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Mercury Bioaccumulation Induces Oxidative Stress and Toxicity to Submerged Macrophyte *Potamogeton crispus* L.

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Industrial wastes and domestic discharges have been recognized as the major sources of toxic metal pollution in aquatic environment. Mercury is a by-product of a number of industrial processes such as chlor-alkali industry (electrolysis), dentistry, paints, pharmaceutical products and agricultural run-off. In India, many aquatic resources have been reported to be contaminated by mercury viz., Kalu river water (0.001 – 0.006 μg ml^{-1}) and sediment (1.5 –140 μg g^{-1} dw) which caused contamination of food chain near Bombay (Mahatre et al. 1980), the water and sediments of low lying wetland area near a chlor-alkali plant at Ganjam ,Orissa contained 4 μg L^{-1} and 41.3 mg kg^{-1} , respectively (Lenka et al. 1992). Aquatic plants have been demonstrated to play significant role in phytoremediation of mercury polluted aquatic ecosystems (Gupta and Chandra 1996). Further, rooted submerged macrophytes have been found very useful in bioremediation and biomonitoring of mercury polluted water bodies as they do not migrate rather attain equilibrium with their surroundings within short time (Gupta and Chandra 1998). However, mercury administers toxic effect in plants either through reacting with the sulfhydryl groups of vital enzymes following metal mercaptide formation (Van Assche and Clijsters 1990) or through the generation of reactive oxygen species like superoxide ($^{\cdot}O_2^-$), hydroxyl radicals ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) (Halliwell and Gutteridge 1984). Further, it has been stated that formation of toxic free radicals due to excess accumulation of mercury disrupt normal functioning of plant and bring about metabolic changes. Besides, mercury has been found to reduce phytomass, total chlorophyll, photosynthesis, nitrogen and phosphorus contents in aquatic plants (Jana and Choudhuri 1984; Mahatre and Chaphekar 1984; Gupta and Chandra 1996,1998; Sinha et al. 1996; Boening 2000).

Potemogeton crispus L. (Potamogetonaceae), a rooted submerged plant, occurring in polluted waters (Ali et al. 1999), accumulated considerable amount of toxic metals (Fe, Pb, Ni, Mn and Cu). Hence, it was considered worthwhile to study the accumulation of a highly toxic metal i.e., Hg^{2+} and induced biochemical changes therein. Present study deals with the mercury accumulation ability of *P. crispus* and mercury induced modification of malondialdehyde content (a measure of lipid peroxidation), potassium leakage, total chlorophyll, protein, cysteine, non protein thiol contents (measure of phytochelatin level) and a key enzyme of nitrogen metabolism i.e., nitrate reductase.

MATERIALS AND METHODS

P. crispus L., collected from freshwater lake Nainital (Nainital, U.P., India), was acclimatized for more than a year in hydroponic tubs. Two months old exponentially growing shoots were cut off from mother plant, washed thrice with running tap water and then with 95% ethanol for 10 min and were grown in 5% Hoagland solution (Light : Dark, 14:10 h, 114 μ mol m^{-2} s^{-1} illumination provided by fluorescent tube light at 25 ± 2°C).Various concentrations (0.0,0.10, 0.25, 0.50, 1.0, 5.0 and 10.0 μM) of Hg^{2+} were supplied exogenously as $HgCl_2$ in 5% Hoagland solution. *P. crispus* plants of same age and weight (approximately 2.0 g) were transferred to 250 ml plastic beakers containing 200 ml Hoagland nutrient medium supplemented with different concentrations of mercury (four sets comprising of three beakers for each concentration and duration). Beakers were placed in growth chamber under the conditions mentioned above. Experimental beakers were aerated 6 h a day. Nutrient solutions were changed after every 24 h period. Plants were harvested after 24, 48, 72 and 96 h, washed thrice with double distilled water and 5 mM EDTA at 4°C for 30

