Journal of Environment Protection and Sustainable Development
Vol. 1, No. 1, 2015, pp. 8-12
http://www.publicscienceframework.org/journal/jepsd

Heavy Metal Accumulation by *Azolla pinnata* of Dal Lake Ecosystem, India

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Abstract

Free floating macrophytes play a significant role in removing different types of metals from the water bodies and carry out its purification. In view of their potential *Azolla pinnata* were collected from Dal Lake, an urban-anthropogenic affected water body of Srinagar city and were studied for accumulation of heavy metals. *Azolla pinnata* were exposed to 4 mg/l concentration of different heavy metals in the form of (Cu) $CuSO_4$, (Pb) $PbNO_3$, (Cr) $K_2Cr_2O_7$, (Cd) $CdNO_3$ and (Zn) $ZnSO_4$ for 10 days experimentation period in laboratory conditions. Atomic absorption spectrophotometric analysis have shown initial concentration of these metals in *Azolla pinnata* as Cu (0.02ppm), Pb (0.085ppm), Cr (0.07ppm), Cd (0.006ppm) and Zn (0.06ppm) and after 10 days period the plant has accumulated Cu (0.90ppm), Pb (0.42ppm), Cr (0.27ppm), Cd (0.042ppm) and Zn (2.1ppm) in the order of Zn>Cu>Pb>Cr>Cd. Present study highlights the fact that *Azolla pinnata* bioaccumulates large concentration of heavy metals, therefore can play an important role in the bioremediation of lake ecosystems and waste water treatment plants which are under heavy stress of anthropogenic pressure.

Keywords

Heavy Metal Accumulation, *Azolla pinnata*, Bioremediation, Dal Lake

Received: February 3, 2015 / Accepted: March 9, 2015 / Published online: March 12, 2015

1. Introduction

Macrophytes are aquatic plants that grow in or near water and can be classify as emergent, submerged or floating plants. There has been considerable interest in using aquatic plants for removal of various pollutants, including heavy metals from water bodies because of their fast growth rate and simple growth requirements (Wang and Lewis, 1997). Moreover, aquatic plants are particularly important in heavy metal pollution studies, since the analysis of these plants can give an indication of the state of water environment to which they have been exposed. Studies had been done in investigating the capabilities of some macrophytes to remove different concentration of heavy metals (Maine *et al.,* 2004, Skinner et al. 2007) in the role as biomonitors of environmental metal levels (Mishra *et al.,* 2007) and in their ability as biological filters of the aquatic environment (Upadhyay *et al.,* 2007). Rapid urbanization, industrialization, population explosion, fertilizer and pesticide use have resulted in heavy metal pollution of land and water resources (He *et al.,* 2004, CPBC, 2008). The increasing load of heavy metals has caused imbalance in aquatic ecosystems and the biota growing under such habitats accumulate huge amount of heavy metals (Zn,Cu,Pb,Cr and Cd etc) which in turn, are being assimilated and transferred to humans within food chain by the process of biomagnification. Many of these heavy metals are highly toxic at exceeded concentration and thus needs modern improving wastewater treatment techniques for their remediation. However, in developing countries like India, lack of technical knowledge, mismanagement of environmental policies and limited financial resources has

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given rise to serious challenge due to these contaminants.

Among various conventional technologies, Phytoremediation has tremendous potential in metal assessment, accumulation and removal from the surrounding waters. Pandit (1984, 99) highlighted importance of number of macrophytes as bioindicators of water quality, based on the kind of species present, number of species and the biomass production. This technology is emerging as a cost effective innovative tool because plants are solar driven and have fast growth rate, simple growth requirements, ability to accumulate toxic substances and fewer negative effects than physical or chemical engineering approaches (Ignjatovic and Marjanovic, 1985; Prasad and Freitas, 2003; Reddy and De Busk, 1986). The principle of phytoremediation system includes: (1) identification and implementation of efficient aquatic plant systems; (2) uptake of dissolved nutrients and metals and creation of favourable environment for a variety of complex chemical, biological and physical processes that contribute to the removal and degradation of nutrients (Billore *et al.,* 1998; Gumbricht, 1993).There are extensive studies on metal accumulation by aquatic plants especially on floatings, such as *Salvinia herzogii* (Maine *et al*., 2004), *Eichhornia crassipes* (Mishra *et al*., 2008), duckweed including *Lemna polyrrhiza, Lemna minor*, and *Spirodela polyrrhiza* (Mishra and Tripathi, 2008). Jain *et al*. (1989) found that *Azolla pinnata* and *lemna minor* removed iron and copper from polluted water. If present at low concentrations the treatment could be done by passing it through ponds containing one or both of these water plants. Contamination removal of the aquatic ecosystems by the use of free floating ones showed a greater efficiency due to their smaller size, rapid multiplication, wide spread occurrence and greater tolerance for heavy metals which makes them excellent choice for phytoremediation process (Maine *et al.,* 2001).

