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Cadmium Accumulation and Its Phytotoxicity in *Potamogeton pectinatus* L. (Potamogetonaceae)*

U. N. Rai, R. D. Tripathi, P. Vajpayee, N. Pandey, M. B. Ali, D. K. Gupta

Ecotoxicology and Bioremediation, National Botanical Research Institute, Rana Pratap Marg, P.B. No. 436, Lucknow-226 001, India

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Cadmium - a heavy metal with density 8.3 g cm^{-3} is a wide spread metal, released into environment by power stations, heating systems, metal working industries, waste incinerators, urban traffic, cement factories and as a by product of phosphate fertilizers (Toppi and Gabbrielli 1999). According to an estimate 29.19 $x10^3$ tonnes cadmium escapes annually into the environment from various sources (Mhatre and Pankhurst 1997). Cadmium enters into food-chain through plants and gets biomagnified. In India, daily Cd intake ranges between 7.8-16.5 µg/person (Rivai *et al* 1990). It is a toxic carcinogen and may cause death if ingested in large doses (Toppi and Gabbrielli 1999). Cadmium in plants causes leaf roll, chlorosis, damage of photosynthetic apparatus particularly the light harvesting complex II and photosystem I and II and reduction in chlorophyll biosynthesis (Reddy 1992).

Cadmium in water bodies originates from natural (weathering of rocks) as well as from anthropogenic sources (industrial effluent, agricultural run-offs). Aquatic plants have ability to accumulate large amount of cadmium and other toxic metals. These plants could be used in designing of cost effective phytoremediation system for cleaning of metal polluted water (Dunbabin and Bowmer 1992; Rai *et al* 1995a; Zayed *et al* 1998; Vajpayee *et al* 2001). It has been reported that submerged plants took up Cd by both adsorption and energy dependent transport (Tripathi *et al* 1995). However, the sorptive capacities are species specific and depend on the growth rate and physiological condition of individual plant. The submerged rooted macrophytes are of great use in planning monitoring and phytoremediation strategies of heavy metal polluted water bodies as they do not migrate and attain equilibrium with their surroundings with in a short period (Guilizzoni 1991; Rai *et al* 1995a).

The main objective of present investigation was to study the cadmium accumulating potential of *Potamogeton pectinatus* L. (a submerged aquatic macrophyte) under the laboratory condition to explore the possible use of this plant in phytoremediation of cadmium polluted water bodies. Phytotoxicity of cadmium on some physiological and biochemical parameters of *P. pectinatus* were also studied and results are presented in this paper.

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Correspondence to: U. N. Rai

MATERIALS AND METHODS

Plants of *Potamogeton pectinatus* L. (Potamogetonaceae), commonly known as funnel-leafed or Sago pond weed, were collected from unpolluted water bodies and acclimatized for more than six months in large hydroponic tubs in natural condition at National Botanical Research Institute, Lucknow. Growing shoots of these plants were cut-off from mother plant and cultured in 250 ml plastic beakers containing 200 ml of 5% Hoagland solution for six weeks under laboratory conditions (light:dark cycle 14:10 h, temperature 25±2°C, 115 μ mol $m^{-2}s^{-1}$ illumination provided through day fluorescent tube light). Various concentrations (0.0, 0.5, 5.0, 25.0, 50.0, 100.0 and 200.0 μM) of Cd were prepared by diluting the stock solution (1000 μM cadmium solution prepared from $CdCl_2$) with 5% Hoagland's nutrient medium. The acclimatized plants (approximately 2 g FW) were transferred to 250 ml plastic beakers containing 200 ml cadmium supplemented medium. Four sets (comprising of three beakers for each concentration), were placed separately in a growth chamber under above mentioned conditions. Plants placed in 5% Hoagland solution without cadmium served as control. Experimental cultures were aerated 6 h a day. One set of each concentration was harvested after 24, 48, 72 and 96 h of the treatment and washed three times with double distilled water. The oven dried (80°C) treated and control plants (1 g each) were digested in HNO_3:$HClO_4$ (3:1, v/v) at 80°C and cadmium concentrations were estimated by flame atomic absorption spectrophotometer at 228.8 nm (Perkin Elmer 2380). The cadmium content in *P. pectinatus* has been reported as measured concentrations. However, a mass balance was performed to account for the adsorption of Cd to plastic vessel wall.

