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Original Research Paper

Biochemical Changes During Lead (Pb) Uptake by the Alga *Pithophora*

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ABSTRACT

Current technologies for cleaning heavy metal contaminated sites like electrolytic, chemical leaching and *in situ* immobilization are all extremely expensive and questionably effective. Bioremediation like the use of algal extract, sequester and/or detoxify heavy metals and other pollutants may offer a cost-effective, less invasive and potentially more effective means of addressing existing heavy metal contamination than those currently practiced. The alga *Pithophora* accumulated high amounts of the heavy metal lead. Under simulated conditions, maximum uptake and bioaccumulation of lead occurred within 6 days. The phytotoxic concentrations of lead reduced chlorophylls, protein, proline, sugar and catalase content and increased the peroxidase activity in the alga.

INTRODUCTION

In nature, metals constitute one of the major environmental pollutants. Pollution by heavy metals in industrial waste effluents is now a global problem. Long term persistence of heavy metals in the environment represents a hazardous problem leading to harmful effects on aquatic as well as human life.

Hashimoto & Furukawa (1985) indicated that the presence of algae in various effluents helped in treatment of such wastewaters. Such information prompted us to use algae for remediation of heavy metals from aquatic bodies. There are several reports of algae accumulating heavy metals in their tissues when grown in polluted waters. Through bioaccumulation process, the concentration of the metals get several folds higher in the organisms than their surroundings, which is called biomagnification. Other reports indicated that carboxyl group on algal cell walls may be responsible for a great portion of metal binding to inactivate algal biomass (Gardea et al. 1990). In live algae, intracellular phosphate has been found to be responsible for metal sequestration (Zhang & Majidi 1994).

Pithophora is related to genus *Cladophora*. These algae were found to clog waterways and lakes with their large mats and filaments. These macro algae are also formed with the removal of nutrients and soluble heavy metals as they form large masses in ponds thereby providing large surface area for binding metals. *Pithophora* was known to accumulate large amounts of arsenic, lead and zinc (Imamul Huq et al. 2004).

Effect of light stress on peroxidase, succinate dehydrogenase and total chlorophyll content in *Andrographis paniculata* has been noted by Kumar et al. (2009). Biochemical assessment of nitrogen fixing cyanobacteria, *Stigonema ocellatum* to acute toxicity of cadmium nitrate was made by Kumar et al. (2009). Acute toxicity of lead nitrate on biochemical changes of nitrogen fixing cyanobacteria, *Aulosira fertilissima* has also been studied by Kumar et al. (2009). The present paper deals with the biochemical changes in the alga *Pithophora* during uptake of the heavy metal lead.

MATERIALS AND METHODS

In the present work, selected algal species *Pithophora* sp. for the metal removal was used. The study aimed at the accumulation of various concentrations of lead (Pb) at different intervals of time by the algae. In *in vivo* experiment, short term toxicity test was designed as per the standard methods (APHA-AWWA-AWPC 1989). In this test to culture the algae, metal accumulation and estimation of biochemical parameters were made.

Culture of algae: The wastewater alga *Pithophora* sp. was collected from Ankleshwar GIDC area. Algae were thoroughly washed by tap water to remove any epiphytic algae attached to it. The media preparation and the culturing methods were carried out following standard methods (APHA-AWWA-AWPC 1989). Duration of incubation period was 3, 6 and 9 days.

Metal accumulation: The stock solution of 1000 ppm of lead was prepared in double distilled water by dissolving lead acetate $(C_2H_3O_2)_2Pb.3H_2O$. For the experiment, 2, 5, 10, 20 and 30 ppm concentration of the metal was used. Algal samples, harvested after 3, 6 and 9 days of incubation, were washed repeatedly, dried and digested in $HNO_3/HClO_4$ mixture (4:1, v/v) for heavy metal analysis. Estimation of lead

was made on ICP [Inductively Couple Plasma Spectrometer, Perkin Elmer Corporation (ICP Optima 3300RL)].

Estimation of biochemical parameters: Peroxidase was determined following Sadasivam & Manickam (1992) and sugar was determined by GOD-POD test. Protein content was determined in accordance with the method of Lowry et al. (1951). Catalase and proline concentrations were determined following Thimmaiah (1999). Chlorophylls *a*,*b* and total chlorophyll concentrations were determined by the procedures of Arnon (1949).

RESULTS AND DISCUSSION

Removal of harmful metals from the environment, particularly from the aquatic water bodies as well as from the industrial wastes is absolutely essential before they enter into food chains. Algae have low and simple nutrient requirements, grow rapidly and easily thrive in aquatic environment and can be well studied physiologically as compared to higher plants. Algae produce oxygen for other aquatic biota and oxidise organic materials present in wastes making wastewater more favourable for release into the environment. There are no reports of dead higher plant materials removing metals, but algae even when dead, efficiently remove metal ions (Morendo-Garrido et al. 1998). The alga selected in the present study is used with a view to provide a useful solution of problems of metal pollution.

