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Efficacy of Bioaccumulation of Heavy Metals by Aquatic Plant *Hydrilla verticillata* Royle

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Abstract: ***The bioaccumulation potential of water plant (Hydrilla verticillata) was studied on metals. The plants were grown for 7 days each in experiment tanks containing a solution of 100 ppm concentration of either Cadmium (Cd), Magnesium (Mg), Copper (Cu) or Calcium (Ca), Iron (Fe), Lead (Pb), Zinc (Zn), Sodium (Na), and Potassium (K),. The change in fresh weight plants was examined. The percentage of removal of the metals by the plant was determined using atomic absorption spectrometry (AAS) on the acid digest of the plant. The biomass reduced insignificantly (P>0.05) in the H. verticillata grown in the test solution and increased by 7.23% (g/g) in the control. Metal uptake occurred to varying degrees. The maximum amount of metal uptake per dry weight of H. verticillata was 12.27ppm of potassium and lowest, 0.02ppm of lead.***

Keywords: *Hydrilla verticillata*, heavy metals, bioaccumulation, aquatic plant

1. Introduction

Water systems in many areas of the world are polluted with toxic metals from industrial effluents, leather, textile dyes, radionuclide, hydrocarbons from oil refineries and pesticides from agricultural industries. Unlike the organic wastes, heavy metals are non-biodegradable, trace and heavy metals such as Arsenic, Selenium, Zinc, Manganese, Lead, Mercury and Cadmium need to be removed from the environment (Alluri et al., 2007).

Most of the remediation technologies in the treatment or removal of metallic wastes are quite expensive and injurious to health. Bioaccumulation, which is the use of plants and their associated microorganisms, is one of the recent technologies which guarantee an effective, economical and sustainable means to achieve this end for developing countries (Macek et al., 2000; Susarla et al., 2002; Xia et al., 2003. Ghosh and Singh, 2005).

Bioaccumulation encompasses five processes of metal removal from soil or water. These processes include: phytoextraction, phytovolatilization, rhizofilteration, phytostabilisation, and phytodegradation. Phytoextraction which is also known as phytoaccumulation is the removal or absorption, concentration and precipitation of the elemental pollutant into the plant material (Salt et al., 1995; Salt et al., 1997; Rulkens et al., 1998). Bioaccumulation involves the extraction, transformation of the pollutant into a volatile and less toxic form which is then transpired into the atmosphere (Ghosh and Singh, 2005). Rhizofilteration is the use of plants to absorb, concentrate and precipitate organic and inorganic pollutants from aqueous sources (Dushenkov et al., 1995; Salt et al., 1995; Flathman and Lanza, 1998; Zhu et al., 1999). Bioaccumulation involves the use of plants (roots) to immobilize the inorganic contaminant through the process of sorption, precipitation, complexation or metal valence reduction in the soil or aqueous environment (Berti and Cunningham, 2000; Ghosh and Singh, 2005). This process has been used to remediate mercury, selenium and tritium (Banuelos, 2000; Henry, 2000; Dushenkov, 2003). Bioaccumulation is the uptake and breakdown of organic molecules to simpler forms by plants using plant enzymes such as the dehalogenases, oxygenases and reductases (Black, 1995; Chaudhry et al., 1998). About 400 plant species have been identified as metal hyperaccumulators (Prasad and Freitas, 2003). Four aquatic plants; Cattail (*Typha domingensis*), duckweed (*Lemna obscura*), Hydrilla (*Hydrilla verticillata* Royle) and Swamp lilly (*Crinum americanum*) have been reported to hyperaccumulate Selenium (Se) (Carvalho and Martin, 2001). *E. crassipes*, *L. minor* and *A. pinnata* have been reported to phytoremediate Cadmium (Cd), Chromium (Cr), Cobalt (Co), Nickel (Ni) and Lead (Pb) (Upadhyay and Tripathi, 2007; Aina *et al.*,2012). The aim of the present study was to evaluate the phyto-remediation potential of water plant (*Hydrilla verticillata*) on some selected heavy metals.

2. Materials and Methods

Plant collection

The 7-8 week *Hydrilla verticillata* plants were collected from local water body, Nellore, Andhra Pradesh, India. Unwanted debris was removed from the plants before being washed with deionised water. The plants were washed thoroughly with tap water followed by deionised water prior to the experimentation. All the plants were grown in 15 lit experimental plastic tubs filled with 10 litres of water. A plant control i,e., plant grown in tap water was also maintained.

