoroughly stirred (to enhance dissolution) using a long glass rod. Nutrient solutions were renewed once in 3 weeks to supplement the essential nutrients that are required for plant growth and this was done after the harvesting of plants and sampling of wastewaters and sediments for heavy metal analysis. 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, fresh water was added (i.e., when required) to maintain the same level (i.e., 300 l) in each tank.

Sampling and analysis of heavy metals

From each tank 2 plants were randomly harvested each week and the plants were separated into aerial tissues (leaves and stems) and roots. The separate parts were oven dried at 70-80°C for 48 hours in order 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 containing 10-15 ml of conc. HCl (which was diluted to a 1:1 ratio) and the ash-conc. 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 colour). Thereafter the resulting solution was cooled and then filtered to a 100 ml volumetric flask. The filtrate was diluted to 100 ml with distilled water prior to heavy metal analysis.

Grab samples of wastewaters were also collected in duplicate at the time of harvesting the plants and the samples were acidified to a pH less than 2 with conc. HNO₃. Simultaneously sediments including detritus produced by senescing plant tissue were also collected at the time of plant harvesting by placing 3 equal sized petri plates at the bottom of each tank and all sediments were carefully collected for analysis. The sediment samples were first filtered, and then oven dried at 70-80°C for 24 hours. All the water and sediment samples were acid digested to reduce the interference caused by organic matter and to convert metals associated with particulates to a free metal form. Acid digestion of water samples were performed in an autoclave at 120°C and 1.5 psig for 30 minutes using a sample volume of 100 ml, 5 volumes of conc. HCl and 1 volume of conc. HNO₃ (ASTM, 1991). Similarly, in the case of sediments acid digestion was carried out using 100 ml of distilled water as for the water samples with 5 volumes of conc. HCl and 1 volume of conc. HNO₃ (ASTM, 1991). The acid digested water and sediment samples were re-filtered and the filtrates were topped up to 100 ml with distilled water prior to heavy metal analysis.

All the wastewater, plant and sediment metal extracts were acidified and stored at a temperature less than 2°C until analysis was performed. Quantitative analysis of Pb,

Cr, Cd and Zn was carried out using a flame GBC 932 Plus AAS at a wavelength of 220.353 nm, 267.716 nm, 214.438 nm and 213.856 nm, respectively. These heavy metals were analyzed in the AAS using an air/acetylene flame.

Variation of heavy metals in different components of the constructed wetlands

A detailed mass balance analysis was carried out for Pb, Cr, Cd and Zn to identify the key mechanisms involved in the removal of these heavy metals from the wastewaters.

Results and discussion

Removal efficiencies of Pb, Cr, Cd and Zn from the wastewaters

Figure 1 presents the removal efficiencies of the tested heavy metals from the metal finishing wastewaters. According to the results obtained the removal efficiencies were high throughout the study period, ranging from 92.1-99.6%, 31.3-100%, 46.3-94.4% and 25.1-94.8% for Pb, Cr, Cd and Zn, respectively. The tank containing Pb reported higher removal efficiencies consistently throughout the study period. Hence the overall removal efficiencies for the 4 heavy metals seemed to be in the order of Pb > Cr > Cd > Zn.

It was noticed that during most periods of time the Cr, Cd and Zn levels remaining in the wastewaters were low (data not shown) with reference to the US Environmental Protection Agency (USEPA) proposed limits of 0.25 mg/l, 0.1 mg/l and 0.5 mg/l, respectively for metal finishing industry (Bhatia, 2005). However, the Pb levels remaining in the wastewater during most time periods were reported to be slightly higher than the UESPA proposed limit of 0.05 mg/l for Pb (data not shown).

