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Chromium-Induced Biochemical Changes in *Eichhornia crassipes* (Mart) Solms and *Pistia stratiotes* L.

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High concentrations of heavy metals in the environment create serious pollution problems. The consequences of elevated levels of trace metals in the ecosphere can be attributed to both natural and anthropogenic sources. These have been investigated intensely in recent years. The aquatic ecosystems of industrialized countries are being continuously contaminated by heavy metals; as a result, water pollution is now a serious global concern.

All living organisms require trace amounts of the metals which are essential for normal metabolic functions (Mertz 1969). High concentrations of some trace metals are known to cause deleterious effects in aquatic plants (Mertz 1969; Taylor 1981). Biological interest in chromium arose from its significant presence in industrial effluents and it is toxic to plants and animals (Langard 1980). High concentrations of this metal occur in wastes from dyeing, tanning, explosives, ceramics, textiles, paints and paper industries.

During the past decade there has been increasing interest in the use of aquatic vascular plants for the removal of pollutants from domestic and sewage effluents. Surface water weeds like water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes) have been shown to have great potential as biological filters for absorbing pollutants, including heavy metals, from waste waters (Jamil et al. 1987). Due to excess accumulation of trace metals in plant tissues, several biochemical processes, such as carbohydrate and nitrogen metabolism and the activity of certain metalloenzymes of the cell, are affected.

The objective of this study was to understand the quantitative uptake and accumulation of chromium by two aquatic weeds, water hyacinth and water lettuce, and their effect on physiological and biochemical parameters.

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MATERIALS AND METHODS

All chemicals used were of analytical grade obtained from Sigma or local firms (SD's, Loba, Bombay, India).

Eichhornia and Pistia plants were collected and maintained in a laboratory pond as stock cultures. From this, plants selected for experimental purposes were approximately of the same size and weight. Two plants were introduced into each 3-liter beaker containing 10, 25 and 50 ppm of soluble solution of $K_2Cr_2O_7$ along with Hoagland's solution (Hoagland and Arnon 1950). Experiments were repeated three times with each concentration. Water samples were collected periodically from zero to 72 hours from each beaker for determining chromium concentrations. Plant samples were separated into leaves, petioles and roots, washed with distilled water, and dried at 80° for 48 hours. The samples were weighed and digested with a mixture of 1:3 (v:v) of concentrated H_2SO_4:HNO_3. After digestion the samples were filtered and analyzed for Cr content using Atomic Absorption Spectrometry (AAS).

Plant samples were also collected after 24, 48 and 72 hours of Cr exposure and were washed with deionized water and used for biochemical estimations. Chlorophyll content was estimated by using the Vernon (1960) method. Protein content was determined by the method of Lowry et al. (1951). Sugar content was determined by the method described by Dubois et al. (1951) and phenols were estimated by the Mallik and Singh (1980) method. Data were subjected to analysis of variance (ANOVA).

For the assay of enzymes, crude homogenates were prepared by grinding 5 g of plant material with 10 ml of extraction buffer (0.1 M phosphate buffer pH 7.4), using a prechilled mortar and pestle at 4°C. The crude extract was filtered through two layers of cheese cloth. The filtrate was centrifuged at 25,000 g for 20 minutes (0-2°C) and the supernatant was dialyzed using cellulose tubing with phosphate buffer (pH 7.4) for 20 hours. After dialysis the homogenate was used for enzyme assays.

Succinic dehydrogenase (SDH) activity was assayed by oxidation of succinate to fumarate using phenazine methosulphate as the electron acceptor (Mallik and Singh 1980). Peroxidase (PR) activity was estimated using pyrogallol as the substrate and the amount of purpurogallin formed during the reaction in the presence of H_2O_2 was followed in a kinetic LKB Spectrophotometer at 420 nm (Kar and Mishra 1976). Polyphenol oxidase (PPO) activity was determined using catechol as the substrate; changes in absorbance at 495 nm were recorded (Mayer et al. (1965). Superoxide dismutase (SOD) activity was assayed by oxidation of NADH in the presence of LDH. The decrease in NADH absorption at 340 nm was recorded in a kinetic LKB spectrophotometer (Oyanagi 1976). Glutathione reductase (GR) activity was determined by the method described by Goldberg and Spoone (1983).

