



Phytoremediation properties of *Hydrilla verticillata*

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Abstract : The research was carried out to estimate the potential of *Hydrilla verticillata* in phytoremediation which can be an innovative approach to check the increased heavy metals pollution in aquatic ecosystem. The present study also emphasized on the ability of different parts, stem, root and leaves of fresh *Hydrilla* plant in terms of tolerance & degradation of lead pollution in water bodies. The plants were treated to 3 different lead (Pb) concentration of 10, 20 & 30 ppm for 15 days. Heavy metal analysis was done with atomic absorption spectroscopy. Significant differences were found in

removing capacity by plant for Pb in all concentrations of 10, 20 and 30 ppm during experiment period. The highest removal ratio for Pb was 7.98 ppm, in the roots 6.67 ppm in leaf and lower removal was 5.28 ppm in stem at concentration of 30 ppm for 15 days. There was accumulation in the root more than the stem & leaf. It was root > leaf > stem > respectively.

Keywords: Phytoremediation; *Hydrilla verticillata*; Accumulation, Atomic spectrophotometer.

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Introduction:

The contamination of the aquatic ecosystem with heavy metals is the frequent environmental problem all over the world and is a serious dilemma that threatens aquatic ecosystem, agriculture and human health (Gupta et al., 2010). Lead is a naturally occurring element found in small amount in earth's crust but it's increased deposition level in vegetation, ground and water surfaces have caused significant injury to the ecosystem. Those heavy metals which are exposed into environment, for example by industrial waste and fertilizers cause serious concern in nature as they are non-biodegradable and accumulate at high level inside the human body (Hall, 2002). Proper treatment of

lead from soil and industrial wastewater is very important. Lead is one of the most abundant toxic metals that pose a serious threat to human beings, animals and phytoplanktons. In human, it is absorbed directly into the blood stream and is stored in soft tissues, bones and teeth (95% in bones and teeth) (David et al., 2003).

Developing countries can't afford high prices involved in these techniques due to having some other investment priorities. So, there is a need of eco-friendly & low-cost remediation technique to clean water & aquatic ecosystem. Phytoremediation is a biological, cost-effective & eco-friendly clean up methodology that uses plants & their associating micro-organisms to degrade, remove or remediate contaminants from soil & water (Mahar et al., 2016) & for the restoration of the water and soil properties (Eid et al., 2012). There are different methods through which phytoremediation takes place like phytoextraction, rhizofiltration, phytostabilization & phytotransformation or phytodegradation.

Hydrilla has leaves in whorls with thin cuticle, which confers a large surface area for uptake of metals from aquatic systems. The application of *Hydrilla verticillata* for metal removal from water body helps in maintenance of water quality and abstains metal transfer to higher organisms through the food chain. Several reports have analysed the potential of *Hydrilla verticillata* for the accumulation of metals & metalloids. *Hydrilla* plant possess significant potential for the greater accumulation of a number of metals like chromium (Cr), cadmium (Cd), mercury (Hg), Zinc (Zn), copper (Cu), iron (Fe), arsenic (As), lead (Pb). It is significant to use native plants for phytoremediation as these plants are frequently preferable in terms of survival, growth & reproduction under environmental stress than to introduce plants from another environment (Galal et

al., 2018). The performance of a phytoremediation process depends on the selection of convenient plants for specific environment (Galal et al., 2018).

Hypothesis:

Hydrilla verticillata is used as an efficient plant for absorption of lead as it possess potential to treat heavy metal polluted water bodies. This plant exhibits different patterns of removal of heavy metals but mostly accumulated at high concentration mainly in the root system. It has been reported that the removal is dependent both on the contact time & the initial metal concentration.

Aims & Objective:

- To test the phytoremediation properties of *Hydrilla verticillata*.
- Comparative study of absorbance of lead by stem, root and leaves of *Hydrilla verticillata* at various concentrations.
- Comparative study of estimation of heavy metals absorbed by plant sample using atomic absorption spectrophotometer.

Materials and Method:

Collection and acclimatization of plant sample : The plants were collected from the pond of college campus and kept in plastic bags containing water and then transferred to the lab. The collected plants were washed well by tap water and small brush to remove sediments and algae while maintaining the root hairs, and the plants were acclimated (for about 10 days) in plastic containers of 1 litre capacity, at room temperature under sunlight.

