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Bioaccumulation and Physiological Changes in *Hydrilla verticillata* (l.f.) Royle in Response to Mercury

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There has been a growing awareness in recent years about the utility of biological methods in tackling the problem of industrial pollution. These methods are both easy and cost effective. In this connection the role of aquatic plants, because of their ability to absorb pollutants, has been recognized worldwide in the treatment of waste water (Brix & Schierup 1989; Rai & Chandra 1989; Garg & Chandra 1990; Sinha & Chandra 1990; Chandra & Garg 1992).

Mercury is one of the most toxic substances in the aquatic environment. Effluents discharged from the industries (chloralkali, paper & pulp, plastic, pesticide etc.) pollute water bodies directly or through seepage. Since most of the water bodies in the country are used for the cultivation of aquatic eatables, the entry of mercury into food chain can not be ruled out.

Hydrilla verticillata, a profusely occurring submergent species, has been found to thrive well in highly polluted water. Also,the plant has shown promise in the removal of heavy metals (Sinha et al. 1993; Gupta & Chandra 1994). This paper reports on studies carried out to evaluate the ability of H. verticillata to accumulate mercury and its toxicity in relation to chlorophyll, protein, in vivo nitrate reductase activity and cysteine content . A study of the effects of Hg on nitrogen, phosphorus and potassium levels in H. verticillata was also made.

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MATERIALS AND METHODS

Samples of H. verticillata were collected from an unpolluted waterbody and grown in large hydroponic tubs for six months. Young shoots were separated from the mother plant and acclimatized in 5% Hoagland solution under the laboratory conditions (providing 115 µ mole m^{-2} s^{-1} light intensity for 14 hr at 25± 2 °C). Plants were treated with five concentrations (0.5, 1.0, 5.0, 10.0 and 20.0 µM) of Hg as mercuric chloride, prepared in 5% Hoagland solution. The experiments were set up in triplicate for each concentration and duration (24,48, 96 and 168 hr). Plants cultured without Hg served as controls. Harvested plants were dried at 80°C for 48 h and digested in HNO_3:$HClO_4$ (3:l v/v).Mercury was measured by mercury anhydride system (detection limit - 0.001 µg/l)attached to a Perkin Elmer Atomic Absorption Spectrophotometer (Model 2380). Chlorophyll content was estimated in 80% chilled acetone extracts of the plants following the method of Arnon (1949).Protein was estimated by the method of Lowry et al (1959) using bovine serum albumin as a standard. The method of Srivastava (1974) was followed for the determination of in vivo NR activity.Treated and untreated plants (300-500 mg) were crushed in 5% chilled $HClO_4$ and cysteine was measured following the method suggested by Gaitonde (1967) .

Nitrogen was measured with a Kjeltec Autoanalyzer 1030. Plant samples were digested in 10 ml H_2S0_4 with copper sulphate tablet as catalyst. Phosphorus was estimated by the vanadomolybdate method suggested by (Kalra & Maynard 1991). Plant samples were digested in an acid mixture of $HN0_3$:$HCl0_4$ (3:l v/v) and O.D. recorded at 470 nm by Milton Roy 1201 Spectrophotometer. Potassium was estimated by flame photometer Mediflame- 127.

Data were subjected to Student's t-test and ANOVA by the method given by Schefler (1969) in order to determine the level of significance within concentration and exposure duration.

