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Heavy metal uptake capacity of *Hydrilla verticillata:* A commonly available Aquatic Plant

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Abstract

Removal of Cr and Cd by Hydrilla verticillata is influenced by concentration of the metals in the test solution . Uptake was higher at low concentration (15mg/l for Cr and 03mg/l for Cd) and decreased thereafter with increase in metal concentration. Cr accumulation was highest in leaves and roots at the concentration of 15mg/l and 20mg/l respectively. Cadmium accumulation was maximum in 3mg/l in both roots and shoots. The chlorophyll and carotenoid content of test plants revealed gradual decrease with increase in concentration of the test solution. Long term metal exposure affected chlorophyll synthesis which indicates inhibition of photosynthesis as a result of higher metallic concentration. This is a clear indication of disruption of chloroplast, thereby reducing photosynthetic activity. Growth was not affected morphologically except dark brown and necrotic spots treated with the solution, which might be an early symptom of metal toxicity.

Keywords: Hydrilla verticillata, cromium, cadmium.

Introduction

Industrial wastes are a matter of great concern all over the world as it pollutes the environment to a great extent. Wastes are generated from different processes and the amount of toxicity from the waste released varies with its own specific industrial processes[1-3]. Waste contains pollutants which are discarded materials, processed materials or chemicals. Pollution could be caused when these materials are released beyond the assimilation capacity of the environment[4]. Heavy metal pollution also results in deletorius health effects during exposure[5]. Tannery effluents are ranked as the highest pollutants among all industrial wastes. They are large contributor of chromium pollution, and to some extent of cadmium pollution along with other heavy metals[6].

Considering the effects on human beings, aquatic organisms and plants, appropriate treatment of the heavy metals from the waste water of great importance[7]. Different methodologies are used for the removal of the heavy metals, such as electro-dialysis, reverse osmosis and absorption etc[8]. However, none of these methodologies could claim to treat the heavy metals in economically feasible manner[9] Contrary to this, phytoremediation, i.e., removal of metals through plants offers one eco-friendly and cost effective methodology for the treatment of heavy metals from waste water[10].

At present, research is oriented towards low cost and eco-friendly technology for water purification. It has been reported that few aquatic macrophytes show the potentiality to absorb metallic ions and deposit them in different parts of plant body depending upon their affinity towards that particular metal[11]. Absorption and accumulation mechanism of the macrophytes render the services of cleaning of water body from the heavy metal contamination. *Hyptis suaveolens* has the ability to accumulate higher amounts of Cr and it may be under stress condition suitable for restoration of Cr contaminated areas as these are fund to flourish on chromium rich dumpsites[12].

Hydrilla verticillata (L.f.) Royle, is a submerged, rooted aquatic plant that forms dense mats in a wide variety of freshwater habitats. It is usually a gregarious plant that frequently forms dense, interwined mats at the water's surface. Approximately 20% of the plant's biomass is concentrated in the upper 10 cm of such a mat[13]. The plants grow and spread quickly. Small fragments of the plant can quickly develop adventitious roots and eventually produce an entire plant.

Hydrilla has very wide ecological amplitude, growing in a variety of aquatic habitats. It is usually found in shallow waters, 0.5 m or greater in depth. In very clear waters it can grow at depths exceeding 10 m. It tolerates moderate salinity up to 33 percent of seawater[14]. It also grows well in both oligotrophic and eutrophic waters, and even tolerates high levels of raw sewage. Sediments with high organic content provide the best growth, although *Hydrilla* also is found growing in sandy and rocky substrates[15].

The present study deals with the response of this prominent aquatic plant towards two important noxious heavy metals, chromium and cadmium contaminating water bodies through industrial waste.

Material and Methods

Experimental set up: About 7-8 week old *Hydrilla* plant material were collected from local water body. 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.

Preparation of chromium solution: The stock solutions of chromium were prepared by dissolving 5.916g analytical grade potassium dichromate ($K_2Cr_2O_3$) in 1000ml of distilled water, From this stock solution, 5, 10, 15, 20 and 25 mg/l chromium solutions were prepared and used in the study.

Preparation of cadmium solution: The stock solutions of cadmium were prepared by dissolving 1.951g analytical grade cadmium chloride ($CdCl_2.2½ H_2O$) in 1000ml of distilled water. From this stock solution, 1, 2, 3, 4 and 5 mg/l cadmium solutions were prepared and used in the study.

Biological treatment: The test plants were allowed to grow in various concentrations of the chemicals, considering the BIS limit[16]. All the experiments were maintained in triplicate in outdoor condition. Plants grown in tap water were taken as control. The plants were taken out of the tubs after 11 days and used for the estimation of chromium and cadmium accumulation and biochemical changes. Loss of water due to evaporation was made up daily by adding tap water to the mark in the tubs.

