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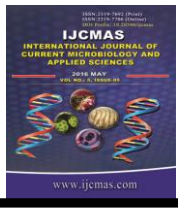

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Studies on the Toxicity of Chromium (VI) to *Pistia stratiotes* L. Plant and its Removal

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A B S T R A C T

Rapid growth of industrialization leads to pollution due to waste water. Many countries have experienced pollution hazards due to toxic metals. Industrial waste effluents containing toxic metals are creating water pollution problems. The present paper reports on the systematic studies of the uptake of Cr (VI) and the effect of the metal on some biochemical constituents of the plant *Pistia stratiotes* L. The biochemical parameters were : Hill activity, Chlorophylls, protein, free amino acid, inorganic phosphorus, RNA, DNA, dry weight and the activities of Protease, RNase and Peroxidase, etc. The effects on the biochemical parameters differed from those in control experiments. The absorption of the metal by the plants gradually increased with increase in concentration of Cr (VI) in the culture medium. The aquatic plant species may be regarded as Cr (VI) accumulators. So, the plant *Pistia stratiotes* L**.** can serve the mankind as the scavenger of Cr (VI) from waste water.

Introduction

Water pollution by toxic chemicals present in industrial waste effluents is a worldwide problem now. Both developed and developing countries are seriously affected due to this water pollution. Many industries like chrome plating, leather, fertilizer, etc. discharge waste effluent containing Cr (VI) into surface waters.

The permissible limit of Cr (VI) in drinking water is 0.05 mg/l. It is irritating to mucous membranes. It causes ulcerations and a variety of respiratory, gastrointestinal, lung and skin complications (Sterrett, 1978). Attempts were made in our laboratory to develop methods for the removal of Cr (VI) (Sen *et al.*, 1979, 1980) from aqueous solutions using hydrous oxides of Sn (IV) and Zr (IV). Aquatic plant *Pistia* has been used in our laboratory for the removal of Hg (II) (De, *et al.*, 1985). *Eichhornia*, *Oedogonium* and *Hydrilla* were also used for the removal of Hg (II) and Cr (VI). The results of all these studies have encouraged us to undertake investigation for the removal of Cr (VI) by *Pistia*. This paper reports the results of the uptake of Cr (VI) by *Pistia* plant and effect of the metal on the changes in some biochemical processes.

Materials and Methods

Plant material

The young plants, *Pistia stratiotes* L., were collected from uncontaminated fresh water pond around Visva-Bharati University campus, Santiniketan, West Bengal, India. The collected plants were grown in a greenhouse at 30 ±2°C. Generally 5 to 10 days were required for the maturity of the plant. Only mature plants were used during this investigation.

Treatment with Cr (VI)

Six plastic tumblers (20 L) were filled with 10 L of culture medium each. The culture medium of pH 7.5 was prepared as per Hewitt (1963) with slight modification necessary for adjustment of lower concentration of nutrients required for aquatic plants. From a stock solution of Na_2CrO_4 different volumes of solution were added to the culture medium separately in order to maintain different concentrations of Cr (VI) (0.5 ppm to 10.0 ppm). In order to check the loss of Cr (VI) from experimental systems due to evaporation and adsorption on the walls of the containers a control experiment without plant was performed simultaneously with the experimental run.

Plastia plants (200 g) were floated in each tumbler. They were cultured in a natural environmental condition in a greenhouse during summer season.

Analysis of uptake of Cr (VI) was made in both culture medium and plants after 1, 2, 3, 4, 5, 6 and 7 days of contact. Biochemical parameters like chlorophyll, protein, free amino acid, DNA, RNA, Hill activity, dry weight and drift of activities of enzymes like protease, catalase, peroxidase and acid and alkaline pyrophosphatases were analysed in the treated plants.

Biochemical Parameters

Total chlorophyll, chlorophyll (a) and chlorophyll (b) were estimated by the method of Knudson *et al.,* (1977). For protein estimation, phenolic compounds were removed by the method of Kar and Mishra (1976); protein was then extracted from the residue after removal of chlorophyll by digesting with 0.5 N NaOH at 80°C for 1 hr and estimated by Folinphenol reagent (Lowry *et al.*, 1951). Free amino acid was extracted according to Dwivedi, *et al*., (1979) and estimated spectrophotometrically by the method of Moore and Stein (1948). DNA and RNA were extracted **(**Cherry, 1962) and RNA was estimated by the method of Markham (1955) and DNA by the method of Burton (1956) as modified by Choudhuri and Chatterjee (1970). The Hill Activity was measured by the method of Kaniuga *et al*., (1978) following the modification by Jana and Choudhuri (1982). Dry weight of the samples was determined by drying the weighed samples at 80^{o} C in a hot air oven for four days and reweighing. Hill activity was expressed as µ mole dichlorophenolindophenol (DCIP) reduced /mg chlorophyll /hr.