Experiment was conducted in a round plastic container containing 1L of tap water. *Hydrilla verticillate* plant was selected for standard experiment. The plant was kept at room

temperature (25 to 30 °C). Tap water was added at regular interval to compensate the water that was lost by plant by transpiration, sampling & evaporation (Favas et al., 2012). The experiments were carried in plastic containers containing 1L of distilled water with 3 different concentrations, 10, 20 and 30 ppm of lead to which plants were exposed respectively for 15 days under ambient sunlight. One set up with *Hydrilla* plant having no concentration of lead was taken as control. All the experimental setups were in triplicates for each of test sample. Morphological changes of plants were monitored visually. Visual assessment of aquatic plant was done by observing changes in the general appearance of the plants such as colour of leaves, stolon & wilting after the completion of retention period of the experiment. Accumulation of lead in leaves, stem and roots was estimated using atomic absorption spectrophotometer at 540 nm wavelength. Aquatic plants showing survival were monitored for their growth rate at the interval of 5 days. The experiments were performed in similar procedure as used for testing survival rates on 5th, 10th & 15th day (Jasrotia et al., 2014).

Preparation of standard stock volume of lead sample : The standard procedure was carried out by using lead nitrate salt. 100 mg of salt was measured and was dissolved in 100 ml of distilled water in a volumetric flask to get 1000 ppm of stock solution.

Preparation of 10 ppm solution : 1 ml of stock solution was taken in a measuring cylinder and transferred to volumetric flask containing 100 ml distilled water. The solution was shaken properly to get the volume of 10 ppm concentration.

Preparation of 20 ppm solution : 2 ml of stock solution was taken in a measuring cylinder and transferred to volumetric flask containing 100 ml

distilled water. The solution was shaken properly to get the volume of 20 ppm concentration.

Preparation of 30 ppm solution : 3 ml of stock solution was taken in a measuring cylinder and transferred to volumetric flask containing 100 ml distilled water. The solution was shaken properly to get the volume of 30 ppm concentration.

Digestion of the sample plant : Leaves, stem and roots of the sample plant were dried in the hot air oven at 35° C for 30 minutes to dry. 1gm of the dry plant sample was digested by 16 ml of mixture of HNO₃ (64%) and H₂O₂ (30%) in ratio of 6:2. The mixture was kept on hot plate for two hours. After cooling the digested samples, 10 ml of distilled water was added. The mixture was filtered through Whatman filter papers (0.45 micrometer), the filtrate was diluted to 50 ml (Senila et al., 2011).

Estimation of Lead concentration using Atomic absorption spectrophotometer: Standard procedure (Saha et al., 2016) was followed to estimate lead from the sample. Diphenylcarbazide (DPC) was used as reagent. Absorption was measured at 540 nm wavelength.

Results and Discussion:

The pattern of lead concentration estimated in the *Hydrilla* organs differed significantly between the sampling intervals. At the end of the retention period of the experiment maximum removal of lead was observed. The allocation of heavy metals in different plant organs depends on their forms, water transport and plant species (Ouzoudou et al., 1995). The present research work reveals that there is much higher accumulation of heavy metals in the root zone than upper parts of plant i.e. stem & leaf. Significant changes were observed in all the test samples. Results of each test sample has been tabulated below.

Table 1. Accumulation of lead in root, stem and leaf of *Hydrilla verticillata* on 5th day

	Absorption				
Tested sample	Plant parts	At 10 ppm.	At 20 ppm.	At 30 ppm.	Control
Sample 1	Leaf	0.73	1.17	2.22	0.08
Sample 2	Stem	0.28	1.09	1.28	0.01
Sample 3	Root	1.07	2.25	3.57	0.14

Table 2. Accumulation of lead in root, stem and leaf of *Hydrilla verticillata* on 10th day

	Absorption				
Tested sample	Plant parts	At 10 ppm.	At 20 ppm.	At 30 ppm.	Control
Sample 1	Leaf	2.46	4.26	5.38	0.08
Sample 2	Stem	1.07	3.12	4.03	0.01
Sample 3	Root	3.21	5.73	6.87	0.14

Table 3. Accumulation of lead in root, stem and leaf of *Hydrilla verticillata* on 15th day

	Absorption				
Tested sample	Plant parts	At 10 ppm.	At 20 ppm.	At 30 ppm.	Control
Sample 1	Leaf	2.46	5.32	6.69	0.08
Sample 2	Stem	1.05	4.20	5.29	0.01
Sample 3	Root	4.65	6.70	7.98	0.14