Bioaccumulation study

This study was conducted to investigate the metal uptake capacity of *Hydrilla verticillata* using such metals as: Potassium (K), Sodium (Na), Zinc (Zn), Lead (Pb), Iron (Fe), Cadmium (Cd), Magnesium (Mg), Copper (Cu) and Calcium (Ca). These metals were of analytical grade obtained from Sigma (Sigma Chemical Co., London) and used in the form of salts; Potassium chloride, Sodium chloride, Zinc sulphate, Lead acetate, Iron sulphate, Cadmium chloride, Magnesium chloride, Copper sulphate and Calcium chloride. A solution of 100 ppm concentration

of each of the salt was maintained. And four liters of each of the salt solution was added into separate bioaccumulation tanks. The test plants were allowed to grow in various concentrations of the chemicals, considering the BIS limit. All the experiments were maintained in triplicate in outdoor condition. The weight of the *H. verticillata* plants were taken before they were introduced into the different solution. The plants were exposed to the different solution for a period of one week with a photo period of 12 hours light and 12 hours dark cycle. The plants were left in the laboratory under the conditions of average temperature ranging between 26 °C and 32 °C, relative humidity between 65 in the night and 87 in the day and the average period of sunlight was 8 h per day. A control experiment was set up with no metal added to tap water. Three replicate experiments tanks were maintained in outdoor condition for each test and the control. After 11 days of metal exposure, the plants were digested for metal extraction and analysis.

Metal extraction from plant

The plants were removed from the bioaccumulation tank after 11 days and digested according to the method of Carvalho and Martin (2001). Each plant was weighed, cut, and blended. The plant was allowed to dry in an oven (Remi, India) at 45 °C for 48 hours. A dry weight was taken and each sample was placed in a 250 ml round bottom flask and 5 ml of 16 M nitric acid and 5 ml of deionized water were added. Each sample was heated for 10 to 15 minutes at 90 °C on a heating mantle. The sample was then allowed to cool and another 5 ml of 16 M HNO_3 was added and heated for the second time at 90 °C for 30 minutes. This step was repeated and 2 ml of deionized water and 3 ml of 30% hydrogen peroxide solution were gently added, and the mixture was heated until effervescence stopped. A 5 ml of 12 M HCl was added and this was refluxed for 10 to 15 minutes. The sample was allowed to cool and then diluted to 100 ml with 6% (v/v) HCl. The sample digest was vacuum filtered using a 0.45 µm Millipore membrane filter. The filtrate obtained was diluted to 100 ml and used immediately for metal analysis.

Metal analysis

Standard solutions of the metals to be analyzed were prepared. The atomic absorption spectrophotometer (AAS203, Chemito Technologies Pvt. Ltd., India) was set with power on for 10 minutes to stabilize. The standard metal solutions were injected to calibrate the AAS using acetylene as the carrier gas. An aliquot of both the metal solution taken from the experiment tank and that obtained from the plant digest were injected and the concentrations were obtained from the AAS.

Data analysis

The weight of *H. verticillata* and metal concentration were given to 2 decimal places and were reported as means ± SEM of triplicate results. Significant differences between metal uptake and control were assessed by a one-way analysis of variance (ANOVA) and the Student's t-test with two-tail probabilities of less than 0.05 considered significant using the SPSS 10 Statistical software.

3. Results

Effect of metals on weight of *Hydrilla verticillata*

The results obtained in this bioaccumulation experiment showed that the metals used in this study reduced the fresh biomass weight with varying degree (Table 1). The percent fresh biomass weight loss was highest; 9.94% with copper metal, 9.22% with cadmium and least 2.10% with magnesium. And the fresh biomass weight in the control experiment increased by 7.23%. However, the change in weight of the test and control plants over the bioaccumulation period was not significant (P>0.05).

Table 1: Biomass (g) of *H. verticillata* 11days after treated to metals

Metals	[1]Initial weight	[1]Final weight	Initial weight-Final weight	% of Weight
Cadmium	190.10 ± 2.41a	172.58 ± 13.69a	-17.52	9.22
Magnesium	213.41 ± 20.72a	209.00 ± 41.36a	-4.41	2.10
Copper	225.86 ± 52.36a	203.40 ± 23.64a	-22.46	9.94
Calcium	240.09 ± 25.63a	231.11 ± 20.78a	-8.98	3.74
Iron	218.21 ± 15.26a	213.10 ± 10.92a	-5.11	2.34
Lead	282.25 ± 45.32a	270.84 ± 17.41a	-11.41	4.04
Zinc	298.47 ± 22.84a	290.10 ± 22.75a	-8.37	2.80
Sodium	274.68 ± 29.63a	263.25 ± 27.14a	-11.43	4.16
Potassium	204.28 ± 42.10a	195.59 ± 10.70a	-8.69	4.25
Control	315.20 ± 20.96a	338.00 ± 24.16a	22.8	7.23

[1]Data represents mean ± SEM of triplicate results. Mean weight of *H. verticillata* plant before and after the experiment for each metal followed by different alphabets differ significantly ($p<0.05$).

