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RESEARCH ARTICLE

REMOVAL OF ZINC, LEAD AND CADMIUM BY WATER HYACINTH (*Eichhornia crassipes*)

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ABSTRACT

Under the present investigation efficiency of the aquatic macrophyte *Eichhornia crassipes* was tested for the removal of selected heavy metals (Zn, Cd, and Pb) in laboratory conditions. The work also aims to characterize the associated micro flora of the rhizosphere system of the *Eichhornia crassipes* and wetland system where it grows naturally. Heavy metal content in water and water hyacinth from wetland system were also studied. The results revealed that the heavy metal concentration of the wetland water ranged from 0.01 to 0.05ppm, from 0 to 0.02ppm and from 0 to 0.05ppm for zinc, cadmium and lead respectively. The order of abundance of heavy metals in the water samples are Zn>Pb>Cd. The abundance patterns of heavy metals in leaf, petiole and root were Pb>Zn>Cd, Cd>Zn>Pb and Cd>Zn>Pb respectively. Six bacterial genera (*Acinetobacter, Alcaligens, Bacillus, Kurthia, Listeria* and *Chromobacterium)* were represented in rhizosphere of *Eichhornia crassipes* and 4 bacterial genera (*Acinetobacter, Bacillus, Listeria* and *Chromobacterium)* were recorded from associated water system. The phytoremediation studies revealed that *Eichhornia crassipes* removed appreciable amount of selected heavy metals during the experiment period. Hundred percentages of Pb and Cd were removed from the experiment tank during 6th and 12th day of the experiment respectively. 90% of zinc was removed during the end of the experiment. Results of the present study concluded that *Eichhornia crassipes* is a hyper accumulator of Zn, Cd and Pb from aqueous solution.

INTRODUCTION

Environment pollution particularly from hazardous heavy metals is an important societal problem. Many of these elements being stable are bio-accumulative, and deriving their safe limits is very difficult. Many industries release heavy metals such as Zn, Cd and Pb and the removal of toxic heavy metals from contaminated wastewater is essential (Yuan *et al*., 2001). They cannot be degraded easily and the cleanup usually requires for their removal (Lasat, 2002). For the treatment of such wastewater containing toxic metals, various chemical and engineering method are available, however, they are expensive and energy consuming (Kum *et al*., 2007). Treatment systems should be low cost and suits to our environment. Cadmium (Cd) is one of the most toxic heavy metals and is considered non-essential for living organisms. Cd has been recognized for its negative effect on the environment where it accumulates throughout the food chain posing a serious threat to human health. Cd pollution has induced extremely severe effects on plants (Baszynski, 1986). Unlike Cd, zinc (Zn) is an essential and beneficial element for human bodies and plants. Complete exclusion of Zn is not possible due to its dual role, an essential microelement on the one hand and a toxic environmental factor on the other (Brune *et al*., 1994). However, Zn can cause nonfatal fume fever, pneumonitis, and is a potential hazard as an environmental pollutant (Hampp *et al*., 1976). Even though zinc is an essential requirement for a healthy body, excess zinc can be harmful, and cause zinc toxicity. Lead (Pb) is a useful and common metal that has been used by humans for thousands of years. It is also a very dangerous poison, particularly for children, when it is accidentally inhaled or ingested. Though lead is found frequently in our environment, it has no known purpose in our bodies. When lead gets inside the body, the body confuses it with calcium and other essential nutrients. This confusion can cause permanent damage to the health of both children and adults. Phytoremediation, the removal of pollutants by the use of plants offers a promising technology for heavy metal removal from waste water (Miretzky *et al*., 2004). Aquatic macrophytes have great potential to accumulate heavy metals inside their plant body. These plants can accumulate heavy metals 100,000 times greater than in the associated water. Therefore, these macrophytes have been used for heavy metal removal from a variety of sources (Mishra *et al*., 2008). *Eichhornia crassipes* (Water hyacinth) is a member of pickerelweed family (Pontederiaceae) that has proven to be a significant economic and ecological burden to many sub-tropical and tropical regions of the world. It is a monocotyledonous, perennial, free floating (except when stranded in the mud) aquatic plant (Wolverton and McDonald, 1979). The plant has been used successfully in wastewater treatment systems to improve water quality by reducing the levels of organic and inorganic nutrients (Delgado *et al*,. 1995). In phytoremediation, the root zone is of special interest. The contaminants can be absorbed by the root to be subsequently stored or metabolized by the plant (Merkl *et al*., 2005).

