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Chromium-Induced Physiologic Changes in *Vallisneria spiralis* L. and Its Role in Phytoremediation of Tannery Effluent

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Chromium (Cr) is the seventh most abundant element on the earth crust (Katz and Salem 1994). It occurs in several oxidation states ranging from Cr^{2+} to Cr^{6+}, with the trivalent and hexavalent states being the most stable and common in terrestrial environments (Zayed et al., 1998). Although, organically chelated Cr^{3+} compound is a co-factor in insuline hormone response controlling carbohydrate metabolism in human, Cr^{6+} is a potent, toxic carcinogen and may cause death if ingested in large doses (Syracuse Research Corporation, 1993). Chromium in waters originates from natural sources, such as weathering and rock constituents, wet precipitation and dry fallout from the atmosphere and runoffs from the terrestrial ecosystem. Chromium concentration in rivers and lakes is usually limited to 0.5-100 nM (Kotas and Stasicka, 2000). However, chromium discharges from industries such as electroplating units, textile, leather tanning and paper into surface water increased its concentration several fold higher than its natural occurrence. According to an estimate, in India, ca 2000-3200 tonnes of elemental chromium escapes into the environment annually from the tanning industry alone (Thiagrajan, 1992). Chromium concentration in tannery effluent usually ranges between 2000-5000 $\mu g\ ml^{-1}$. In developing countries like India, the effective management of effluent discharged by the tanneries has become a formidable task. Hence, contamination of water resources through these chromium rich effluents is posing serious health hazards to human beings and animals.

Aquatic plants have shown tremendous capacity to reduce the level of toxic metals (Cr, Pb, Cd, Hg etc), nutrients (N and P), biological oxygen demand (BOD) and total suspended solids from polluted waters (Kadlec and Kadlec, 1979; Nixon and Lee, 1986; Dunbabin and Bowmer, 1992; Rai et al., 1995; Vajpayee et al., 1995; Zayed et al., 1998). However, the use of these plants in designing low cost treatment system is still at experimental stage and is currently a burgeoning area in environmental management. Studies have shown that the uptake of metals largely depend on the chemical speciation of metal and life form of the macrophytes i.e., floating, rooted submerged, emergent species etc. Rooted submerged plants have great importance in such phytoremediation strategies due to their soil binding roots, rhizomes and stolens which help facilitate colonization by benthic algae, other microbes and invertebrates (Qian et al., 1999). Since *Vallisneria spiralis* L. (Hydrocharitaceae) is a rooted submerged and high biomass yielding plant, the

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present study was conducted to evaluate Cr accumulation and toxicity in relation to photosynthetic pigments, cysteine and protein contents and its possible role in bioremediation of chromium rich tannery effluent, through Cr accumulation.

MATERIALS AND METHODS

Plants of *Vallisneria spiralis* L. were collected from an unpolluted water body from Lucknow and acclimatized in hydroponic tubs under natural condition at National Botanical Research Institute, Lucknow. Young plants were detached from mother plants, planted in sterilized acid washed sand, containing 5% Hoagland solution in plastic containers of 2 L capacity. Cultures were placed in a growth chamber (light :dark cycle 14:10 h, temperature 28±2°C, 115 μ mol $m^{-2}s^{-1}$ illumination provided through day fluorescent tube light). Various concentrations (0.0,0.1, 1.0, 2.5, 5.0 and 10.0 μg ml^{-1}) of Cr (VI), were prepared by adding the required aliquots of 1000 μg ml^{-1} stock solution of $K_2Cr_2O_7$ to the 5% Hoagland solution. Plants of *V. spiralis* were uprooted and transferred (approximately 3.5 g) to 250 ml plastic beakers containing 200 ml chromium supplemented medium. Three sets, each of six concentrations were placed separately in a growth chamber under conditions mentioned above. Plants placed in 5% Hoagland solution without chromium served as control. Experimental cultures were aerated 6 h a day. One set of each concentration was harvested after 24, 48 and 72 h of the treatment and washed three times with double distilled water. The oven dried (80°C) plant tissues (leaves, rhizomes and roots) of treated and control plants were digested in HNO_3:$HClO_4$ (3:1, v/v) at 80°C and chromium concentration was estimated by a Flame Atomic Absorption Spectrophotometer (Perkin Elmer 2380).

