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Chromium accumulation in submerged aquatic plants treated with tannery effluent at Kanpur, India

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Abstract

Aquatic macrophytes have been widely studied because of their capability of absorbing contaminants from water and their subsequent use in biomonitoring. This study presents a comparison of Cr accumulating potential of submerged aquatic plants viz *Vallisneria spiralis* and *Hydrilla verticillata*. These plants were treated with various concentrations of treated tannery effluent collected from UASB, Jajmau, Kanpur under repeated exposure in controlled laboratory conditions in order to assess their maximum bioaccumulation potential. The maximum accumulation of 385.6 and 201.6 μg g^{-1} dry weight was found in roots of *V. spiralis* and the whole plants of *H. verticillata*, respectively at 100% concentration after 9th day of effluent exposure. The chlorophyll and protein content of both species decreased with increase in effluent concentration and duration. At highest concentration and duration a maximum reduction of 67.4 and 62.66% in total chlorophyll content, 9.97 and 4.66% in carotenoid content and 62.66 and 59.36% in protein content was found in *V. spiralis* and *H. verticillata* respectively. Anatomical studies in both *V. spiralis* and *H. verticillata* was carried out to assess the effects of metal accumulation within the plants. Changes in the anatomical structures of both plants exhibits the capacity of these species to act as indicator of effluent toxicity. The high accumulation potential of Cr by both plants revealed their capability to remove pollutants from effluent.

Key words

Vallisneria spiralis, Hydrilla verticillata, Chromium, Biomonitoring, Bioremediation, Tannery effluent

Introduction

In recent years, aquatic plants have been frequently used for biomonitoring of various water pollutants (Maine *et al*., 2001; Dhote and Dixit, 2009). The submerged plants have been recommended for remediation of wastewater by several workers (Elankumaran *et al*., 2003). Wang *et al*. (2009) suggested that submerged rooted plants have potential to extract heavy metals from water as well as sediments, whereas rootless plants extract metals rapidly only from water. *Vallisneria spiralis* and *Hydrilla verticillata* have been found growing luxuriantly in polluted water bodies (Srivastava and D'Souza, 2009). Tanning industry is one of the major sources of aquatic pollution in India. There are about 2500 tanneries in our country and nearly 80% of them are engaged in chrome tanning process (Sinha *et al*., 2006). In Kanpur district of Uttar Pradesh itself, there are about 300 tanneries along the bank of river Ganga. It is a prominent center for leather processing, especially for the manufacture of saddlery products. The wastewater discharged from these industries contains various pollutants, including high amount of chromium (1.07 to 7.80 mg l^{-1} Cr). Cr (VI) is a toxic, powerful, epithelial irritant and an established human carcinogen by International Agency for Research on Cancer and World Health Organization (WHO, 1990). In plants, it interferes with several metabolic processes causing phytotoxicity like reduced growth, chlorosis, ultrastructural effects on organelles, chromatin condensation, swelling of mitochondria etc. and finally leading to plant death (Dazy *et al*., 2008).

Plants growing in these areas accumulate high amounts of metals within their tissue, but long-term exposure may alter their growth pattern, cellular structure and metabolic activities. The present work is an effort to study the response of two submerged plants *viz. Vallisneria spiralis* and *Hydrilla verticillata* to tannery effluent at Kanpur, India.

Materials and Methods

Experiments were conducted with tannery effluent collected from Up flow Anaerobic Sludge Blanket (UASB) treatment plant, Jajmau, Kanpur, which has a capacity of 36 MLD and receives effluent from about 400 industries. The physicochemical properties of the effluent were determined using standard methods of APHA (2005). The effluent was allowed to settle down for a week and filtered. The settled filtered effluent (100%) was diluted with tap water so as to have a 75, 50 and 25% of the original concentration. Plants of *V. spiralis* and *H. verticillata* (approximately 100 g fresh weight) were individually treated with different concentrations of effluent for 2 and 7 days. Two sets of each experiment were kept in 250 ml plastic beakers for each effluent concentration and harvested after 7 and 14 days. The harvested plants were washed thoroughly with distilled water, oven dried (80°C) and digested with HNO_3:$HClO_4$ (3:1 v/v) to estimate the Cr concentration by a flame atomic absorption spectrophotometer (Perkin Elmer 2380).

