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BIOACCUMULATION POTENTIAL OF *PISTIA STRATIOTES* AND ITS RESPONSE TO TANNERY EFFLUENT EXPOSURE

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ABSTRACT

Pistia stratiotes was explored as bioindicator potential investigation to treated tannery effluent containing Cr (VI).The root system was found to accumulate a high amount of Cr to the extent of (23.64 64 µgg-^{1}fw) with respect to the shoot system (11.22 µgg-^{1}fw) for an 2.36 ppm concentration of Cr. The chlorophyll and protein content of the plant decreased in correspond to effluent concentration and duration. At highest concentration and duration a maximum reduction of 34.12% in total chlorophyll content and 23.24% in protein content was recorded in *Pistia stratiotes*. An anatomical study was carried out to assess the effects of metal accumulation within the plants. Changes in the anatomical structures exhibits the capacity of these species to act as indicator of effluent toxicity. The high Cr accumulation capacity of plants indicated ***a*** noticeable improvement of overall removal of chromium from effluent which clearly advocates the benefit of potential application to alleviate Cr (VI) uptake by aquatic macrophyte.

Keywords: Chromium, Bioindicator, Tannery effluent, *Pistia stratiotes,* anatomical variation.

INTRODUCTION

The increasing pace of industrialization in public and private sectors along with urbanization explosion and green revolution are reflected in varying degree of polluted water, soil and air. Water pollution is a major global problem [1]. Tanning industries are one of the key sources to pollute our environment. There are more than 2500 tanneries in the country and nearly 80% are engaged in the chrome tanning process. Jajmau, Kanpur in U.P is a major industrial town (about 400 tanneries) located on the bank of river Ganga specialized in processing hide in to heavy leather [2]. Unfortunately only fraction of chromium is utilized in tanning process and rest is discharged as byproduct of waste water treatment [3]. The enormous pollution load along with the toxic nature of waste water makes the tanneries a potential threat to the areas in the vicinity of their locations. The chromium (Cr VI) is one of most problematic pollutant discharge by tanning industry [4,5]. Cr (VI) compounds are toxic, mutagenic and even carcinogenic in nature [6,7]. Plants undergo significant morphological and metabolic changes in response to metal toxicity which are the expression of metal induced alteration at the cell tissue and organ levels, in turn are the result of a direct interaction of toxic metals with structural components at these sites [8].

Toxicity tests are an important tool in monitoring programme for controlling the quality of effluent discharges. The use of aquatic macrophytes in water quality studies has been considered appropriate because they are commonly exposed to water pollution [9]. The toxic effects of contaminants on aquatic vegetation growing in constructed wetlands are usually estimated from changes in some population, biological and physiological parameters of plants [10,11]. The anatomical structures were also affected by heavy metals [12]. Toxicity tests constitute a valuable help in assessing the capacity of these plants to withstand the inflow of a certain wastewater, which is crucial for their successful application in a phytoremediation. These aquatic plants are also suitable for wastewater treatment because they have tremendous capacity of absorbing nutrient and heavy metals [13]. Among water plants *Pistia stratiotes* also concentrate heavy metals.

In the present investigation, an attempt of has been made to evaluate Cr accumulation and toxicity in relation to photosynthetic pigment, protein content, histo-chemical studies and its possible role in phytoremediation of Cr rich tannery effluent.

MATERIAL AND METHODS

In India, Kanpur (U.P.) lies in Indo-Gangetic plains between the parallels of 26 º 28’ N and 80º 24’ E. about 400 tanneries are the major centers for the possessing of raw hides. The discharge from these

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industries wastewater is being treated in an up flow anaerobic sludge blanket (USAB) treatment plant having a capacity of 36 MLD. The physico-chemical properties of the effluent were determined using standard methods of APHA [14]. The treated wastewater is being used more than two decades for the irrigation purpose in an area of 2100 acre. Due to long term irrigation from contaminated wastewater, the above mentioned site is selected for the present study.