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min for desorption of cell surface bound Hg^{2+} following Tripathi et al. (1995). Plant samples were digested in HNO_3: $HClO_4$ (3:1, v/v) at 60°C in a water bath. Metal analysis was performed using mercury hydride system (MHS-10) attached with Perkin Elmer Atomic Absorption Spectrophotometer (AAS 2380). The mercury test concentrations were analytically confirmed by estimating the Hg^{+2} in test solutions as described above. The standard reference material of mercury ($HgCl_2$ E- Merck, Germany) was used to provide calibration and quality assurance for each analytical batch. The efficiency of digestion of plant samples and mercury test concentrations was determined by adding standard reference material of mercury ($HgCl_2$ E- Merck, Germany) to the samples. After addition of standard mercury solution samples were digested and mercury was estimated as described above. Mean recovery of mercury was 96 ± 9%. The detection limit of mercury in HNO_3 using the cold vapour technique was 1 ng L^{-1}. Replicate (n = 3) analyses were conducted to assess precision of the analytical techniques. . Duplicate analysis for each test concentration varied by no more than 5%. The level of lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA) content determined by thiobarbituric acid (TBA) reaction following the method of Heath and Packer (1968). Potassium leakage was estimated in treated solution by using flame photometer (Mediflame) as per procedure given in APHA (1985). Chlorophyll was extracted in chilled 80% acetone by using method of Arnon (1949). Protein content of leaf material was estimated following Lowry et al. (1951) using bovine serum albumin as standard. For assay of nitrate reductase (NR) activity, plants were incubated in 10 mM KNO_3 for 72 h prior to mercury exposure. *In vivo* NR activity was assayed in leaves by the method of Srivastava (1974). The enzyme activity was expressed as nmol NO_2^- $h^{-1}g^{-1}$ fw. Cysteine and non-protein thiol contents were measured in mercury treated plant tissues following methods of Gaitonde (1967) and Ellman (1959), respectively Multivariate analysis of variance (MANOVA) in complete randomized block design involving seven mercury concentrations and four treatment durations was performed to assess the variability and reliability of the data (Gomez and Gomez 1984). Duncan multiple range test (DMRT) was used to find out the variation between two concentrations. Further, correlation analysis between various pairs of parameters has been done and results are presented in matrix table.

RESULTS AND DISCUSSION

P. crispus plants treated by different concentrations (0.0, 0.10, 0.25, 0.50, 1.0, 5.0 and 10.0μM) of Hg^{2+} have shown significant accumulation (MANOVA; $P<0.05$) of metal inside plant tissues. The metal uptake potential of *P. crispus* was positively correlated with concentration of Hg^{2+} in nutrient solution (correlation coefficient significant ($P<0.05$) at df =n-2 = 5, Table 5).The mercury concentration of plant increased significantly (DMRT, $P<0.05$) with increase in concentration and exposure duration (Table 1). The interaction between concentration and treatment duration was also significant at 5% level. MDA content is considered to be a measure of lipid peroxidation in plants. During present study, significant (MANOVA; $P<0.05$) increase in MDA content of *P. crispus* was observed initially at 0.10 μM Hg^{2+} after 24 h exposure which was found to be elevated with increase in Hg^{2+} concentration and duration of exposure. However, in comparison to control, highest concentration of Hg^{2+} and treatment duration used in the experiment, resulted in approximately five-fold increase in MDA contents. (Table 2). Mercuric ions also induced K^+ leakage in test plant at 0.10 μM level after 24 h exposure which further increased with increase in concentration (Table 2). Further, membrane permeability (**K^+** leakage) increased in a concentration and duration dependent manner (MANOVA, $P<0.05$). The MDA contents and K^+ leakage in mercury treated plants were positively correlated (correlation coefficient (r) significant ($P<0.05$) at df = n-2 = 5, Table 5). A significant decrease (MANOVA, $P<0.05$) in total chlorophyll content was observed at a very low concentration of Hg^{2+} (0.10 μM) after 24 h exposure. The loss in total chlorophyll content was more following further increase in concentration and treatment duration (Table 3). Finally, about 65% decrease in total chlorophyll content was observed after 96 h exposure at 10.0 μM Hg^{2+}. Results revealed that mercury (up to 0.25 μM) has significant stimulatory effect (DMRT test, $P<0.05$) on protein content up to 72 h (Table 3). However, The stimulatory effect of mercury on protein content was limited to 0.1μM after 96 h exposure(DMRT, $P<0.05$). A decrease in protein content was observed at all the treatment durations when concentration of Hg^{2+} in treatment medium was > 0.25 μM. Maximum decrease in protein content (60%) was observed after 96 h exposure at10.0 μM Hg^{2+}.

Table 1. Mercury uptake by *P. crispus* from mercury supplemented nutrient solution.