The chlorophyll and carotenoid contents of fresh leaves were estimated by the method of Amon (1949) using 80% acetone. The chlorophyll and carotenoid concentration in mg g^{-1} of fresh leaves was calculated using the formula given by Duxbury and Yentsch (1956). Protein content of leaf tissues was estimated by the method of Lowry *et al*. (1951) using egg albumin as standard. The plant parts were harvested after 7 days and cut into 10-15 cm pieces and preserved in formalin-acetic acid alcohol (FAA) a lethal chemical preservative. Manual sectioning was done to study the plant material in cross sections. After sectioning, the material was passed through alcohol series and stained with safranin and fast green stains, and mounted in glycerine on glass slides. 100 sections of untreated and treated plant parts from each treatment were analyzed. The best five transverse sections were selected for study of anatomical characteristics. Microscope with 10x ocular and 10x objectives was used for all observations.

To confirm the variability of data and validity of results, the data were subjected to analysis of variance (ANOVA) and to determine the significance difference between treatments, least significant difference (LSD) was performed (Gomez and Gomez, 1984), wherever required.

Results and Discussion

The physico-chemical analysis of the effluent revealed that it was slightly alkaline (pH 8.6) with biochemical oxygen demand (BOD) 471 mg l^{-1}, chemical oxygen demand (COD) 1361 mg l^{-1}, total dissolved solids (TDS) 4315 mg l^{-1}, total suspended solid (TSS) 421 mg l^{-1} and Cr content was 2.16 mg l^{-1} (Table 1). Thus the

Table - 1: Physico-chemical characteristics of treated tannery effluent collected from UASB, Jajmau, Kanpur

Parameters	Values
Colour	Greyish black
Odour	Foul smell
pH	8.6±0.29
Electrical conductivity	12.34±0.23
TDS	4315±51.4
TSS	421±7.6
TS	4630±10.1
BOD	471±10.3
COD	1361±17.3
Cromium (Cr)	2.16±0.71

Values are given in mg l^{-1} except pH, temperature, colour, odour. Mean±SE (n=3)

Table 2: Accumulation of chromium (Cr) dry weight in different aquatic plants collected from Jajmau, Kanpur

Plants	Concentration of Cr (µg g^{-1})
Alternanthera sessiles	R(811.0±11.2) > S(241.8±19.6) > L(187.1±16.5)
Ipomoea spp.	L(26.4±2.8) > R(18.6±1.8) > S(11.2±0.9)
Cyanodon dactylon	L(543.0±21.1) > S(504.2±30) > R(443.0±26.1)
Brassica campestris	R(49.2±5.1) > S(20.1±3.4) > L(15.3±1.9)
Vallisneria spiralis	R(63.7±6.3) > L(35.4±0.8) S = NA[a]
Cyperus rotundus	R(215.1±24.1) > S(30.5±3.2) L = NA[a]

R = Root, S = Shoot, L = Leaves. All the values are mean of three replicates + S.D. [a]NA = Plant parts not available

Table - 3: Accumulation of Cr (µg g^{-1} dry weight) in roots and leaves of *V. spiralis* at different concentration of treated tannery effluent after 2nd and 7th day

Concentrations (%)	Accumulation of of Cr (µg g^{-1} dry wt.)			
	Roots		Leaves	
	2nd day	7th day	2nd day	7th day
25	110.6±20.1	175.3±21.3	53.0±7.8	91.4±9.8
50	160.4±23.7	249.7±30.6	87.3±9.4	143.5±20.6
75	197.3±25.4*	330.2±33.4*	114.6±13.7*	196.4±26.3*
100	220.1±28.3*	385.6±37.3*	135.1±19.3*	210.3±28.1*

All values are mean of triplicate ±S.D. ANOVA *Significant ($p<0.01$) compared to 25% . LSD*= $p<0.01$ as compared to 25%

