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MODULATION OF ANTIOXIDANT DEFENCE SYSTEM FOR DETOXIFICATION OF OXIDATIVE STRESS CAUSED BY TANNERY EFFLUENT IN *EICHHORNIA CRASSIPES*

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Abstract

A study was performed to evaluate capability of aquatic macrophyte *Eichhornia crassipes* plants to combat oxidative stress caused by tannery effluent treatment. For this purpose, tannery effluent was collected from "Up flow Anaerobic Sludge Blanket" (UASB) Jajmau, Kanpur. Plants of *Eichhornia crassipes* were exposed to various concentrations of tannery effluent (0.0, 25, 50, 75 and 100%) for 2 and 7 days durations. Plants accumulated significant ($p<0.01$) amount of Cr (a major constituent of tannery effluent) in a concentration duration dependent manner; which was more in roots (220 mg g^{-1} dw) than in leaves (83 mg g^{-1} dw). Tannery effluent induced oxidative stress was evident by increased level of MDA content. To combat oxidative stress plants showed alleviated level of antioxidants as its defense mechanism. Among enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity increased over to control upto 2^{nd} days of treatment however on exposure of long duration i.e. 7 days these antioxidants declined in correspond to various treatments. Similarly non enzymatic antioxidants (carotenoid and ascorbic acid content) which scavenged free radicals efficiently also showing elevation at all concentrations up to 2 days exposure over unstressed plants, thereafter responded in decreasing manner due to ill effects of treatments. Therefore it can be concluded, *E. crassipes* can be utilized as a biomonitoring as well as phytoremedial tool using aforesaid biomarkers for pollution caused by tannery effluent.

Keywords: Tannery effluent, Oxidative stress, Antioxidants, Biomarkers, *Eichhornia crassipes.*

Introduction

Indiscriminate discharge of untreated or partially treated waste water directly or indirectly into aquatic bodies may render water resources unwholesome and hazardous to man and other living systems (Bakare *et al*., 2009; Olorunfeme *et al*., 2010. Tannery effluents are ranked as the highest pollutants among all industrial wastes. India is the third largest producer of leather in the world having about 3000 tanneries with annual processing capacity

of 0.7 million tonnes of hides and skin. In Uttar Pradesh (India) Jajmau, Kanpur is a major industrial town (about 400 tanneries) located on the bank of river Ganga which specialized in processing hide into heavy leather (Sinha *et al*., 2008; Gupta *et al*., 2010). Nowadays, majority of tanning industries favour chrome tanning for processing leather. Unfortunately only fraction of chromium (Cr) is utilized in tanning process and rest is discharged as byproduct of wastewater treatment (Hafez *et al.,* 2002). Therefore the treated wastewater discharged from tanning industries contains high level of BOD, COD, electrical conductivity and heavy metals especially Cr above permissible limit as recommended by various regulatory agencies making it potentially toxic (Lal, 2009. Usually tanning industries discharge their wastewater into nearby rivers and indirectly is being used for irrigation of crops and vegetables. This practices has ultimately led to movement of potentially toxic metals from water to plant system and ultimately to human beings (Sinha et al., 2008). It is well known that Cr (VI) is a potent carcinogen to humans and animals as it enters cells via surface transport system and gets reduced to Cr (III) inducing genotoxicity (Matsumoto *et al*, 2006. Thus Cr loaded effluent used for irrigation disrupts several physiological and cytological processes in cells (Shanker *et al*., 2005; Chidambaram *et al*., 2009) leading to reduced root growth, biomass, seed germination, early seedling development (Irfan and Akinici, 2010, and induces chlorosis, photosynthetic impairment and finally plant death (Seoccianti *et al* .,2005; Akini and Akini, 2010. In plants, like other heavy metals Cr induces phytotoxicity because of the induction of free radical (FR) and reactive oxygen species (ROS) which reacts with lipids, proteins, photosynthetic pigments and nucleic acids, causing lipid peroxidation, membrane damage, metabolite degradation, inactivation of enzymes and finally leading to cell death (Heath and Packer, 1968; Piotrowska et al., 2009. To scavenge these highly reactive molecules, plants possess a defense mechanism like antioxidants that may be of enzymatic or non-enzymatic nature (Appel et al., 2004). These antioxidants have recently been used as principal biomarkers that offer more complete and biologically more relevant information on the potential impact of toxic pollutants on the health of organisms (Ferrat et al., 2003).