Mercury (μM)	Treatment duration (h)			
	24	48	72	96
Metal accumulation (μg/g dw)				
0.1	3.24^{f} ±0.59	6.65^{f} ±0.86	20.29^{f}±1.13	27.64^{f}±1.41
0.25	7.63^{e} ±0.77	15.70^{e} ±1.04	25.76^{e}±1.71	32.31^{e}±1.15
0.5	13.62^{d} ±0.94	19.38^{d} ±0.67	33.40^{d}±1.59	46.83^{d}±0.79
1.0	17.32^{c} ±0.56	23.95^{c} ±0.94	42.53^{c}±1.28	56.39^{c}±2.14
5.0	25.90^{b} ±1.60	36.65^{b} ±1.04	59.77^{b}±1.79	77.01^{b}±1.25
10.0	39.25^{a} ±1.89	53.26^{a} ±1.55	84.76^{a}±2.52	124.98^{a}±2.6
MANOVA of mercury uptake				
Source of variation	df	Sum of squares	Mean of squares	F*
Replications	2	5.76	2.88	
Treatments	23	55066.63	2394.20	
Duration (A)	3	20057.49	6685.83	1044.36
Hg^{2+} conc. (B)	5	30194.48	10064.83	1572.18
A x B	15	4814.66	320.98	50.14
Error	46	294.48	6.40	
Total	71	55366.87		

Mean ± SD (n=3); * Significant at 5% level; Different superscripts on means denote significant difference ($P<0.05$) between means in column according to DMRT. Mercury was below detection limit in control samples.

The activity of NR (key enzyme of nitrogen metabolism) was stimulated significantly (DMRT, $P < 0.05$) by mercury concentrations < 0.5 μM upto 72 h (Figure 1A). However, when treatment duration was extended upto 96 h, relatively low levels of stimulation of NR activity were recorded. The inhibition in NR activity was found concentration and duration dependent (concentration x duration, significant at 5% level, Table 4). Mercury treatment (0.10 – 5.0 μM) increased non-protein thiol contents of test plant up to 72 h treatment duration (Figure1B). However, 5 μM Hg^{2+} was found inhibitory following 96 h exposure. While highest concentration (10 μM) used in the experiment, reduced non- protein thiol contents significantly (DMRT, $P<0.05$) at all treatment durations. Similarly, mercury exposure (<10 μM) to test plant for 24 h caused accumulation of cysteine which increased with elevated mercury concentration in the medium up to 72 h treatment duration (Figure1C). However, when exposure period was extended up to 96 h, 5 μM Hg^{2+} also significantly reduced the cysteine content following various treatment durations being maximum during 96 h exposure. Plants exposed to highest concentration of mercury (10 μM) always exhibited significant reduction in cysteine content after 24 h. Non-protein thiol and cysteine were positively correlated to each other (correlation coefficients (r) significant ($P<0.05$)at df = n-2=5, $P<0.05$, Table 5). The interaction between concentration and duration was significant for both non protein thiol and cysteine contents of mercury treated plants at df = 30 (MANOVA, $P < 0.05$, Table 4).

Production of high biomass is an essential feature for selecting a species for phytoremediation purpose. *P. crispus* is a fast growing plant, produces high biomass and have shown potential to accumulate 15-132 ng Hg^{2+} g^{-1} dw in field conditions (Seigel et al. 1985). However, during present experimental conditions *P. crispus* collected from Nainital lake, was found to accumulate quite higher amount of mercury (125 μg g^{-1} dw at 10 μM Hg^{2+} after 96 h exposure) showing its greater phytoremediation potential. The surface fresh water in India has mercury contamination in the range of 0.0001-0.006 μg/ml (Mahatre et al. 1980; Lenka et al. 1992). The first effect levels of Hg^{2+} in this plant ranged between 0.1-10μM (stimulatory/inhibitory) which are much higher than mercury level in surface water (Table 6). This ability of *P. crispus* to tolerate high levels of

Table 2. Effect of Hg^{2+} on MDA content and K^+ leakage in *P. crispus*.

