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Research Article

# AZOLLA FILICULOIDES LAM AS A PHYTOTOOL FOR REMEDIATION OF HEAVY METALS FROM SEWAGE

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**ABSTRACT**

Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing significance of ecological, evolutionary, nutritional and environmental reasons. Water pollution is a global problem. The heavy metals are omnipresent in our environment, and higher concentration of heavy metals poses a serious threat to the aquatic ecosystem. The remediation of aquatic environment by aquatic plants i.e. phytoremedition is an emerging area of research. In the present investigation laboratory experiments were conducted to study the accumulation profile of heavy metals in *Azolla filiculoides* Lam., exposed to 25%, 50%, 75% and 100% concentration of sewage, at the interval of 4 days for 12 days. The results revealed that the accumulation of heavy metals in test plant varies with sewage concentration and duration of exposure and directly proportional to its concentration and duration of exposure. It is evident from the present investigation that the *Azolla filiculoides* Lam., can be used as phytotool for remediation of heavy metals from sewage.

**Keywords**: *Azolla filiculoides,* Phytoremediation, sewage, Heavy metals and Accumulation.

## INTRODUCTION

The term "Heavy Metal (HM)" refers to any metallic element that has a relatively high atomic density greater than 4g /cm$^3$ or 5 times more than water [1] and is toxic or poisonous even at lower concentration [2]. However, chemical properties of HMs are the most influencing factors compared to their density. It also changes the physicochemical characteristic of water bodies [3]. These include Lead (Pb), Cadmium (Cd), Nickel (Ni), Cobalt (Co), Zinc (Zn), Chromium (Cr), Iron (Fe) Arsenic (As) and Silver (Ag). The aquatic plants play an important role in balancing the water bodies by accumulating large quantity of heavy metals [4,5]. Several submerged, emergent and free-floating aquatic macrophytes are known to accumulate heavy metal [6,7]. Researchers have proved the capabilities of many aquatic plants and algae for reclamation of wastewater [8,9]. Bioaccumulation of HMs was also well demonstrated by using aquatic macrophytes such as *Echhornia, Lemna*, *Spirodella, Nasturtitum*, *Ipomea; Pistia stratiotes* and algae for renovation of different types of domestic sewage, dairy and animal waste waters [10-12]. These macrophytes are important components of the aquatic ecosystem not only as a sources food for aquatic invertebrates, but also act as an efficient accumulator of heavy metals [13,14]. Similarly, *Ceratophyllum demersum* L. is a free floating aquatic perennial macrophytes growing slow flow running water, rich nutrients water bodies, which is important source of food for aquatic invertebrates, fish and herbivorous aquatic birds [15].

*Azolla filiculoides* a free floating aquatic macrophyte belonging to pteridophytes has been intensively studied during the last few years due to its potential use as green manure in rice fields of Asia and Africa and as a feed supplement for aquatic and terrestrial animals [16]. In fact, a symbiotic association between *Azolla filiculoides* and a heterocyst's blue-green algae *Anabena azollae* has been used as biofertilizer because of its nitrogen-fixing capacity [17,18]. On the other hand, it has a negative effect on aquatic ecosystem due to its capability of colonizing rapidly to form a dense mat over water surfaces. The present study was conducted to evaluate the capability of *Azolla filiculoides* to absorb and accumulate the heavy metals from different concentrations of sewage.

## MATERIALS AND METHODS

### Collection of sewage samples and experimental plant

The sewage samples were collected from the Hubblli main sewage channel near Bidnal, brought to the laboratory in plastic cans and stored under dark condition until their use. Experimental plant *Azolla filiculoides* Lam. were sampled from the pond at Shivagiri, Dharwad, Karnataka, India. The samples were brought to the laboratory in small polythene bags and maintained under laboratory conditions. The young and healthy *Azolla filiculoides* plants were selected and acclimatized for two weeks in plastic tubs of (10 liters capacity) containing Hoagland's solution.

### Experimental Design

The 50 g of plant material was sampled from the original stock maintained under laboratory conditions and introduced into separate plastic tubes containing 25, 50, 75 and 100% of sewage. The control plants were maintained in tap water. All experiments were carried out in triplicate under well aerated conditions.