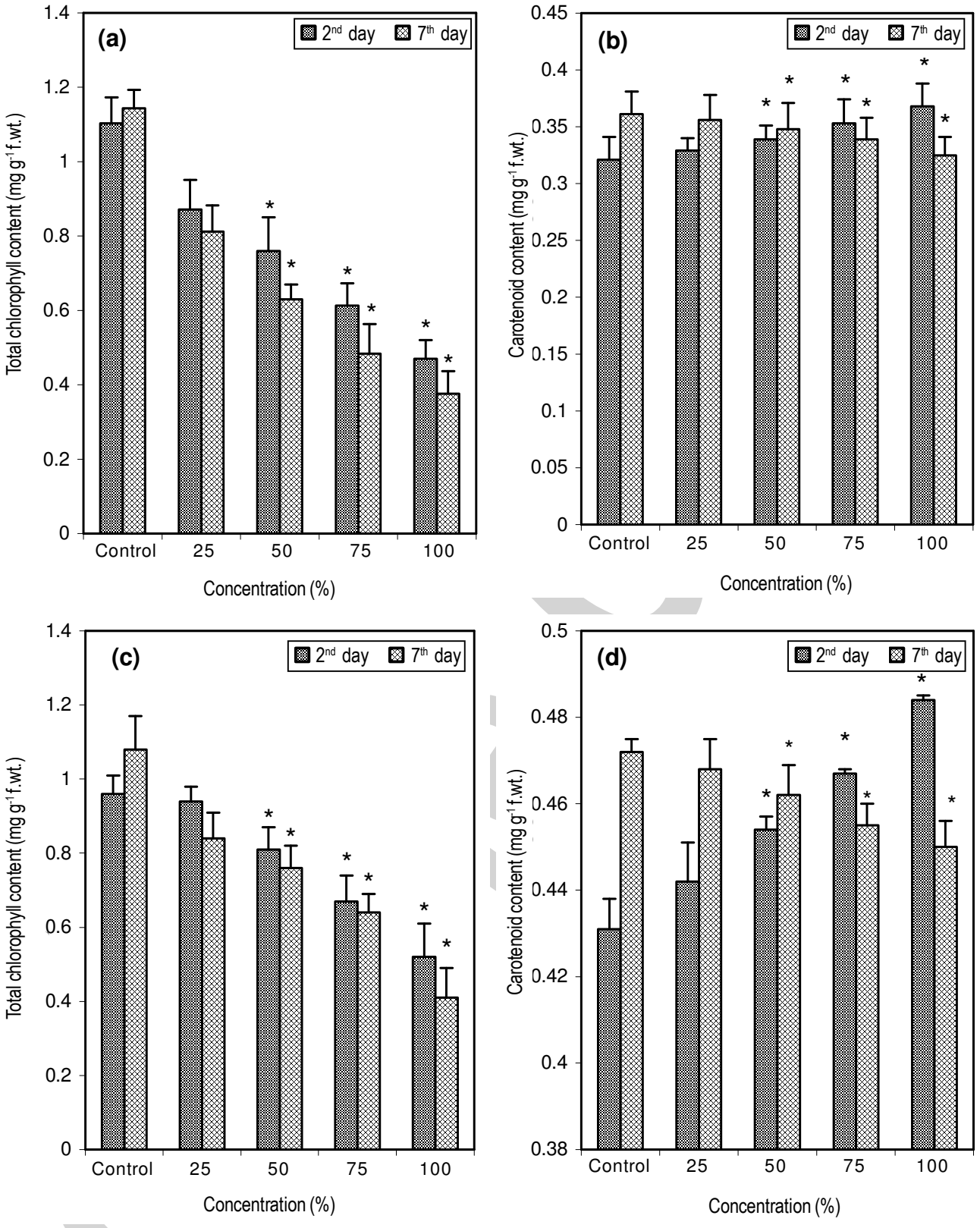

Fig. 1: Effect of different concentrations of tannery effluent on (a) total chlorophyll content (mg g^{-1} f.wt.), (b) carotenoid content (mg g^{-1} f.wt.) in *V. spiralis* and (c) total chlorophyll content (mg g^{-1} f.wt.), (d) carotenoid content (mg g^{-1} f.wt.) in *H. verticillata* at various exposures (days). All values are mean of triplicates +S.D.* = Significance ($p<0.01$) compare to control. (f.wt. = Fresh weight)