Mercury (μM)	Treatment duration (h)			
	24	48	72	96
MDA content (μ mol/g fw)				
0.0	$3.546^{g}\pm0.16$	$3.787^{g}\pm0.13$	$4.000^{g}\pm0.11$	$4.369^{g}\pm0.21$
0.1	$4.010^{f}\pm0.13$	$4.290^{f}\pm0.15$	$4.961^{f}\pm0.15$	$5.186^{f}\pm0.12$
0.25	$4.412^{e}\pm0.14$	$5.202^{e}\pm0.23$	$5.470^{e}\pm0.28$	$6.087^{e}\pm0.16$
0.5	$6.076^{d}\pm0.16$	$6.299^{d}\pm0.18$	$6.418^{d}\pm0.25$	$7.114^{d}\pm0.18$
1.0	$7.540^{c}\pm0.16$	$8.139^{c}\pm0.31$	$8.932^{c}\pm0.24$	$10.44^{c}\pm0.90$
5.0	$8.942^{b}\pm0.21$	$9.755^{b}\pm0.24$	$10.77^{b}\pm0.67$	$15.18^{b}\pm0.59$
10.0	$11.24^{a}\pm0.63$	$13.39^{a}\pm0.59$	$14.41^{a}\pm0.70$	$21.63^{a}\pm1.36$
MANOVA of MDA contents				
Source of variation	df	Sum of squares	Mean of squares	F*
Replications	2	4545.92	10332686.17	
Treatments	27	1468.91	4.40	
Duration (A)	3	140.26	46.75	78.56
Hg^{2+} conc. (B)	6	1197.90	199.65	335.46
A x B	30	130.75	7.26	12.20
Error	54	32.14	0.60	
Total	83	1501.05		
K^+ leakage (μmol/g fw)				
0.0	$14.20^{g}\pm0.71$	$14.32^{g}\pm0.70$	$14.39^{g}\pm0.68$	$14.80^{g}\pm0.73$
0.1	$15.30^{f}\pm0.61$	$15.88^{f}\pm0.64$	$17.30^{f}\pm0.80$	$18.52^{f}\pm0.89$
0.25	$16.92^{e}\pm0.80$	$18.20^{e}\pm0.88$	$21.96^{e}\pm1.09$	$25.32^{e}\pm1.20$
0.5	$19.23^{d}\pm0.89$	$21.30^{d}\pm1.03$	$24.30^{d}\pm1.19$	$29.20^{d}\pm1.40$
1.0	$22.10^{c}\pm1.11$	$25.10^{c}\pm1.23$	$27.12^{c}\pm1.28$	$33.50^{c}\pm1.60$
5.0	$26.32^{b}\pm1.25$	$29.80^{b}\pm1.30$	$35.12^{b}\pm1.71$	$39.62^{b}\pm1.92$
10.0	$32.10^{a}\pm1.50$	$37.10^{a}\pm1.70$	$39.12^{a}\pm1.83$	$44.22^{a}\pm2.21$
MANOVA of K^+ leakage				
Source of variation	df	Sum of squares	Mean of squares	F*
Replications	2	44532.7	991580498.58	
Treatments	27	6305.52	233.54	
Duration (A)	3	824.22	274.74	111.83
Hg^{2+} conc. (B)	6	5242.15	873.69	355.62
A x B	30	239.15	13.29	5.40
Error	54	132.67	2.46	
Total	83	6438.18		

Mean ± SD (n=3); * Significant at 5% level; Different superscripts on means denote significant difference ($P<0.05$) between means in column according to DMRT; Mercury was below detection limit in control samples;fw : fresh weight.

mercury make the plant highly suitable for phytoremediation of mercury polluted water bodies. Further, mercury shows speciation in waters showing inorganic and organic forms. In solution culture the same accumulation factor was found for both inorganic and organic mercury in contrast to results obtained during field studies (Mortimer, 1985). Many other aquatic plants have also been reported to concentrate mercury from ambient environment (Lenka et al. 1992; Gupta and Chandra 1996, 1998). However, the accumulation of metals in plants is often accompanied by an induction of a number of cellular changes, some of which are directly related to metal tolerance capacity of the plants. Mercury accumulation in *P. crispus* caused lipid peroxidation and an increase in MDA level and potassium leakage was observed. Our findings are in contrast to the results of Sinha et al. (1996) who reported reduced MDA contents in a mercury treated wetland plant (*Bacopa*

monnieri) However, present findings are in agreement with Somashekaraiah et al. (1992) who reported an increase in MDA level of cadmium treated germinating seedlings of *Phaseolus vulgaris*. Similar to our finding mercury induced loss in K^+ content of *Hydrilla verticillata* was reported by Gupta and Chandra (1998). A decline in total chlorophyll content was observed at very low concentration of mercury (0.25 μM) after 24 h treatment which was found to reduce further in a concentration-duration dependent manner. Further, total chlorophyll content was found to be negatively correlated with increased MDA content and potassium leakage. It has been reported that peroxidation reactions in the photosynthetic tissues are initiated either by excited chloroplasts or oxygen species deriving from the superoxide anion radical (Kunert and Elder 1985). Metal ions block the formation of excited chlorophyll, which in turn causes the production of free radicals.