Macrophytes are aquatic plants that grow in or near water and can be classify as emergent, submerged or floating plants. There has been considerable interest in using aquatic plants for removal of various pollutants, including heavy metals from water bodies because of their fast growth rate and simple growth requirements, which are favorably compared to those of fish (Lewis 1995, Wang 1991). Moreover, aquatic plants are particularly important in heavy metal pollution studies, since the analysis of these plants can give an indication of the state of water environment to which they have been exposed (Hellawell 1986). Dal lake is the central civilization and is among the most beautiful and national heritages. It has played a major role in the economy of the state of Jammu and Kashmir through its attraction of tourists as well as its utilization as water and food. During the last few years grave concern have been voiced by many researchers and people of the different walks of life over the deteriorating condition of the Dal lake. The cumulative impact of these human greed's resulted significant alterations to the entire lake ecosystem. The lake is under the impact of different anthropogenic stresses. It receives a lot of untreated sewage from house boats, residential areas and commercial areas situated in its vicinity. Run offs from floating gardens as well as from catchment areas are also equally responsible for its deterioration. The increasing human activities in four basins of Dal lake is mainly responsible for the degradation of its entire ecosystem.

Plenty of work has been carried out by different researchers over different ecological aspects from time to time viz. Dass, 1970; Vass and Zutshi, 1979; Zutshi and Ticku, 1990; Yousuf and Parveen, 1992; Zutshi and Gopal, 2000; Kundenger and Abubaker, 2004; Qadri and Yousuf, 2005; Iqbal et al., 2008; Yaqoob and Pandit, 2009; Saba and Wanageo, 2010, Khan et al., 2012 and Mushtaq et al., 2013 and Mushtaq et al., 2014. However, assessment of heavy metal and their uptake and consequences on different basins of Dal lake have been scantly reported till date. This paper presents a study of uptake of heavy metals by the free floating macrophyte *Azolla pinnata* from different concentrations. *Azolla pinnata* is a small free floating aquatic fern which lives in swamps, ditches, and even in lakes and rivers where the water is not turbulent (Lumpkin and Plucknett, 1982). It doubles its biomass in less than two days in laboratory conditions and 5-10 days in normal field conditions (Boyd, 1970).

2. Material and Methods

The *Azolla pinnata* plants used in this study were collected from the freshwater habitat of Dal Lake, Srinagar (Latitude 34^0 07′ N and longitude 74^0 52′ E, alt. 1580 m) and transported to the laboratory in clean plastic bags. Plants were carefully washed using tap water and then distilled water for one minute, to remove visible debris.Wet weight of the plants were registered with a digital balance. 10 g of healthy plants were exposed to 4 mg/l concentration of 5 different heavy metals in the form of (Cu) $CuSO_4$, (Pb) $PbNO_3$, (Cr) $K_2Cr_2O_7$, (Cd) $CdNO_3$ and (Zn) $ZnSO_4$ for 10 days experimentation period in 5 litre trays in laboratory conditions. During experimental period following parameters were maintained; pH 7.0 ± 0.2, water temperature 25 ± 2.0ºC. All trays were exposed to enough light for detention time of 10 days. Plants with uniform size and growth stage were selected to reduce errors in the experiment. To analyse initial and final concentration of different metals, plants were carefully dried at 70^0 C in hot air oven for 48 hours and then powdered and digested in 10 ml concentrated HNO_3 till white fumes start appearing. The digested sample was made to 50 ml with deionised water and filtered through N0 42 filter paper and finally concentration of heavy metals were determined using atomic absorption spectrophotometer (Model Elemental AS-AAS 4141).

The percentage removal efficiency was calculated according to (Tanhan *et al.,*2007).

$$\%\ \text{efficiency} = \frac{C_1 - C_0}{C_0} \times 100$$

C_0 and C_1 are initial and final concentrations of metal in medium (ppm).