Collection of plants and acclimatization

Laboratory Studies: The young plants (*Pistia stratiotes*) were collected from a nearby pond and acclimatized hydroponically in a flat bottom HDPE tray. The plant did not possess any prehistorical exposure in chromium contaminated environment. Plants harvested from the tray were subsequently washed in single distilled water and finally in deionised water. They were grown hydroponically in 10% Hoagland solution [14] as a nutritional supplement at pH 7 ± 0.2 in approximately 10,000 lux/m^2 light intensity under controlled environment provided with fluorescent tubes. The experiment was carried out at ambient temperature (25 ± 5 0C) with an 8h photoperiod. Approximate 60 g of equal sized plants were placed in the aquarium and allowed to retain in 1.5 litre volume of Hoagland solution. Volume of solution was adjusted with distilled water after aliquots of solution were taken for analysis and to make up the loss due to evaporation.

Experimental setup

Preparation of gradient solution of tannery effluent:

Experiments were conducted with tannery effluent collected from up flow anaerobic sludge blanket (USAB) treatment plant, Jajmau; Kanpur The effluent was allowed to settle down for a week and filtered. The settled filtered effluent (100%) was diluted with tap water (having Cr concentration below detectable limit i.e. < 0.004 mg l^{-1}) so as to have a 75, 50 and 25% of the original concentration.

Chromium estimation

Plants of *P. stratiotes* (approximately 60 g fresh weight) were individually treated with different concentrations of effluent for 2, 4 and 6 days. Two sets of each experiment were kept in 250 ml plastic beakers for each effluent concentration and harvested after 2, 4 and 6 days. The harvested plants were washed thoroughly with distilled water, oven dried (80^{o}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). Plants growing luxuriantly in the site of effluent collection were also collected for estimation of Cr concentration.

Biochemical assay

The chlorophyll contents of fresh leaves were estimated by the method of Arnon [15] using 80% acetone. Protein content of leaf tissues was estimated by the method of Lowry *et al* [17] using egg albumin as standard.

Histological profile

The plant parts (Root, rhizome and leaf) of *P. stratiotes* were harvested after 6 days and cut into 10-15cm pieces and preserved in formalin-acetic acid alcohol (FAA) a lethal chemical preservative. Manual sectioning was done to study the structural variations in cross sections. After sectioning, the material was passed through alcohol series and stained with safranin and fast green stains, and mounted in glycerin on glass slides. 100 cross-sections of root, rhizome and leaves of untreated and treated plants from each treatment were analyzed for anatomical variations. The best five transverse sections were selected for study of anatomical characteristics. Digital images were obtained using a digital camera (Nikon P-5100) linked to an optical microscope (Nikon –E-200 with 10x ocular and 10x objectives).

To confirm the variability of data and validity of results, the data were subjected to an analysis of variance (ANOVA) and to determine the significance difference between treatments, least significant difference (LSD) was performed [18] wherever required.

RESULTS

The physico-chemical analysis of the effluent revealed that it was slightly alkaline (pH 8.2) with biochemical oxygen demand (BOD) 473 mg l^{-1}, chemical oxygen demand (COD) 1371 mg l^{-1}, total dissolved solids (TDS) 4325 mg l^{-1}, total suspended solid (TSS) 405mg l^{-1} and Cr content was 2.34 mg l^{-1} (Table 1).

P. stratiotes grows in various concentration of effluent and exposure duration, accumulated significant amount (ANOVA, $p \leq 0.05$) of Cr. The result showed a concentration and duration dependent accumulation of Cr in plant tissues. However maximum bioconcentration of Cr was accumulated in roots of *P. stratiotes* (23.64 μgg-^{1}fw) (Fig-1), followed by shoot (11.22 μgg-^{1}fw) (Fig-2) in 100% effluent after 6 days of exposure.

Total chlorophyll content in leaves decreased corresponds to effluent concentration and duration with respect to their control (Fig. 3). The maximum inhibition of 34.12% at 100% effluent concentration after 6 days treatment was recorded.