Table 3. Effect of Hg^{2+} on total chlorophyll and protein content of *P. crispus*.

Hg concentration	**Duration of exposure (h)**			
(μM)	**24**	**48**	**72**	**96**
Total Chlorophyll (mg/g fw)				
0.0	$3.416^{a}\pm0.25$	$3.432^{a}\pm0.04$	$3.498^{a}\pm0.07$	$3.454^{a}\pm0.03$
0.1	$3.094^{b}\pm0.19$	$2.955^{b}\pm0.04$	$2.871^{b}\pm0.03$	$2.789^{b}\pm0.03$
0.25	$3.037^{b}\pm0.06$	$2.610^{c}\pm0.01$	$2.749^{c}\pm0.06$	$2.591^{c}\pm0.06$
0.5	$3.013^{b}\pm0.04$	$2.622^{c}\pm0.02$	$2.498^{d}\pm0.04$	$2.410^{d}\pm0.05$
1.0	$2.827^{c}\pm0.10$	$2.394^{d}\pm0.02$	$2.224^{e}\pm0.06$	$2.099^{e}\pm0.04$
5.0	$2.376^{d}\pm0.03$	$2.170^{e}\pm0.03$	$1.975^{f}\pm0.03$	$1.891^{f}\pm0.08$
10.0	$1.804^{e}\pm0.10$	$1.730^{f}\pm0.07$	$1.543^{g}\pm0.08$	$1.216^{g}\pm0.06$
MANOVA of Total chlorophyll				
Source of variation	df	Sum of squares	Mean of squares	F*
Replications	2	478.28	114374.5	
Treatments	27	28.74	1.06	
Duration (A)	3	2.28	0.76	153.57
Hg^{2+} conc. (B)	6	25.43	4.24	857.56
A x B	30	1.03	0.57	11.55
Error	54	0.27	0.0049	
Total	83	29.00		
Protein (mg/g fw)				
0.0	$8.12^{c}\pm0.05$	$8.14^{c}\pm0.06$	$8.07^{c}\pm 0.08$	$8.36^{b}\pm0.09$
0.1	$8.77^{b}\pm0.05$	$8.73^{b}\pm0.07$	$8.66^{b}\pm 0.15$	$8.83^{a}\pm0.08$
0.25	$9.85^{a}\pm0.12$	$9.99^{a}\pm0.13$	$9.98^{a}\pm 0.08$	$7.52^{c}\pm0.12$
0.5	$7.80^{d}\pm0.06$	$7.59^{d}\pm0.05$	$7.34^{d}\pm 0.06$	$7.12^{d}\pm0.15$
1.0	$7.34^{e}\pm0.13$	$7.28^{e}\pm0.15$	$6.73^{e}\pm 0.06$	$6.56^{e}\pm0.08$
5.0	$7.10^{f}\pm0.06$	$7.00^{f}\pm0.04$	$6.37^{f}\pm0.03$	$6.19^{f}\pm0.04$
10.0	$5.57^{g}\pm0.12$	$4.50^{g}\pm0.10$	$3.68^{g}\pm0.11$	$3.34^{g}\pm0.11$
MANOVA of protein				
Source of variation	df	Sum of squares	Mean of squares	F*
Replications	2	4046.82	8188367	
Treatments	27	240.76	8.92	
Duration (A)	3	5.69	1.90	22.33
Hg^{2+} conc. (B)	6	227.30	37.88	221.99
A x B	30	7.77	0.43	19.37
Error	54	1.75	0.32	
Total	83	242.51		

Mean ± SD (n=3); * Significant at 5% level; identical superscript denote no significant (P>0.05) difference between means in a column (total chlorophyll/protein) according to DMRT; Mercury was below detection limit in control samples; fw : fresh weight.