Bioconcentration factor (BCF) was determined and calculated using the formula

BCF = Final metal concentration in biomass / Initial metal concentration in biomass

3. Results and Discussion

The present analysis has revealed the role of free floating macrophyte (*Azolla pinnata*) in phytoremediation technology. This macrophyte has showed an excellent performance in removing the metals and was able to remove huge amount of heavy metals in 10 days experimentation period. As presented in table-I the removal efficiency shown by healthy pre weighed *Azolla pinnata* was comparatively higher for Zn and Cu and lower for Cr and Cd. the biosorption of metal ions was depended on the experimental conditions particularly pH and concentration of metal ions in the medium. It was observed that bio concentration factor (BCF) was highest for Cu and lowest for Cr. Results also found *Azolla pinnata* as hyper accumulator of Cu, Zn and moderate accumulator of Pb, Cr and Cd. Hyper bio accumulator factor in *Azolla pinnata* may be due to highest accumulation capability of heavy metal from a system. The bioabsorption capacity of the plant was decreasing in the order of Zn>Cu>Pb>Cr>Cd. A comparison between initial and final metal concentration within the plant has showed that the average final concentration was higher than the initial concentration. In current investigation *Azolla pinnata* showed lowest accumulation for chromium and is supposed to be one of the most difficult metals to remove from the water due to the fact that macrophytes do not require this element for any physiological purpose (Elsharawy *et al*., 2004).

Table I. Changing concentration of different heavy metals in *Azolla pinnata*

Metal	Initial conc.	Final conc.	Total removal	Removal efficiency	BCF
Cu	0.02ppm	0.90ppm	0.88ppm	4400	45
Pb	0.085ppm	0.42ppm	0.335ppm	394.12	4.94
Cr	0.07ppm	0.27ppm	0.2ppm	285.71	3.857
Cd	0.006ppm	0.042ppm	0.036ppm	600	7
Zn	0.06ppm	2.1ppm	2.04 ppm	3400	35

Increasing environmental awareness and concern is required to extend its exploitation in phytoremediation area because the fern can accumulate variety of pollutants and heavy metals. Most of the studies on phytoremediation (Saxena, 1995; Maine *et al.,* 2004; John *et al.,* 2008; Mishra and Tripathi, 2008) have been conducted in laboratory or greenhouse settings using metal-enriched nutrient solutions and showed impressive results with high metal uptake or accumulation. Mashkani and Ghazvini (2009) suggested pretreatment of *Azolla* modified the surface characteristics, which will inturn, improve its biosorption process. The results of present analysis coincide with the previous studies and suggested that *Azolla pinnata* is an efficient macrophyte that could be very helpful for the treatment of various metal pollutants present in contaminated waters. Jain et al. (1989) found that A. pinnata removed the heavy metals iron and copper from polluted water. If present at low concentrations the treatment could be done by passing it through ponds containing these water plants. *Azolla pinnata* removed 92.7, 83.0, 59.1, 65.1, 95.0, 90.0 and 73.1% of the initial Fe, Zn, Cu, Mn, Co, Cd and Ni, respectively from mixture of waste waters. Arora et al., (2006) compared A. filiculoides with A. microphylla and A. pinnata for its phytoaccumulation potential of Cd, Cr, and Ni. They recorded that Cd, Ni and Cr content (ppm) in tissues was in the following order: A. microphylla>A. filiculoides>A. pinnata; A. pinnata> A. microphylla> A. filiculoides and A. pinnata>A. filiculoides>A. microphylla, respectively

4. Conclusion

Plants can take up heavy metals by their roots, or even by stems and leaves, and accumulate them in organs, the accumulation depends on the specific metal element and plant species, and the environmental condition. Therefore, plant systems are used to remove heavy metals from wastewater and provide a good performance. From the above results, it has been concluded that the *Azolla pinnata* is a potential plant for accumulation of heavy metals from contaminated waste water. During the experimentation this free floating macrophyte has successfully removed the metals, without production of any toxicity. The percentage removal efficiency and bio concentration factor indicates that this plant is potent tool in the abatement of heavy metal pollution in aquatic ecosystems receiving industrial effluents and municipal wastewater.

Acknowledgment

We are grateful to Director, Centre of Research for Development (CORD) and Head P. G. Department of Environmental science, University of Kashmir for facilitating the opportunity to carry out the research work

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