LONG TERM PERFORMANCE OF A CONSTRUCTED WETLAND PLANTED WITH TYPHA ANGUSTIFOLIA

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Abstract: Constructed wetlands are often utilized for wastewater treatment, including removal of metals. The removal mechanism is phytoremediation, in which plants are manipulated to immobilize the pollutants. Physico-chemical properties of substrate and its potential for removal of pollutants also affect the performance of constructed wetlands. Many engineering studies of treatment by wetlands use a black box approach. This paper discusses the Zn uptake by Typha angustifolia and its distribution within the shoot (leaves and stem) and root. This was investigated by introducing different operation conditions to the constructed wetland during acclimatization with nutrients, shock loading of Zn at high concentration, acclimatization with nutrients, and gradual variation of Zn loading with incremental increases. The relative changes in the substrate (clay tile) were also investigated by means of XRD, FT-IR and SEM analysis. The results revealed that the clay tile is a promising adsorption material for Zn. In addition, Typha angustifolia is found to contribute to metal trapping into the senescing plants via rhizodeposition and act as a catalyst for biochemical reactions. The interesting feature noted in the wetland was that after nearly two years of operation, almost all clay tiles around the rhizosphere of Typha angustifolia were disintegrated completely, back to the original clay, suggesting that nature helps to sustain the plants by providing an environment with less toxicity. It could be concluded that the long term adaptation of wetland plants to heavy-metal rich environments would not help the sequestration process, and they cannot be considered to be heavy-metal sinks in the aquatic system once this stage is reached.

Keywords: Clay tile, Constructed wetlands, *Typha angustifolia*, Zn

1. Introduction

Heavy metal pollution of water resources due to various anthropogenic activities is becoming a potential environmental problem in the industrial areas in Sri Lanka. The use of constructed wetlands to remove heavy metal containing wastewaters has been considered as an environmentally friendly alternative as opposed to the currently available physical and chemical methods, which are commercially not feasible, either because of high capital and operating costs or the difficulty in treating the chemical sludge being generated. However, in constructed wetlands processes such as various physicochemical, phytoremediation and microbial immobilization are known to govern heavy metal removal. Thus, many engineering studies of treatment by wetlands use a black box approach as the distribution and processes of pollutant removal by the different components are largely unknown.

In this context, the present paper principally discusses the Zn uptake by *Typha angustifolia* and its distribution within the shoot (leaves and stem) and root and any other processes happening in the wetland. To accomplish this, two objectives were defined as:

1. Investigation of Zn concentration in different components of wetland viz plant (*Typha angustifolia*) and substrate (clay tile).
2. Determination of Zn removal mechanism through constructed wetlands.

2. Materials and Methods
2.1 Design and operation of constructed wetland

In this study a plug flow, continuous type SSF wetland having a 1% slope with a maximum depth of 0.6 m and an area of 15 m x 1 m was constructed in an outdoor area at the premises of the University of Moratuwa, Sri Lanka. Perforated tubes (1 inch diameter) were permanently placed at a depth of 0.48 m at the points P-1, P-2 and P-3 in the wetland which are

at a distance of 0.21 m, 7.5 m and 14.8 m, respectively from the inlet end (Fig. 1).

Burnt clay tiles were obtained from the premises of the University of Moratuwa, Sri Lanka. The clay tile material was washed with tap water (pH: 6.2-7.2) several times under high pressure in order to remove soluble inorganic salts and any adhering materials. After thorough washing, the tile material was sun dried and then manually crushed into sizes in the range of 25-50 mm.
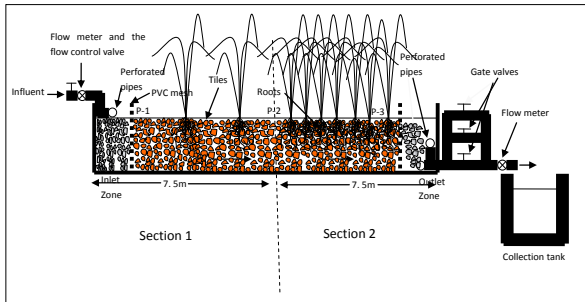