Although the removal efficiencies for the 4 tested heavy metals were high throughout the study period, the pattern of removal efficiencies did not follow a uniform trend. This scenario was more evident in the case of Cr, Cd and Zn. In other words reductions in removal efficiencies were noticed

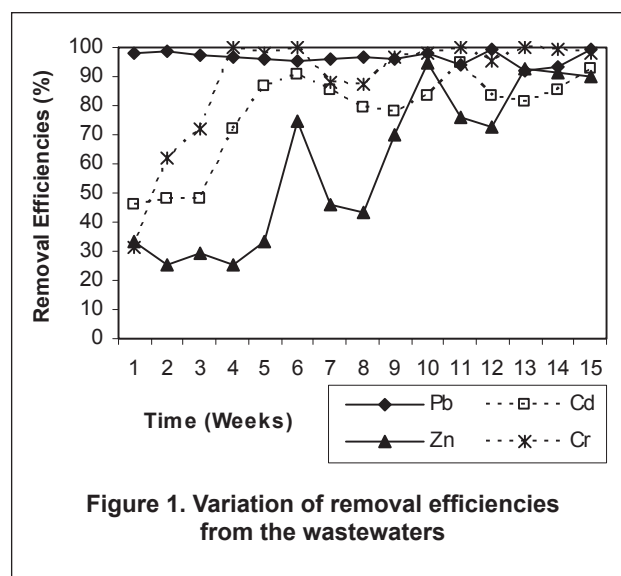


Figure 1. Variation of removal efficiencies from the wastewaters

during certain time periods (Figure 1). These observations therefore suggest that in constructed wetlands removal of heavy metals from wastewaters does not occur continuously unlike in the case of biochemical oxygen demand (BOD) and TN. In other words it seems that the mechanisms involved in the removal of heavy metals are not the same throughout the study period.

Mechanisms of heavy metal removal in the constructed wetlands

Removal of Pb

According to the mass balance analysis a substantial amount of the added Pb was removed through the formation of Pb containing sediments from the period of 1st week (Figure 2). Further the removed Pb remained more or less immobilized in the sediments throughout the study period, thereby elucidating that any desorption of Pb^{2+} back to the wastewater column was insignificant although acidic pH conditions (pH range of 2.64-5.1) were reported throughout the study period (data not shown). Therefore these observations manifested that Pb was removed due to precipitation followed by sedimentation of flocculated particles and subsequently the Pb ions having a strong adsorption potential due to its smaller ionic radius remained strongly bound to the sediments (Matagi et al., 1998).

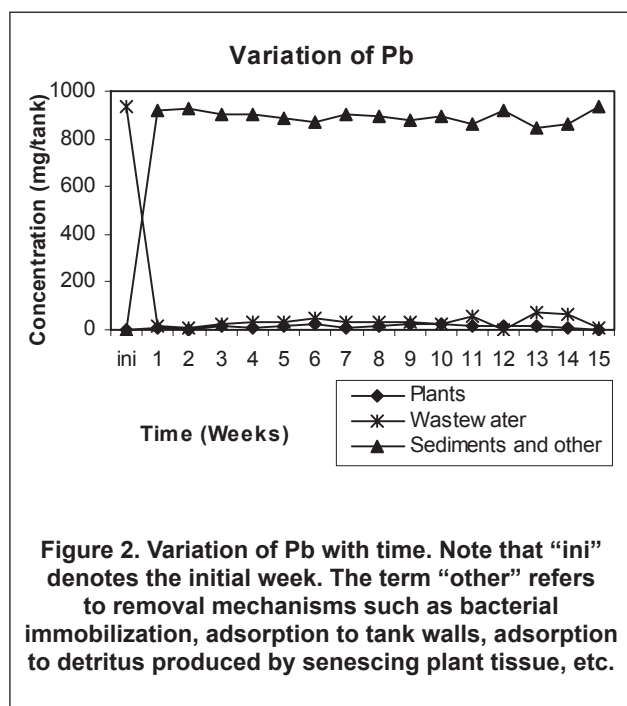
However, the present study manifested that in constructed wetlands comprising water hyacinth stands any removal of Pb is not attributed to chemical precipitation of $Pb(OH)_2$ which has a maximum solubility in the acidic conditions (pH range of 2.64-5.1) observed in this study. Therefore extracellular precipitation mediated by the fine root hairs seemed to play a dominant contribution to Pb removal (Dushenkov et al.,