### Analysis of heavy-metal accumulation

The plants were harvested at 4, 8 and 12 days of exposure to sewage, thoroughly washed with distilled water and dried at $80^0$ C in an oven for 24 hours to achieve constant weight. The dried plant material was blended to obtain powder and used for mixed acid digestion [19]. One gram powder was taken in a digestion tube containing 2 ml of 60% perchloric acid, 5ml of conc. $HNO_3$ and 0.1 ml of Conc. $H_2SO_4$. After gentle swirling, it was digested slowly on moderate heat with a gradual increase in temperature. The mixture was digested till the appearance of white fumes. The digested samples were cooled and diluted with double distilled water and filtered through Whatman Filter Paper No.42 into 100 ml volumetric flask and volume made to mark by adding distilled water. The estimation of heavy-metal content in both treated and control plants were carried out using Atomic Absorption Spectrophotometer (GBC 932 Plus, Australia) with Air Acetylene Oxidizing flame and Metal hallio cathode lamps.

### Statistical analysis

The statistical analysis was done using IBM (SPSS windows software) version 20 followed by one way ANOVA and Tukey test.

## RESULTS AND DISCUSSION

The accumulation of heavy metals by experimental plant from the sewage is directly proportional to the increasing concentration of sewage and duration of exposure. The rate of accumulation of heavy metals increased with the duration of the experiment. The accumulation of the iron (Fe) was a maximum at all concentrations of sewage followed by the manganese (Mn) and occurs in the following order Fe > Mn >Pb >Cr >Ni >Cu > Cd. The accumulation potentional of experimental plant was more in the concentrated medium showing the amelioration potentional of heavy metals. Like all living organisms, plants are also sensitive to both the deficiency and excess availability of some heavy-metal ions occurring as potentional micronutrients in the aquatic ecosystem. At higher concentration's ions such as Fe, Mn, Pb, Cr, Ni, Cu and Cds are poisonous to the metabolic activities. Among various water pollutants, heavy metals are of major concern because of their persistent, bio-accumulative and biomagnification nature [20-23]. *Azolla* plants are known to absorb and store the considerable amount of metals [24,18]. The free-floating aquatic plants viz., *Salvinia molesta, Pistia strateotes, Echhornia, Spirodella; Boccopa monira* have been evaluated for their performance to remove heavy metals from different concentrations of sewage [25,26]. The toxic effect of sewage on plant depends upon the type of the metal accumulated, its concentration and stability.

The results revealed that the accumulation of chromium, nickel, cadmium, lead, copper, iron and manganese was found to be accumulated in the following order 54.43 to 80.21 mg/g, 22.57 to 40.73mg/g, 16.17 to 89.20mg/g, 15.50 to.93mg/g, 408 to 942mg/g and 89.20 to 382.10mg/g 10mg/g respectively (Table 1 and fig 1; A-G). Chromium stress is one of the important factors that affect photosynthesis in terms of $CO_2$ fixation, Electron transport, photophosphorylation and enzyme activities [27]. Nickel (Ni) is a transition metal found in natural soil in trace concentrations except in serpentic soil. However, Ni concentration is increasing in certain areas by human activities. *Pistia stratoites* (water lettuce) is an aquatic plant that grows rapidly with an extensive root system which enables the heavy-metal removal and exhibited different patterns of lead removal from the media and accumulated in high concentrations, mainly in the root system [28]. Lead contaminates water by the corrosion of household plumbing system and erosion of natural deposits (US EPA 2005). Presence of higher concentration of heavy metals in plants signifies the biomagnifications Uysal and Taner [29] examined the ability of the *L. minor* to remove soluble lead under different pH values (4.5-8.0) and temperature (15-35°C) exposed to different Pb concentrations 0.1-10.0 mg/L for 7 days. Gallardo et al., [30] found that after one week of exposure lead accumulation by hydrilla which showed maximum uptake (98%) of Pb.

The Results of Benarya [31] revealed that metal content was higher in the whole plant than in the extracted fractions indicating the contribution of both apoplast and symplast to the proportional accumulation of lead in the *Azolla* Leaf. Analysis of accumulation of heavy metals in plants has practical value in outlining ore deposits of variety of metals and phytoremediation studies. It is evident from the present investigation that *Azolla filiculoides* found to be an excellent tool for phytoremidation heavy metals from aquatic environment.