RESULTS AND DISCUSSION

The accumulation of Hg by H. verticillata varied

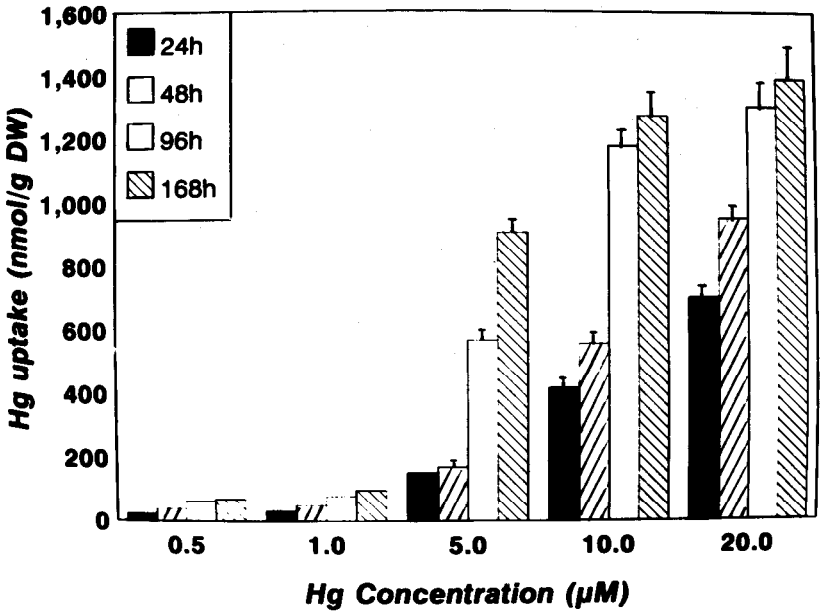

Figure 1 Mercury accumulation in H. verticillata at different test concentrations and durations.
F value (concentration) = 22.02
F value (exposure) = 6.66*, * = P < 0.01.

considerably at the various test concentrations and durations (Fig.1). Uptake was not high during the first 48 hr at the lower concentrations (0.5-1.0 µM). However, it increased with the increase in metal concentration and treatment duration. At the highest metal concentration tested (20.0 µM Hg), plants accumulated 1388.75 n mole / g dw Hg after 168 hr.

Table la summarizes the effect of different Hg concentration on chlorophyll content. Reduction in chlorophyll content at the lowest Hg concentration (0.5 µM) tested was not significant, however,it gradually increased with the increase in metal concentration. At 20.0 µM Hg, the reduction was ca.23% for 168 hr.

Decrease in protein content was dose and duration dependent (Table 1b) . The decrease was quite marked above 1.0 µM Hg.

Table 1 (a-d). Effect of Hg on chlorophyll, protein, in vivo NR activity and cysteine content in H. verticillata.at varying lengths of time.

(a) Hg Conc. (μM)	Chlorophyll content (mg/g FW) 24hr	48hr	96hr	168hr
0.0	0.78±0.01	0.79±0.018	0.86±0.022	0.87±0.021
0.5	0.78±0.01	0.76±0.02	0.68±0.014^{c}	0.64±0.007^{e}
1.0	0.71±0.014^{c}	0.67±0.008^{c}	0.60±0.006^{d}	0.57±0.006^{e}
5.0	0.63±0.009^{e}	0.58±0.008^{e}	0.55±0.007^{e}	0.50±0.004^{e}
10.0	0.60±0.007^{e}	0.51±0.006^{e}	0.48±0.003^{e}	0.44±0.004^{e}
20.0	0.52±0.004^{e}	0.42±0.003^{e}	0.26±0.003^{e}	0.21±0.002^{e}
(b)	Protein content (mg/g FW)			
0.0	5.98±0.32	6.01±0.37	6.10±0.38	6.22±0.38
0.5	5.92±0.36	5.68±0.29	5.40±0.34	5.13±0.35^{a}
1.0	5.70±0.34	5.28±0.29	5.04±0.32^{a}	4.90±0.29^{a}
5.0	5.13±0.30^{a}	4.88±0.24^{a}	4.64±0.28^{b}	4.40±0.20^{b}
10.0	4.75±0.26^{b}	4.29±0.20^{b}	3.79±0.18^{c}	3.50±0.16^{c}
20.0	4.24±0.20^{c}	3.68±0.19^{c}	3.29±0.18^{c}	2.64±0.12^{d}
(c)	NR activity (% control)			
0.0	100±5.74	100±5.72	100±5.72	100±5.70
0.5	102±5.26	97±5.64	90±5.70	82±5.52^{a}
1.0	95±5.66	90±5.48	79±4.78^{a}	62±4.44^{c}
5.0	83±5.10^{a}	78±4.71^{b}	61±4.62^{c}	59±3.14^{c}
10.0	72±4.99^{b}	63±4.76^{b}	52±3.01^{d}	45±2.64^{d}
20.0	64±4.99^{c}	58±3.10^{c}	41±2.18^{d}	38±2.11^{e}
(d)	Cysteine content (n mole/g FW)			
0.0	58.80±3.19	60.16±3.64	64.40±3.66	64.80±3.71
0.5	64.30±3.26	69.18±3.67	79.91±3.66^{b}	82.48±4.04^{b}
1.0	68.40±3.21^{a}	79.31±3.71^{b}	83.78±3.78^{b}	88.69±4.11^{b}
5.0	74.60±3.44^{b}	73.52±3.61^{a}	72.46±3.64	60.80±3.51
10.0	62.41±3.20	51.74±3.18	40.88±2.14^{c}	36.84±2.04^{c}
20.0	53.72±3.17	40.41±3.01^{c}	28.24±1.96^{d}	26.22±1.62^{d}

Each value represents Mean ±S.E. (n=3); a=P<0.10, b=P<0.05, c=P<0.025, d=P<0.0l, e=P<0.005 (t- test, one sided).