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The removal of pollutants and the consequent wastewater purification are the results of a series of processes, which involve reaction and interaction among substratum, microorganisms and plants, and hence the process is aptly termed as 'rhizosphere treatment' as the root zone micro flora plays a chief role in pollutant removal. Aquatic macrophytes are generally not considered as the main mode of remediation in 'rhizosphere treatment' technique. Rather, the plant creates a niche for rhizosphere microorganisms to carry out the degradation. Rhizospheric microorganisms are well known for their coexistence with plants and for providing nutrition to plants (Uroz *et al*., 2007). *Eichhornia crassipes* showed increased removal efficiency of heavy metals through the activity of its rhizospheric

Bacteria (So *et al*., 2003). By characterizing the microbial communities of the rhizosphere, a significant contribution was made to clarifying the process mechanism that contributes to the germ removal. The main aim of the present study was to evaluate the effectiveness of *Eichhornia crassipes* for removing selected heavy metals (Zn, Cd and Pb) from solution. For this water samples were collected from the selected *Eichhornia* beds of Kuttanad wetland (Part of Ramsar site Vembanadu-coal wetland) for the basic understanding of heavy metals contamination and microbial diversity of the system. The study also aims to identify the root zone associated bacteria of *E. crassipes* from the Kuttanad wetland.

METHODOLOGY

Experimental Plant

The aquatic macrophyte *Eichhornia crassipes* (Water hyacinth) was selected to check heavy metal removal capacity from water under laboratory conditions. Water hyacinth is a perennial aquatic weed spread all over the world, considered noxious and extremely invasive for freshwater environments. These species carry out their entire life cycle as free-floating plant, only the root system is completely submerged.

Collection of *E. crassipes* and water samples

E. crassipes of uniform size were collected from the water bodies of selected *Eichhornia* beds (Kainady, Poovam, and Nedumudi) of Kuttanad wetland ecosystem. The plants were then transferred to a sterile polythene cover for heavy metals analysis. The root region of *E. crassipes* was washed in to a sterile polypropylene bottles using sterilized water for the isolation of rhizosphere associated bacteria. Water samples were also collected using sterile polypropylene bottles from the selected *Eichhornia* bed. The plant, water and rhizosphere samples were transported to the laboratory in an ice box and stored at 4ºC until analysis.

Analysis of heavy metals

The collected plants were first washed with tap water and then with deionised water, allowed to drain off excess water and the plants were divided in to three parts: petioles, leaves and roots. Subsequently, the plant parts were dried in the oven for 24 hours at 70°C, for preparation to ascertain the accumulation of heavy metal (Hamizah, Morad and Fizani, 2011). 0.2 g of dried ground plant samples were taken in digestion tubes and digested by Nitric- Perchloric Acid Digestion method as described by USEPA, 1995 (APHA, 1995). The collected water samples were also digested by the same method. The digested plant and water samples were analysed for Cu content by Voltametric trace metal analyzer (Metrohm 797 VA Computrace) using HMDE (Hanging Mercury Drop Electrode) method.

Isolation and identification of bacteria

Bacterial strains were isolated from rhizosphere and growing water bed of *E. crassipes*. Isolation and enumeration of bacteria were carried by standard serial dilution plate technique. Serially diluted samples were sow in Nutrient Agar and incubated at 37°C for 24- 48 hours. Bacterial colonies from Nutrient agar were isolated, purified and maintained as a pure culture which were characterized and identified up to genus level by morphological tests as per Bergey's Manual of Determinative Bacteriology: 9th edition and 8th edition (Buchanan and Gibbons, 1974). Morphological tests carried out for the identification of the isolates are Gram's staining, cell shape and arrangement, pigment production, O/F glucose tests, Endospore staining, Motility, Catalase, Oxidase etc.

Phytoremediation study

Fibre tanks of 150 litres capacity filled with the water from selected *Eichhornia* beds of Kuttanad wetland were used for removal studies. The plants with uniform size were put in experiment tank and control tank was maintained without any plants for each heavy metal. There was no supplement of heavy metal ions in the experiment and control tanks but the contamination of selected heavy metals in collected water samples were analysed and the values was considered as initial (0th day) concentration. Experimental setup was maintained in duplicate for 15 days. The volume of water in each tank was kept constant and the change in volume due to evapo transpiration was compensated by the addition of deionised water. Every 3 days interval, the water samples were collected from the experimental set up and were analyzed for Zn, Cd and Pb content.