Similarly protein content declined in treated plants as compared to control (Fig. 4). The maximum reduction of 23.24 % was noticed at 100% effluent after 6 days.

Anatomical studies

After 2nd day of treatment with 100% effluent concentration the plants became fragile and their old leaves showed softening of tissues as compared to control plant. Plants turned yellowish brown in colour from green.

The cell size of epidermis and cortical region in the root of treated plant decreased in comparison with the unstressed plant. Vascular bundles were also reduced (Fig.5 a-b).Fig. 6 a-b showed the cross section view of rhizome of control and treated plants. On comparing with control, the treated rhizome showed a reduced and distorted epidermal, cortex and vascular bundle regions. The upper surface view of leaf showed that the cell alongwith trichomes were deformed (Fig-7 a,b).

Table 1. Physico-chemical properties of tannery effluent collected from Ganga Pollution Control Board, Jajmau, Kanpur

Parameters	Ganga Pollution Control Board, Jajmau, Kanpur (Site II)	
	Untreated	**Treated**
Colour	Black	Grey
pH	9.0±0.49	8.4±0.39
Odour	Pungent	Slight pungent
Temperature (^oC)	26±2.4	24±2.2
EC (dSm^{-1})	14.30±0.47	12.02±0.33
TDS	8860±79.3	4215±61.4
TS	12930±31.2	4640±15.6
TSS	4070±19.4	425±8.6
BOD	1275±12.9	478±12.1
COD	3187±17.6	1369±19.3
Nitrate	67.2±5.9	58.6±7.2
Na	129.7±7.1	86.40±1.3
K	276±9.7	217±8.2
Cr	8.2±0.74	2.36±0.78

All the values are in mg l^{-1} except colour, pH, odour and temperature.**All the values are mean of triplicate ±S.D.

Fig. 1. Chromium accumulation (mg g^{-1} dw) in roots of *P.stratiotes* at different concentrations and durations. All the values are mean of triplicate +SD. *LSD (P<0.01) as compared to control.

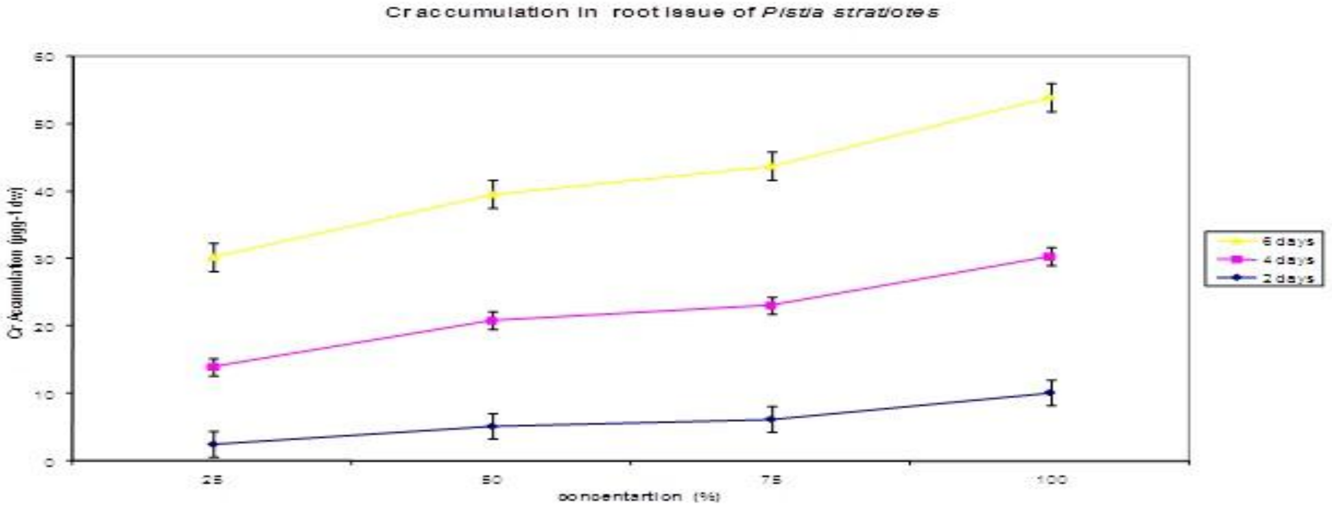

Fig. 2. Chromium accumulation (mg g^{-1} dw) in shoot of *P.stratiotes* at different concentrations and durations. All the values are mean of triplicate +SD. *LSD (P<0.01) as compared to control.