Biomass of the plants was determined on dry weight (DW) basis. The photosynthetic pigments were extracted in 80% acetone (v/v) and estimated as per procedure of Arnon (1949). However, carotenoids were calculated by the formula given by Duxbury and Yentsch (1956). Protein and cysteine contents in leaves and roots were estimated following the method of Lowry et al. (1951) and Gaitonde (1967), respectively.To study the bioremediation potential of tannery effluent by *V. spiralis* effluent was collected from inlet and outlet source of a common tannery effluent treatment plant situated at district Unnao (U.P.). Physico-chemical analysis of both type of effluents (inlet and outlet) was carried out as per procedures described in APHA (1985) and the concentrations of chromium in treated and untreated effluent were estimated by digesting the 50 ml sample in HNO_3: $HClO_4$ (3:1, v/v) as described above. Tannery effluent collected from outlet of treatment plant was used for treatibility studies. Various concentrations of tannery effluent (25, 50, 75 and 100%) were prepared adding double distilled water. One litre effluent of each concentration was treated by 14-g plants of *V. spiralis* for 7 d under natural conditions. Plants were harvested after 7 d of treatment washed three times by double distilled water and oven dried (80°C). Chromium concentration in plant tissues and treated effluent was determined as above. During present study, the chromium test concentrations were analytically confirmed by estimating the Cr in test solutions as described above. The standard reference material of chromium (E-Merck, Germany) was used to provide

calibration and quality assurance for each analytical batch. The efficiency of digestion of plant samples and chromium test concentrations was determined by adding standard reference material of Cr (E- Merck, Germany) to the samples. After addition of standard chromium solution, samples were digested and Cr was estimated as described above. Mean recovery of chromium was 96 ± 9%. The detection limit of chromium in HNO_3 using Atomic Absorption Spectrophotometer (Perkin Elmer 2380) was 0.002 μg ml^{-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%.

A two way analysis of variance in complete randomized block design involving six chromium concentrations and three durations was performed to confirm the validity of data except in case of treatibility studies where one way ANOVA in complete randomized block design was done (Gomez and Gomez, 1984). The variation between means was tested using Duncan's multiple range test. Correlation coefficient (r) was calculated to find out correlation between various parameters and Cr concentrations.

RESULTS AND DISCUSSION

V. spiralis grows on chromium enriched nutrient medium and different plant parts accumulated significant amount (ANOVA, $P< 0.05$) of chromium (Table 1). Maximum amount of Cr was accumulated in roots of *V. Spiralis* (1050 μg g^{-1} DW), followed by leaves (697μg g^{-1} DW) and least in rhizomes (437 μg g^{-1} DW) in nutrient solution containing 10.0 μg ml^{-1} Cr after 72 h of treatment. Biomass of the plant was not found affected up to concentration of 2.5 μg ml^{-1}chromium when exposed for 24 h (Table 2). However, chromium concentration > 2.5 μg ml^{-1} significantly (DMRT, $P < 0.05$) reduced the biomass. An increase in treatment duration enhanced the chromium toxicity and 0.1 μg ml^{-1} Cr caused 7% decrease in biomass after 48 h. It has been observed that chromium toxicity to biomass was concentration and duration dependent (ANOVA, $P < 0.05$). Approximately, 64% loss in biomass was recorded after 72 h exposure of 10.0 μg ml^{-1} Cr to *V. spiralis*. Chl a and Chl b contents of *V. spiralis* were negatively correlated (correlation coefficient (r) significant at 5% level at df (n –2) = 4) with the concentration of Cr in nutrient solution (Table 7).