RESULT AND DISCUSSION

Heavy metal content in *Eichhornia* plant and growing water bed

The heavy metal concentration of the water samples ranged from 0.01 to 0.05ppm, from 0 to 0.02 and from 0 to 0.05ppm for zinc, cadmium and lead respectively (Fig.1). The order of abundance of heavy metals in the water samples are Zn>Pb>Cd. Zinc and lead levels are within the limits of standards but cadmium values showed above the permissible limit. Canal systems in Kuttanad wetlands are interconnected with river systems and it receives run off from paddy fields and different types of agriculture systems. It also receives the domestic and municipal sewage from the nearby townships. That may be the reason for the presence of heavy metals in canal systems. Low concentration of heavy metals in the wetland system probably attributed to water hyacinth abundantly in these wetlands covering almost the entire water surface. Heavy metal content such as zinc, cadmium and lead in different parts of *Eichhornia crassipes* collected from different locations were studied. The abundance patterns of heavy metals in leaf, petiole and root were Pb>Zn>Cd, Cd>Zn>Pb and Cd>Zn>Pb respectively. Metal accumulation in wetland plants are affected by many factors. In general, variations in plant species, the growth stage of the plants and the element characteristics control absorption, accumulation, and translocation of metals. Furthermore, physiological adaptations also control toxic metal accumulations by sequestering metals in the roots (Guilizzoni, 1991). During cultivation, the excess application of synthetic chemical fertilizers, herbicides and fungicides are common in Kuttanad (Thampatti and Padmakumar, 1999). Floating plants have the ability to accumulate the heavy metals from surrounding water and it mainly accumulates in the aerial parts (Valittuto *et al.,* 2006).

Table 1. Bacterial genera isolated from rhizosphere of *Eichhornia crassipes* and water

S.No.	Rhizosphere	Water
1	*Acinetobacter*	*Acinetobacter*
2	*Alcaligens*	*Bacillus*
3	*Bacillus*	*Chromobacterium*
4	*Kurthia*	*Listeria*
5	*Listeria*	
6	*Chromobacterium*	