Metal uptake capacity by *Hydrilla verticillata*

The various metals assayed in the experiment were found present in the acid digest of both the control and test plants. However, the concentration of the metals (Potassium, Lead, Cadmium and Copper) in the test plants differed significantly when compared to the control (Table 2). Due to the disparity in weight of the plants used in each experiment, the metal uptake capacity was expressed as concentration of metal uptake per dry weight of the plants (Table 3).

Table 2: Metal concentration (ppm) in *H. verticillata* grown in control and treated solutions

Metals	Concentration in Control	Concentration in treated solution
Cadmium	2.68 ± 0.70a	20.36 ± 4.02b
Magnesium	8.46 ± 2.30a	7.42 ± 2.10a
Copper	0.85 ± 0.26a	7.55 ± 0.72b
Calcium	32.21 ± 3.98a	32.64 ± 4.01a
Iron	19.48 ± 2.78a	18.06 ± 2.82a
Lead	2.40 ± 0.70a	0.64 ± 0.22b
Zinc	3.85 ± 1.12a	4.50 ± 0.77a
Sodium	3.90 ± 0.70a	3.98 ± 1.10a
Potassium	219.32 ± 7.52a	136.30 ± 4.76b

[1]Data represents mean ± SEM of triplicate results. Mean metal concentration in plants between test and control experiment followed by different alphabets differ significantly ($p<0.05$).

Table 3: Heavy metal uptake capacity (ppm) per dry weight of *H. verticillata*

Sample	[1]Dry weight of test plant (g)	Heavy metals	[1]Concentration of metal in test plant (B)	[1]Concentration of metal in control plant (A)	Concentration of metal uptake in plant (B-A)
1	20.40 ± 3.59	Cadmium	0.95 ± 0.10a	0.46 ± 0.15b	0.49
2	3.95 ± 1.85	Magnesium	1.90 ± 0.21a	0.48 ± 0.18b	1.42
3	15.32 ± 2.75	Copper	0.45 ± 0.05a	0.10 ± 0.04b	0.35
4	16.90 ± 2.89	Calcium	1.80 ± 0.18a	1.71 ± 0.35a	0.03
5	14.35 ± 2.47	Iron	1.23 ± 0.03a	1.20 ± 0.12a	0.03
6	3.24 ± 1.20	Lead	0.12 ± 0.08a	0.10 ± 0.05a	0.02
7	12.10 ± 3.11	Zinc	0.46 ± 0.07a	0.33 ± 0.05b	0.13
8	7.40 ± 1.23	Sodium	0.65 ± 0.14a	0.42 ± 0.08b	0.23
9	5.30 ± 1.25	Potassium	24.32 ± 0.95a	12.05 ± 1.52b	12.27

[1]Data represents mean ± SEM of triplicate results. Dry weight of control plant (g) = 18.47 ± 1.76. Mean between test and control experiment for each analyte followed by different alphabets differ significantly ($p<0.05$).

4. Discussion

The results (Table 2) showed that the *Hydrilla verticillata* can bioaccumulation metals such as Potassium, Sodium, Zinc, Lead, Iron, Cadmium, Magnesium, Copper and Calcium. *H. verticillata* has been reported to bioaccumulation some of these metals (Carbonell et al., 1998; Zhu et al., 1999; Ingole and Bhole, 2003; Mahmood et al., 2005; El-Gendy et al., 2006; Tiwari et al., 2007; Upadhyay and Tripathi, 2007). However, the reduction in the concentration of potassium, lead, iron and magnesium in the acid digest of the test plants could be as a result of the fact that the average weight of the control plants is higher than the test plants. But when the metal uptake capacity was expressed as concentration of metal uptake per dry weight of the plants (Table 3), the highest metal uptake capacity was observed with potassium and the least with lead. More so, all the metals assayed were found to be removed by *H. verticillata* but at different degrees (Table 3). The amount of metal removed (Table 3) with respect to lead (Pb), Copper (Cu) and Zinc (Zn) were lower than those reported in literature (El-Gendy et al., 2006). However, this may be due to the effect of the concentration of the metal in the plant growth medium. Studies have shown that the bioaccumulation efficiency of metals greatly depends on the concentration of such metals in solution, and the higher the concentration of the metals in the solution the lower the removal efficiency (Carvalho and Martin, 2001; Ingole and Bhole, 2003; Keith et al., 2006).

5. Conclusion

The study showed that *Hydrilla verticillata* could effectively bio-filtered contaminated water containing metals such as Potassium (K), Sodium (Na), Zinc (Zn), Lead (Pb), Iron (Fe), Cadmium (Cd), Magnesium (Mg), Copper (Cu) and Calcium (Ca), thus; reducing the environmental hazard that could arise from untreated waste water to the ecosystem. Future study will examine the potential of *H. verticillata* as a bio-agent to bioaccumulation of different heavy metals and other toxic materials from industrial wastewater in India.

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