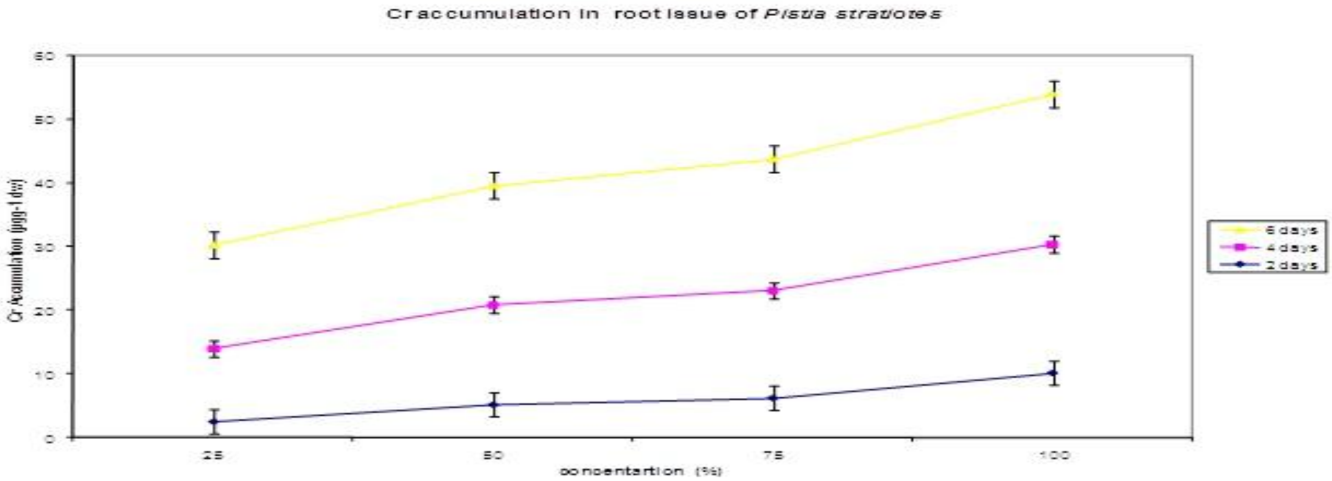

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Fig. 3.Effect of various concentrations and durations of tannery effluent on total chlorophyll in *P.stratiotes*. All the values are mean of triplicate +SD. *LSD (P<0.01) as compared to control.