The photosynthetic pigments were extracted in 80% chilled acetone (v/v) and estimated as per procedure of Arnon (1949). However, carotenoid concentrations in these extracts were calculated by the formula given by Duxbury and Yentsch (1956). Protein was estimated following the method of Lowry *et al* (1951) using bovine serum albumin as reference. The lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA) content determined by the thiobarbuteric acid (TBA) reaction following the method of Heath and Packer (1968). Cysteine and non-protein thiol contents were estimated following Gaitonde (1967) and Ellman (1959), respectively.

During present study, the cadmium test concentrations were analytically confirmed by estimating the cadmium in test solutions as described above. The standard reference material of cadmium (E-Merck, Germany) was used to provide calibration and quality assurance for each analytical batch. The efficacy of digestion of plant samples and Cd test concentrations was determined by adding reference material of cadmium to the samples. After addition of standard cadmium solution, samples were digested and cadmium was estimated as above. Mean recovery of cadmium was 97 ± 8%. The detection limit of cadmium in residue was 0.0002 μg ml^{-1}. Replicate (n=3) analyses were conducted to assess precision of the analytical techniques. Triplicate analysis for each test concentration varied by no more than 5%. A two way analysis of variance in complete randomized block

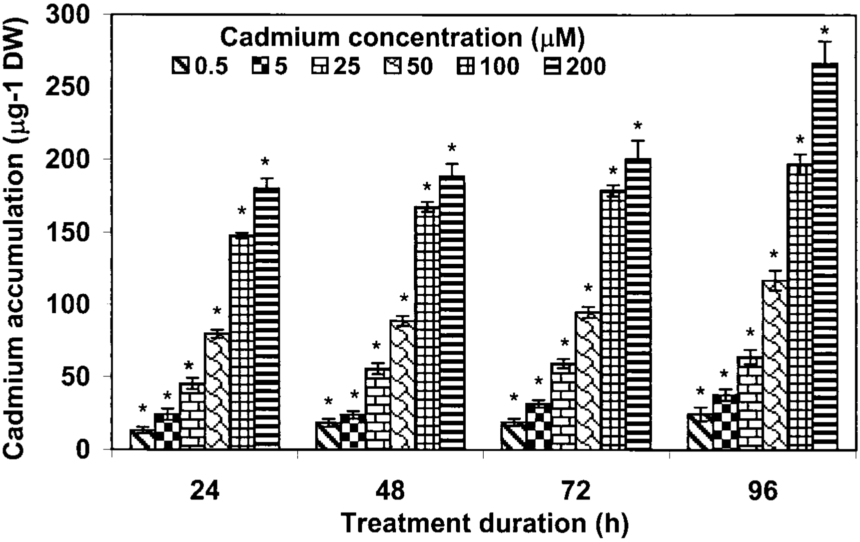

Figure 1. Cadmium accumulation (μg g^{-1} dw) in *P. pectinatus*. Values are mean ± SD (n=3); ANOVA p<0.05; replication df = 2, treatment df = 27; error df = 54, total df = 55). $F_{conc.}$ = 2581 (df=6), $F_{duration}$ = 120.18 (df=3), $F_{conc.\ X\ duration}$ = 18.82 (df=18); cadmium concentration in control was below the detection limit (0.0002 μg ml^{-1}). * = LSD p < 0.01 (from control at each treatment duration).

high amount of Cd. Thus, *P. pectinatus* could survive in metal polluted lakes (Ali *et al* 1999). Adams *et al* (1980) reported the luxuriant growth of *P. pectinatus* in lakes having 0- 4.15 μg/L Cd (0.466 μM) in their water. Several submerged aquatic plants viz., *Hydrilla verticillata*, *Ceratophyllum demersum* and *Pistia stratiotes* etc. have also been reported to accumulate large amounts of cadmium (Rai *et al* 1995a b; Tripathi *et al* 1995, 1996).