In recent years, aquatic plants have been frequently used for biomonitoring of various water pollutants (Dhote and Dixit, 2009). *Eichhornia crassipes*, a free-floating aquatic plant has been widely studied due to its tendency to bioconcentrate and biomagnify heavy metal contaminants present in water bodies (Xia and Ma, 2006). The plant possesses a well-developed root system and a good metal tolerating capacity (Dhote and Dixit, 2009. It has been found growing luxuriantly in water bodies containing moderate level of heavy metals like Cr, Pb, Zn, Cu etc. In this context, the present study was undertaken to evaluate the effect of Cr biosorption, lipid peroxidation and understand the biochemical detoxification strategies adopted by *E. crassipes* against oxidative stress by Cr.

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. During study period two times effluent sample was collected in two numbers of polyethylene containers of 5 litres each. 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. Plants of *Eichhornia crassipes* (approximately 100 g fresh weight) were 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 2^{nd} and 7^{th} days. The harvested plants were washed thoroughly with distilled water, oven dried (80^oC) 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.

Roots and leaves were dried at 80^oC and digested in mixture of HNO_3 : $HClO_4$ (3:1 ratio) using Microwave Digestion System MDS 2000 and Cr content was estimated by GBC Avanta Σ Atomic Absorption Spectrophotometer using air acetylene gases at 357.9 nm wavelength.

The level of lipid peroxidation was measured following the Heath and Packer (1968). Leaves of the control and treated plants were homogenized in 3 ml of 100 mM of EDTA in presence of polyvinyl polypyrrolidone (PVP). The homogenate was centrifuged at 12,000 g for 15 min. at 4^oC. All steps in the preparation of enzyme extract were carried out at 0-4^oC. This supernatant was used to measure the activities of superoxide dismutase, catalase and peroxidase.

The activity of SOD was measured by the method of Nishikimi and Rao (1972) and Catalase was estimated in the plant parts following the method of Aebi (1984) while Peroxidase was measured following the method of Curtis (1971), modified by Kato and Shimizu (1987).

Antioxidants Carotenoids were calculated by following the methods of Duxbury and Yentsch (1956) and Ascorbic acid content was estimated by the method of Keller and Schwager (1977).

Statistical analysis

To confirm the variability of data obtained and validity of results, all the data were subjected for the statistical analyses using Two Way Analysis of Variance (ANOVA) and least significant difference (LSD) (Gomez and Gomez 1984).

Results

Physico-chemical analysis (as in Table 1) revealed characteristics of the tannery effluent. The effluent was dark brown in colour having high BOD (494 mgl-1) and COD (1382). It was slightly alkaline (pH 8.5) in nature. The effluent was contaminated with high concentrations of Cr (2.32 mgl^{-1}) along with other metals (table-1). Physico-chemical analysis of effluent showed its toxic nature due to high BOD, COD low dissolved oxygen (DO) and presence of heavy metals.

E. crassipes accumulated Cr in a dose and exposure dependent manner (Fig. 1a, b). Plants exposed to 100% tannery effluent accumulated highest amount of Cr after 7 days of treatment. The perusal of data indicated that Cr accumulation was more in roots than in

leaves of *E. crassipes*. The maximum accumulation of 220 and 83 μg g^{-1} dw Cr was found in roots and leaves of *E. crassipes* treated with 100% tannery effluent after 7 days.

MDA content, a phytotoxic product of lipid peroxidation increased gradually in proportion to increased Cr concentration as compared with unstressed plants (Fig- 2). The highest stimulation of MDA level (126.04 %, $p<0.01$) was recorded in the leaves of *E. crassipes* exposed to 100% tannery effluent after 7 days treatment.

Super oxide dismutase (SOD) activity increased substantially upto 50% tannery effluent after 7 days in comparison to unstressed plants (Fig. 3). Maximum enhancement of 137.5 was observed at 100% tannery effluent after 2 days. However, declined significantly ($P<0.01$) at higher concentration and duration in comparison to unstressed plants.

Compared to unstressed plants, catalase activity enhanced upto 100% treatment for 2 days exposure and decreased at higher concentration and duration. The maximum inhibition of 77.84 % was recorded at 100% concentration after 7days exposure treatment (Fig. 4).

POD activity also showed similar trend as SOD at same concentration and duration. The maximum induction of 121.31 % was observed at 100% tannery effluent treatment after 2 days exposure. Further a significant ($p<0.01$) decrease was recorded at higher concentration and duration (Fig.5).