Metal concentration: The collected test plants were, taken out of the tubs after 11 days wiped out with 0.01N HCl and then washed with distilled water in order to remove the adsorbed ions. Both leaf and root portion were separated out and oven dried with 80^{o}C for 48 hours. The known weight of powdered samples was acid digested and the levels of Cr and Cd were estimated[17].

Estimation of Photosynthetic pigment: Chlorophyll and carotenoid estimation were carried out after 11 days of exposure in test solution. 0.5g of fresh leaf material was taken and ground with 10 ml of 80% acetone in pastle and mortar. The homogenate was centrifused at 3000rpm for 20 minutes. The supernatant was saved and re–extracted. The supernatant was used for chlorophyll and carotenoid estimation. The absorbance was read at 645 nm, 663 nm and 480nm.

Statistical analysis: The Mean and SD were calculated. The data were expressed in Mean± SD in triplicate.

Results and Discussion

Amount of removal of Cr and Cd by test plant material has been found to be influenced by concentration of the metal in the test solution (table-1). Metal uptake was higher at low concentration (15mg/l for Cr and 03mg/l for Cd) and decreased thereafter with increase in metal concentration. This is probably due to toxicity of concentration. Similar results were obtained in various other aquatic plant species[18,19].

Bioaccumulation of Cr and Cd in the body of *Hydrilla vrticillata*: There was an increase in metal accumulation in leaves and roots (table-2, figure-1, 2). Plants treated with 15mg/l solution for 11 days accumulated highest level of metal in leaves for Cr solution, 20mg/l highest in roots. Cadmium accumulation was maximum in 3mg/l in both roots and shoots. In the present study, bioaccumulation of both Cr and Cd in root is significantly lower than leaf in all concentrations. Uptake of metal depends on factors such as level of contamination, plants' ability to intercept, metal availability and interaction between plant habitat and climatic condition[20].

Phytotoxicity: The chlorophyll and carotenoid content of test plants revealed gradual decrease (table-3, figure-3,4) with increase in concentration of the test solution. Long term metal exposure was here found to affect chlorophyll synthesis which indicates inhibition of photosynthesis as a result of higher metallic concentration. This is a clear indication of disruption of chloroplast, thereby reducing photosynthetic activity. Growth was not affected morphologically except d ark brown and necrotic spots treated with the solution, which might be an early symptom of metal toxicity. Inhibition of root and shoot length, reduction in chlorophyll and carotenoid in plants treated with metallic solution was previously reported[21,22].

World Health Organisation, 1990, has reported hexavalent chromium, Cr (VI) to be a toxic, painful skin irritant and a carcinogen. In plants, it interferes with several metabolic processes causing phytotoxicity such as reduced growth, chlorosis, ultrastructural effects on organneles, chromatin condensation and swelling of mitochondria etc. which leads to plant death.

Table-1
Percentage of removal of Cr. and Cd from aqueous solution by *Hydrilla verticillata* after 11 days of retention

Initial Conc of Cr (mg/l)	Initial conc of Cd (mg/l)	Final conc of Cr (mg/l)	Final conc of Cd (mg/l)	Percentage of removal of Cr	Percentage of removal of Cd
05.00	01.00	0.95±0.02	0.005±0.00	76.60	99.50
10.00	02.00	5.72±0.05	0.087±0.00	73.80	95.60
15.00	03.00	6.79±0.02	0.186±0.00	66.05	95.30
20.00	04.00	2,24±0.00	0.181±0.00	38.05	64.02
25.00	05.00	2.60±0.00	0.192±0.00	32.65	64.00

Values represent Mean± SD of three replicates.

Table-2
Accumulation of Cr and Cd in root and leaf (mg/g) of *Hydrilla verticillata* in different concentration after 11 days of retention period

Heavy metal	Conc of aqueous metal soln (mg/l)	Accumulation of Cr and Cd in *Hydrilla* (mg/g dry wt)	
		Leaf	Root
Cr	05.00	0.58±0.00	0.165±0.02
	10,00	1.45±0.02	0.370±0.02
	15.00	1.77±0.00	0.390±0.02
	20.00	1.90±0.01	0.310±0.00
	25.00	1.35±0.00	0.310±0.00
Cd	01.00	0.80±0.00	0.250±0.00
	02.00	1.27±0.04	0.785±0.15
	03.00	2.26±0.02	1.060±0.00
	04.00	1.90±0.01	0.826±0.10
	05.00	0.65±0.01	0.657±0.00