The metal accumulation in plants is often accompanied by induction of variety of cellular changes and some of them directly or indirectly contribute to metal tolerance capacity of the plant. The toxic effects of metal might be attributed to oxidative breakdown of chlorophyll, carotenoids, proteins, membrane structures and substitution of metal co-factors (Toppi and Gabbrielli 1999). In the present study, photosynthetic pigments (Chl a, Chl b and total chlorophyll) were found negatively correlated with Cd level in the nutrient medium (Table 3). Cadmium inhibits the formation of chlorophylls by reacting with essential thiol groups in both the protochlorophyllide reductase and the enzymes involved in the light dependent synthesis of δ-aminolevulinic acid (Stobart *et al* 1985). Besides, decline in chlorophylls was concomitant with increase in MDA level. Lipid peroxidation in plants initiated either by excited chloroplast or oxygen radicals deriving from the superoxide and hydroxy radicals (Kunert and Elder 1985). Metal ions could check the formation of excited chlorophyll, which inturn causes the production of free radicals. Hence, decline in chlorophylls might be attributed to the phytotoxic effects on the lipid peroxidation (Somashekaraiah *et al* 1992). It could be inferred from present observations that reduction in chlorophylls was design involving seven cadmium concentrations (including control) and four treatment duration was performed to confirm the validity of results and variability of data (Gomez and Gomez 1984). The comparison between means has been done using LSD (Least Significant Difference) test. Correlation coefficient (r) was calculated to find out correlation between various parameters and Cd concentrations.

RESULTS AND DISCUSSION

P. pectinatus accumulated significant amounts (ANOVA $p< 0.05$) of cadmium in a concentration duration dependent manner (Fig. 1). Maximum amount of Cd (266.3μg g-1 DW) was accumulated when plants were growing in nutrient solution containing 200 μM cadmium (LSD, $p< 0.01$). Results indicated significant loss in photosynthetic pigments (Chlorophyll a, chlorophyll b, total chlorophyll) of *P. pectinatus* plants exposed to different concentrations of Cd for 24-96 h. Further, decrease in total chlorophyll content was concomitant with increase in MDA content (Table 4). The toxicity of Cd to photosynthetic pigments was maximum after 96h exposure to 200 μM cadmium (Table 1). The lower concentrations of cadmium (0.5-25 μM) enhanced the level of carotenoid at all the treatment duration. However, reduction in carotenoid contents was observed when plants were exposed to cadmium concentration >25 μM. The toxicity to carotenoid content was concentration and duration dependent. The maximum reduction in carotenoid content was recorded after 96 h exposure of 200 μM cadmium (Table 1).

Cadmium has stimulatory effect on protein content of *P. pectinatus* exposed to different concentrations of Cd for 24-96 h (Table 2). However, the adverse affect of cadmium on protein content was first noticed at 100 μM Cd in nutrient solution after 24 h exposure which enhanced with increased cadmium concentration and treatment duration (LSD, $p< 0.05$). Cadmium induced lipid peroxidation (enhanced MDA levels) in *P. pectinatus* at all treatment concentrations and duration (ANOVA $P<0.05$). Maximum level of MDA (25 μ mol g^{-1} FW) was observed when plants were exposed to 200 μM cadmium for 96 h (Table 2). Cadmium treated test plants accumulated significant (ANOVA $p<0.05$) amount of cysteine in a concentration-duration dependent manner upto 50 μM Cd exposure for 96 h (Table 2). The toxicity of cadmium to cysteine was first observed when plants were exposed to 100 μM Cd for 24 h and increased with level of cadmium in nutrient solution and treatment duration (LSD, $p <0.01$ at 200 μM Cd). Further, the data showed a significant (LSD, $p < 0.05$) increase in NP-SH content of cadmium (0.5-50 μM) treated plants at all the treatment duration (Table 2). However, Cd concentration >50 μM in nutrient medium had an adverse effect on NP-SH content. The lowest NP-SH content (6.44 μ mol g^{-1} FW) was observed when plants were grown in medium supplemented with 200 μM cadmium for 96 h.