For enzyme assays, the protease, catalase and peroxidase were extracted (Kar and Mishra, 1976) in phosphate buffer (pH 6.5). The protease activity was estimated by the method of Snell and Snell (1971) with little modification (Biswas and Choudhuri, 1978). Residual protein was measured by Folin- phenol reagent (Lowry *et al.*, 1951). Catalase activity was measured by the method of Biswas and Choudhuri, (1978) while peroxidase activity by the method of Kar and Mishra (1976) using pyrogallol as co-substrate. Extraction and measurement of the activities of acid and alkaline pyrophosphatases were done

according to the methods of Kar and Mishra (1975).

For all enzyme assays, zero time control was taken as a blank and the activities of enzymes except protease were expressed as

$$\left(\frac{\Delta A \; X}{TV \;\; T \;\; X \atop v} \right)$$

where ΔA is the absorbance of the sample after incubation minus the absorbance at zero time control; TV is the total volume of the filtrate, (t) is the time (hr) of incubation with substrate and (v) is the volume of filtrate taken for incubation (Fick and Qualsett, 1975). Protease activity was expressed as µg of protein hydrolysed /hr/g fresh weight.

Each experiment was carried out ten times and the mean values with standard deviation are given in the tables.

Results and Discussion

Uptake of Cr(VI) by *Pistia*

Pistia plants, water and sediment samples collected from the selected pond were analyzed for Cr (VI) and it was observed that they were free from detectable Cr (VI) contamination.

At 20 ppm of Cr (VI), the plants died after 3 days but at 0.5, 1.0, 5.0 and 10.0 ppm the study was undertaken upto 7 days and it is evident from the results that the plants were affected to a greater extent in case of 5.0 and 10.0 ppm concentration of Cr (VI). The day by day removal of Cr (VI) from the culture medium is represented, while the results of 3 and 5 days of contact are tabulated in Table I. The results of 6 and 7 days of contact did not show any significant change. It is evident that the plants took up maximum Cr (VI) and accumulated it mainly in roots within 5 days. The Cr (VI) content in shoots is negligible upto 3 days and then increased slightly with the time of exposure. Actually, 90 to 95% of Cr (VI) was accumulated in the roots in each case. When the tumbler contained 5.0 and 10.0 mg per 10 L the Cr (VI) was totally absorbed by the plants. Percent removal of Cr (VI) gradually decreased with increasing concentrations. At lower concentrations, however, the accumulation of Cr (VI) occurred mainly in roots. The possible explanation is that probably Cr (III) is formed due to reduction and forms complexes with COOH groups (Sigel, 1973) in the roots thus preventing it from passing into the shoot system.

Toxic Effects of Cr (VI) on Changes in some Biochemical Parameters of Pistia

From Table II, it is evident that at 10 ppm of Cr (VI), Chlorophyll a and b decreased with the time of exposure. At 0.5 and 1.0 ppm concentrations there was no significant change in these parameters over control with time. The results suggest that at 10 ppm the Cr (VI) lowered the chlorophyll by decreasing the synthesis of chlorophyll as possibly by increasing chlorophyllase activity. The lower ration of chlorophyll a to b was obtained due to a greater decline in chlorophyll a compared to chlorophyll b. It also suggests that activities of both the photosystems (PI and PII) may be equally active and C3 type of metabolism was prevalent in this species (Black and Mayne, 1970). Fig. 2 shows that Cr (VI) affected least Hill activity at 0.5 and 1.0 ppm concentration. However, Hill activity declined with increasing contact time in days at >5 ppm of Cr (VI). Our results reveal the disorder of chloroplast membrane and inactivation of electron transport of photosystem II (Shioi *et al.*, 1978) at >5 ppm of Cr (VI).