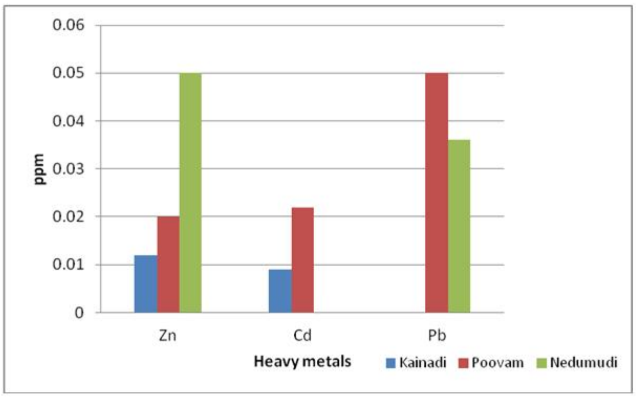

Fig. 1. Heavy metals concentrations in water samples

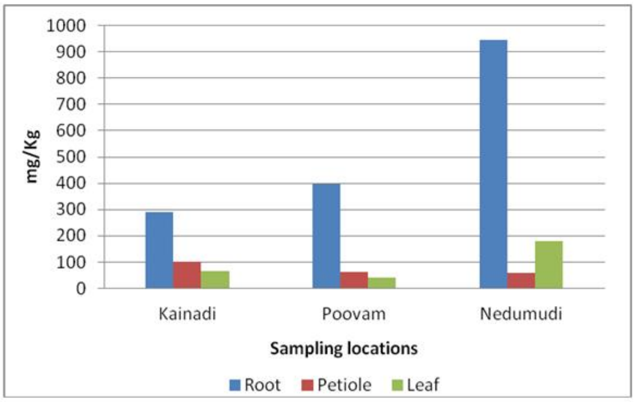

Fig. 2. Zinc content in plant parts of *Eichhornia crassipes*

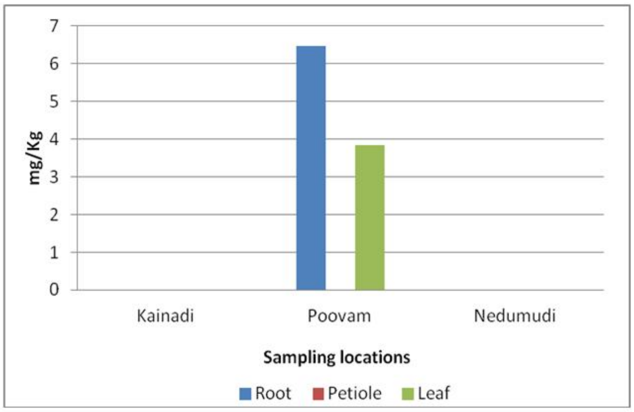

Fig. 3. Cadmium content in plant parts of *Eichhornia crassipes*

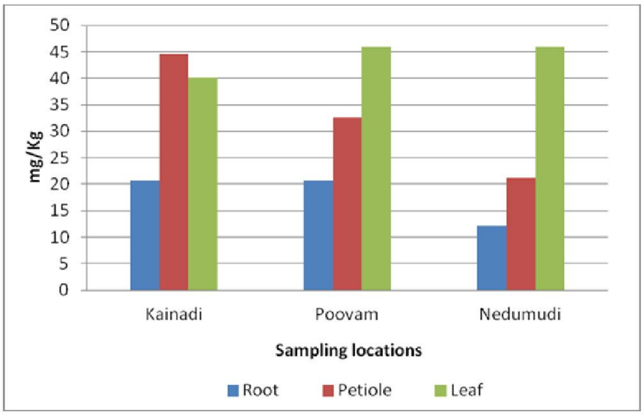

Fig. 4. Lead content in plant parts of *Eichhornia crassipes*

Bacteriological studies

In the present study culturable bacteria from the rhizosphere of *Eichhornia crassipes* and the *Eichhornia* growing water body were isolated, enumerated and identified up to genus level. The microbial load in rhizosphere ranged from 210×10^3 to 248×10^3 and associated water system was 213×10^2 to 92×10^3 (Fig.5). Microbial load in rhizosphere was higher than that of growing water body and these results in tune with the reports of Zhan *et al.* (1993). Six bacterial genera from the rhizosphere of *E. crassipes* were identified which belongs to *Acinetobacter, Alcaligens, Bacillus, Kurthia, Listeria* and *Chromobacterium* (Table. 1). Zhan *et al*. (1993) also reported most of the genera identified during the preset study from the root zone of water hyacinth. Four bacterial genera were identified from the water belongs to *Acinetobacter, Bacillus, Listeria* and *Chromobacterium* (Table.1). Maya *et al.* (2011) reported most of the bacterial genera identified during the present study from the water samples of Kuttanad wetland, which belongs to *Bacillus*, *Listeria*, *Kurthia*, *Carnobacterium* and *Staphylococci.*

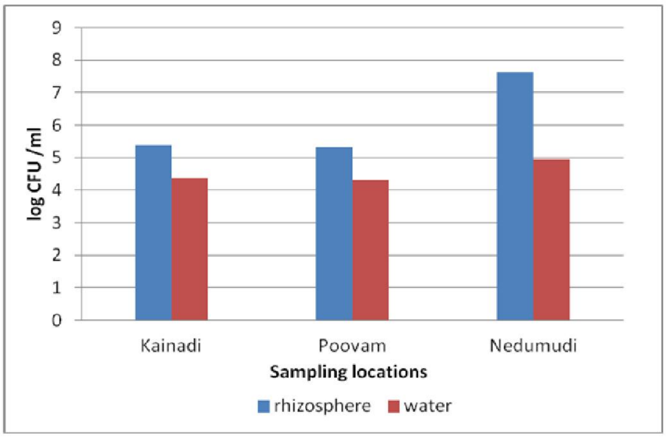

Fig. 5. Load of heterotrophic plate count of rhizosphere associated bacteria and water of *Eichhornia crassipes*

Phytoremediation of heavy metals using *Eichhornia crassipes*

In phytoremediation studies, percentage removal of selected heavy metals from water by *Eichhornia crassipes* in an experimental set up were studied. The phytoremediation studies showed that *Eichhornia crassipes* removed considerable quantity of heavy metals during the experiment period. Zen percentage of Pb and Cd were removed from experiment tank during 6^{th} and 12^{th} day of the experiment respectively. 90% of zinc was removed during the end of the experiment. (Figure 6, 7 and 8).