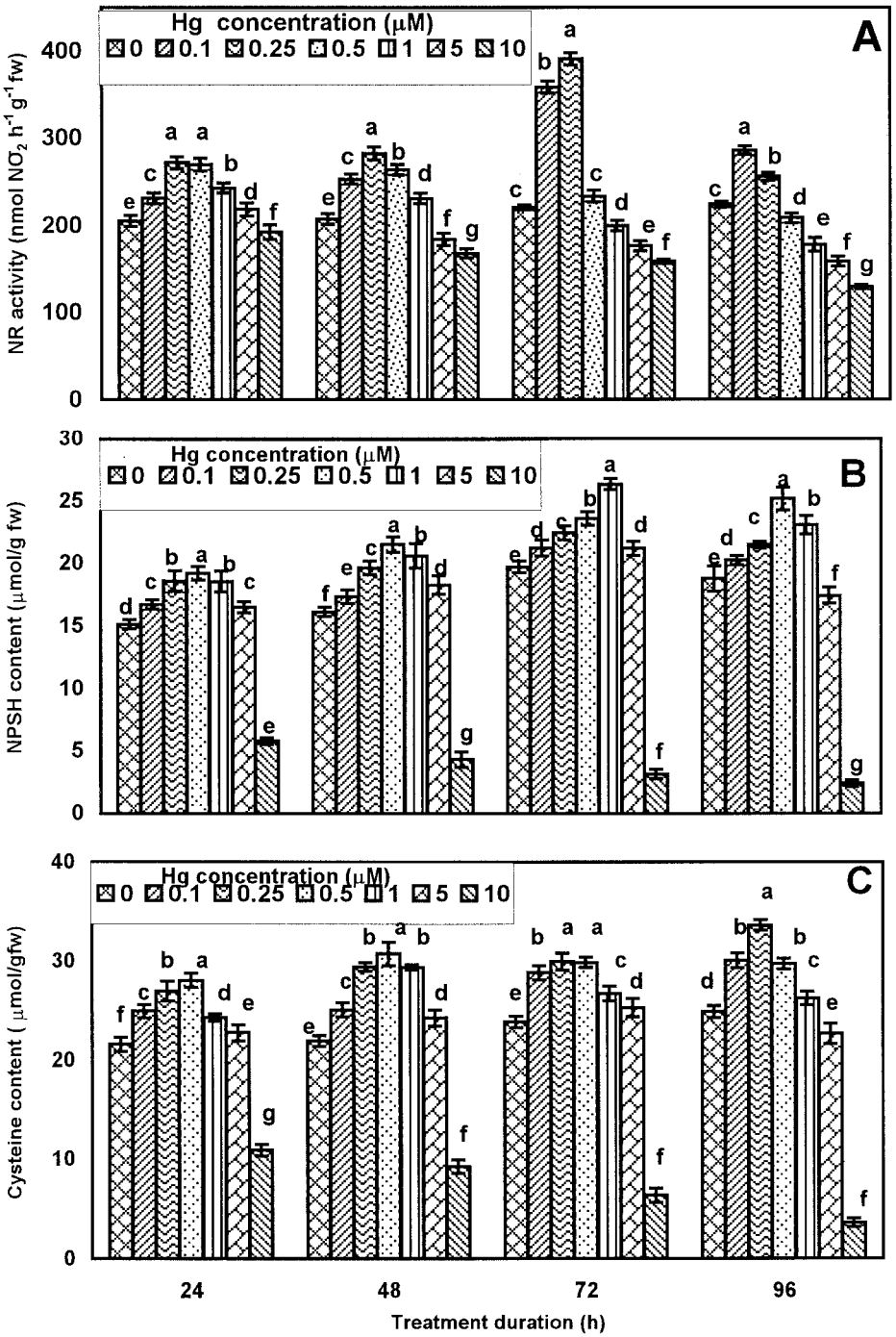

Figure 1. Effect of Hg^{2+} on NR activity (A), NPSH (B) and cysteine (C) contents of *P. crispus*. Identical superscript denote no significant difference ($p<0.05$) between means at each treatment duration according to DMRT. fw : fresh weight

Table 4. The multivariate analysis of variance in complete randomized blocks for nitrate reductase activity, nonprotein thiol and cysteine contents of *P. crispus* studied under Hg^{2+} stress