Figure 1: Schematic diagram of the SSF constructed wetland comprises of *Typha angustifolia*

The operation of the wetland was done in four phases. Phase I, where the wetland was acclimatized with nutrients (nitrogen and phosphorus) which were added as urea $(NH_2)_2CO$ and A.R. grade KH_2PO_4, respectively, varying in the range of 31.0-77.6 mg/l and 1.0-10.4 mg/l, respectively for a period of 97 days. This was carried out in order to allow plant growth and more biomass production. The acclimatization was carried out until the plants exhibited signs of optimum nutrient removal. After the acclimatization period, where Phase II was begun with shock loading of Zn at high concentration of 135 mg/L in order to have intense phytotoxicity. Distribution of wastewater to the constructed wetland was done only after sufficient dilution to the desired levels (Table 1). The wetland was operated at a HRT of 7 days during the period of 98-140 days and, on 141st day wetland operation was temporarily suspended as the plants exhibited signs of intense phytotoxicity. Therefore, in the phase III the wetland was again acclimatized with nutrient levels varying in the range of 34.67-48.83 mg/l and 0.16-8.66 mg/l, of total nitrogen and total phosphorus respectively until growth resumed and the plants showed some considerable biomass production (i.e. until the 189th day). Thereafter, (i.e. from 190th day) during phase IV, the wetland was fed at low concentration of Zn (10 mg/L) and operation was carried out at a HRT

of 3.5 days while increasing the subsequent Zn levels up to 185 mg/L. Operation of the wetland continued up to 546th day and thereafter operation was terminated as it was observed that, from the 525th day there was no further removal. During this phase, plants exhibited signs of intense phytotoxicity by way of chlorosis (i.e. from 273th day onwards).

Table 1: Characteristics of wastewater

Parameter	Diluted wastewater
pH	4.90 -7.64
Electrical conductivity (μS/cm)	426.67-1593.67
COD (mg/L)	3.68-36.80
Zn (mg/L)	5.21-194.89
Fe (mg/L)	0.95-23.48
SO_4^{2-} (mg/L)	23.05-179.46

Table 2: Different phase of wetland operation

Phases	Time (days)	Operational condition
Phase I	0-97	Acclimatization with nutrients, no Zn loading
Phase II	98-140	Shock loading of Zn at 135 mg/L reduced gradually
Phase III	141-189	Acclimatization with nutrients, no Zn loading
Phase IV	190-546	Gradual increases of Zn load from 10 mg/L to 185 mg/L

2.2 Characterization of substrate material

The mineral constitution of the clay tile was determined using X-ray diffraction (XRD) techniques (SIEMENS D5000 X-Ray Diffractometer, USA). The functional groups present in the clay tile samples were also characterized by Fourier Transform Infrared (FTIR) Spectroscopy (Nicolet FTIR spectrophotometer, USA). The IR spectra were determined in the wavelength range of 4000-400 cm^{-1}. Scanning Electron Microscopy (SEM) analysis was carried out using the LEO 1420VP Electron Scanning Microscope with the 100×, 500× and 1000× of image magnifications for morphological characterization.
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2.3 Zn mass balance variation in plant and substrate

Plants

Three plants were randomly harvested from the wetland every week at the points P-1, P-2 and P-3. The harvested plants were separated into shoots (i.e. leaves and stems) and roots (below ground parts). The separated parts were then oven dried at 70-80ºC for 48 hours in order to determine the dry weights. Oven dried 1 g samples of each part was ashed in a muffle furnace at 550ºC for 4 hours. The ash was transferred to a 100 ml pyrex conical flask containing 10-15 ml of conc. HCl. Then 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 Zn analysis (Jayaweera et al., 2006; Jayaweera et al., 2007a; Jayaweera et al., 2007b).