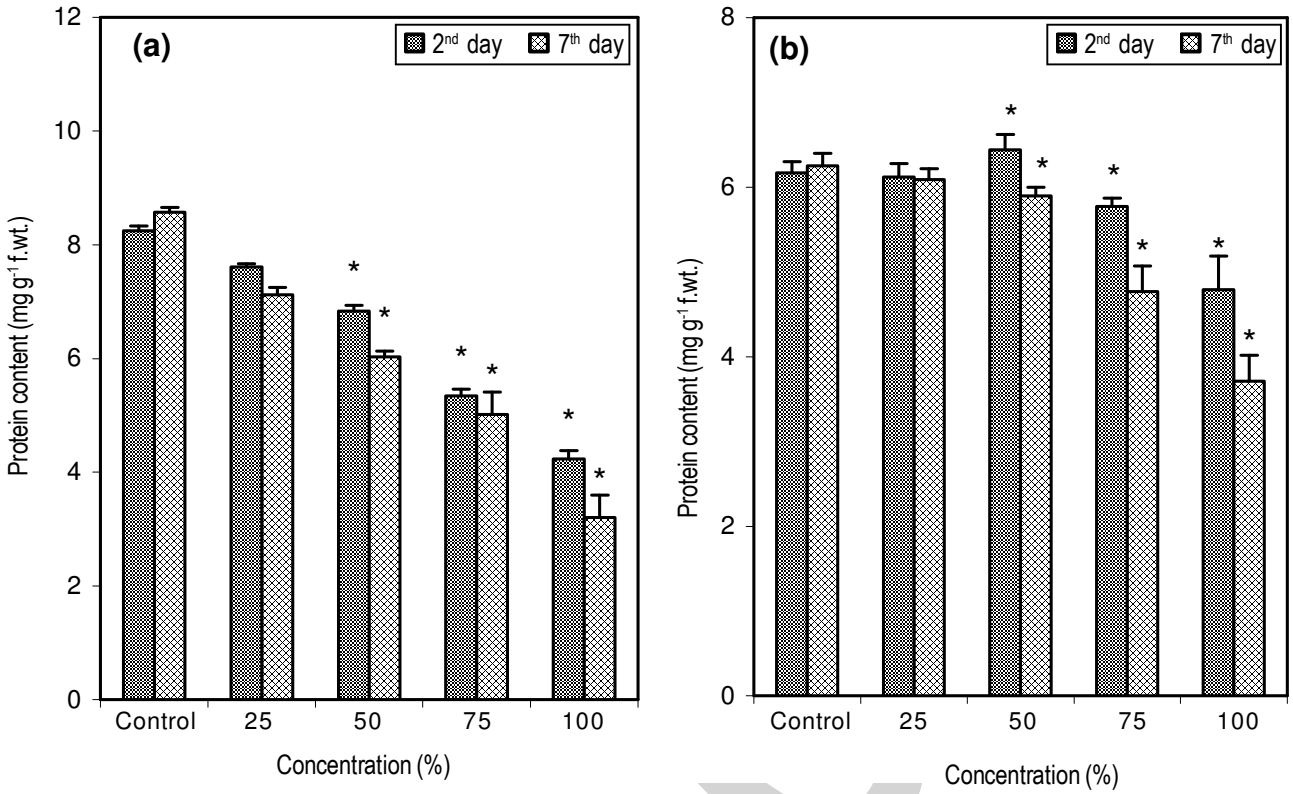

Fig. 2: Effect of different concentrations and duration (day) of tannery effluent on protein content (mg g^{-1} f.wt.) in V. spiralis and (b) *H. verticillata* at various exposures (days). All values are mean of triplicates ±SD. ANOVA significant at $p<0.01$. LSD * = $p<0.01$ as compared to control, f.wt. = Fresh weight