Values represent Mean± SD of three replicates

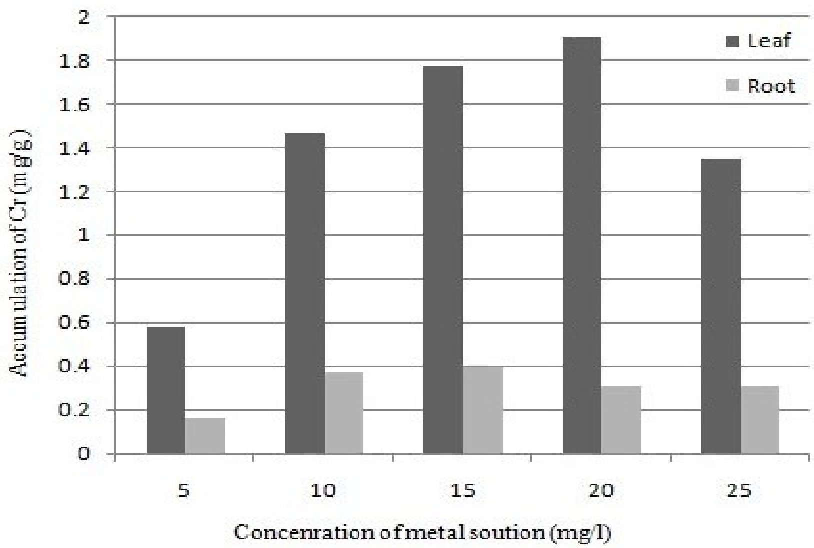

Figure-1
Accumulation of Cr in root and leaf (mg/g) of *Hydrilla verticillata*

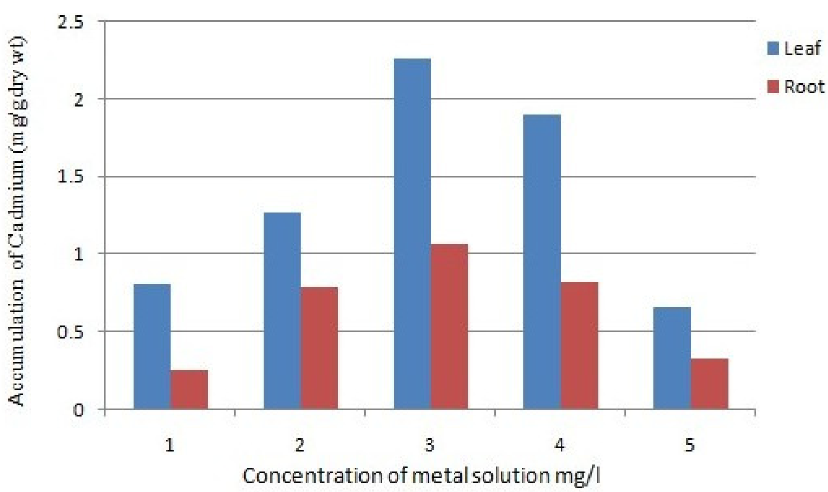

Figure-2
Accumulation of Cadmium in root and leaf (mg/g) of *Hydrilla verticillata*

Table-3
Effect of Cromium and Cadmium accumulation on chlorophyll and carotenoid content of *Hydrilla verticillata* after 11 days of retention period

Heavy metal	Conc of aqueous metal solution (mg/1)	Pigment content (mg/g) fresh wt of *H.verticillata* treated with aqueous soln of metal	
		Chlorophyll	Carotenoid
Cr	05.00	0.23±0.00	0.07±0.00
	10,00	0.26±0.04	0.09±0.02
	15.00	0.22±0.00	0.07±0.01
	20.00	0.18±0.01	0.05±0.00
	25.00	0.08±0.00	0.05±0.00
Cd	01.00	0.23±0.00	0.07±0.00
	02.00	0.30±0.02	0.13±0.02
	03.00	0.26±0.02	0.10±0.01
	04.00	0.17±0.04	0.04±0.00
	05.00	0.05±0.00	0.04±0.00

Values represent Mean± SD of three replicates.