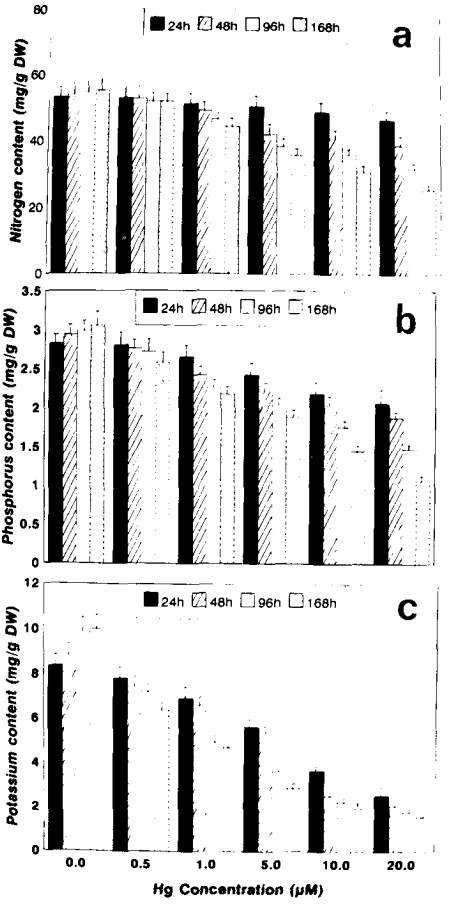

Figure 2 a- c. Effect of Hg on nitrogen,phosphorus and potassium content in H. verticillata at varying lengths of time.

In vivo nitrate reductase (NR) activity was at par with the control at the lowest test concentration (0.5μM Hg) during the first 24 hr. With the increase in background metal concentration, the enzyme activity decreased considerably (Table 1c). The reduction to the extent of ca. 38% was recorded at 20.0 μM for 168 hr.

An increase in cysteine content was observed upto

1.0 µM Hg. At higher test concentrations the decrease was more. Cysteine content was reduced by 40% at 20.0 µM Hg after 168 hr (Table 1 d).

Figure 2 a - c Reduction in N, P, K. content was negligible at lower Hg concentrations (0.5, 1.0 µM). Decrease in N,P,K. content was linear with the increase on Hg concentration and treatment duration. It was maximum at 20.0 µM Hg after 168 hr.

The results of present study show that the plants of H. verticillata are capable of accumulating Hg effectively. Plants subjected to higher metal concentrations showed substantial uptake of Hg during the first 96 hrs. Similar observations were made on duckweed and water velvet (Mo et al.1989; Jain et al. 1989). Duckweeds showed faster accumulation of Hg during the first three days of treatment and at higher background concentration of the metal.

Submerged plants (Elodea nuttali, Ceratonhyllum demersum) are reported to accumulate more metal than the floating ones because of their large surface/biomass ratio (Werff et a1.1982; Guilizzoni 1991). These reports hold good in the case of H. verticillata, because of finely divided leaves, plants are able to accumulate Hg substantially.

Plants of H. verticillata showed high level of tolerance to Hg. They grew well in medium containing upto 5.0 µM Hg for 168 hr. No significant phytotoxicity was observed at lower (0.5 and 1.0 µM) ambient Hg concentration. Reduction in chlorophyll content, protein, cysteine and N.P.K. was observed above 1.0 µM test concentrations. Jana & Choudhury (1982) reported insignificant toxicity at lower concentrations of Hg in H. verticillata. This tolerance shown by the plants might be due to increased level of cysteine under Hg stress condition.

Some aquatic vascular plants (Potamogeton ,Equisetum) have been found effective in the monitoring of metal pollution because of their selective nature of accumulation of metal ions (Ray & White 1976). The

results obtained presently conform with the above observations and suggest the suitability of H. verticillata in monitoring Hg pollution on account of its uptake potential, tolerance and common occurrence in Hg polluted waters.

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