Somashekar (1982) reported *Pithophora* as one of the dominant algae in the effluents from electroplating industry where it could accumulate 15mg/L of metals. It was also investigated in the present work that *Pithophora* accumulated higher level of lead. The differential absorption capabilities of the metal ions in different algal groups depend on several other external factors such as pH, background metal ion concentration, light intensity, chemical make up of the medium as well as growth phase and cell division stages (Skipnes et al. 1975). *Pithophora* showed more affinity towards Pb ions and this might be the reason that lead is accumulated in higher amounts in the cells. In present work accumulation of Pb ions was higher with 8.96 mg/L at 5 ppm concentration of 6 days and lower with 0.28 mg/L at 10 ppm concentration of 6 days (Table 1, Fig. 1).

In *Pithophora*, peroxidase activity increases after 3 days of exposure to Pb (Table 2). There are reports suggesting the increase in peroxidase activity under metal ion stress. Mukherji & Maitra (1976) demonstrated the stimulated activities of catalase, peroxidase and IAA oxidase under conditions of Pb toxicity in rice seedlings. The increased peroxidase activity is a response to an increase in peroxides, which reduce disruption of plasma membrane by lipid peroxidation.

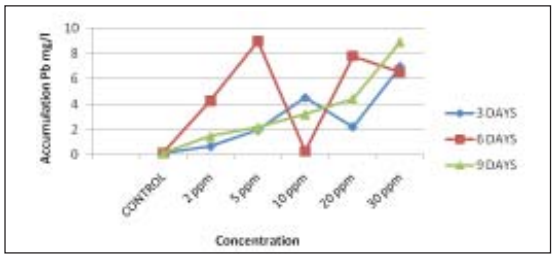

Fig. 1: Comparison of accumulation of Pb ion at different durations of incubation period in *Pithophora.*

In the present study, the activity of catalase decreased in Pb ion treatments after each day of exposure in *Pithophora* (Table 3). The increased exposure periods lower the enzyme activity because of loss of pigment, protein and proline induced due to metal ions interfering with the physiological activity of the cells.

Earlier, it was reported that exposure of several types of stresses viz., heat, salinity, osmotic stress, metal exposure, results in alterations in protein synthesis. In the present study

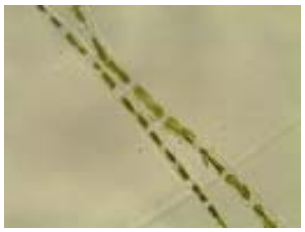

Fig. 2: Pithophora after accumulation of 2 ppm Pb on 6 day.

Fig. 3: Pithophora after accumulation of 5 ppm Pb on 6 day.

Fig. 4: Pithophora after accumulation of 10 ppm Pb on 6 day.

Fig. 5: Pithophora after accumulation of 20 ppm Pb on 6 day.

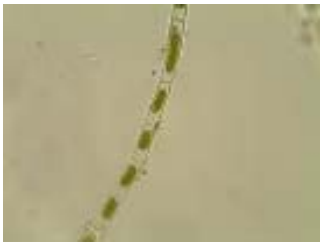

Fig. 6: Pithophora after accumulation of 30 ppm Pb on 6 day.

Table 1: Uptake of Pb ions at various concentrations (mg/L) after 3, 6 and 9 days of exposure by *Pithophora* sp.

No.	Concentration (Pb)	3 Days	6 Days	9 Days
1	Control	0.1352	0.1352	0.1352
2	2 ppm	0.6685	4.2451	1.4714
3	5 ppm	1.9631	8.9600	2.1775
4	10 ppm	4.5061	0.2808	3.2154
5	20 ppm	2.2129	7.7945	4.4331
6	30 ppm	6.9655	6.5344	8.9289

Table 2: Estimation of peroxidase (mg/g) at various Pb concentrations in *Pithophora* sp.

No.	Concentration (Pb)	3 Days	6 Days	9 Days
1	Control	0.890	0.890	0.889
2	2 ppm	0.891	0.739	0.729
3	5 ppm	0.893	0.740	0.735
4	10 ppm	0.898	0.746	0.731
5	20 ppm	0.900	0.751	0.743
6	30 ppm	0.911	0.779	0.771

Table 3: Estimation of catalase (mg/g) at various Pb concentrations in *Pithophora* sp.