RESULTS AND DISCUSSION

A linear increase in the uptake of Cr by water hyacinth and water lettuce was observed with increasing concentrations. Percent absorption, however, gradually decreased as the initial Cr concentration increased.

The bioaccumulation pattern of chromium ions in the different tissues of the plants - leaves, petioles and roots of Eichhornia and leaves and roots of Pistia - are presented in Figs. 1 and 2.

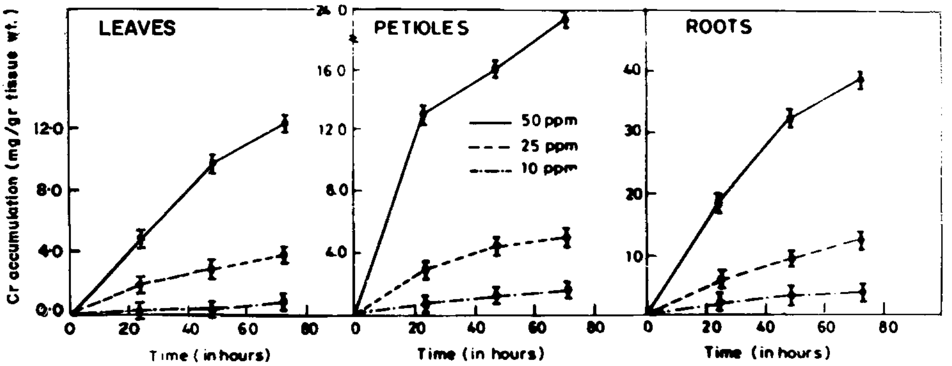

Figure 1. Accumulation of Cr in water hyacinth (Eichhornia crassipes) plants.

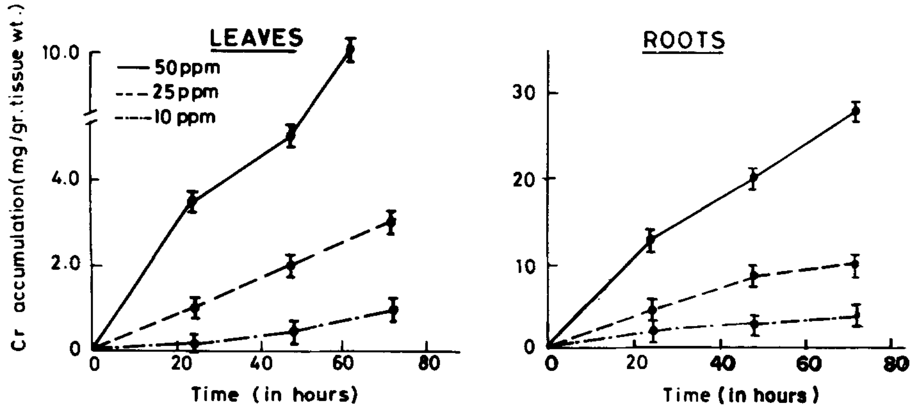

Figure 2. Accumulation of Cr in water lettuce (Pistia stratiotes) plants.

The accumulation of Cr in leaves, petioles, and roots was found to increase with increasing concentrations and exposure period. A similar type of increase in water hyacinth was reported by Kay et al. (1984). The order of metal accumulation in the tissues was roots>petioles>leaves in water hyacinth and roots>leaves in water lettuce. Tatsuyama et al. (1977) also reported similar results with Cd in water hyacinth plants.

Heavy metal accumulation in vascular plants is known to produce significant physiological and biochemical responses (Pahlsson 1989). Low concentrations (10 ppm) of Cr showed no morphological changes, but plants exposed to 25 and 50 ppm showed chlorosis and yellowing of petioles in water hyacinth, and chlorosis and detachment of leaves from the roots in water lettuce.

Changes in biochemical parameters of both plant species, i.e., chlorophyll, sugars, proteins and phenol contents, are presented in Tables 1 and 2.

Table 1. Effect of Cr on biochemical parameters of water hyacinth.