Tables III and IV show the effect of Cr (VI) on changes in protein, free amino acid, DNA and RNA after 3 and 5 days of contact. Protein content decreased in the shoot system with the treatment of Cr (VI) at 10 ppm, while in root system the value remained more or less same as the control. Below 5 ppm, the protein content showed no significant change over control. In all the treatments, free amino acid decreased in roots with increasing concentration of Cr (VI), while in shoots it increased with concentration. So Cr (VI) exerted its adverse influence more in roots than in shoots. DNA content remained the same as that in control in roots and shoots. At 10 ppm the RNA content decreased of control roots and shoots. At bellow 5 ppm the values were more or less the same as that in control. A decrease in RNA content with time at 10 ppm was due to the fact that Cr (III), produced due to reduction of Cr (VI), was tied with DNA and RNA molecules and changed the conformation of nucleic acids. It can be stated here that the binding of Cr (III) with RNA prevents its degradation by RNase. At 10 ppm, the dry weight increased in both roots and shoots; while below 1 ppm the value remained the same as that in control (Table V).

Table.1 Absorption of Cr(VI) by *Pistia* Plants after 3 and 5 Days of Contact*

Initial absorption by plants Amount of Cr(VI) (mg/101)	Cr(VI) in culture medium after (mg/101)		Cr(VI) Absorbed by Plants					
			Root (mg/g fresh wt)		Shoot (mg/g fresh wt)		Whole plant (mg/g fresh wt)	
	3	5	3	5	3	5	3	5
5	1.03 ± 0.02	0.00 ±0.00	0.05 ±0.003	0.06 ±0.001	trace	0.001 ±0.001	0.017 ± 0.001	0.021 ±0.001
10	3.55 ± 0.05	1.54 ±0.01	0.08 ±0.007	0.106 ± 0.009	0.0006 ± 0.0001	0.0017 ±0.0001	0.027 ±0.002	0.037 ±0.003
50	40.65 ±0.4	33.75 ± 0.5	0.116 ±0.001	0.203 ±0.003	0.004 ± 0.001	0.007 ±0.0008	0.041 ±0.0007	0.073 ±0.003
100	88.1 ± 0.9	78.2 ±0.8	0.14 ± 0.004	0.173 ±0.005	0.0072 ±0.0002	0.012 ± 0.0009	0.052 ±0.005	0.100 ± 0.007

*Each value is the mean of ten replicates ± S.D.

Table.2 Effect of Toxicity of Cr VI) on Chlorophyll Content (mg/g Fresh wt) in the Leaves of *Pistia* after Different durations of Contact

Treatment (ppm	Chlorophyll a			b			a : b		
	Contact Time in Days								
	0	3	5	0	3	5	0	3	5
Control	0.789 ± 0.002	0.785 ± 0.003	0.780 ± 0.005	0.185 ± 0.003	0.183 ± 0.002	0.182 ± 0.004	4.26 ± 0.006	4.28 ± 0.003	4.28 ± 0.005
0.5	0.789 ± 0.002	0.784 ± 0.002	0.778 ± 0.006	0.185 ± 0.003	0.183 ± 0.001	0.182 ± 0.002	4.26 ± 0.006	4.28 ± 0.004	4.27 ± 0.003
1.0	0.789 ± 0.002	0.782 ± 0.004	0.775 ± 0.005	0.185 ± 0.003	0.181 ± 0.002	0.180 ± 0.002	4.26 ± 0.006	4.32 ± 0.003	4.30 ± 0.005
5.0	0.789 ± 0.002	0.735 ± 0.004	0.679 ± 0.007	0.185 ± 0.003	0.176 ± 0.004	0.169 ± 0.005	4.26 ± 0.006	4.17 ± 0.008	4.02 ± 0.009
10.0	0.789 ± 0.002	0.678 ± 0.005	0.583 ± 0.008	0.185 ± 0.003	0.175 ± 0.004	0.164 ± 0.003	4.26 ± 0.006	3.87 ± 0.007	3.55 ± 0.005

* Each value is the mean of ten replicates ± S. D.

