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Phytoremediation Ability of Aquatic Fern (*Azolla pinnata*) in Lead and Zinc

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ABSTRACT

Heavy metals are environmental pollutants and their toxicity is a problem in increasing significance of ecological, evolutionary and nutritional reasons. They are ubiquitous in environment and higher concentrations poses serious threat to the aquatic ecosystem. The remediation of aquatic environment by aquatic plants known as phytoremediation is an emerging area of research. Hence, an effort was made to understand the role of aquatic fern *Azolla pinnata* in absorption and level of accumulation so as to understand the extent of phytoremediation value in aquatic environmental studies.

Accumulation profile of heavy metals in *Azolla pinnata* exposed to 25%, 50%, 75% and 100% dilutions of sewage and aquaculture effluents was carried out. Atomic absorption spectrophotometric analysis of sewage effluent accumulation in *Azolla pinnata* showed initial concentration of metals such as Pb and Zn, were 0.085 and 0.060 ppm respectively. After 42 days of exposure, then metals were accumulated in *Azolla pinnata* concentration to an extent of 0.933 and 0.074 ppm respectively. The order of concentration accumulated in the plant is Pb>Zn. The plant when exposed to aquaculture effluent, the accumulation concentration of Pb and Zn were 0.853 and 0.914 ppm in the order of Zn>Pb. Results revealed that the accumulation of heavy metals in test plant varied with different effluents concentrations and the duration of exposure was unfirmed that it is directly proportional to its concentration. It is evident from the present investigation that *Azolla pinnata* could be used as phyto-tool for remediation of heavy metals from different effluents. In order to confirm the exact amount of accumulation of heavy metals, various other effluents could be exposed to varieties of plants which are having phytoremediation may be experimented.

Keywords: *Azolla pinnata*; Sewage effluent; Aquaculture effluent; Phytoremediation

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INTRODUCTION

Increased agriculture and aquaculture activities, industrialization and urbanization have resulted in the discharge of untreated wastes containing heavy metals. In recent years, this has become a global problem and the aquatic systems are severely affected as the large amount of wastes easily enter into water bodies through runoff and result in contaminations of water.

Unlike other types of contaminants, heavy metals cannot be eliminated from the environment and pollutants are of great concern as they cause deterioration of life sustaining quality of water and thus damage both aquatic fauna and flora [1-4]. The Sewage contains nutrients but when it is not optimally used may cause eutrophication in receiving waters. Hence, better discharging into water bodies could be useful for irrigation or fooder cultivation [5]. Generally, heavy metals remove or eliminate physically or to transform into non-toxic form with the help of various conventional technologies including electrocoagulation-flotation reverse osmosis, electro dialysis, chemical precipitation, ultra filtration, chemical oxidation, and reduction, electrochemical treatment, adsorption, coagulation-flocculation, ion exchange, etc. [6,7]. All these methods are costly and not eco-friendly, hence in order to overcome this, a special technique has been adopted and one such method is bioremediation, among them is phytoremediation which plays an potential role. The term phytoremediation originate from Greek word phyton meaning plant and Latin word remediare means remediate and refers to the treatment of polluted water or soil by plant species [8]. Different phytoremediation techniques such as phytoextraction, phytostabilization, phytovolatilization, rhizodegradation, phytodegradation and rhizofiltration have been extensively determinated (**Figure 1**) [9].

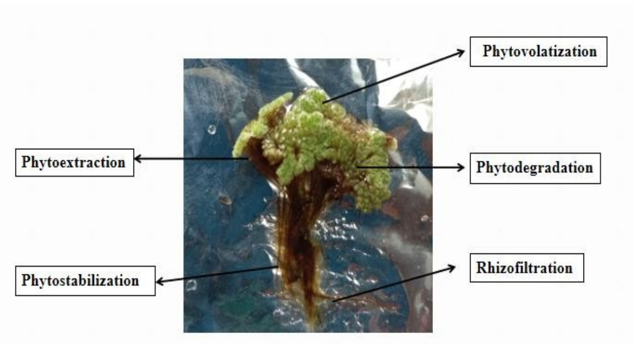

Figure 1: Phytoremediation mechanisms.

Phytoremediation is one of the biological wastewater treatment methods by using plant based system for removing the contaminants from various natural resources. To clean up the contaminant water, selection of an appropriate and efficient plant system should have high uptake of both organic and inorganic pollutants, grow well in polluted water and be easily controlled in quantitatively propagated dispersion [10]. It is a promising clean up technology for wastewater. The remediation techniques utilizes specific planting rearrangement, floating plant systems and numerous others configuration [11].