Although aquatic plants accumulate large quantities of Cd, it is not required for growth of these plants. During present investigation, *P. pectinatus* accumulated

Table 1. Effect of cadmium on photosynthetic pigments (mg g^{-1} FW) of *P. pectinatus*

Cd (μM)	Treatment duration (h)			
	24	48	72	96
		Chlorophyll a		
0.0	1.803±0.047	1.826 ±0.039	1.843±0.055	1.847±0.063
0.5	1.783±0.042	1.762± 0.051	1.746±0.056	1.684±0.051
5.0	1.745±0.055	1.710±0.048	1.668±0.047	1.596±0.055
25.0	1.687±0.073	1.608±0.064	1.581±0.036	1.546±0.047
50.0	1.623*±0.076	1.498*±0.041	1.477*±0.043	1.463*±0.037
100.0	1.481*±0.040	1.357*±0.053	1.302*±0.032	1.251*±0.028
200.0	1.459*±0.048	0.985*±0.032	0.960*±0.029	0.927*±0.022
		Chlorophyll b		
0.0	1.052±0.041	1.069 ±0.039	1.070 ±0.035	1.086±0.068
0.5	1.025±0.042	0.985± 0.033	0.964±0.049	0.933±0.043
5.0	0.992±0.055	0.976±0.048	0.957±0.047	0.873±0.040
25.0	0.941±0.043	0.878±0.034	0.842*±0.036	0.808**±0.034
50.0	0.898±0.036	0.813*±0.035	0.784**±0.043	0.741**±0.030
100.0	0.832*±0.026	0.773**±0.033	0.758**±0.032	0.712**±0.027
200.0	0.816*±0.018	0.770**±0.013	0.744**±0.029	0.661**±0.012
		Total chlorophyll		
0.0	2.856±0.140	2.890 ±0.131	2.912±0.115	2.928±0.118
0.5	2.806±0.130	2.746± 0.126	2.712±0.116	2.615*±0.116
5.0	2.735±0.136	2.694±0.122	2.621±0.117	2.460**±0.110
25.0	2.629±0.126	2.489*±0.116	2.423**±0.110	2.350**±0.114
50.0	2.529*±0.121	2.411**±0.113	2.345**±0.115	2.210**±0.108
100.0	2.311**±0.113	2.130**±0.101	2.069**±0.098	1.963**±0.098
200.0	2.271**±0.110	1.757**±0.082	1.704**±0.076	1.588**±0.080
		Carotenoids		
0.0	0.874±0.035	0.837 ±0.033	0.838±0.039	0.840±0.027
0.5	0.943±0.043	0.961± 0.045	0.936±0.033	0.966±0.032
5.0	1.016±0.55	1.027 ±0.032	1.028±0.030	1.067±0.033
25.0	1.210**±0.043	1.269**±0.041	1.170**±0.049	1.213**±0.041
50.0	0.941±0.022	0.822±0.033	0.829±0.029	0.701±0.024
100.0	0.794±0.021	0.673±0.021	0.778±0.021	0.614±0.026
200.0	0.662**±0.021	0.611**±0.018	0.551**±0.025	0.412**±0.018