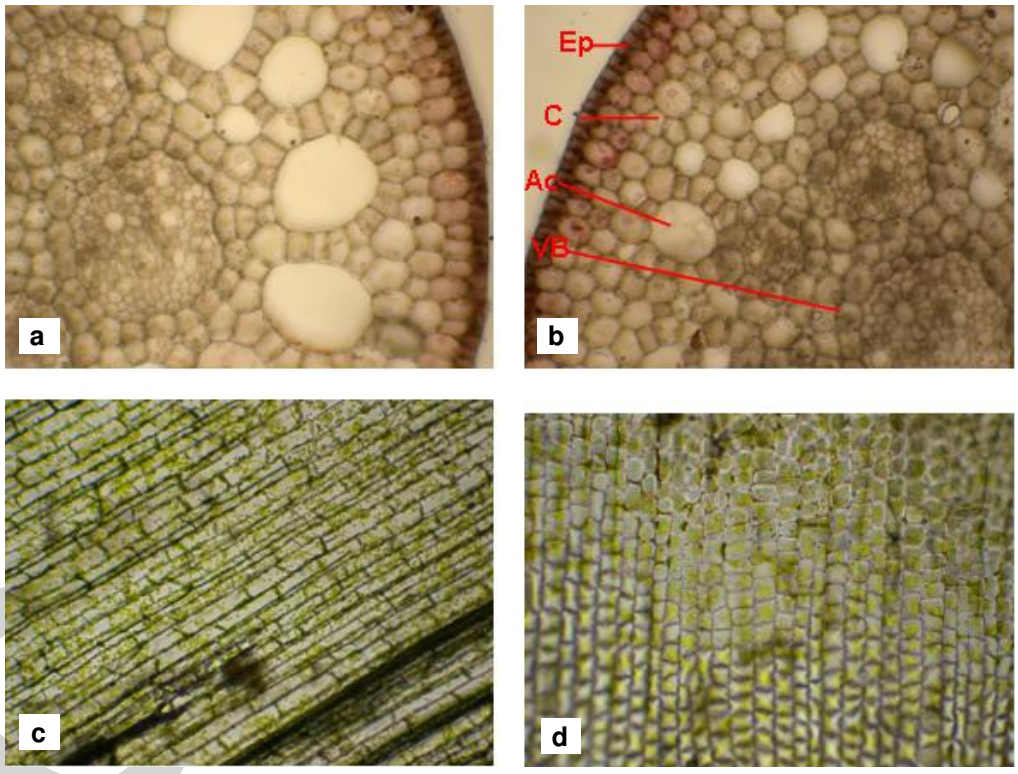

Fig. 3: Cross-sections (10X) of rhizome of control (a) and treated (b) and upper surface view of leaf of control (c) and treated plants (d) of *V. spiralis*. Ep = Epidermis; C = cortex; Ac = Air chambers; VB = vascular bundles