1995; Choo et al., 2005). Our visual observations showed the presence of white precipitates on the side walls and bottom of the tank and even on the root surfaces, thereby suggesting that these precipitates are probably some lead phosphate ($Pb_3(PO_4)_2$) complexes. Similar mechanisms involving extracellular root-mediated precipitation have been reported in the case of water hyacinth for Fe^{2+} and Mn^{2+} under flooded conditions (Vesk and Allaway, 1997; Vesk et al., 1999; Soltan and Rashed, 2003), for Pb^{2+} at levels exceeding 70 mg/l in hydroponically grown *Brassica juncea*, Sunflower (*Helianthus annuus* L.) and various species of grass (Dushenkov et al., 1995), for Ni^{2+} at 500 μ M in *Berkheya coddii* (Robinson et al., 2003) and for Al^{3+} in Norway spruce (*Picea abies* [L.] Karst.) (Heim et al., 2000) and in *Phragmites australis* (Batty et al., 2002). According to Batty et al. (2002) this mechanism is attributed to the leakage of O_2 from the roots mediating the precipitation of the metals, hence to avoid phytotoxicity.

It should be noted that the rate of Pb removal from the wastewater through flocculation and subsequent sedimentation was high during the period of initial to first weeks (Figure 2), hence elucidating that the water hyacinth roots secreted organic exudates (possibly peptides) to result in rapid coagulation and subsequent flocculation into large, denser flocs (Dushenkov et al., 1995; Choo et al., 2005). Also any secretion of extracellular polymeric substances or extracellular biopolymers (ECPs) by metal immobilizing bacterial biofilms occurring in the water hyacinth roots possibly further enhanced the flocculation of Pb rich colloids and subsequently rapid sedimentation (You et al., 2005). Moreover the addition of divalent cations such as Ca^{2+} and Mg^{2+} as nutrients may have improved any bioflocculation by acting as a bridge between the negatively charged sites of the ECPs, a phenomenon known as cation bridging model (You et al., 2005).

Removal of Cr

The pattern of Cr removal from the wastewater seemed to be more or less similar to Pb removal (Figure 3). However, Cr removal was attributed to the precipitation of Cr as Cr_2O_3 and $Cr(OH)_3$ following the reduction of Cr^{6+} to Cr^{3+} . It should be noted that any Cr^{3+} ions formed readily precipitates when pH is above 4 (Chen and Cutright, 2003) and it seems that any reduction of Cr^{6+} to Cr^{3+} was mainly a consequence of the secretion of root exudates such as organic acids by the water hyacinth, considering the fact that the pH remained acidic in the range of 2.89-5.5 throughout the study period (data not shown). Further any Cr removal may have been due to the reduction of Cr^{6+} to Cr^{3+} mediated by the bacterial biofilms occurring in the roots of water hyacinth with subsequent precipitation of Cr_2O_3 and $Cr(OH)_3$ (Faisal and Hasnain, 2005). In fact recent studies manifested that water hyacinth roots harbour bacteria of the genera *Pseudomonas* and *Bacillus* in significant numbers (Jayaweera et al., 2005). It is noteworthy that these bacterial types have an ability to



reduce Cr⁶⁺ to Cr³⁺ (Ishibashi et al., 1990; Wang and Xiao, 1995).

Since reduction of Cr⁶⁺ to Cr³⁺ and subsequent precipitation of Cr was noticed the yellow colour of the wastewater disappeared and turned colourless around the period of 3-4th weeks. Simultaneously the rate of formation of Cr containing sediments showed a marked increase up to the period of 4th week. However, some solubilization of Cr containing sediments was noticed in the period of 7th week with a simultaneous increase in the Cr levels in the wastewater (Figure 3). The mechanisms behind this observation remain unclear, though solubilization of Cr containing sediments for subsequent uptake by algal proliferations (which was observed in this tank from the period of 7th week) may be a possibility. Nevertheless, immobilization of Cr in the form of sediments occurred from the period of 8th week and then remained more or less stabilized throughout the study period. One possibility to account for this particular observation is that any Cr⁶⁺ present in the wastewater during the period of 7th week may have been reduced by humic material produced by the necrosing and senescing plant tissue. Therefore once the Cr³⁺ was formed it remained strongly bound to the organic matter due to its strong adsorption potential (Figure 3).