Values are Mean (n=3) ± SD; ANOVA P<0.05; chlorophyll a : $F_{conc.}$ = 21.717 (df=6), $F_{duration}$ =3.299(df=3), $F_{conc.\ X\ duration}$ = 0.6494^{ns} (df=18); chlorophyll b : $F_{conc.}$ =20.900 (df=6), $F_{duration}$ =15.015(df=3), $F_{conc.\ X\ duration}$ =2.351(df=18); total chlorophyll: $F_{conc.}$ =367.085 (df=6),, $F_{duration}$ = 30.265 (df=3), $F_{conc.\ X\ duration}$ =2.230 (df=18); carotenoids : $F_{conc.}$ = 12.491(df=6), $F_{duration}$ =2.379(df=3) $F_{conc.\ X\ duration}$ =0.2358^{ns} (df=18); in all cases replication df = 2, treatment df = 27; error df = 54, total df =55; ns = p>0.05; * = LSD p < 0.05, ** = LSD p < 0.01.

Table 2. Effect of cadmium on protein, MDA, cysteine and non-protein thiol (NP-SH) contents of *P. pectinatus*

Cd (µM)	Treatment duration (h)			
	24	48	72	96
Protein (mg g^{-1} FW)				
0.0	11.69±0.481	11.89 ±0.572	11.78±0.409	12.11±0.579
0.5	14.07±0.678	14.60± 0.493	16.09**±0.789	17.03**±0.889
5.0	16.61±0.542	17.23**± 0.725	18.10**±0.885	22.62**±1.102
25.0	18.95**±0.555	19.16**±0.885	20.39**±0.886	14.39±0.506
50.0	14.22±0.740	14.01±0.335	13.88±0.443	11.99±0.336
100.0	8.73*±0.539	8.04*±0.744	7.10**±0.223	5.78**±0.455
200.0	7.77*±0.606	6.35**±0.849	5.22** ±0.408	3.70**±0.431
MDA (µ mol g^{-1} FW)				
0.0	6.92±0.201	6.92 ±0.139	7.15±0.259	7.67±0.225
0.5	7.66±0.362	7.75± 0.223	8.09±0.288	11.30*±0.430
5.0	8.43±0.305	8.53±0.332	8.77 ±0.255	11.52*±0.555
25.0	9.76± 0.407	9.86±0.398	10.18±0.347	13.11**±0.634
50.0	11.54**±0.459	12.51**±0.356	12.68**±0.436	16.43**±0.793
100.0	12.04**±0.536	13.94**±0.530	16.49**±0.432	20.25**±0.898
200.0	17.39**±0.668	19.25**±0.889	22.28**±1.020	25.18 **±1.136
Cysteine (µ mol g^{-1} FW)				
0.0	33.00 ±1.60	33.12 ±1.20	33.07±1.30	34.20±1.55
0.5	52.32**±2.60	52.32**± 2.50	60.24**±2.30	62.72**±2.60
5.0	54.34**±2.15	54.34**±2.55	64.80**±2.50	66.30**±2.88
25.0	58.59**±2.78	58.29**±2.70	67.68**±2.70	69.06**±3.10
50.0	63.23**±2.98	63.23**± 2.75	72.50**±2.88	74.32**±3.21
100.0	30.62*±1.20	29.62*±1.30	28.26*±1.10	27.46*±1.11
200.0	28.18**±1.30	22.18**±1.25	20.83**±0.99	18.19**±0.86
NP-SH ((µ mol g^{-1} FW)				
0.0	16.90±0.55	17.64 ±0.65	18.26±0.75	19.41±0.76
0.5	20.78*±0.78	21.54*± 0.76	22.57**±1.02	25.00**±1.11
5.0	22.16**±1.03	23.56 **±1.02	24.34**±1.10	28.57**±1.20
25.0	25.57**±1.10	26.09**±0.88	32.51**±1.36	34.20**±1.50
50.0	31.49**±1.22	32.51**±1.30	35.70**±1.42	38.19**±1.88
100.0	13.15*±0.56	11.64**±0.55	10.23**±0.43	9.34**±0.39
200.0	12.01**±0.48	10.15**±0.46	7.78**±0.36	6.44**±0.20