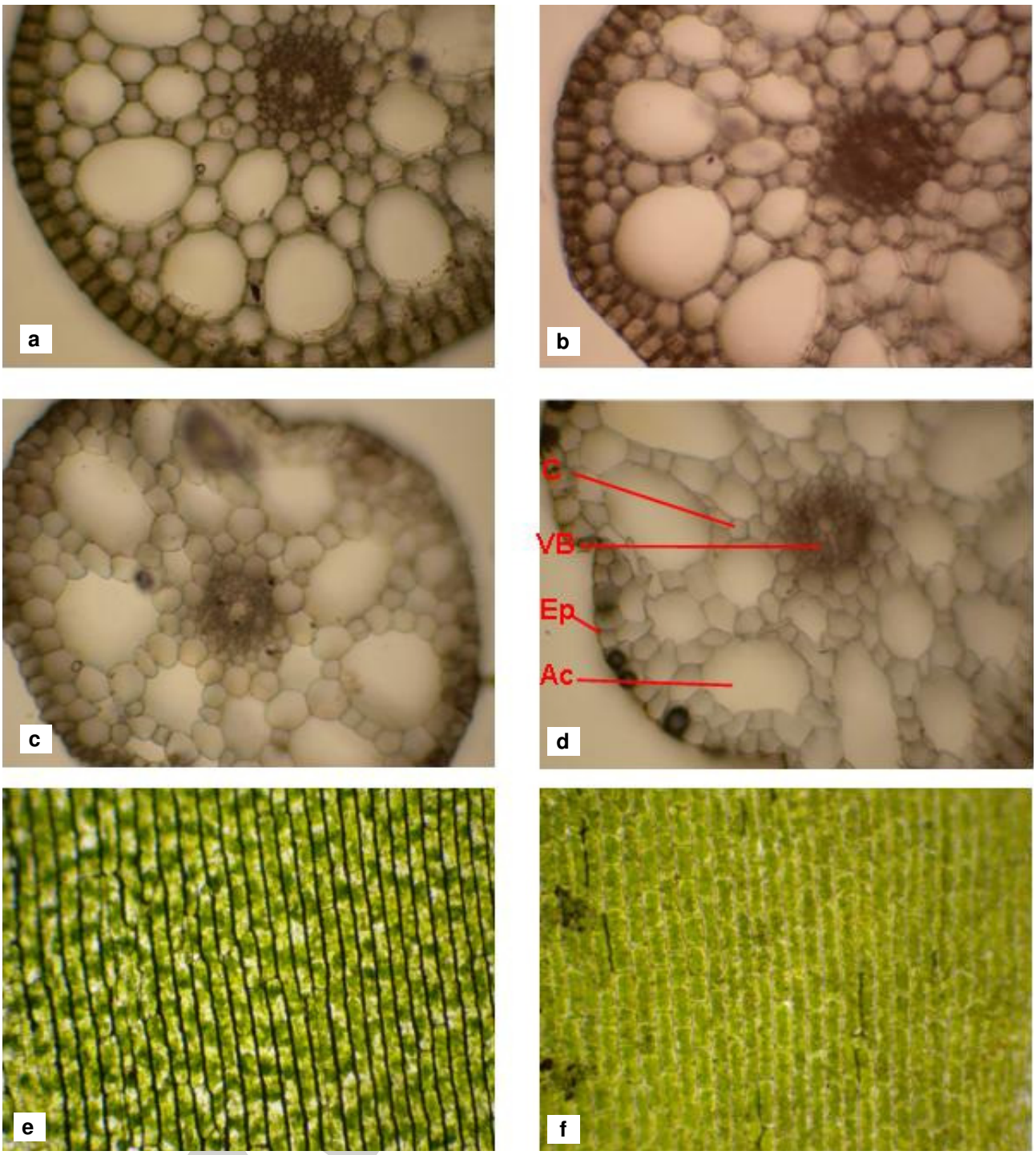

Fig. 4: Cross-sections (10 X) of stem of control (a) and treated (b), rhizome of control (c) and treated (d) and upper surface view of leaf of control (e) and treated (f) plants of *H. verticillata*. Ep = Epidermis; C = Cortex; Ac = Air chambers; VB = Vascular bundles

effluent was alkaline, with a low dissolved oxygen (DO) level, and high BOD, COD and metal content. Alkaline nature of tannery effluent may be due to the presence of high concentration of salts. However, high concentrations of metals (Cr) were found in the tannery effluent, which may be toxic to the plants.

Accumulation of Cr in the plants collected from the vicinity of the discharge point of UASB (Up flow Anaerobic Sludge Blanket), Jajmau, Kanpur, was more in roots than in shoots of most species except for *Ipomoea* and *C. dactylon* (Table 2). Pollutant concentration in plant tissues was higher than in the surroundings because of a continuous metal absorption by plants for a long time (Sinha *et al*., 2002).

Table - 4: Accumulation of Cr (μg g^{-1} dry weight) in whole plant of *H. verticillata* at different concentration of treated tannery effluent after 2nd and 7th day

Concentrations (%)	Accumulation of of Cr in plant (μg g^{-1} dry wt.)	
	2nd day	7th day
25	42.1±5.3	74.3±8.1
50	87.0±7.9	132.4±12.3
75	134.6±13.5*	178.0±19.6*
100	178.3±21.8*	201.6±20.6*

All values are mean of triplicate ±S.D.* = Significant ($p<0.01$) compared to 25%. LSD*= $p<0.01$ as compared to 25%