Chromium also reduced total chlorophyll content in a concentration–duration dependent manner. However, an increase in carotenoid content in leaves of the test plant was observed during present study (Table 3). Chromium also caused toxicity to protein content of *V. spiralis* leaves and roots in concentration–duration dependent manner. However, toxicity was more prominent in roots than leaves (Table 4). Similarly, reduction in cysteine contents of leaves and roots of chromium treated test plant was noted. Cysteine content in leaves and roots of chromium treated plants was negatively correlated with concentration of chromium in nutrient medium (Table 7). Raw tannery effluent and effluent processed through treatment plant were analyzed for physico-chemical properties and chromium content. Raw tannery effluent was slightly alkaline (pH 7.8) having

Table 1. Chromium accumulation by *Vallisneria spiralis* from nutrient medium containing different concentration of Cr.

Cr ($\mu g\ ml^{-1}$)	Cr accumulation ($\mu g\ g^{-1}$ DW)		
	24 h	48 h	72 h
	Leaves		
0.1	$4.20^{e}\pm0.17$	$6.00^{e}\pm0.30$	$6.90^{e}\pm0.35$
1.0	$75.60^{d}\pm3.78$	$83.4^{d}\pm3.17$	$91.8^{d}\pm4.59$
2.5	$151.8^{c}\pm7.60$	$173.4^{c}\pm7.7$	$182.4^{c}\pm9.12$
5.0	$303.8^{b}\pm14.5$	$340.5^{b}\pm15.5$	$360.4^{b}\pm17.0$
10.0	$625.5^{a}\pm31.3$	$675.9^{a}\pm32.8$	$697.5^{a}\pm34.9$
	Rhizomes		
0.1	$3.00^{e}\pm0.14$	$3.90^{e}\pm0.15$	$5.40^{e}\pm0.17$
1.0	$31.8^{d}\pm1.5$	$40.5^{d}\pm1.03$	$47.4^{d}\pm2.36$
2.5	$87.7^{c}\pm4.4$	$109.5^{c}\pm5.5$	$120.6^{c}\pm5.03$
5.0	$170.4^{b}\pm7.6$	$202.4^{b}\pm10.1$	$239.5^{b}\pm11.6$
10.0	$332.4^{a}\pm14$	$362.4^{a}\pm16.5$	$437.1^{a}\pm20.8$
	Roots		
0.1	$9.00^{e}\pm0.35$	$12.0^{e}\pm0.50$	$15.6^{e}\pm0.76$
1.0	$121.5^{d}\pm4.08$	$130.8^{d}\pm5.50$	$135.0^{d}\pm5.60$
2.5	$242.4^{c}\pm11.1$	$256.8^{c}\pm11.8$	$271.5^{c}\pm13.6$
5.0	$484.8^{b}\pm22.1$	$502.4^{b}\pm20.1$	$540.3^{b}\pm33.1$
10.0	$955.0^{a}\pm47.1$	$1001.8^{a}\pm44.3$	$1050.8^{a}\pm52.2$

Mean ± SD (n = 3); ANOVA,P <0.05; different superscripts denote significant ($P< 0.05$) difference between the means in a column. Control samples : Cr below the detection limit (0.002 $\mu g\ ml^{-1}$).

Table 2. Effect of Cr on biomass production by *Vallisneria spiralis*.

Cr ($\mu g\ ml^{-1}$)	Biomass (g DW) at different treatment duration (h)		
	24 h	48 h	72 h
0.0	$0.358^{a}\pm0.018$	$0.361^{a}\pm0.015$	$0.420^{a}\pm0.021$
0.1	$0.356^{a}\pm0.016$	$0.336^{b}\pm0.013$	$0.320^{b}\pm0.014$
1.0	$0.352^{a}\pm0.012$	$0.332^{bc}\pm0.014$	$0.315^{bc}\pm0.014$
2.5	$0.351^{a}\pm0.015$	$0.328^{c}\pm0.012$	$0.310^{c}\pm0.013$
5.0	$0.315^{b}\pm0.013$	$0.307^{d}\pm0.014$	$0.290^{d}\pm0.011$
10.0	$0.214^{c}\pm0.010$	$0.198^{e}\pm0.008$	$0.150^{e}\pm0.006$

Mean (n=3) ±SD; ANOVA, $P<0.05$; Identical superscripts denote no significant ($P<0.05$) difference between the means in a column.