Plant part	Cr Conc. (ppm)	(mg/g dry wt)			
		Chlorophyll	Sugar	Protein	Phenol
Leaf	Control	29.94	47.00	140.00	18.80
	10	22.56**	21.21**	108.00**	10.65
	25	16.92**	18.29**	99.00**	8.21*
	50	11.07**	14.02**	93.00**	7.00**
Petiole	Control	-	33.00	54.92	10.47
	10	-	13.02**	41.61**	5.29**
	25	-	11.12**	38.93**	3.33**
	50	-	10.23**	30.64**	2.01**
Root	Control	-	22.34	96.80	12.90
	10	-	12.34**	73.21	8.21
	25	-	10.13**	71.63*	5.81*
	50	-	8.92**	66.69**	3.63**

Significant change from control P** = 0.01 and P* = 0.05

Chlorophyll content declined with Cr accumulation. The reduction of chlorophyll content was directly related to the accumulation of Cr in the leaves (Tables 1 and 2). Stiborova et al. (1986) also reported the reduction of chlorophyll with heavy metal accumulation in maize plants.

With increasing concentrations of Cr in the tissues, a decline in sugar and protein content was observed in both plants (Tables 1 and 2).

Table 2. Effect of Cr on biochemical parameters of water lettuce.

Plant part	Cr Conc. (ppm)	(mg/g dry wt.)			
		Chlorophyll	Sugar	Protein	Phenol
Leaf	Control	15.43	28.15	143.00	11.20
	10	9.97**	22.64*	95.21**	19.21**
	25	8.75**	18.30**	88.21**	21.43**
	50	7.04**	12.43**	86.32**	23.21**
Root	Control	-	18.74	86.02	8.24
	10	-	10.64**	66.21**	11.31**
	25	-	8.32**	64.00**	13.00**
	50	-	6.43**	60.21**	15.21**

Significant change from control P** = 0.01 and P* = 0.05

Maximal reduction in the sugars and protein contents was observed with accumulation 12.6 ± 2.5 mg Cr in leaves, 23.0 ± 4.0 mg Cr in petioles and 40.0 ± 5.0 mg Cr in roots of water hyacinth and 10.0 ± 2.2 mg Cr in leaves and 28.0 ± 5.0 mg, in roots of water lettuce. The decrease in the total carbohydrate levels in the heavy-metal treated plants can be attributed to the reduced rates of photochemical activities and chlorophyll formation (Abdul Razak 1985). Significant decline in protein content suggests the possible interference of metal ions in the protein synthesis of the plants.

Whereas phenol content decreased in water hyacinth and increased in water lettuce with Cr treatment, Fry (1979) reported that metal accumulation disrupted cell membranes causing injury and increase in the concentration of several phenolic compounds. Our experiments confirm this belief in Pistia as leaves were more easily detached from roots with increase in exposure period.

Enzyme activities varied differently with Cr accumulation in different parts of the two plants (Figs. 3, 4). In water hyacinth, succinic dehydrogenase (SDH) activity was inhibited in leaves but stimulated in petioles and roots. Glutathione reductase (GR) and peroxidase (PR) activities were found to decrease in leaves and petioles and increase in roots. The increase in the activity may be due to the accumulation of excess Cr in the root tissues. Polyphenol oxidase (PPO) activity, however, decreased in all parts with Cr accumulation. SOD activity decreased in leaves and increased in roots, but in petioles the activity decreased initially and increased with 5.0 ± 1.0 mg Cr accumulation (Fig. 3).

In water lettuce SDH, PR and PPO activities were stimulated with Cr accumulation in leaves and roots (Fig. 4), signifying an increase in respiration. It is well known that chronic treatment of Cr leads to oxidative damage and increase in dehydrogenase activity (Jin and