Parameters	Source of variation	df	Sum of squares	Mean square	F *
Nitrate reductase activity	Replications	2	3792596	7191893163224.68	
	Treatments	27	271461	10054.11	
	Duration (A)	3	19754.63	6584.88	36.50
	Hg^{2+} conc. (B)	6	181543.6	30257.27	167.73
	A x B	30	70162.74	3897.93	21.60
	Error	54	9740.98	180.39	
	Total	83	281202		
Non protein thiol	Replications	2	22574.76	254809828.77	
	Treatments	27	3203.28	118.64	
	Duration (A)	3	172.55	57.52	58.14
	Hg^{2+} conc. (B)	6	2881.14	480.19	485.39
	A x B	30	149.60	8.31	8.40
	Error	54	53.42	0.99	
	Total	83	3256.7		
Cysteine	Replications	2	41816.87	874325347.158	
	Treatments	27	4590.99	170.04	
	Duration (A)	3	38.66	12.89	15.90
	Hg^{2+} conc. (B)	6	4282.61	713.77	880.99
	A x B	30	269.72	14.98	18.50
	Error	54	43.75	0.81	
	Total	83	4634.74		

* Significant at 5% level

Therefore, the decrease in chlorophyll content may also account for phytotoxic consequence of lipid peroxidation (Somashekaraiah et al. 1992). Besides, mercury has also been reported to impair the δ-aminolevulinic acid metabolism by inhibiting δ-aminolevulinic acid dehydratase activity (Prasad and Prasad 1987). In the present study reduction in total chlorophyll content was probably achieved both by reaction with constituent biosynthetic enzymes as well as peroxide mediated degradation. It is clear from Table 6 that out of various parameters studied, total chlorophyll was most sensitive to Hg^{2+} toxicity.

NR, a key enzyme of nitrogen metabolism, catalyses the first step in the assimilatory reduction of nitrate to ammonia, reduces nitrate to nitrite using NADH as an electron donor. In the present study it was observed that lower concentrations of mercury (< 0.5 μM) stimulate NR activity. However, higher concentrations significantly reduced the NR activity. Gupta and Chandra (1998) reported reduction in NR activity by 0.5 μM and higher concentrations of mercury in another submerged plant *Vallisneria spiralis*. Stimulation of NR activity by lower concentrations of Cd has earlier been reported in *Hydrilla verticillata* (Garg et al.1997; Rai et al.1998). The cause of hormesis (stimulation) of NR activity under metal stress is still unknown but role played by signalling molecules like H_2O_2, jasmonic acid, salicylic acid etc. could not be ruled out (Sinha et al.1994; Leon et al. 1995; Xiang and Oliver 1998). Further, it has been suggested by *in vivo* experiments on leaf NR, that activity of the enzyme was reduced by oxidative environments, e.g., light, hyperoxia and treatments with oxyradicals propagating compounds (Kennis and Trippi 1986; Kennis et al. 1992). The present study also inferred that *in vivo* effects of toxic levels of mercury to NR were more complex as this metal is not only a potent sulfhydryl reagent, but also enhances the generation of active oxygen species. Results of present study revealed that adverse effects of mercury induced oxidative damage are not prominent at lower level of mercury (0.1μM, Table 6). This might be attributed to the induction of some stress proteins. Increased level of protein up to 0.25 μM concentration also support our contention. Toxic metals have been reported to induce a number of proteins, which play important role in preventing plant from toxic effects of metals

(Reddy 1992). Higher concentrations (0.50 –10.0 μM) of mercury cause a significant decrease in protein content of *P. crispus*. The decrease in protein contents in test plant by higher concentrations of Hg^{2+} was probably due to adverse effects of active oxygen species. Active oxygen species are reported to fragment a number of proteins (Davies 1987). A decrease in protein content by 0.5 μM and higher concentration of mercury has been reported in several other aquatic plants (Gupta and Chandra 1996; Sinha et al. 1996).

Table 5. Correlation coefficient matrix of responses of various parameters studied in mercury treated plants of *P. crispus* after 24 h, 48h (bold), 72h (bold italics) and 92 h (parenthesis).