Values are Mean ± SD (n=3); ANOVA P<0.05; protein: $F_{conc.}$ = 36.717 (df=6), $F_{duration}$ =0.154 ^{ns} (df=3), $F_{conc.\ X\ duration}$ = 0.6494 ^{ns} (df=18); MDA $F_{conc.}$ =65.60 (df=6), $F_{duration}$ =20.16(df=3), $F_{conc.\ X\ duration}$ =1.180 (df=18); cysteine: $F_{conc.}$ =120.40 (df=6), $F_{duration}$ =2.841(df=3), $F_{conc.\ X\ duration}$ = 2.450 (df=18); NP-SH: $F_{conc.}$ =220.05, $F_{duration}$ =7.878 (df=3), $F_{conc.\ X\ duration}$ = 4.229 (df=18); in all cases replication df = 3, treatment df = 27; error df =54; total df =55; ns = p>0.05; * = LSD p < 0.05, ** = LSD p < 0.01.

Table 3. Correlation coefficient (r) of various responses of *P. pectinatus* under Cd stress

Parameter	Correlation coefficient (r) at different treatment duration (h)			
	24	48	72	96
Cd uptake	0.956	0.939	0.937	0.973
Chlorophyll a	-0.919	-0.990	-0.982	-0.968
Chlorophyll b	-0.885	-0.811	-0.774	-0.781
Total chlorophyll	-0.910	-0.971	-0.954	-0.927
Carotenoids	-0.691*	-0.700*	-0.743*	-0.858
Protein	-0.543*	-0.615*	-0.681*	-0.675*
Cysteine	-0.504*	-0.506*	-0.579*	-0.664*
MDA	0.978	0.982	0.992	0.960
NP-SH	-0.517*	-0.621*	-0.632*	-0.683*

Tabular value of r at 5% level (df = 5) = 0.754; * = insignificant

Table 4. The summary of the physiological responses of *P. pectinatus* exposed to various concentrations of cadmium based on statistical analysis of the data.

Parameters	Concentrations of the Cd (μM) exhibiting first effects at different treatment durations (h)							
	Stimulatory				Inhibitory			
	24	48	72	96	24	48	72	96
Chlorophyll a	-	-	-	-	50	50	50	50
Chlorophyll b	-	-	-	-	100	50	25	25
Total chlorophyll	-	-	-	-	50	25	25	0.5
Carotenoids	25	25	25	25	100	100	100	100
Protein	25	5	0.5	0.5	100	100	100	100
MDA	50	25	25	0.5	-	-	-	-
Cysteine	0.5	0.5	0.5	0.5	100	100	100	100
NP-SH	0.5	0.5	0.5	0.5	100	100	100	100

probably achieved both by reaction with responsible biosynthetic enzymes as well as lipid peroxidation mediated degradation. During present study, lower concentration of Cd enhanced the carotenoid biosynthesis. Carotenoids have long been recognized as important cellular antioxidants (Devi and Prasad 1998). Carotenoid can interact with singlet oxygen and free radicals preventing the initiation of potentially leathal processes such as lipid peroxidation. Cadmium induced lipid peroxidation in *P. pectinatus* is evident from enhanced levels of MDA. Probably the increased carotenoid level in test plant is a part of strategy adopted by the plant to counteract the toxic effect of free radicals generated under Cd stress. Kenneth *et al* (2000) reported that many plants have quite large difference in the absolute level of the carotenoid and synthesize zeaxanthin in response to stress condition.