Among non-enzymatic antioxidants, total carotenoid and ascorbic acid content increased significantly ($p<0.01$) at all concentration and duration with respect to their control values (Fig. 6 a, b).

It showed that lower doses had stimulatory effect on the activity of plants. On the other hand higher concentration and duration of tannery effluent had inhibitory effect in dose-duration dependent manner over to control.

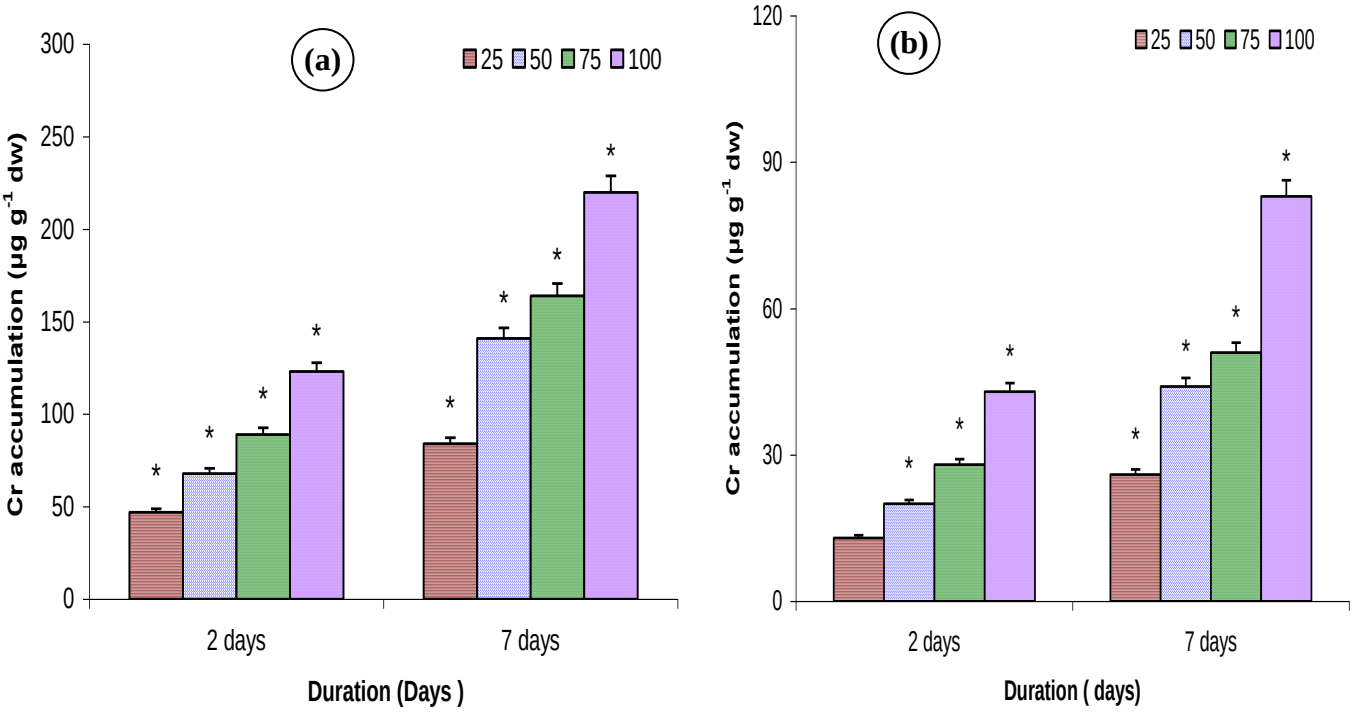
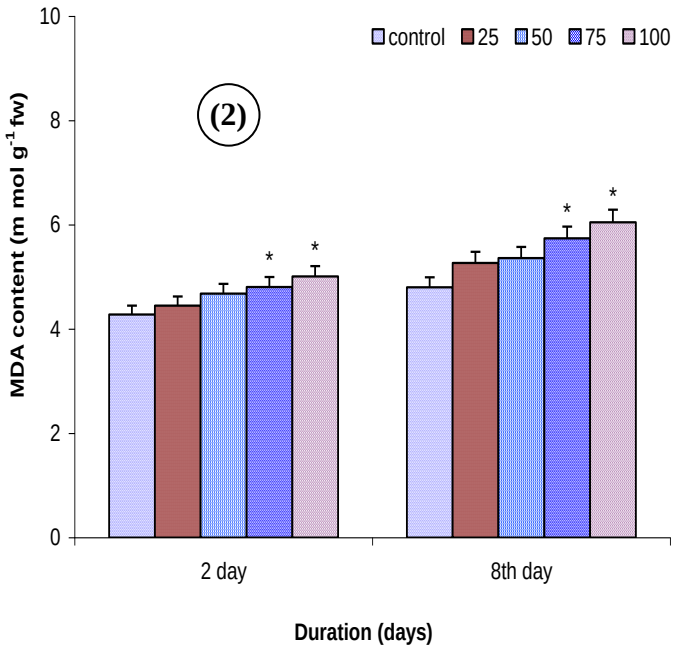