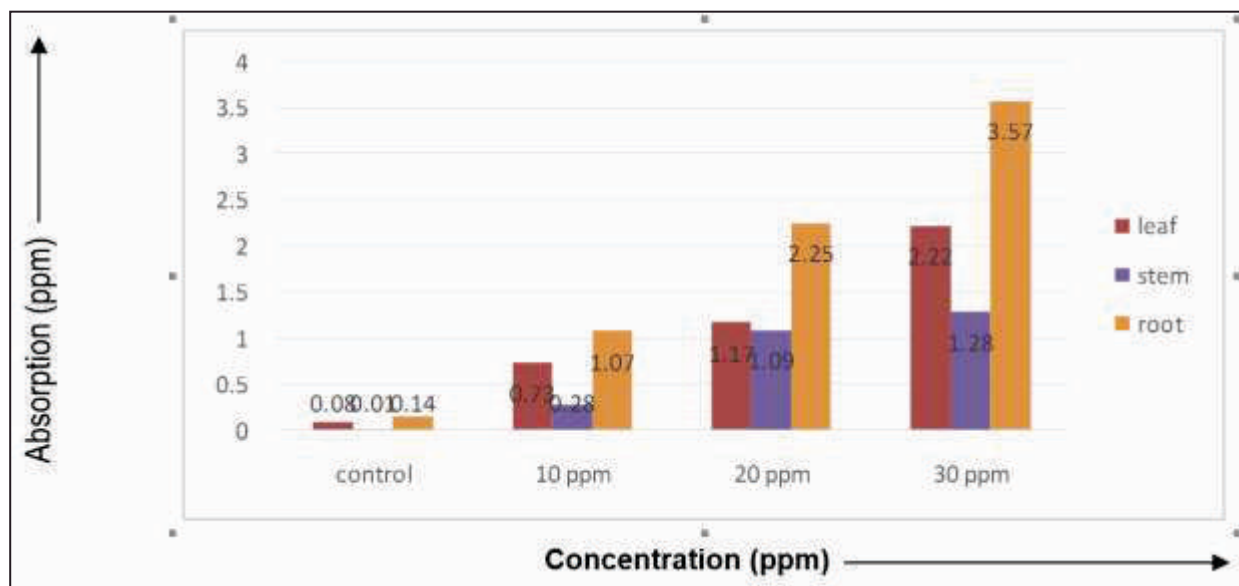


Fig. 1. Accumulation (ppm) of lead in plant tissue, after 5 days of experiment period

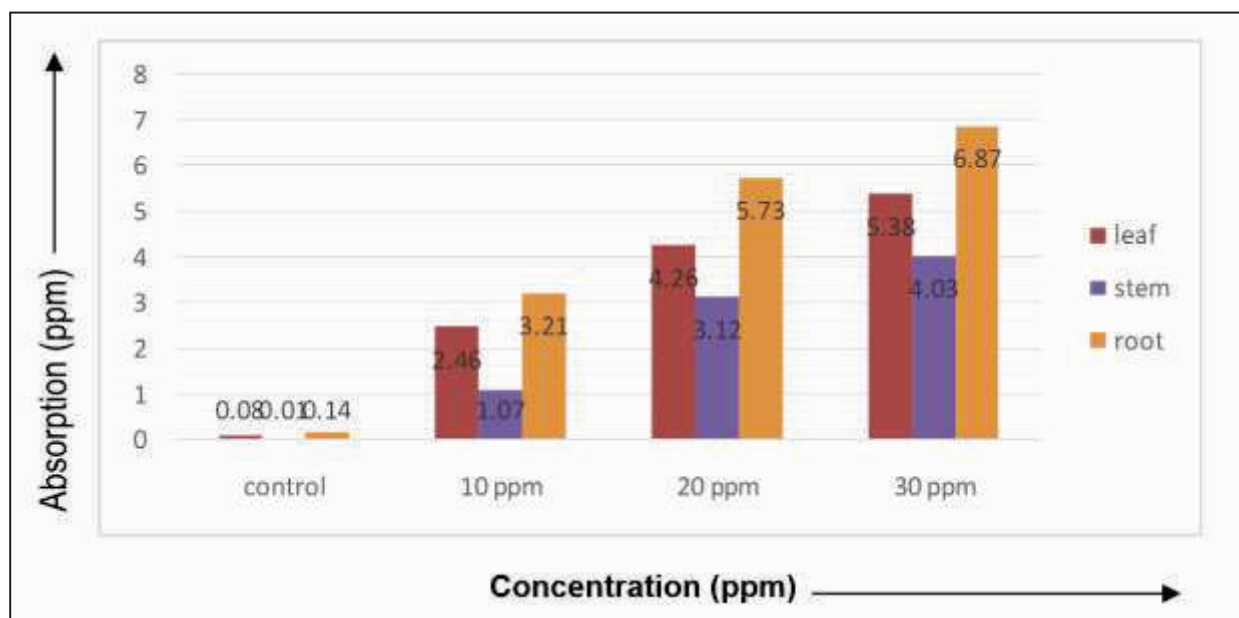


Fig. 2. Accumulation (ppm) of lead in plant tissues, after 10 days of experiment period