Cadmium has been reported to induce stress proteins in *Oryza sativa* (Reddy 1992). Further, it has been demonstrated that the DNA of Cd stressed cells produces specific mRNA transcripts, which regulate the synthesis of stress proteins (Toppi and Gabbrielli 1999; Czarnecka *et al* 1988). Therefore, increased protein content in *P. pectinatus* treated by Cd (0.5-50 μM) might be attributed to the synthesis of stress proteins. The higher concentrations of cadmium (>50 μM) have toxic effect on protein contents of *P. pectinatus*. The decline in protein content under cadmium stress in aquatic plants has been reported (Rai *et al* 1995b, 1998). A decrease in protein content in the presence of Cd may be due to the breakdown of soluble proteins or due to the increased activity of protease or other catabolic enzymes which were activated and destroyed the protein. The increased lipid peroxidation is considered to be an indication of increased oxidative stress, which is often observed under metal stress condition in a variety of plants (DeVos *et al* 1992). In the present study, the concentration of MDA was significantly increased by treatment with Cd as compared to control, indicating enhanced lipid peroxidation in Cd exposed plants. The level of MDA was found positively correlated to the concentration of Cd in the nutrient solution (Table 3). Results of the present study indicate that plant possess a cytoplasmic detoxification mechanism comprised of the enhanced levels of non-protein thiols and cysteine contents to tolerate the high concentrations of Cd. Induction of the sulfate reduction enzyme ATP sulfurylase and adenosine 5' phosphosulphate sulpho transferase during Cd stress leading to accumulation of more cysteine has been reported (Nussabaum *et al* 1988). Possibly this may be essential to meet the high demand of reduced S to sustain required threshold of phytochelatin synthesis. It may be likely that Cd at various concentrations may activate the sulphate reduction enzymes in a similar way. The decrease in cysteine content at higher concentration may be ascertained to the toxic effect of Cd on the enzymatic protein content. Plants of *H. verticillata* and *P. stratiotes*, growing under Cd stress conditions have been reported to synthesize phytochelatins which is accompanied by increased levels of cysteine and non-protein thiols (Rai *et al* 1995b; Tripathi *et al* 1996). Varying responses to Cd induced oxidative stress are probably related both to levels of Cd supplied in the medium and to concentration of non-protein thiols already present or induced by Cd treatment. Thiols possess strong antioxidative properties and they are consequently able to counteract oxidative stress (Pichorner *et al* 1993).

P. pectinatus appears to be an ideal candidate for the phytoremediation of cadmium polluted water bodies as it accumulated significant amounts of Cd and showed tolerance through increased levels of non-protein thiols, cysteine and carotenoids. However, before using the plant for phytoremediation, it is necessary to study the effect of environmental variables that may be encountered in the field studies viz., light, temperature and pH etc.

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REFERENCES

Adams FS, Atchinson GJ, Vetter RJ (1980) The impact of an industrially contaminated lake on heavy metal levels in its effluent stream.

Hydrobiologia 54: 313-355
Ali MB, Tripathi RD, Rai UN, Pal A, Singh SP (1999) Physico-chemical characteristics and pollution levels of lake Nainital (U.P., India): Role of macrophytes and phytoplankton in biomonitoring and phytoremediation of toxic metal ions. Chemosphere 39: 2171-2182
Arnon DI (1949) Copper enzyme in isolated chloroplast polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24: 1-15
Czarnecka L, Nagao RT, Key JL, Gurley WB (1988) Characterization of gmhsp26-A, a stress gene encoding a divergent heat shock protein of soybean: heavy metal-induced inhibition of intron processing. Mol Cell Biol 8: 1113-1122
Devi SR, Prasad MNV (1998) Copper toxicity in *Ceratophylum demursum* L. (Coontail), afree loating macrophyte: Response of antioxidant enzymes and antioxidants. Plant Sci 138: 157-165
DeVos CHR, Vonk MJ, Vooijs R, Schat H (1992) Glutathione depletion due to copper induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. Plant Physiol 98: 853-858
Dunbabin JS, Bowmer KH (1992) Potential use of constructed wetlands for treatment of industrial waste waters containing metals. Sci Total Environ 111: 151-168
Duxbury AC, Yentsch CS (1956) Plankton pigment monomograph. J Air Pollut Control Assoc 16: 145-150
Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82: 70-77
Gaitonde MK (1967) A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring aminoacids. Biochem J 104: 627-633
Gomez KA, Gomez AA (1984) Statistical Procedures for Agricultural Research. John Willey & Son, New York
Guilizzoni P (1991) The role of heavy metal and toxic materials in physiological ecology of submerged macrophytes. Aquat Bot 87: 87-109
Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125: 189-198
Kenneth E, Pallett KE, Young AJ (2000) Carotenoids. Antioxidants in Higher Plants. CRC Press pp. 60-81
Kunert KJ, Elder M (1985) Leaf aging and lipid peroxidation : The role of the antioxidants vitamin C and E. Physiol Plant 65: 85-88
Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin-phenol reagent. J Biol Chem 193: 265-275
Mhatre GN, Pankhurst CE (1997) Bioindicators to detect contamination of soils with special reference to heavy metals, In: Pankhrust, CE, Doube MB, Gupta, VVSR (eds.), Biological Indicators of Soil Health and Sustainable Productivity. CAB International, New York, pp. 349-369
Nussabaum S, Schmutz D, Brunold C (1988) Regulation of assimilating sulphate reduction by cadmium in *Zea mays* L. Plant Physiol 88: 1407-1410
Pichorner H, Korori SAA, Thur A, Ebermann R (1993) The two and the four electron transfer to molecular oxygen mediated by plant peroxidase in the