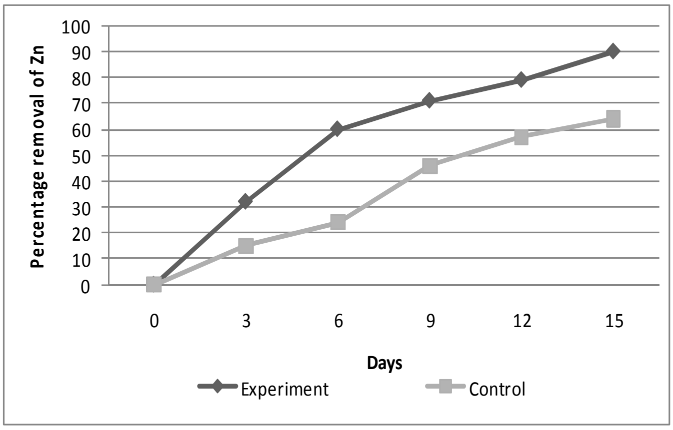

Fig. 6. Percentage removal of Zinc from water

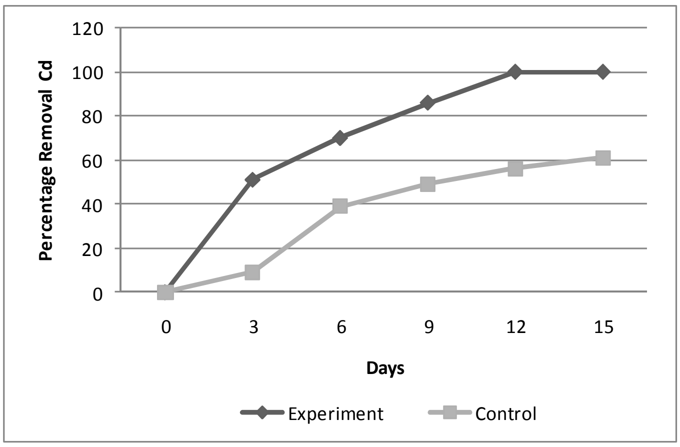

Fig. 7. Percentage removal of Cd from water

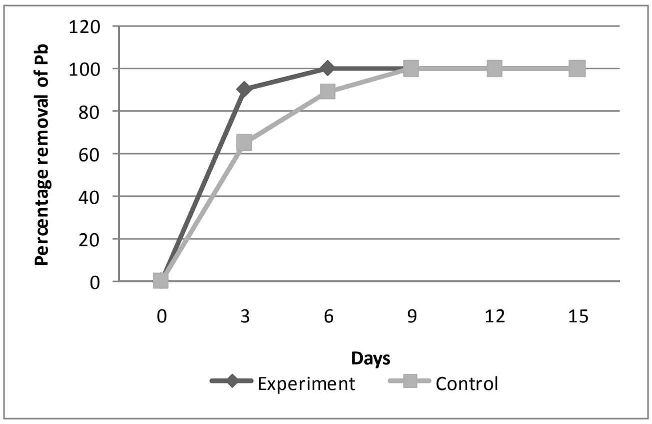

Fig. 8. Percentage removal of Lead from water

The results of present study in tune with the observation of Maria *et al.* (2001) were 72% of cadmium was removed during experimental period. The results of Cd and Zn removal were agreed with the results of Mishra and Tripathi (2008) were more than 90% of Cd and Zn removed during experimental period. Ritusmita *et al.* (2010) reported that more than 90% of lead was removed by water hyacinth. Felix *et al.* (2010) observed that the accumulation potential of Zn by water hyacinth was higher than that of Pb and Cd and the present study disagreed with this observation in which the accumulation potential of Pb is higher than that of Zn and Cd. Only around 60% of Zn and Cd were removed from the control tank during the end of the experiment but 100% of Pb was removed from control tank during the 9th day of the experiment. The reduction of heavy metals in control tank probably attributed to the presence of microbes and adsorption by tank wall.

Conclusions

Results of the present study showed that *Eichhornia crassipes* is a hyper accumulator of Zn, Cd and Pb from aqueous solution. Based on the results, it can be proposed that *Eichhornia crassipes* can act as powerful agents of Zn, Pb and Cd removal from water. Since this species grow abundantly in wetlands covering almost the entire water surface, the ability to absorb Zn, Pb and Cd as demonstrated here shows that species will effectively remove Zn, Pb and Cd in the field.

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