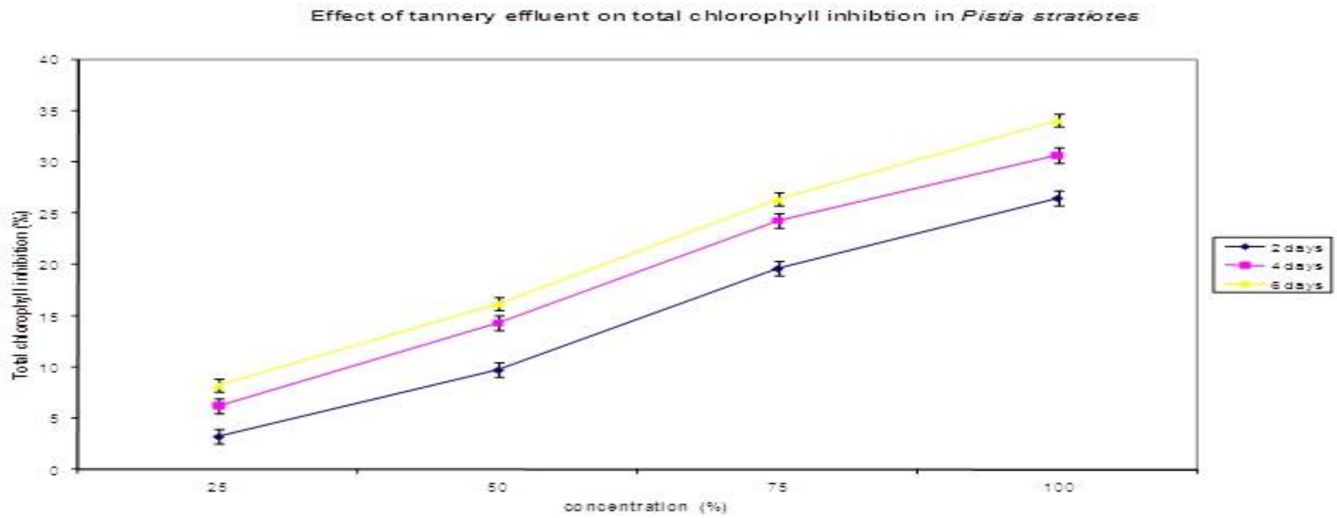


Fig. 4. Effect of various concentrations and durations of tannery effluent on protein content in *P.stratiotes*. All the values are mean of triplicate +SD. *LSD (P<0.01) as compared to control.

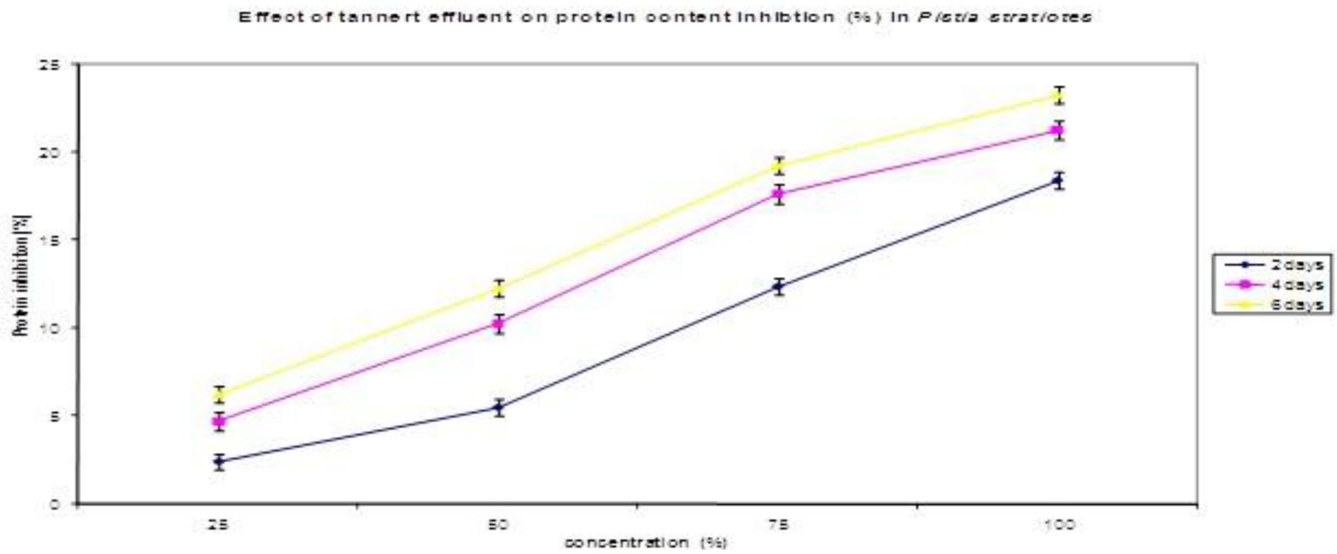


Fig. 5. Cross-sections (10X) of root of control (A) and treated plants of *P.stratiotes* (B);; C = cortex; VB = vascular bundles