presence of thiols, in; Welinder KG, Rasmussen SK, Penel C, Greppin (eds.) Plant Peroxidases: Biochemistry and Physiology University of Geneva, pp. 131-136

Rai UN, Gupta M, Tripathi RD, Chandra P (1998) Cadmium regulated nitrate reductase activity in *Hydrilla verticillata* (l.f.) Royle. Water Air Soil Pollut 106: 171-177

Rai UN, Sinha S, Tripathi RD, Chandra P (1995a) Wastewater treatability potential of some aquatic macrophytes: removal of heavy metals. Ecol Eng 157: 1-8

Rai UN, Tripathi RD, Gupta M, Chandra P (1995b) Induction of phytochelatin under cadmium stress in water lettuce (*Pistia stratiotes* L). J Environ Sci Hlth 30(9): 2017-2026

Reddy GN (1992) Cadmium induced bio-chemical changes in *Scenedesmus quadricauda* and *Oryza sativa*. Ph.D. Thesis submitted to the University of Hyderabad, Hyderabad, India

Rivai IF, Koyama H, Suzuki S (1990) Cadmium content in rice and its daily intake in various countries. Bull Environ Contam Toxicol 44: 910-916

Somashekaraiah BV, Padmaja K, Prasad ARK (1992) Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. Physiol Plant 85: 85-89

Stobart AK, Griffiths WT, Ameen-Bukhari I, Sperwood RP (1985) The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Physiol Plant 63: 293-298

Toppi LSD, Gabbrielli R (1999) Resonse to cadmium in higher plants. Environ Exp Bot 41: 105-130

Tripathi RD, Rai UN, Gupta M, Chandra P (1996) Induction of phytochelatins in *Hydrilla verticillata* (l.f.) Royle under cadmium stress. Bull Environ Contam Toxicol 56: 505-512

Tripathi RD, Rai UN, Gupta M, Yunus M, Chandra P (1995) Cadmium transport in submerged macrophyte *Ceratophyllum demerssum* L. in presence of various metabolic inhibitors and calcium channel blockers. Chemosphere 31: 3783-3791

Vajpayee P, Rai UN, Ali MB, Tripathi RD, Yadav V, Sinha S, Singh SN (2001) Chromium induced physiologic changes in *Vallisneria spiralis* L. and its role in phytoremediation of tannery effluent. Bull Environ Contam Toxicol 67: 246-256

Zayed A, Gowlthaman S, Terry N (1998) Phytoaccumulation of trace elements by wetland plants: I Duckweed. J Environ Qual 27: 715-721