The capacity of vascular aquatic plans to assimilate nutrients from polluted water has been recognized for several years. According to Rogers and Davis, nutrient removal efficiency of a system containing plant will depend on the type of aquatic plant, growth rate of plant, nutrient composition of water and physico-chemical environment in the water.

The significance of the study is useful in detecting heavy metal contamination thereby *Azolla* could be one of the tools in determining its effect on food chain. The study enables to understand effect of water contamination and variation of physico-chemical characteristics of different wastewaters. The following objectives were considered for the investigation:

- To assess the phytoremediation efficacy of aquatic fern, *Azolla pinnata* to different concentration of effluents.
- To investigate accumulation ability of heavy metals in aquatic fern, *Azolla pinnata*.

MATERIALS AND METHODS

Azolla Procurement

The floating Aquatic fern, *Azolla pinnata* was collected from ICAR-Krishi Vigyan Kendra, Dakshina Kannada, Mangaluru and brought to the laboratory of department of aquatic environment management and maintained in ambient conditions. The species was identified using standard literature [12]. The young and healthy *Azolla pinnata* plants selected were acclimatized for two weeks in polythene tubs (10 L capacity) containing Hoagland's solution.

Collection of Water Samples

Domestic sewage was collected from:

- Sewage treatment plant located at Bajal area near Jappinamogeru, Mangaluru and
- Aquacultue waste water was collected from shrimp farm drainage point located at Kumta taluk, Uttara Kannada district.

The collected wastewater samples were transported to the laboratory in polythene cans (5 L) and used for the experiment following dilution at different concentrations.

Analyses of Wastewater Sample

Physico-chemical characteristics such as water temperature, Turbidity, Total Dissolved Solids (TDS), Electrical Conductivity (EC), Total Suspended Solids (TSS), pH, Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Ammonia (NH_4), Nitrite (NO_2), Nitrate (NO_3) and Phosphate (PO_4) were analyzed following the procedure of standard methods [13].

Experimental Design

About 50 g of plant material was taken from the original stock maintained under laboratory conditions and introduced into separate glass aquarium (25 liters capacity with a depth of 9 feet) containing known concentrations of different wastewaters.

A control was also maintained separately filled with tap water. Experiments for Sewage and aquaculture effluent samples were carried out in five concentrations in triplicates as shown in Figure 2.

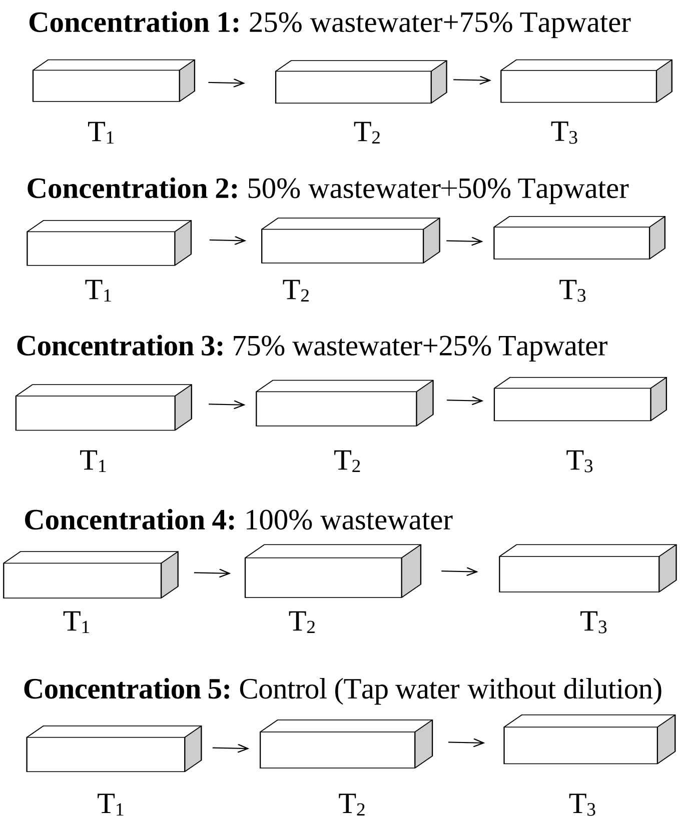

Figure 2: Experimental set up analysis of heavy-metal accumulation.