Table.3 Effect of Toxicity of Cr (VI) on Changes in Protein and Free Amino Acid (mg/g Fresh wt) in *Pistia* after Different Durations of Contact *

Root, shoot and Cr (VI) With concerntration (ppm)	Protein			Free Amino Acid		
	Contact Time in Days					
	0	3	5	0	3	5
Root control	16.2 ± 0.002	15.7 ± 0.003	15.5 ± 0.004	0.29 ± 0.003	0.35 ± 0.005	0.36 ± 0.004
0.5	16.2 ± 0.002	15.6 ± 0.004	15.4 ± 0.003	0.29 ± 0.003	0.37 ± 0.006	0.34 ± 0.006
1.0	16.2 ± 0.002	15.4 ± 0.002	15.2 ± 0.003	0.29 ± 0.003	0.32 ± 0.003	0.30 ± 0.002
5.0	16.2 ± 0.002	14.8 ± 0.005	14.5 ± 0.006	0.29 ± 0.003	0.30 ± 0.008	0.29 ± 0.007
10.0	16.2 ± 0.002	14.3 ± 0.004	14.0 ± 0.003	0.29 ± 0.003	0.29 ± 0.001	0.28 ± 0.005
Shoot control	12.5 ± 0.003	12.9 ± 0.002	13.4 ± 0.001	0.07 ± 0.004	0.08 ± 0.002	0.08 ± 0.004
0.5	12.5 ± 0.003	13.2 ± 0.004	13.8 ± 0.005	0.07 ± 0.004	0.08 ± 0.005	0.09 ± 0.006
1.0	12.5 ± 0.003	13.5 ± 0.002	14.1 ± 0.001	0.07 ± 0.004	0.09 ± 0.003	0.10 ± 0.001
5.0	12.5 ± 0.003	12.1 ± 0.006	11.5 ± 0.007	0.07 ± 0.004	0.10 ± 0.007	0.12 ± 0.008
10.0	12.5 ± 0.003	11.2 ± 0.009	9.9 ± 0.008	0.07 ± 0.004	0.11 ± 0.005	0.13 ± 0.009

* Each value is the mean of ten replicates ± S. D.

Table.4 Effect of Toxicity of Cr (VI) on Changes in DNA and RNA (mg/g Fresh wt) in *Pistia* after different Durations of Contact *

Root, shoot and Cr (VI) with Concentration (ppm)	DNA			RNA		
	Contact Time in Days					
	0	3	5	0	3	5
Root control	2.7 ± 0.02	2.7 ± 0.04	2.8 ± 0.01	15.8 ± 0.04	17.9 ± 0.06	18.4 ± 0.08
0.5	2.7 ± 0.02	2.7 ± 0.05	2.6 ± 0.04	15.8 ± 0.04	18.0 ± 0.03	18.7 ± 0.05
1.0	2.7 ± 0.02	2.7 ± 0.02	2.6 ± 0.01	15.8 ± 0.04	18.2 ± 0.03	19.3 ± 0.01
5.0	2.7 ± 0.02	2.7 ± 0.05	2.6 ± 0.05	15.8 ± 0.04	15.3 ± 0.09	15.1 ± 0.09
10.0	2.7 ± 0.02	2.6 ± 0.03	2.6 ± 0.04	15.8 ± 0.04	15.0 ± 0.07	14.7 ± 0.06
Shoot control	2.6 ± 0.03	2.7 ± 0.02	2.7 ± 0.05	17.7 ± 0.05	18.5 ± 0.03	19.6 ± 0.04
0.5	2.6 ± 0.03	2.6 ± 0.05	2.6 ± 0.04	17.7 ± 0.05	18.6 ± 0.06	19.8 ± 0.05
1.0	2.6 ± 0.03	2.6 ± 0.01	2.7 ± 0.01	17.7 ± 0.05	18.7 ± 0.04	20.1 ± 0.07
5.0	2.6 ± 0.03	2.6 ± 0.04	2.7 ± 0.03	17.7 ± 0.05	17.6 ± 0.07	17.2 ± 0.03
10.0	2.6 ± 0.03	2.6 ± 0.05	2.6 ± 0.05	17.7 ± 0.05	17.1 ± 0.06	16.5 ± 0.09

* Each value is the mean of ten replicates ± S. D.