Removal of Cd

The results of the present study elucidated that phytoremediation of Cd by water hyacinth played a significant contribution to Cd removal. It was noticed that accumulation of Cd by water hyacinth optimized during the period of 3rd and 11th weeks. However, the highest plant biomass accumulation of 164.4 mg/kg dry weight was evident during the 3rd week (Figure 4).

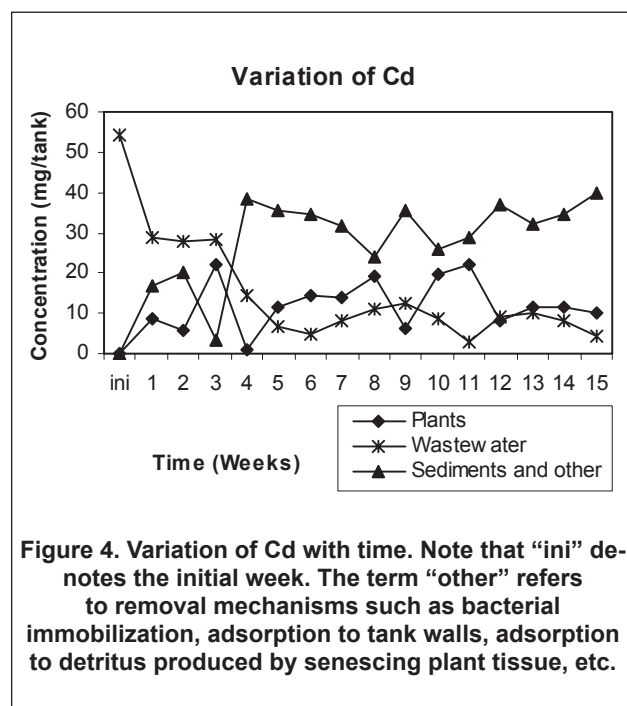
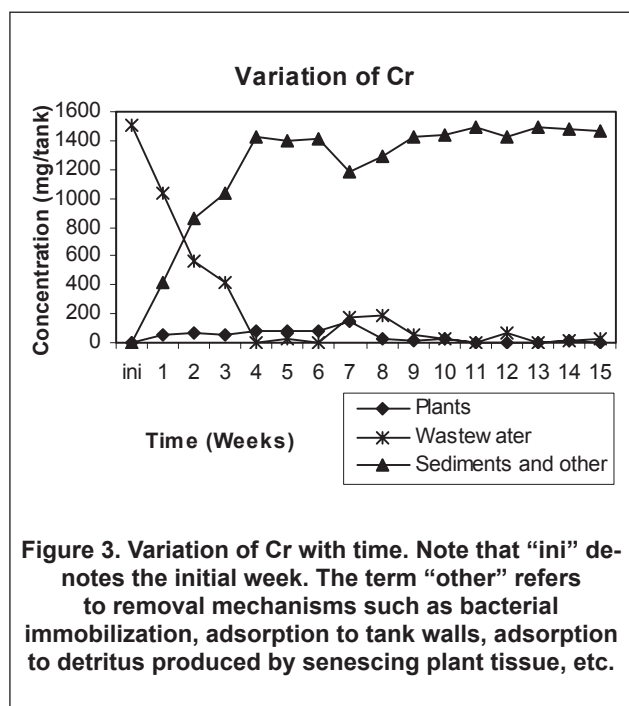
Analysis of the water hyacinth plant tissues revealed that

most of the Cd were localized in the roots than in the aerial tissues justifying that rhizofiltration was the main mechanism of phytoremediation (data not shown). Further the plants appeared to be healthy and no signs of phytotoxicity such as chlorosis of leaves and stunted growth were evident at the end of the study. Therefore it appeared that the plants were able to synthesize phytochelatins particularly in the roots in order to effectively detoxify Cd as demonstrated in other studies (Ding et al., 1994).