Concentration 1: 25% wastewater+75% tap water

Concentration 2: 50% wastewater+50% tap water

Concentration 3: 75% wastewater+25% tap water

Concentration 4: 100% wastewater

Concentration 5: Control (tap water without dilution)

Samples were drawn from each tank at weekly intervals for analysis of physico-chemical characteristics of wastewater. The plants were harvested from at 7, 14, 21, 28, 35, 42 days of exposure to different concentration of both the effluents. The plant was thoroughly washed with distilled water and dried at 80°C in an oven for 24 hours to achieve constant weight. The dried plant material was then blended to obtain powder and used for mixed acid digestion. One gram powder was taken in a digestion tube containing 2 ml of 60% perchloric acid, 5 ml 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 was made up to the mark by adding distilled water. The estimation of heavy metal contents in both treated and control plants were carried out using atomic absorption spectrophotometer with air acetylene oxidizing flame and metal hallio cathode lamps at fisheries research and information center (Inland), Hebbal, Bengaluru.

Statistical Analysis

The data obtained were subjected to One Way Analysis of Varience (ANOVA) and Duncan test using SPSS package (16th editionn) to draw the inference at 95% confidence at $p<0.05$ significance.

RESULTS AND DISCUSSION

The present research 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 42 days of experimental period. Physico-chemical analysis of the effluents exposed with *Azolla pinnata* showed the presence of metal ions Lead (Pb) and Zinc (Zn) in different concentrations but within the permissible limit given by CPCB (Table 1).

Table 1: Physico-chemical analysis of the effluents.

Parameters	Units	Sewage effluent	Aquaculture effluent
Water temperature	°C	28.15	27
pH	No units	7	6.35
EC	S/m	391	7.71
Turbidity	NTU	3.06	2.31
DO	mg/l	7.13	5.43
COD	mg/l	55.5	24.8
TDS	mg/l	650	1220
NH_3	µg-at./l	5.5	15.4

NO_2-N	µg-at./l	3.22	3.04
NO_3-N	µg-at./l	9.91	11.1

Accumulation of lead in *Azolla pinnata* exposed to sewage and aquaculture effluents: In sewage effluent the higher level of lead accumulation was observed in the *Azolla pinnata* under treatment as compared to the test plant in control group (**Figure 3**).

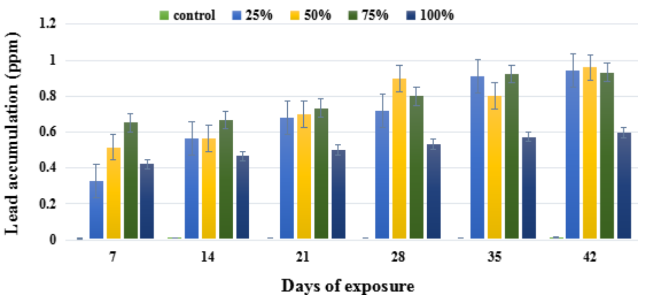

Figure 3: Lead accumulation in *Azolla pinnata* on exposure to different concentrations of sewage effluent.

The concentration of lead in the roots and shoots kept on increasing with period of exposure. After 7 days the accumulation was highest in 75% (0.653 ppm) treatment and after 42 days of exposure the highest were recorded in the 25% (0.943 ppm) treatment, possibly due to the high translocation factor. Accumulation of llead by *Azalla pinnata* was correlated with damage caused by the loss of essential nutrients capacity. *Azolla pinnata* showed the maximum removal percentage of lead from sewage effluent on 42nd day. At an initial accumulation concentration of lead in *Azolla pinnata* was 0.085 ppm. Sharma observed, after 30 days of exposure of lead, the accumulated lead increased to 0.945 in *Azolla mexicana [14]*. Thus, the concentration of the accumulated Heavy metal (Lead) in the *Azolla pinnata* was higher when compared to control.

As the days advanced the accumulation of lead also showed increased trend in aquaculture effluent in all the treatments (**Figure 4**).

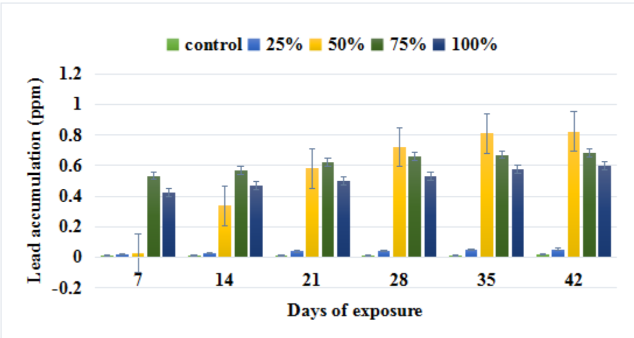

Figure 4: Lead accumulation in *Azolla pinnata* on exposure to different concentrations of aquaculture effluent.