low dissolved oxygen (0.24 $\mu g\ ml^{-1}$), high biochemical oxygen demand (1230 $\mu g\ ml^{-1}$), chemical oxygen demand (2460 $\mu g\ ml^{-1)}$ and chromium content (1.307 $\mu g\ ml^{-1}$). Tannery effluent processed by treatment plant has pH: 7.6; DO: 0.60 $\mu g\ ml^{-1}$; BOD: 330 $\mu g\ ml^{-1}$; COD: 660 $\mu g\ ml^{-1}$ and Cr : 1.051 $\mu g\ ml^{-1}$ (Table 5) used in the experiment to study the bioremediation potential of chromium by *V. spiralis*. Roots accumulated maximum amount of Cr from all the grades

(25, 50, 75, and 100%) of tannery effluent, followed by leaves and least in rhizomes (Fig 1). While plants of *V. spiralis* accumulated approximately 95% of Cr from 25% tannery effluent.

Table 3. Effect of chromium on photosynthetic pigments (mg g^{-1} FW) of *Vallisneria spiralis*.

Cr ($\mu g\ ml^{-1}$)	Treatment duration (h)		
	24 h	48 h	72 h
	Total chlorophyll		
0.0	$1.0054^{a}\pm0.050$	$1.0848^{a}\pm0.052$	$1.1260^{a}\pm0.056$
0.1	$0.9155^{b}\pm0.046$	$0.9324^{b}\pm0.044$	$0.8558^{b}\pm0.028$
1.0	$0.8126^{c}\pm0.038$	$0.7133^{c}\pm0.034$	$0.6756^{c}\pm0.026$
2.5	$0.6540^{d}\pm0.030$	$0.6207^{d}\pm0.031$	$0.5630^{d}\pm0.026$
5.0	$0.5989^{e}\pm0.028$	$0.5495^{e}\pm0.026$	$0.4043^{e}\pm0.023$
10.0	$0.4089^{f}\pm0.022$	$0.4184^{f}\pm0.021$	$0.2899^{f}\pm0.021$
	Chlorophyll a		
0.0	$0.7734^{a}\pm0.039$	$0.7059^{a}\pm0.032$	$0.8019^{a}\pm0.038$
0.1	$0.6507^{b}\pm0.032$	$0.6491^{b}\pm0.027$	$0.6451^{b}\pm0.020$
1.0	$0.5569^{c}\pm0.021$	$0.4643^{c}\pm0.023$	$0.4892^{c}\pm0.016$
2.5	$0.4669^{d}\pm0.023$	$0.4291^{d}\pm0.015$	$0.4010^{d}\pm0.012$
5.0	$0.4592^{e}\pm0.020$	$0.3639^{e}\pm0.018$	$0.2677^{e}\pm0.010$
10.0	$0.2934^{f}\pm0.015$	$0.2779^{f}\pm0.013$	$0.2052^{f}\pm0.014$
	Chlorophyll b		
0.0	$0.3012^{a}\pm0.015$	$0.3789^{a}\pm0.016$	$0.3241^{a}\pm0.016$
0.1	$0.2648^{b}\pm0.012$	$0.2833^{b}\pm0.010$	$0.2143^{b}\pm0.011$
1.0	$0.2557^{c}\pm0.010$	$0.2490^{c}\pm0.012$	$0.1864^{c}\pm0.007$
2.5	$0.1871^{d}\pm0.008$	$0.1916^{d}\pm0.008$	$0.1620^{d}\pm0.004$
5.0	$0.1397^{e}\pm0.006$	$0.1856^{e}\pm0.007$	$0.1355^{e}\pm0.004$
10.0	$0.1155^{f}\pm0.004$	$0.1406^{f}\pm0.006$	$0.0847^{f}\pm0.002$
	Carotenoids		
0.0	$0.1870^{f}\pm0.008$	$0.2199^{f}\pm0.012$	$0.2210^{f}\pm0.011$
0.1	$0.2101^{e}\pm0.010$	$0.2404^{e}\pm0.010$	$0.2810^{e}\pm0.014$
1.0	$0.2611^{d}\pm0.012$	$0.3560^{d}\pm0.013$	$0.4361^{d}\pm0.015$
2.5	$0.3472^{c}\pm0.015$	$0.4350^{c}\pm0.016$	$0.5412^{c}\pm0.022$
5.0	$0.4022^{b}\pm0.020$	$0.5132^{b}\pm0.026$	$0.6122^{b}\pm0.030$
10.0	$0.5130^{a}\pm0.023$	$0.6210^{a}\pm0.030$	$0.7334^{a}\pm0.036$

Values are mean ± SD (n=3); ANOVA, $P<0.05$; different superscripts denote significant ($P<0.05$) difference between the means in a column.