Clay tile

Clay tiles were collected at the points of P-1, P-2 and P3 and oven dried at 105±2°C for 2 hours (ASTM: D3976, 1991). Then the samples were ground until all material was finer than 100 mesh (150 μm). Then total digestion of 1 g of clay tile samples were done according to the ASTM: D 4698, 1991. Digested samples were then filtered and filtrates were topped up to 100 ml with distilled water prior to Zn analysis. In addition, sediments and detritus were also collected from the bottom of the wetland at the points P-1, P-2 and P-3, by carefully removing the clay tile from the top-most surface. These samples were first filtered or washed, and then oven dried at 105±2°C for 2 hours prior to the acid digestion.

A detailed mass balance analysis was carried out, and the following mass balance equation was used as described in Dortch and Gerald (1995).

$$QC = \frac{W_L}{1+K\tau} \qquad (1)$$

Where

Q   = total water flow rate exiting the wetland
C   = pollutant concentration of flow exiting the wetland
WL   = total loading of pollutant entering the wetland
K   = bulk loss or removal rate of the pollutant due to physical, chemical or biological processes

τ = hydraulic residence time

In each analysis three replicates were tested and blank run was carried out. All glassware was first rinsed with HNO_3 (1+1), and then with distilled water, before each use. All Zn extracts were stored at a temperature less than 2ºC until analysis was performed. Quantitative analysis of Zn was carried out using a flame atomic absorption spectrophotometer (AAS, GBC 932 Plus, Australia) at a wavelength of 213.9 nm by using an air/acetylene flame.

3. Results and Discussion

3.1 Zn mass balance variation in plant

This study shows Zn concentrations in the rhizomes throughout the study period were higher than those in the leaves of *Typha angustifolia*. Under normal condition, mechanism of heavy metal accumulation within the plants occurred mainly through compartmentalization and sequestration within the root, xylem loading and transport, distribution of metal in the aerial parts and sequestration and storage in leaf cells. This accumulation can be dependent on plant species, growth stage of the plants, pH and heavy metals concentration in the water etc. (Deng et al., 2004; Mishra et al., 2008). However, the contaminants may be released back, either directly by excretion from the leaves (Burke et al., 2000; Weis et al., 2004) or when plant becomes detritus.

However, the interesting feature noted in this study was that following nearly two years of operation (phase IV), almost all clay tiles around the rhizosphere of *Typha angustifolia* have begun to dissolve. When this observation was first made the plants in the wetland seemed to have almost perished completely but not died at all. This phenomenon can be judged as a response of the plant's (*Typha angustifolia*) rhizosphere in an event of long-term exposure to very high heavy metal loading which is reckoned to be a rare phenomenon. Under stressed conditions of heavy metal, plants are adjusted to respond by several different mechanisms.

The first line of defensive response under stressed condition is, increasing of reactive oxygen species in plants. Heavy metals cause formation of reactive oxygen species (ROS) in chloroplasts and peroxisomes. The enzyme superoxide dismutase (SOD), located in the

chloroplasts, counteracts this effect and transforms free O_2 to H_2O_2 and dissolved oxygen (Mittler, 2002).

In addition, plants have a propensity to act as a catalyst for biochemical reactions involving organic acids such as citrate, oxalate, malate, malonate, fumarate, and acetate (Ryan et al., 2001). These weak acids act as anions to chelate metallic ions thus decreasing their phytotoxicity. Organic ligands are rich in cysteine and non-protein thiols, such as phytochelatins and metallothioneins. These phytochelatins are chelate metals and form complexes, which are then transported into the vacuoles (Pal and Rai, 2010). Metallothioneins and metallochaperones contribute to maintain the homeostasis, bind metals, and protect against oxidative stress (Palmer and Guerinot, 2009).

However, adaptability to such a metal stress is multigenic, and plants have different mechanisms for detoxification (Palmer and Guerinot, 2009; Sharma and Dietz, 2009; Pal and Rai, 2010). Most macrophytes play a role in maintaining oxidizing conditions by shoot-to-root oxygen transport. Such conditions promote formation of iron oxides, hydroxides and oxyhydroxides, such as the iron-plaques, and consequently result in metal removal by adsorption and co-precipitation.