Table.5 Effect of Toxicity of Cr (VI) on Changes in DNA and RNA (mg/g Fresh wt) in Pistia after different Durations of Contact *

Root, shoot and Cr (VI) With concentration (ppm)	Dry Weight (in mg/g Fresh wt) Contact Time in Days		
	0	3	5
Root control	80.1 ± 0.001	80.1 ± 0.001	80.1 ± 0.001
1.0	80.1 ± 0.001	80.5 ± 0.005	80.8 ± 0.006
5.0	80.1 ± 0.001	83.2 ± 0.004	84.8 ± 0.003
10.0	80.1 ± 0.001	88.2 ± 0.006	96.8 ± 0.008
Shoot control	79.6 ± 0.003	79.6 ± 0.003	79.6 ± 0.003
1.0	79.6 ± 0.003	79.8 ± 0.005	79.9 ± 0.003
5.0	79.6 ± 0.003	84.5 ± 0.002	86.2 ± 0.001
10.0	79.6 ± 0.003	90.1 ± 0.004	97.4 ± 0.005
		91.2 ± 0.002	100.5 ± 0.001

* Each value is the mean of ten replicates ± S. D

Table.6 Effect of Toxicity of Cr (VI) on Changes in the Activities of Protease, Catalase, Peroxidase and Acid, Alkaline Pyrophosphatases in Pistia After 5 Days of Contact *

Root, shoot and Cr (VI) With concentration (ppm)	Protease (µg protein/hr/g fresh wt)	Catalase (enzyme unit/hr/g fresh wt)	Peroxidase (enzyme unit/hr/g fresh wt)	Pyrophosphatase (enzyme unit/unit/hr/g fresh wt)		
				Acid	Alkaline	Acid: alkaline
Root Initial activity	275 ± 1.5	22 ± 0.1	18 ± 0.09	40 ± 0.02	88 ± 0.04	0.45 ±0.01
Control	341 ± 1.58	16 ± 0.04	22 ± 0.08	45 ± 0.01	86 ± 0.21	0.52 ± 0.02
0.5	277 ± 1.41	14 ± 0.09	27 ± 0.12	43 ± 0.01	85 ± 0.09	0.51 ± 0.03
1.0	251 ± 1.55	12 ± 0.2	29 ± 0.15	48 ± 0.03	84 ± 0.08	0.57 ± 0.02
5.0	182 ± 1.47	6 ± 0.01	35 ± 0.19	60 ± 0.08	72 ± 0.1	0.88 ± 0.01
10.0	127 ± 0.9	4 ± 0.02	46 ± 0.16	73 ± 0.09	61 ± 0.09	1.19± 0.02
Shoot Initial activity	450 ± 2.9	20 ± 0.2	86 ± 0.21	357 ± 0.27	812 ± 0.13	0.44 ±0.03
Control	464 ± 2.8	17 ± 0.04	88 ± 0.27	398 ± 0.35	800 ± 0.93	0.50 ± 0.04
0.5	448 ± 1.9	13 ± 0.03	87 ± 0.09	388 ± 0.12	796 ± 0.35	0.49 ± 0.03
1.0	430 ± 1.98	11 ± 0.01	90 ± 0.05	395 ± 0.15	792 ± 0.42	0.50 ± 0.01
5.0	325 ± 1.35	5 ± 0.02	96 ± 0.53	402 ± 0.43	512 ± 0.73	0.78 ± 0.12
10.0	132 ± 1.2	3 ± 0.005	99 ± 0.41	452 ± 0.41	301 ± 0.17	1.50 ± 0.16

* Each value is the mean of ten replicates ± S. D.

Toxic effect of Cr (VI) on the activities of protease, catalase, peroxidase and acid and alkaline pyrophosphatases after 5 days of contact is shown in Table VI. In all the treatments activities of protease, catalase and alkaline pyrophosphatase decreased in both root and shoot while an opposite trend in the activities of peroxidase and acid pyrophosphatase as well as the ratio of acid to alkaline pyrophosphatase activity were observed with increasing concentration of Cr (VI).

The increased activity of enzymes may be due to senescence of the plants and increased activity of a number of hydrolytic enzymes as suggested by Thomas and Stoddart (1980). A decrease in enzymatic activity may be due to formation of protein complex with Cr (III) changing the conformation and solubility of the protein.

Experiments conducted on the uptake of Cr (VI) by *Pistia* may enable one to understand the behavior of the metal toxicant discharge into the aquatic environment. From the foregoing results it is evident that at 10 ppm concentration, Cr (VI) promotes senescence of *Pistia* plants by general inhibition of biosynthesis of cellular metabolites, impairing the degradation of the biochemical processes and also interfering with the nucleic acid biosysthesis and protein systhesizing machinery in the plant.

At concerntrations below 5 ppm Cr (VI), the metabolic activities of the plant are least affected, probably because there is a route by which the metal ion may be accumulated in the vacuolar system of the plant. It may be suggested that *pistia* plant can utilized as the scavenger of Cr (VI) from waste water.

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