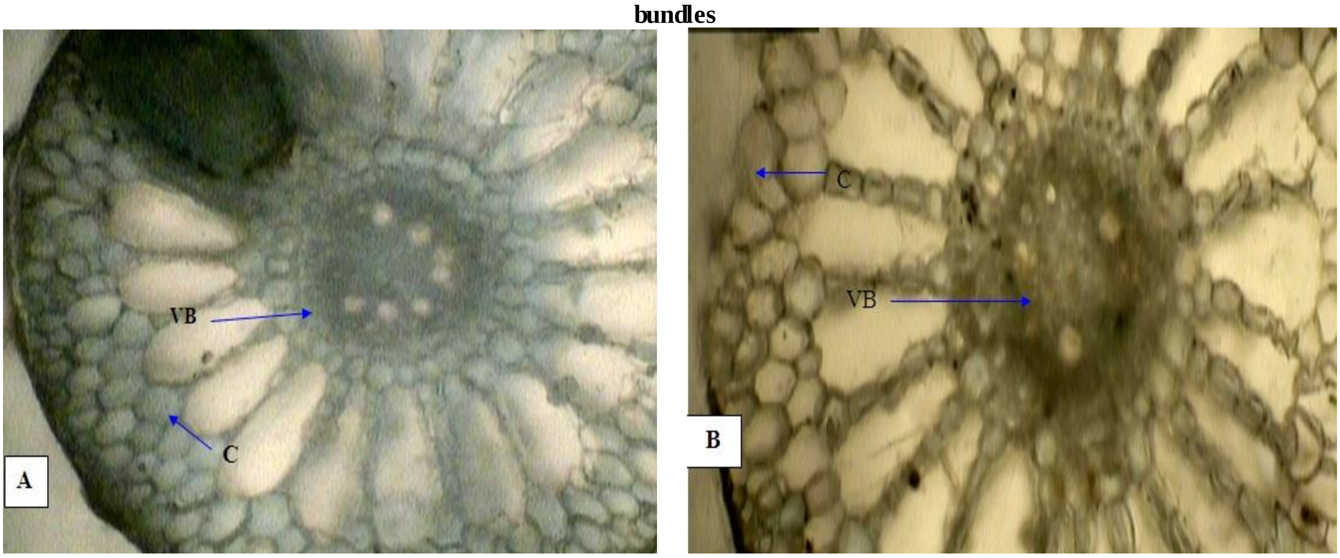


Fig. 6. Cross-sections (10 X) of rhizome of control (A) and treated (B), plants of *P.stratiotes*. VB = Vascular bundles, C= cortex;