Nevertheless, it should be noted that phytoremediation of Cd did not occur continuously as the plants also demonstrated various mechanisms of avoiding phytotoxicity from a very high accumulation of Cd. Basically the results manifested that root-mediated precipitation of Cd (possibly Cd as Cd₃(PO₄)₂) significantly occurred during the period of 2nd, 4th, 9th, 12th, 14th and 15th weeks with a simultaneous increase in the accumulation of Cd containing sediments and a reduction in the phytoremediation efficiencies. In other words it seems that Cd rich colloids were formed inside the root cells and involved a release of root exudates, probably some peptides to flocculate and subsequently settle the Cd rich colloidal particles. In addition, water hyacinths demonstrated some signs of active effluxing (Figure 4).

Removal of Zn

Phytoremediation by water hyacinth mainly through the process of rhizofiltration also played an important contribution to Zn removal as was observed in the case of Cd (Figure 5). It was evident that phytoremediation of Zn optimized at the 10th week with plant biomass accumulation of 775.5 mg/kg dry weight. It seems that water hyacinth has an ability to synthesize phytochelatins to effectively detoxify Zn also. Nevertheless, phytoremediation of Zn was also not a continu-



ous mechanism to govern Zn removal. This is because the plants actively effluxed Zn²⁺ back to the wastewater column particularly during the period of 7th, 8th, 11th, 12th and 14th weeks as mechanisms of avoiding phytotoxicity (Figure 5). Also during these periods of active effluxing it seems that the plants formed Zn rich colloids (possibly as Zn₃(PO₄)₂) inside the roots and then released to the wastewater column possibly with some root exudates for subsequent flocculation and sedimentation (Figure 5).

In addition to removal of Zn by the water hyacinth, some immobilization of Zn in the form of sediments was noticed during the first few weeks of the study (Figure 5). This formation of Zn containing sediments during the first few weeks too may have contributed to removal through complexation with ECPs produced by bacterial biofilms present in the water hyacinth roots. However, the accumulation of Zn containing sediments gradually increased from the period of 7th week and after the period of 11th week immobilization as Zn containing sediments was relatively significant (Figure 5). This phenomenon was mainly due to detritus production by the senescing plant tissue particularly from the roots. Also it should be noted that in comparison with other tanks there was a significant increase in the wastewater pH from the 9th week to a value of around 6.14 at the 15th week (data not shown). Therefore there was a possibility that adsorption of Zn²⁺ or hydroxide precipitation through secretion of NH₃ or organic bases by bacterial biofilms occurring in the water hyacinth roots or on side walls of the tank also played a significant contribution to Zn removal with time (Chen and Cutright, 2003).

However, there was no evidence to manifest that any significant proliferation of sulphate reducing bacteria (SRB) occurred with time (as was not observed in other tanks),

hence Zn removal was not attributed to dissimilatory SO₄²⁻ reduction. This is because the redox potential (Eh) in the wastewater column did not decrease to low values in the range of -200 mV to -100 mV with time (data not shown) and any generation of H₂S (with its characteristic rotten egg odour) was undetected.

Disposal of water hyacinth containing heavy metals and settled metal precipitates

Although batch type constructed wetlands comprising water hyacinth plants are effective in removing heavy metals from wastewaters, careful management is necessary to ensure the sustainability. In this respect complete harvesting of plants showing signs of phytotoxicity and even senescing would be of paramount importance in order to minimize recycling of accumulated metals through mechanisms such as active effluxing. This is very important in the case of batch type constructed wetlands designed to remove Cd and Zn considering the fact that the phytoremediation was a significant mechanism. Moreover some studies have shown that water hyacinth enriched with trace amounts of heavy metals could be utilized for biogas production (Verma et al., 2006), hence the problem of disposal of the plants produced in the phytoremediation process could be significantly reduced.