Chromium accumulation: Chromium accumulation in plants of *V. spiralis* and *H. verticillata* treated with different concentrations (0.0, 25, 50, 75 and 100%) of tannery effluent was concentration and duration dependent. In *V. spiralis* roots accumulated more Cr (385.6 μg g^{-1} dry weight) than shoots (210.3 μg g^{-1} dry weight) (Table 3). However, whole plant of *H. verticillata* accumulated substantial amount of Cr in their tissues (Table 4). The maximum absorption of Cr was found at 100% effluent after 7 days of treatment in both the plants. Earlier studies have shown that the Cr is retained largely in the roots and play a major role than shoots in the uptake of metal ions (Fritioff and Greger, 2007; Shukla *et al*., 2009). Low molecular weight proteins like phytochelatins are present in roots that bind metals and prevent further translocation of metals to shoots. The oxidation state of Cr, the pH and presence of humic substances also affect Cr uptake and transport in plant tissues (Sinha *et al*., 2002). Once the metal ions enter roots, they are either stored in roots or translocated to shoots through xylem (Jabeen *et al*., 2009). We have observed significant amount of Cr in shoots of *V. spiralis* possibly due to a constant contact of shoots with water. The leaves may directly uptake Cr from water in addition to translocation from roots. Our findings substantiate those of Vajpayee *et al*. (1995); Sinha *et al*. (2002) and Shukla *et al*. (2009) on various aquatic plants.

Total chlorophyll content in leaves of *V. spiralis* and *H. verticillata* decreased with increase in effluent concentration and duration with respect to their control (Fig. 1). The maximum inhibition of 67.4 and 62.66% was observed in *V. spiralis* and *H. verticillata*, respectively at 100% effluent concentration after 7 days treatment in both the plants. Cr toxicity affects photosynthesis by causing distortion of chloroplast ultrastructure, inhibiting synthesis of photosynthetic pigment in chlorophyll content and enzymes of the Calvin cycle. Reduction in chlorophyll content in the present study might be due to impaired δ-aminolevulinic acid dehydrates activity leading to reduced photosynthetic pigments, as observed earlier in Cr-treated aquatic plants (Vajpayee *et al*., 2000; Mishra *et al*., 2009). In plants induction of enzymatic and non enzymatic antioxidants are usually studied as metal detoxifying responses (Diwan *et al*., 2008, 2010). In the present study, carotenoid content in treated plants of *V. spiralis* and *H. verticillata* increased at all effluent concentrations after 2nd day and upto 50% concentrations after 7 days of exposure. The increase may be attributed for protection from free radical formation against environmental stress (Prasad *et al*., 2001; Hou *et al*., 2007) whereas decrease at higher concentration and duration may be due to excessive generation of reactive oxygen species (ROS).

The protein content declined in treated plants of both the species, as compared with the control (Fig. 2). The maximum reduction of 62.66 and 59.36% occurred in *V. spiralis* and *H. verticillata*, respectively, at 100% effluent after 7 days. This could be due to increased activity of protease and other catabolic enzymes or fragmentation of proteins due to toxic effects of reactive oxidative stress, as suggested by Mishra *et al*. (2007).

Anatomical studies: After 2nd day of treatment with 100% effluent concentration both the plants became fragile and their old leaves showed softening of tissues as compared to control plant. *V. spiralis* did not grow as well in the effluent as *Hydrilla* did. The cell size of epidermis (ED), cortical region (C) and air chamber (Ac) in the rhizome of treated plant of *V. spiralis* decreased in comparison with the control. Vascular bundles (VB) were also reduced (Fig. 3a-b). In the upper surface view of *V. spiralis* leaf, elongated cells became shorter and widened in treated plants than in the control (Fig. 3c-d).

Fig. 4a-b shows the cross section view of rhizome of control and treated *H. verticillata* plants. On comparing with control, the treated rhizome showed a reduced and distorted epidermal (ED) and cortex regions (C), though the number of air chambers (Ac) increased. Deposition of metal ions in the cells was also observed alongwith highly reduced vascular bundles (VB). The cross sectional view of stem (Fig. 4c-d) in the treated *H. verticillata* plants showed increased number of air chambers (Ac) together with dark-stained epidermal (ED) cells and vascular bundle (VB), which might be due to deposition of metal (Warrier and Saroja, 2008). The upper surface view of leaf of *H. verticillata* showed that the cell wall was not prominent and the green pigments were diffused in leaf tissue of treated plants compared with the control (Fig. 4e-f).

Aquatic plants vary in their ability to accumulate metal in their tissues. Both *H. verticillata* and *V. spiralis* may be used as bioaccumulators because of their efficiency in metal absorption and as bioindicators of chromium pollution due to their specific anatomical responses to the pollutant.

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