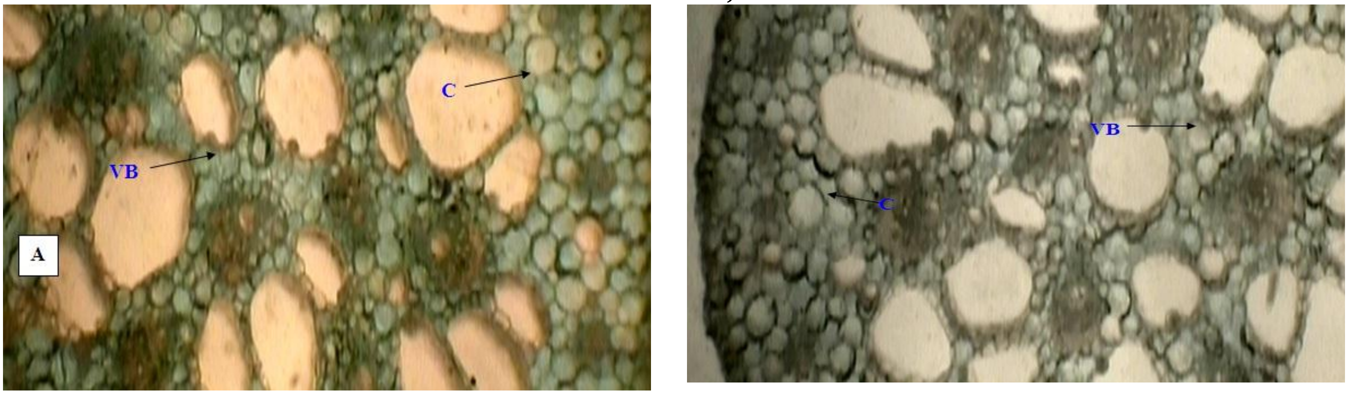
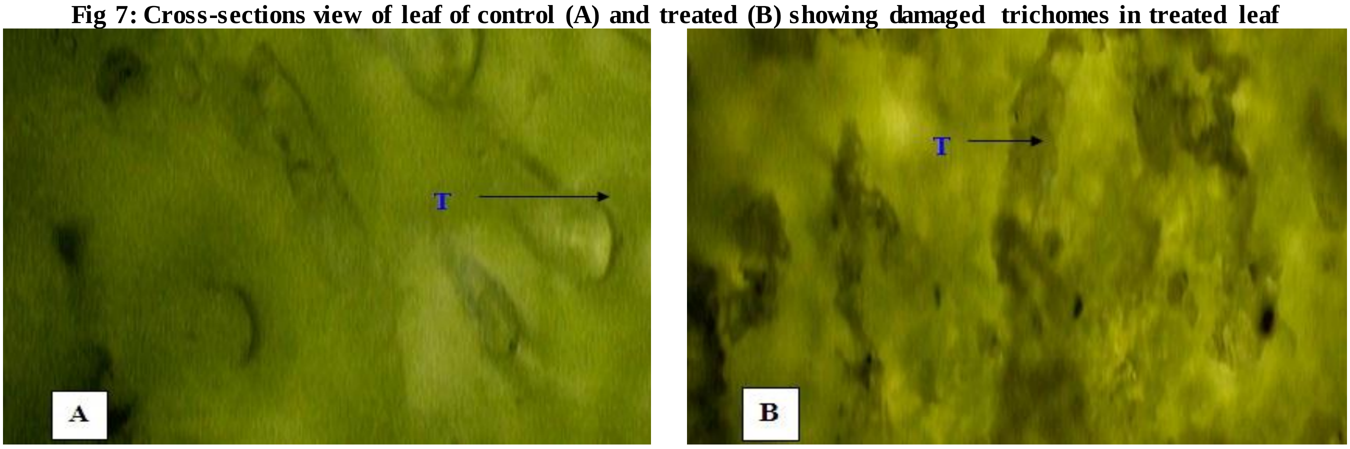


Fig 7: Cross-sections view of leaf of control (A) and treated (B) showing damaged trichomes in treated leaf



DISCUSSION

Table: 1 exhibited that 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.

Root and shoot both tissue bioconcentrate substantial amount of metal however metal concentration was significantly ($p \leq 0.05$) higher in roots than in leaves. Present result is well collaborated with other authors who studied free floating macrophytes [19]. Earlier various studies have shown that the Cr is largely retained in the roots of *P. stratiotes* [20]. The oxidation state of the Cr, pH and presence of humic substances affect the plant uptake and transport.

Cr toxicity affects photosynthesis by causing distortion of chloroplast ultra structure, 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 [21, 22].Similarly protein inhibition may 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 Gupta *et al* [23].

Anatomical variation in the various tissue of the plant could be due to ill effect of tannery effluent and are well collaborated with earlier reports on anatomical variations in aquatic plants exposed to pollutants [7].

CONCLUSION

Aquatic plants vary in their ability to accumulate metal in their tissues. Having promising metal accumulation potential advocated plant's practical utility in phytoremediation of effluent. So the plant may be used as bioaccumulators because of their efficiency in metal absorption and as bioindicator of chromium pollution due to their specific anatomical as well as biochemical responses to the pollutant.

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