No.	Concentration (Pb)	3 Days	6 Days	9 Days
1	Control	25.4	25.3	25.1
2	2 ppm	27.0	23.2	22.9
3	5 ppm	26.5	21.7	21.1
4	10 ppm	25.0	20.7	20.3
5	20 ppm	24.2	20.2	19.5
6	30 ppm	23.0	20.0	19.1

Table 4: Estimation of protein (mg/g) at various Pb concentrations in *Pithophora* sp.

No.	Concentration (Pb)	3 Days	6 Days	9 Days
1	Control	10.92	10.61	10.56
2	2 ppm	10.87	10.76	10.67
3	5 ppm	11.56	11.02	10.88
4	10 ppm	11.03	10.56	10.35
5	20 ppm	10.69	10.07	9.01
6	30 ppm	10.11	9.80	9.00

Table 5: Estimation of proline (mg/g) at various Pb concentrations in *Pithophora* sp.

No.	Concentration (Pb)	3 Days	6 Days	9 Days
1	Control	0.883	0.880	0.877
2	2 ppm	0.863	0.861	0.857
3	5 ppm	0.861	0.851	0.846
4	10 ppm	0.858	0.822	0.820
5	20 ppm	0.843	0.819	0.810
6	30 ppm	0.839	0.811	0.808

Table 6: Estimation of sugar (mg/g) at various Pb concentrations in *Pithophora* sp.

No.	Concentration (Pb)	3 Days	6 Days	9 Days
1	Control	20.7	20.7	20.7
2	2 ppm	24.5	23.2	22.5
3	5 ppm	23.0	22.8	21.7
4	10 ppm	24.0	23.6	23.8
5	20 ppm	20.2	21.4	22.1
6	30 ppm	19.2	19.9	20.9

Table 7: Estimation (mg/g) of chlorophyll *a*, *b* and total chlorophyll at various Pb concentrations in *Pithophora* sp.

No.	Concentration	3 days			6 days			9 Days		
	(Pb)	Chl-*a*	Chl-*b*	Total chl	Chl-*a*	Chl-*b*	Total chl	Chl-*a*	Chl-*b*	Total chl
1	Control	0.217	0.158	0.375	0.216	0.158	0.374	0.216	0.157	0.373
2	2 ppm	0.217	0.165	0.382	0.213	0.162	0.375	0.212	0.158	0.370
3	5 ppm	0.211	0.167	0.378	0.208	0.159	0.367	0.202	0.159	0.361
4	10 ppm	0.212	0.147	0.359	0.205	0.145	0.350	0.201	0.144	0.345
5	20 ppm	0.216	0.161	0.377	0.212	0.160	0.372	0.210	0.146	0.356
6	30 ppm	0.213	0.151	0.364	0.207	0.149	0.356	0.205	0.145	0.350

it was observed that the algae showed enhanced protein level after first exposure period i.e., after 3 days at 5 ppm concentration, which is higher than the others (Table 4). Enany & Issa (2001) investigated the accumulation of proline and correlated it with protein content. *Pithophora* showed reduction in the amount of protein at 20 ppm and 30 ppm Pb level. This might be due to degradation of protein at extreme concentration of the metal ions. The degradation of protein content was reported after 6 and 9 days in *Pithophora*.

It was found that the proline content decreases day by day after the treatment with *Pithophora*. After treatment proline accumulation has highest value of 0.863 mg/g at 3 days at 2 ppm concentration and lower value of 0.808 mg/g after 9 days at 30 ppm exposure (Table 5). As a result of less proline amount after the metal exposures, the cells have lower survival and less protected.

The sugar content has decreased almost continuously in *Pithophora* except on 3 days at 2 ppm, when it was higher

than others. The increased concentration of metal ion has resulted in lower sugar content of 19.2 mg/g at 30 ppm after 3 days of treatment in *Pithophora* (Table 6).

The chlorophyll pigment of the algae *Pithophora* under study was affected by heavy metal exposure (Table 7). Inhibition in chlorophyll contents in the algae supports the earlier findings, which suggested that the impaired metabolic status imposed by toxic metals, might be associated with either induction or activation of specific degrading enzymes leading to the breakdown of cell organelles and macromolecules (Nag et al. 1984).

CONCLUSION

The study proves that algae can sequester metal ion in their cells with differential uptake capacity towards absorption of different metals. As metals hinder normal biological processes in algae, they affect various biochemical parameters like enzymes catalase and peroxidase, protein, proline, sugar and chlorophylls in the algae . As a response to heavy metal stress, proline accumulation was found in the algae. Prolonged exposure to metal ions caused inhibitory effect on the synthesis of chlorophylls, protein and proline contents of the algae. Peroxidase activity, in general, exhibited a rise in *Pithophora*, while the activity of catalase decreased. Therefore the survival of the algae under heavy metal stress might be due to increased activity of peroxidase and not due to catalase.

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