Thermodynamic data on the speciation of oxalate at different temperatures, and their complexes with Al in solution, and kinetic data on the rate of decomposition of this organic acid have been studied by Bell and Palmer, 1994; Bell et al., 1994; Harrison and Thyne, 1994; Ganor et al., 2001. Oxalic acid is a simple carboxylic acid that is relatively abundant in soil and groundwater, with concentrations ranging from 10^{-5} to 10^{-3} M in upper soil horizons (Graustein et al., 1977; Crossey, 1991).

3.2 Dissolution of substrate (clay tile)

The mineral dissolution can also be explained as a surface process and therefore it is more appropriate to express the dependence on the initial concentrations (of ions) adsorbed on the surface of tile. However, the dissolution of clay minerals has been the subject of scientific discussions for several decades with no profound reasoning.

The proton-promoted dissolution of kaolinite has been studied by several researchers (Carroll-Webb and Walther, 1988; Carroll and Walther, 1990; Chin and Mills, 1999). The overall dissolution reaction of kaolinite under acidic conditions can be expressed as:

$$Al_2Si_2O_5(OH)_4 + 6H^+ \rightarrow 2\,Al^{3+} + 2H_4SiO_4 + H_2O$$
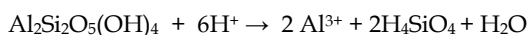
Low-molecular-weight organic acids can especially be found in the rhizosphere. They are produced from decomposition of organic material into soils such as dead litter and roots and the residues of fungi and bacteria. Furthermore, root exudates and leaf washings also contain these kinds of acids. Such organic acids can occur in higher than millimolar concentrations during microbial processes and in root exudates under stressed condition of heavy metals. This phenomenon could also trigger the above-mentioned reaction thereby there could be complete dissolution.

Protons from their dissociation decrease the pH value of systems or free cations at the mineral surface to shift the equilibrium toward dissolution. Anions from their dissociation may affect mineral weathering by affecting the saturation state of the solution with respect to the minerals and the speciation in solution of ions such as Zn, which affects mineral dissolution rate. Moreover, Chin and Mills (1991) have showed the effect of organic acids on the kaolinite dissolution rate under acidic conditions, and found that Al was released faster than Si.

Fig. 2 depicts the structural changes taken place in the form of morphology changes with the loss of sharp edges.

The effect of organic ligands (oxalate, malonate, succinate, salicylate, phthalate, and benzoate) on the dissolution of Al_2O_3 and iron oxides has been studied by some with limited success (Wieland and Stumm, 1992; Ganor and Lasaga, 1994; Chin and Mills, 1991; Ganor and Lasaga, 1994).
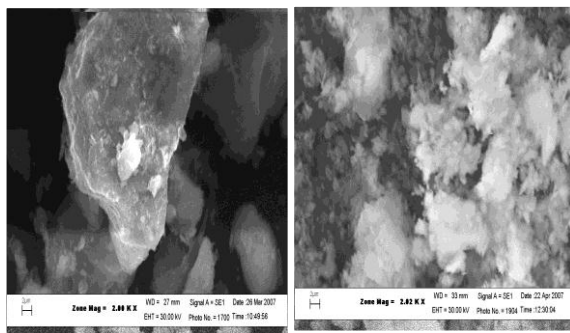
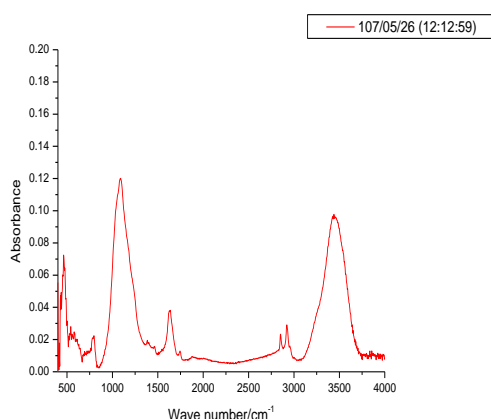Figure 2: SEM analysis of untreated clay tile
and dissolved clay tile