**Table 1: Influence of different concentrations of sewage treatment on accumulation of heavy metals by *Azolla filiculoides***

| Days | Concentration (Percentage) | Chromium (µg/g tissue) | Nickel (µg/g tissue) | Cadmium (µg/g tissue) | Lead (µg/g tissue) | Copper (µg/g tissue) | Iron (µg/g tissue) | Manganese (µg/g tissue) |
|---|---|---|---|---|---|---|---|---|
| 4th day | Control | 33.70±0.20 | 22.57±0.21 | 2.27±0.12 | 16.17±0.15 | 15.50±0.26 | 408.30±1.28 | 89.20±0.10 |
| | 25 | 54.43±0.21 | 24.60±0.10 | 2.40±0.20 | 35.77±0.15 | 16.37±0.29 | 384.97±0.47 | 94.40±0.20 |
| | 50 | 62.27±0.31 | 27.43±0.15 | 3.37±0.25 | 44.10±0.78 | 16.43±0.25 | 557.20±1.11 | 106.30±2.07 |
| | 75 | 63.33±0.42 | 29.11±0.15 | 3.40±0.20 | 48.50±0.36 | 17.30±0.10 | 800.17±0.67 | 286.40±0.70 |
| | 100 | 64.50±0.26 | 30.67±0.25 | 3.53±0.15 | 80.37±0.06 | 18.43±0.25 | 912.00±2.00 | 331.00±1.00 |
| 8th day | Control | 40.30±0.20 | 25.43±0.15 | 2.50±0.10 | 18.30±0.10 | 17.50±0.10 | 414.33±1.03 | 91.20±0.20 |
| | 25 | 58.17±0.29 | 26.40±0.20 | 2.63±0.12 | 16.70±0.28 | 18.23±0.06 | 395.47±0.75 | 97.30±0.10 |
| | 50 | 68.33±1.00 | 28.47±1.12 | 3.17±0.06 | 45.37±0.15 | 19.30±0.10 | 566.23±2.74 | 112.97±0.12 |
| | 75 | 71.23±0.40 | 33.53±0.83 | 3.57±0.15 | 49.03±0.32 | 19.63±0.23 | 810.60±0.70 | 311.13±0.95 |
| | 100 | 77.23±0.49 | 39.13±0.67 | 3.90±0.10 | 87.20±0.10 | 21.33±0.12 | 922.00±1.00 | 362.03±1.05 |
| 12th day | Control | 40.50±0.10 | 28.53±0.15 | 2.80±0.10 | 19.30±0.10 | 18.57±0.25 | 424.27±0.35 | 93.17±0.15 |
| | 25 | 67.97±0.47 | 36.93±0.42 | 2.73±0.06 | 42.67±0.21 | 19.20±0.20 | 404.67±0.21 | 99.17±0.15 |
| | 50 | 72.50±0.10 | 37.50±0.26 | 3.80±0.10 | 48.60±0.10 | 21.73±0.15 | 577.20±0.92 | 125.67±0.25 |
| | 75 | 77.50±0.26 | 39.37±0.15 | 4.20±0.10 | 51.80±0.46 | 23.77±0.32 | 831.30±0.61 | 293.23±0.95 |
| | 100 | 80.21±0.26 | 40.73±0.15 | 4.83±0.06 | 89.20±0.10 | 24.93±0.25 | 942.77±0.49 | 382.10±0.20 |

Each value represents three replicates mean values ± standard error with significant $p<0.05$ level.