	Hg^{2+} conc.	Hg uptake	MDA	K^+ Leakage	Total chlo.	Protein	NRA	NPSH	Cysteine
Hg^{2+} conc.									
Hg uptake	0.950 **0.960** ***0.970*** **(0.980)**								
MDA	0.910 **0.941** ***0.910*** **(0.972)**	0.992 **0.992** ***0.979*** **(0.971)**							
K^+ leakage	0.941 **0.930** ***0.890*** **(0.833)**	0.997 **0.991** ***0.928*** **(0.953)**	0.995 **0.998** ***0.971*** **(0.928)**						
Total chl.	-0.964 **-0.854** ***-0.828*** **(-0.853)**	-0.979 **-0.981** ***-0.985*** **(-0.983)**	-0.960 **-0.962** ***-0.949*** **(-0.930)**	-0.980 **-0.970** ***-0.970*** **(-0.971)**					
Protein	-0.833 **-0.871** ***-0.882*** **(-0.900)**	-0.846 **-0.813** ***-0.838*** **(-0.908)**	-0.882 **-0.881** ***-0.903*** **(-0.933)**	-0.860 **-0.868** ***-0.820*** **(-0.855)**	0.796 **0.771** ***0.787*** **0.851**				
NRA	-0.638* **-0.796*** ***-0.617**** **(-0.806)**	-0.404* **-0.624*** ***-0.569**** **(-0.813)**	-0.434* **-0.713*** ***-0.682**** **(-0.883)**	-0.455* **-0.699*** ***-0.630**** **(-0.869)**	0.467* **0.525*** ***0.535**** **(0.780)**	0.668* **0.822** ***0.858*** **(0.921)**			
NPSH	-0.866 **-0.843** ***-0.845*** **(-0.912)**	-0.666* **-0.608*** ***-0.628**** **(-0.736*)**	-0.635* **-0.659*** ***-0.669**** **(-0.812)**	-0.680* **-0.620*** ***-0.555**** **(-0.554*)**	0.733* **0.503*** ***0.496**** **(0.619*)**	0.717* **0.785** ***0.708*** **(0.746*)**	0.793 **0.704** ***0.378**** **(0.592*)**		
Cysteine	-0.878 **-0.843** ***-0.900*** **(-0.934)**	-0.704* **-0.589*** ***-0.700**** **(-0.809)**	-0.691* **-0.652*** ***-0.781*** **(-0.884)**	-0.727* **-0.615*** ***-0.647**** **(-0.670*)**	0.755 **0.473*** ***0.584**** **(0.701*)**	0.781 **0.758** ***0.845*** **(0.909)**	0.845 **0.796** ***0.590**** **(0.785)**	0.981 **0.986** ***0.950*** **(0.941)**	

Tabular r=0.754 (5%), 0.0.874 (1%)at df (n-2 =5); * insignificant at 5% level.

Results revealed that *P. crispus* has developed some defense mechanisms to tolerate mercury toxicity. An increase in cysteine and non-protein thiol contents following Hg^{2+} treatment was recorded during present study. The high amount of non-protein thiol, which represents the major proportion of phytochelatins (PCs), may be attributed to the enhanced level of phytochelatin during metal stress. The increase in non-protein thiols under mercury stress was almost completely due to the newly formed PCs (Gupta et al. 1998). Since phytochelatins, with the structure $(\gamma\text{-Glu-Cys})_n$-Gly (n= 2-11) have been described as major component of toxic metal detoxification in plants, algae and some yeast species and also work as bioindicator of metal stress (Mehra and Tripathi

Table : 6 A summary table of first effect levels of mercury to various parameters of *P crispus* exposed to different concentration of Hg^{2+}.

Parameters	Concentrations of the Hg^{2+} exhibiting first effects (μM)	
	Stimulatory	Inhibitory
K^+ Leakage	0.1	-
MDA	0.1	-
Total chlorophyll	-	0.1
Protein	0.1	0.5
Nitrate reductase activity	0.1	0.5
Non protein thiol	0.1	10
Cysteine	0.1	10

1999), the oxidative stress induced by mercury in *P. crispus* might be due to synthesis of phytochelatins which depleted the glutathione pool. DeVos et al.(1992) also reported that induction of phytochelatins under metal stress results in oxidative stress because of reduced glutathione contents. It could be concluded from present study that *P. crispus* plants accumulated appreciable amount of Hg^{2+} in a concentration – duration dependent manner and might be used for phytoremediation of mercury polluted water bodies.

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