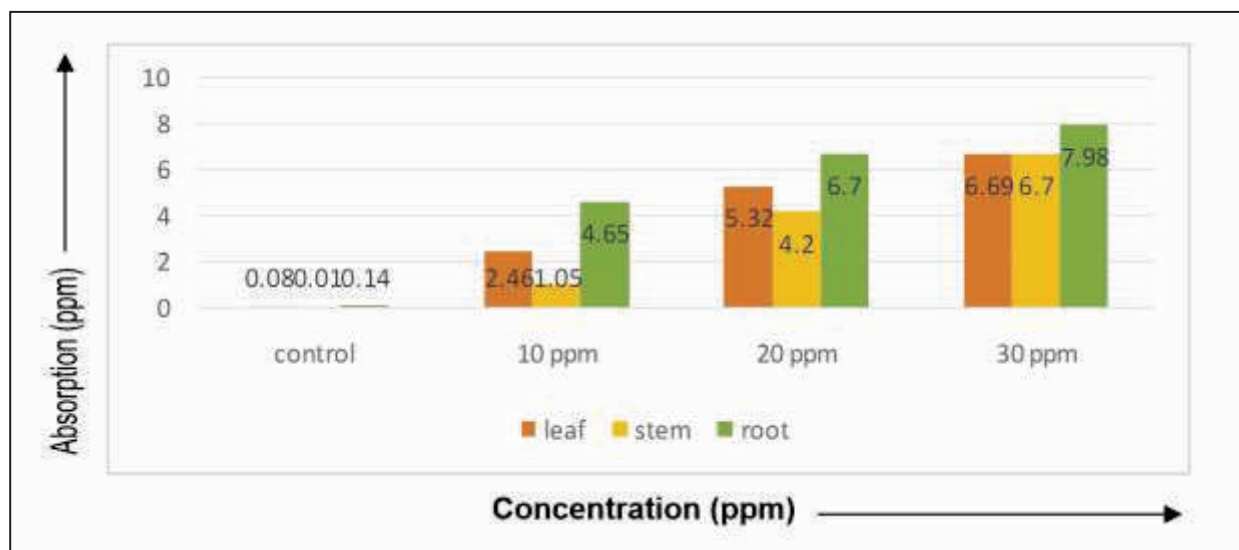


Fig. 3. Accumulation (ppm) of lead in plant tissues, after 15 days of experiment period

Exposure of *Hydrilla verticillata* plant to different concentrations of lead element, an increase in the accumulation of the element in the root was significantly observed. As the days passed morphological changes in the plant was also noticed. Significant decline in the growth was observed at the end of the retention period of the experiment.

As shown in table 1 and 2 maximum absorption of lead was measured at 30 ppm in the root sample and minimum absorption was measured in the stem sample. Highest Concentration of lead was found to be 7.98 at the end of 15th day in the root sample at 30 ppm as shown in table 3. The stem sample showed least absorption of lead which was recorded as 5.29 at 30 ppm. Higher deposit of lead in the roots of

Hydrilla verticellata plant is consistent with as reported by David, 2003, who studied the stock of Cadmium and chromium in *Hydrilla verticellata* plant and concluded that the *Hydrilla verticellata* was able to stock metals more in the roots in the comparison to the other parts of the plant. The results of this research work also showed that *Hydrilla verticellata* plant stores maximum lead in the roots which was recorded as 3.57, 6.87 and 7.98 at 30 ppm on 5th, 10th and 15th day respectively. Remarkable morphological changes were observed in form of wilting of leaves, decrease in number of leaves and change in colour of the plants as shown in figure 4. The high accumulation of heavy metals in root may be ascribed to complexation of heavy metals with the sulfhydryl groups, creating in less heavy metals translocation to shoot system (Singh et al., 2014). The higher concentration in root than shoot system, perhaps, occurred because the roots are finest organ to get in contact with the heavy metals. In addition, there are many reports on the synthesis of phytochelators, which can sequester heavy metals, therefore, greater accumulation occurred in the root system (Eid and Shaltout 2014). These results indicate that the weed can remove the heavy metals by bio-accumulation. It has been reported that the removal is dependent both on the contact time and the initial metal concentration.

Conclusion:

It is evident from this study that the aquatic plant confers the phytoremediation properties. The absorption of lead also increases at different concentration in the time interval of 5, 10, 15 days. The high accumulation of heavy metals in root equally confines the greater absorption occurred in root region at 3 different concentration in comparison to stem and leaves. Heavy metals in our environment is persistent pollutant which needs

absolute elimination for a completely remedial objective. Comprehensive interaction, transport and chelator activities regulate the storage & accumulation of heavy metals by aquatic plants. *Hydrilla* has proved to be the potential accumulator of numerous heavy metals like Ag, Mg, Pd, Cd which might be due to the presence of transporter for different metals and due to variable specificity of a transporter for distinct metals. The use of aquatic plants in phytoremediation like other conventional physical and chemical techniques doesn't require any post-filtration & can be applied to treat a large volume of polluted water bodies & soil. Based on present analysis, the benefits of using aquatic plant to treat contaminants are huge, because this technology not only treats the contaminants but is cost-effective and visually pleasing as well as being advantageous for the sustainability of the whole ecosystem.

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