They were able to ameliorate only 59% of Cr from 100% tannery effluent after 7 days. Thus, *V. spiralis* was highly suitable for bioremediation of effluents containing low level of Cr. Results also indicated that plant could tolerate chromium contamination as it was growing well in Cr containing solution culture and tannery effluent. However, approximately 64% reduction in plant biomass

Table 4. Effect of chromium on protein content of *Vallisneria spiralis*.

Cr ($\mu g\ ml^{-1}$)	Protein (mg g^{-1} FW)		
	24 h	48 h	72 h
	Leaves		
0.0	$7.60^{a} \pm 0.38$	$7.85^{a} \pm 0.36$	$8.10^{a} \pm 0.36$
0.1	$6.50^{b} \pm 0.38$	$6.43^{b} \pm 0.38$	$6.16^{b} \pm 0.38$
1.0	$6.01^{c} \pm 0.30$	$6.20^{c} \pm 0.31$	$5.84^{c} \pm 0.25$
2.5	$5.12^{d} \pm 0.26$	$4.80^{d} \pm 0.20$	$4.66^{d} \pm 0.20$
5.0	$3.10^{e} \pm 0.14$	$2.98^{e} \pm 0.14$	$2.80^{e} \pm 0.12$
10.0	$2.56^{f} \pm 0.12$	$2.28^{f} \pm 0.11$	$2.03^{f} \pm 0.10$
	Roots		
0.0	$6.58^{a} \pm 0.30$	$6.84^{a} \pm 0.32$	$6.90^{a} \pm 0.30$
0.1	$5.39^{b} \pm 0.30$	$5.20^{b} \pm 0.30$	$5.06^{b} \pm 0.30$
1.0	$4.93^{c} \pm 0.22$	$4.82^{c} \pm 0.22$	$4.69^{c} \pm 0.20$
2.5	$3.96^{d} \pm 0.16$	$3.80^{d} \pm 0.16$	$3.60^{d} \pm 0.16$
5.0	$2.53^{e} \pm 0.12$	$2.39^{e} \pm 0.11$	$2.21^{e} \pm 0.11$
10.0	$1.97^{f} \pm 0.09$	$1.70^{f} \pm 0.07$	$1.52^{f} \pm 0.05$

Values are mean (n=3) ±SD; ANOVA,$P<0.05$; different superscripts denote significant ($P<0.05$) difference between the means in a column.

Table 5. Effect of chromium on cysteine content of *Vallisneria spiralis*.

Cr ($\mu g\ ml^{-1}$)	Cysteine (n mol g^{-1} FW)		
	24 h	48 h	72 h
	Leaves		
0.0	$90.50^{a} \pm 3.53$	$91.02^{a} \pm 4.30$	$91.80^{a} \pm 4.40$
0.1	$80.59^{b} \pm 4.02$	$75.20^{b} \pm 3.70$	$70.23^{b} \pm 2.98$
1.0	$64.30^{c} \pm 3.22$	$60.10^{c} \pm 2.98$	$55.97^{c} \pm 2.40$
2.5	$58.40^{d} \pm 2.82$	$50.30^{d} \pm 2.40$	$47.16^{d} \pm 2.50$
5.0	$45.30^{e} \pm 2.15$	$40.20^{e} \pm 1.98$	$36.40^{e} \pm 1.75$
10.0	$39.60^{f} \pm 1.90$	$33.20^{f} \pm 1.60$	$30.10^{f} \pm 1.51$
	Roots		
0.0	$110.5^{a} \pm 4.98$	$111.2^{a} \pm 5.50$	$112.5^{a} \pm 4.62$
0.1	$90.72^{b} \pm 4.30$	$87.29^{b} \pm 3.98$	$81.34^{b} \pm 3.90$
1.0	$75.58^{c} \pm 3.70$	$71.72^{c} \pm 3.40$	$65.47^{c} \pm 3.20$
2.5	$66.52^{d} \pm 3.20$	$62.27^{d} \pm 2.96$	$53.10^{d} \pm 2.50$
5.0	$51.38^{e} \pm 2.46$	$46.30^{e} \pm 2.10$	$41.74^{e} \pm 2.07$
10.0	$44.31^{f} \pm 2.20$	$40.28^{f} \pm 2.01$	$33.18^{f} \pm 1.60$