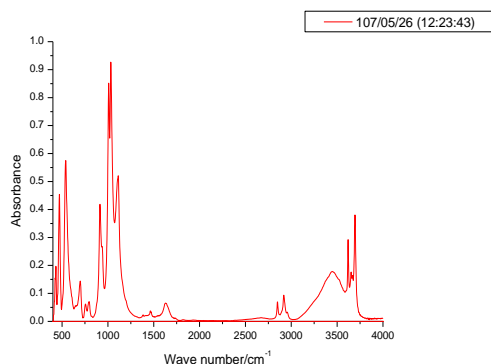Figure 3: FT-IR analysis of treated clay tile

Figure 4: FT-IR analysis of dissolved clay tile

Figs 3 – 6 showed the complete structural change taken place for the tile particles back to original kaolinite with unknown processes.

Under normal conditions, there has been no observation to note such structural changes from most stable oxidized state to somewhat weak state without strong de-bonding mechanisms. The formation of very strong oxidants such as H_2O_2 by the mediation of roots of the plants may have strong link towards breaking the strong ligands back to the original

clay. Nevertheless it was evident that similar observations are reported elsewhere (Mittler, 2002) mainly through the involvement of plants.
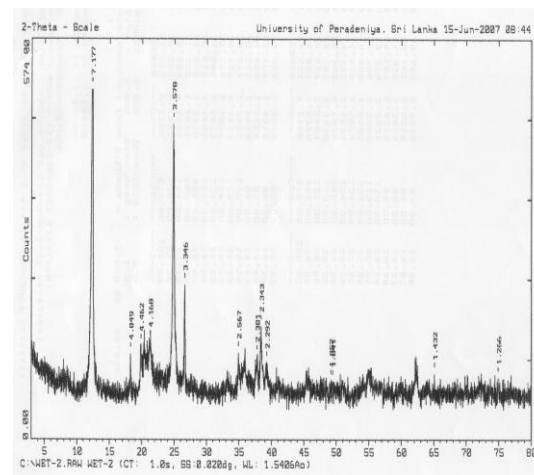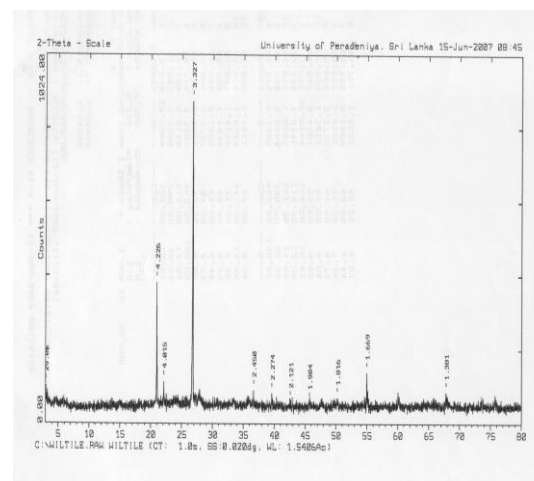

Figure 5: XRD analysis of dissolved clay tile

Figure 6: XRD analysis of treated clay tile

It therefore appears that plants could secrete different chemicals or acids making the substrate very weak in adsorbing heavy metals or make the environment conducive for structural change easily thereby there is less toxicity effect on to the plant growth.

As these observations are found to occur after a considerable period of time, its behaviour is hardly reported or completed omitted in many experiments, pilot studies etc.

CONCLUSION
It could be concluded that the long term adaptation of wetland plants to heavy-metal rich environment would not help reduce the sequestration process there by they can not be thereafter considered to be heavy-metal sinks in

the aquatic system. In other words the plants would survive from intense phytotoxicity by creating an environment with lesser heavy metal persistence though high loading rates are applied.

The exact hypothesis for dissolution of tiles are yet to be postulated even though it is real hence the defence mechanism adopted by plants for their own survival seems to be amazing

Finally long-term application of wetland concept needs to be revisited for removal of heavy metals and with the observation of this study it is not recommended or short term designs preferably one having operations less than a year seems to be more realistic for the removal of heavy metals.

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