Whereas the maximum accumulation were observed in the 75% (0.683 ppm) concentration on 42nd day of exposure. As the older shoot remobilize the lead content to younger leaves from older senescing parts and Lead accumulated in the poplast and in the symplast of *Azolla* plants exposed to various concentrations of the heavy metal. Robertz observed similar results in *Azolla caroliniana*. Muramoto and Oki have carried out similar experiments for the removal of lead using water hyacinth [15].

Accumulat on of zinc in *Azolla pinnata* exposed to sewage and aquaculture effluents: In sewage effluent the higher level of zinc accumulation was observed in the *Azolla pinnata* under treatment as compared to the test plant in control group (**Figure 5**). After 7 days the accumulation was highest in 100% (0.423 ppm) treatment and after 42 days of exposure the highest were recorded in the 50% and 75% (0.933 ppm) concentration. The concentration of zinc remaining in sewage effluents increases with the passage of time and then remains almost constant due to saturation. As soon as the saturation state is reached, it seems that it is not possible for these plants to further absorb zinc significantly. Similar results have also been reported by Wolverton and Donnald, Chigbo et al., Tatsuyama et al., who used water hyacinth for removal of zinc [16,17].

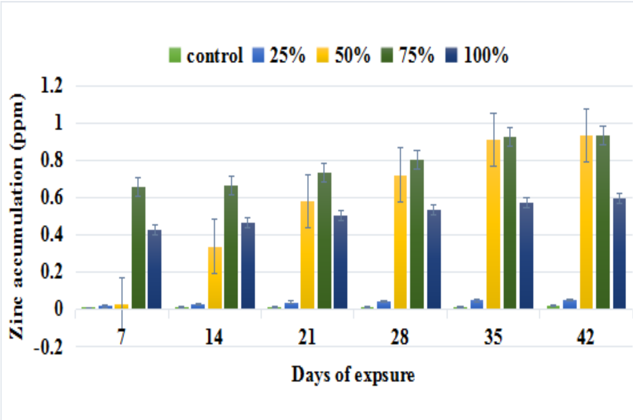

Figure 5: Zinc accumulation in *Azolla pinnata* on exposure to different concentrations of sewage effluent.

As the days increases the accumulation of zinc was increased in aquaculture effluent almost in all treatments, whereas the maximum accumulation were observed in the 75% (0.914 ppm) concentration on 42nd day of exposure. Maximum accumulation of zinc could be due to electro chemical gradient which generate by electron transport and also due to activates the insoluble to the soluble state in effluent Thereby increases the ability of aquatic plants to absorb zinc. Mishra and Tripathi reported that with increasing zinc in aquatic plants were able to remove and accumulate high amounts of metals [18]. *Azolla* species also removed substantial amounts of these metals, indicating that this species can be used effectively to remove Zn (**Figure 6**).

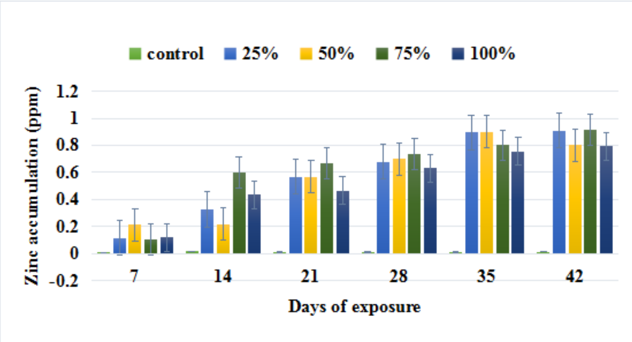

Figure 6: Zinc accumulation in *Azolla pinnata* on exposure to different concentrations of aquaculture effluent.

CONCLUSION

In all, the aquatic fern, *Azolla pinnata* a candidate species had been found to be an effective floating aquatic plant in absorbing heavy metals at different concentrations of selected effluents. The current studies suggest that, dilution of two different effluents. The current study suggest that, dilution of two different effluents at varying concentrations in accordance with earlier trials, standardization becomes a reference for recommendations to take up further studies.

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