Values are mean (n=3) ±SD; ANOVA,$P<0.05$; Identical superscripts denote no significant difference ($P<0.05$) between the means in a column.

was recorded when plants were exposed to 10 $\mu g\ ml^{-1}$ Cr (VI) for 72 h. Reduced phytomass due to Cr toxicity has also been reported by Mc Grath (1982) and Tripathi and Smith (2000). The chromium removing potential of *V. spiralis* was quite promising as plant could accumulate significant amount of chromium in

Table 6. Physico-chemical characteristics of tannery effluent.

Parameters	Raw	After processing by treatment plant
pH	7.8 0± 0.38	7.6 ± 0.28
Temperature (°C)	26.5 ± 1.22	26.8±1.30
Dissolved Oxygen (DO)	0.24 ± 0.01	0.60±0.02
Biochemical Oxygen Demand (BOD)	1230 ± 61.5	330.0±15.5
Chemical Oxygen Demand (COD)	2460 ±123	660.0±21.0
Chromium	1.307± 0.06	1.05±0.05

Values are given in μg ml^{-1} otherwise stated.

different plant parts from both chromium containing culture solutions and tannery effluent (Fig 1). However, the translocation of Cr from roots to shoots was restricted, as concentration of Cr in roots was highest. This is in agreement with earlier reports on chromium uptake by aquatic plants (Sen et al., 1987; Gupta et al., 1994; Vajpayee et al., 1999 Vajpayee et al., 2000). The ability of *V. spiralis* to tolerate high chromium levels makes the plant highly suitable for constructing wetlands for phytoremediation of chromium-polluted waste water. Removal of 95% chromium from 25% tannery effluent by *V. sipralis* confirms our earlier finding that aquatic plants are very effective in bioremediation of diluted tannery effluent (Vajpayee et al., 1995). Chromium uptake by aquatic plants influenced many biochemical and physiological processes inside the plants. Photosynthetic pigments of plants belonging to different groups exhibit differential tolerance to metals (Rai et al., 1992). During present study, decline in Chl a, Chl b and total chlorophyll contents were recorded. It has been reported that Cr (VI) causes toxicity to δ-aminolevulinic acid dehydratase (an enzyme involved in chlorophyll biosynthesis) by impairing δ- amino levulinic acid (ALA) utilization (Vajpayee et al.,2000). Further, authors suggested that Cr (VI) could exchange the Mg from active sites of the enzyme resulting into phaeophytin. Thus, depleted chlorophyll contents in Cr treated plants might be attributed to both altered chlorophyll biosynthesis and replacement of Mg ions. Besides, it has also been reported that chromium inhibits chlorophyll biosynthesis by creating nutrient imbalances (Barcelo et al., 1986). Earlier reports suggested that effects of heavy metals on carotenoid content were plant and metal specific. Chromium induced degradation of carotenoids has been reported in some plants (Baszynski et al., 1981; Rai et al., 1992). In contrast to these reports, an increase in carotenoid content was observed in chromium treated plants of *V. spiralis*.