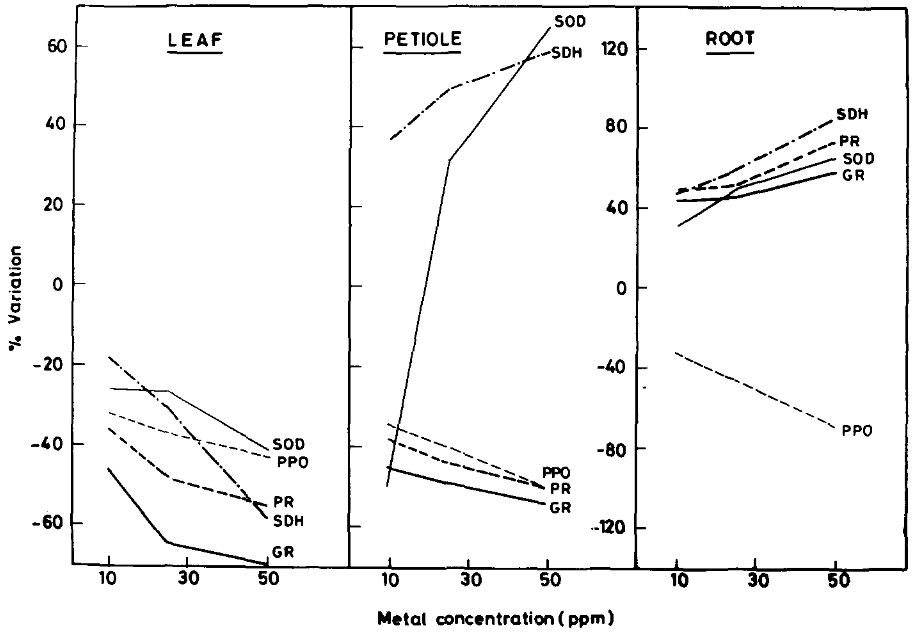

Figure 3 . Percent variations in enzyme activities of water hyacinth (Eichhornia crassipes) exposed to Cr (after 72 hrs).

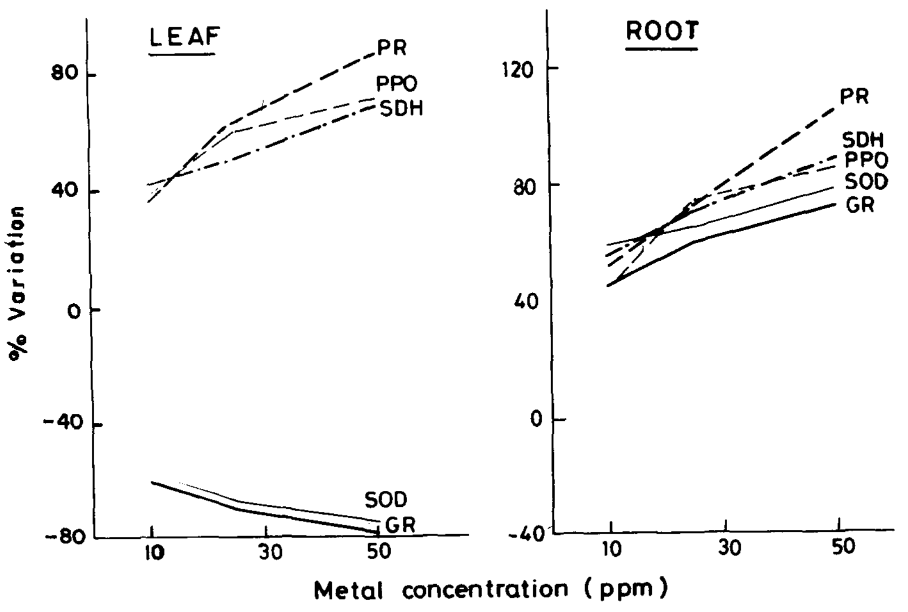

Figure 4 . Percent variations in enzyme activities of water lettuce (Pistia stratiotes) exposed to Cr (after 72 hrs).

Yong 1990). The SOD and GR activities decreased in leaves and increased in roots.

Metal accumulation in both plants apparently led to changes in the metalloenzymes by displacement or replacement of metal ions, resulting in changes in both photosynthetic and respiratory activities. An increase in respiration led to yellowing of the leaves (Tetley and Thimann 1974). This was confirmed in the present study as the respiratory enzyme (SDH and PPO) activities increased in leaves, particularly in water lettuce.

It is evident, therefore, that high concentrations of chromium bioaccumulation in plant tissues induced significant biochemical changes which were responsible for the inhibition of chlorophyll synthesis resulting in the loss of photosynthetic activity.

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