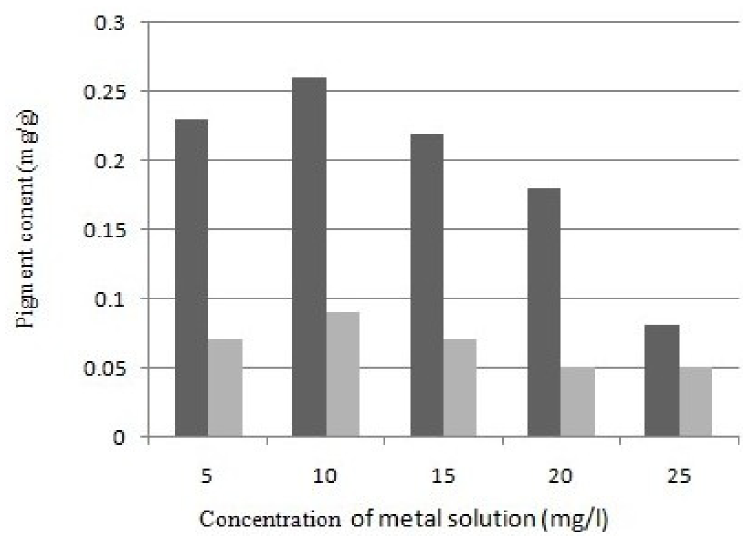

Figure-3
Effect of Cromium accumulation on chlorophyll and carotenoid content of Hydrilla verticillata

Cadmium (Cd), although considered as non-essential for normal life processing of living organisms is one of the very toxic heavy metals. It has been recognized for its negative effect on the environment where it accumulates throughout the food chain posing a serious threat to human health as well as toxic effects on plants[23].

A large number of aquatic plants, such as water hyacinth (*Eichornea cressipes*), Duck weeds (*Lemna sps.)*, small water fern (*Azolla sp*.), *Pistia stratiotes*, *Nelumbo nucifera* have been used for the removal of heavy metals from waste water[24]. All the above species take up metals from water, producing an internal concentration of several folds, greater than surrounding environment. *Hydrilla verticillata* known to grow profusely in polluted as well as natural water bodies. In the present study this plant was found to successfully remove both C chromium and cadmium and reveals strong potential of this plant to uptake Cr and Cd from aqueous solution. However, higher concentration become toxic and decrease plant uptake as well as inhibits plant growth.

Conclusion

Based on these investigations, *Hydrilla* can be ideally used as possible application in reclamation and revegetation of adversely affected water environments, biological monitoring of the state of pollution of the environment, explore the possibility of recognizing the local plant communities as indicator plants and for phyto-remediation. It may be suggested to be an economically cheap technology for the removal of chromium and cadmium from polluted water.

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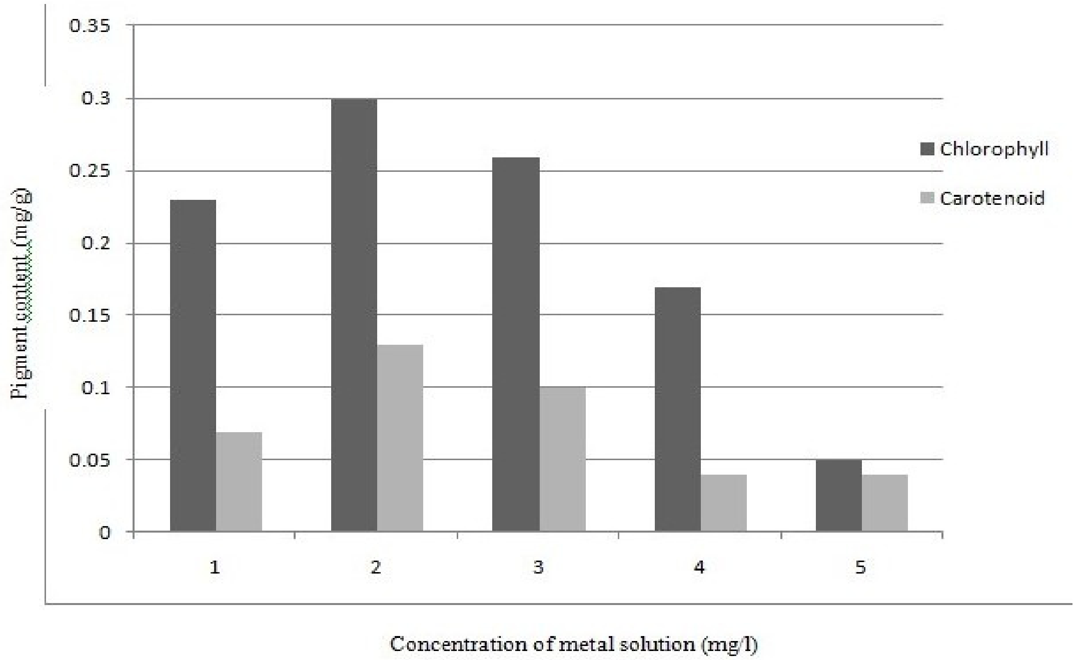

Figure-4
Effect of Cadmium accumulation on chlorophyll and carotenoid content of *Hydrilla verticillata*

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