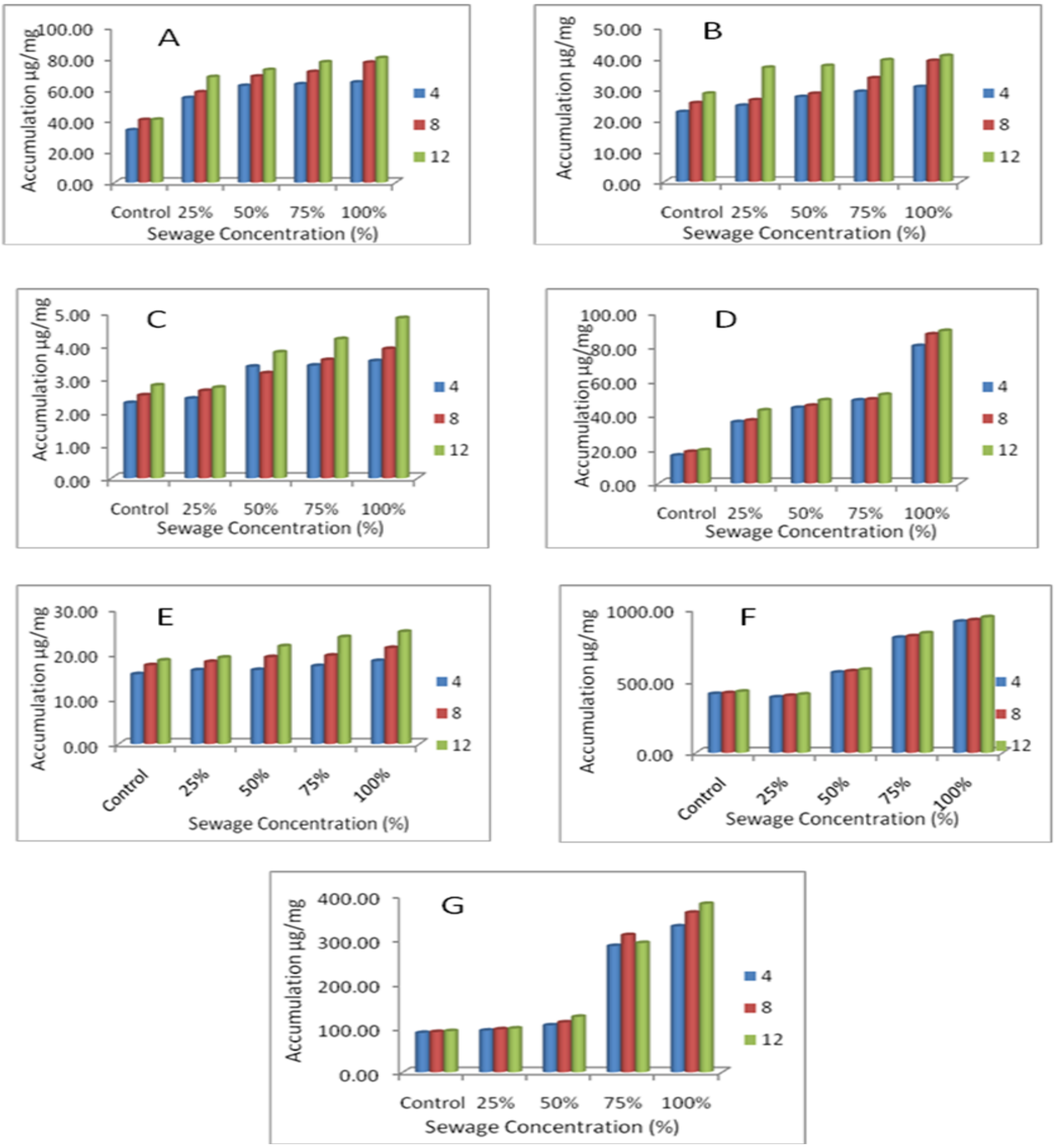


**Fig. 1: Accumulation of Heavy metals in *Azolla filiculoides.***

1. **Chromium, B) Nickel, C) Cadmium, D) Lead, E) Copper, F) Iron and G) Manganese**

## 7. CONCLUSION

*Azolla filicoloides* Lam. demonstrated the good accumulation profile of heavy metals at all concentrations of sewage throughout the experiment. The rate of heavy metal accumulation increased with increasing concentration of heavy metals being directly proportional to sewage concentration and duration of exposure. The experiment revealed that the *Azolla filiculoides* accumulates a maximum amount of Fe. Followed by Mn, Pb, Cr, Ni, Cr and Cd. Accumulation potentional was more in higher concentration showing amelioration potential of heavy metals. From the present investigation, it is evident that the aquatic macrophyte *Azolla filiculoides* Lam., can be used as an effective phyto tool for remediation of sewage and domestic waste water.

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