Other heavy metals such as Zn, Cd and Cu treatment to a sea grass (*Halophila ovalis*), in giant duckweed Cr increases carotenoid levels (Tripathi and Smith 2000;Ralph and Burchett, 1998). Our results are in agreement with Ralph and Burchett (1998). Increased carotenoid concentration for the protection from free radical formation is a common response to xenobiotics (Foyer and Harbinson, 1994; Devi and Prasad, 1998).Chromium has been reported to induce oxidative stress in aquatic plants (Roy et al., 1992). Therefore, enhanced carotenoid content

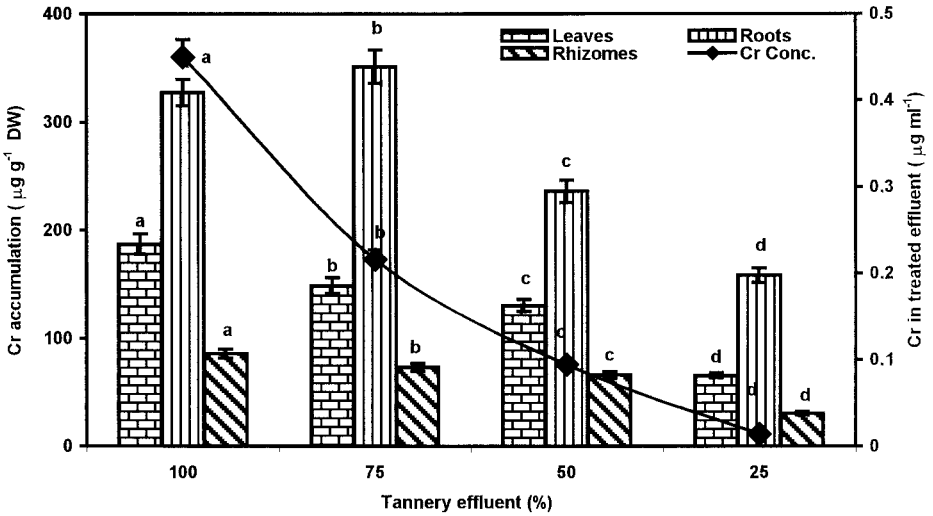

Figure 1. Chromium removal by *Vallisneria spiralis* from tannery effluent after 7 d of treatment; Mean ± SD; different superscripts on bars (each plant part) denote significant ($P<0.05$) differences between means.

Table 7. Correlation coefficient (r) of various responses of *Vallisneria spiralis* under Cr stress.

Parameter		Correlation coefficient (r)*		
		24 h	48 h	72 h
Cr uptake	Leaves	0.999	0.999	0.999
	Roots	0.999	0.999	0.999
	Rhizomes	0.999	0.999	0.999
Biomass		-0.967	-0.962	-0.908
Chlorophyll a		-0.900	-0.863	-0.859
Chlorophyll b		-0.913	-0.813	-0.822
Total chlorophyll		-0.940	-0.855	-0.855
Carotenoid		0.969	0.942	0.909
Protein	Leaves	-0.923	-0.854	-0.903
	Roots	-0.911	-0.899	-0.888
Cysteine	Leaves	-0.873	-0.851	-0.825
	Roots	-0.858	-0.838	-0.809

Tabular value of r at 5% level (df=4) =0.811;*significant at 5% level.

may be attributed to the strategy of plant to overcome the chromium induced oxidative stress. Chromium reduces protein content in plants by inhibiting nitrate reductase activity (Solomonson and Barber, 1990; Vajpayee et al., 1999; Vajpayee et al., 2000). A positive correlation between NR activity and protein content has been demonstrated in earlier studies (Rai et al., 1992). Cysteine, a –SH containing amino acid, plays a key role being the key constituent of

phytochelatins. Decrease in cysteine content might be to decreased activities of the sulphate reduction enzymes, ATP sulphurylase and adenosine 5' phosphosulphate sulphotransferase under metal stress.

It may be concluded from this study that plants of *V. spiralis* remove chromium effectively by surface adsorption or absorption and incorporate them into their own system or store them in a bound form. Chromium toxicity reduces total chlorophyll, Chl a, Chl b, protein and cysteine contents of plant as primary effects before it finally affects biomass as its secondary late effects. However, carotenoids of plant showed higher values in chromium treated plants. In view of effective removal of Cr from the tannery effluent by *V. spiralis*, the present study confirms the suitability of *V. spiralis* in constructing wetlands for amelioration of chromium containing wastewater. However, the safe disposal of contaminated plants in cemented vaults like contaminated soil is recommended (Moffat, 1995).

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