In addition, the settled precipitates (sediments) need to be regularly removed and disposed of in order to prevent any possible dissolution of the sediments or desorption of sediment adsorbed metal ions particularly under highly acidic conditions. In this respect it is of paramount importance to chemically fix the sediments with cement and then carry out the USEPA defined toxicity characteristic leaching protocol (TCLP) test prior to disposal in secure landfills. Alternately the removed sediments after adequate dewatering may be utilized in the building construction industry particularly in the manufacturing of bricks and tiles (Weng et al., 2003).

Conclusion

This study concludes that batch type constructed wetlands comprising aquatic plants such as water hyacinth are a promising low cost option for removing the tested heavy metals within the ranges investigated. Removal efficiencies from the wastewaters were high throughout the study period in the order of Pb > Cr > Cd > Zn. However, it was noticed that removal of heavy metals is not a continuous process in batch type constructed wetlands comprising water hyacinth stands suggesting that heavy metal removal mechanisms are not the same throughout the study period.

According to the results of this study phytoremediation by water hyacinth mainly through rhizofiltration was an important mechanism in the removal of Cd and Zn, though phytoremediation was not a continuous mechanism due to the fact that the plants demonstrated various active mechanisms of avoiding metal phytotoxicity. Nevertheless, the ability of water hyacinth to remove Cd and Zn in appreciable amounts

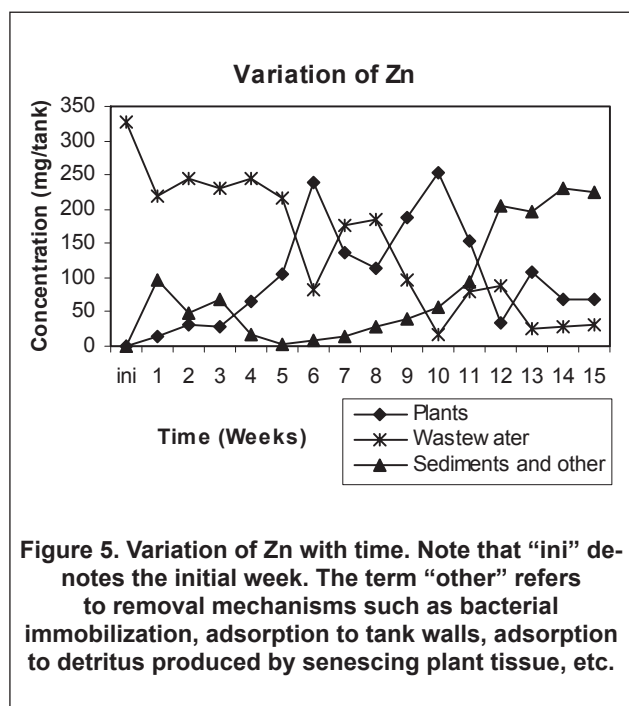


Figure 5. Variation of Zn with time. Note that “ini” denotes the initial week. The term “other” refers to removal mechanisms such as bacterial immobilization, adsorption to tank walls, adsorption to detritus produced by senescing plant tissue, etc.

without showing signs of phytotoxicity elucidated that water hyacinth has an inherent ability to synthesize phytochelatins particularly in the roots and hence to sequester these 2 heavy metals. However, the results of this study manifested that phytoremediation does not play a dominant contribution in the removal of certain heavy metals such as Pb and Cr. Nevertheless, the roots of aquatic plants such as the water hyacinth seemed to play an important role in indirectly removing such heavy metals through the secretion of organic exudates. These root exudates seemed to serve as effective coagulants and flocculants for Pb and Cr, and even as reducing agents for Cr⁶⁺. Further any symbiotic bacterial biofilms present in the roots possibly plays a crucial role in immobilizing such heavy metals, though the present study did not evaluate the role of root associated bacteria in heavy metal removal. In this respect special emphasis will have to be paid in the identification of different extracellular biopolymers (ECPs) specifically produced for different heavy metals so that the real mechanisms could be understood more precisely.

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