Figure 1a-b. **Accumulation of Cr in (a) roots and (b) leaves of *E. crassipes* at various concentrations and durations of tannery effluent. All the values are mean of triplicate ±SD. *LSD (P<0.05).**



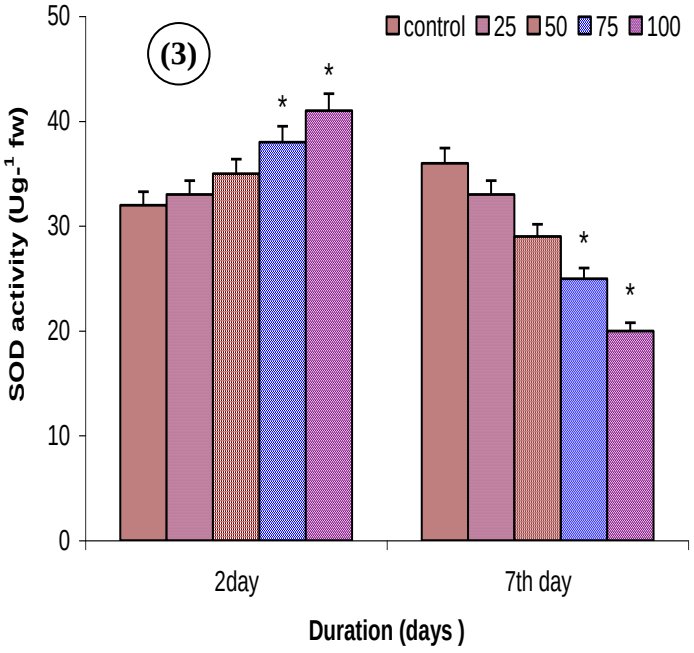
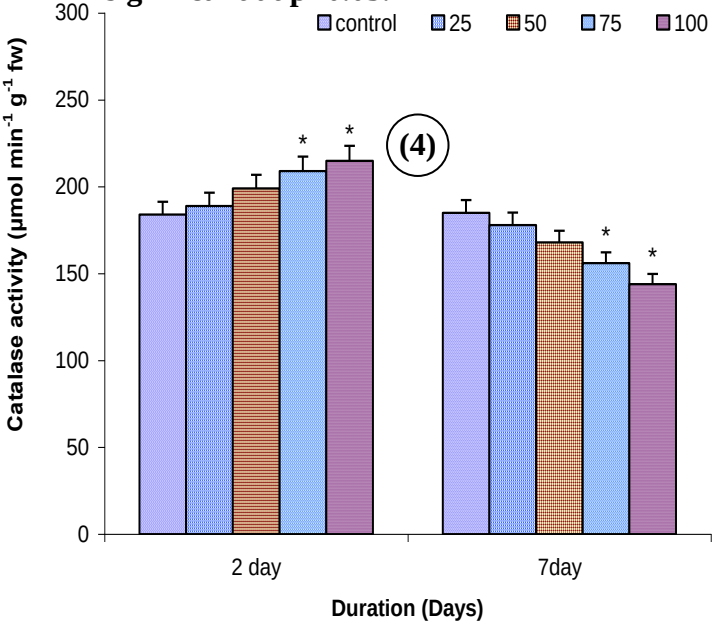


Figure 2. **Effect of tannery effluent on MDA content in *E. crassipes* at different durations (days). All the values are mean of triplicate ±SD. *LSD (P<0.05).**

Figure 3. **Effect of various concentrations of tannery effluent on SOD activity in *E. crassipes* at different durations (days). Values are mean of three replicates ± SD. *LSD significant at p<0.05.**



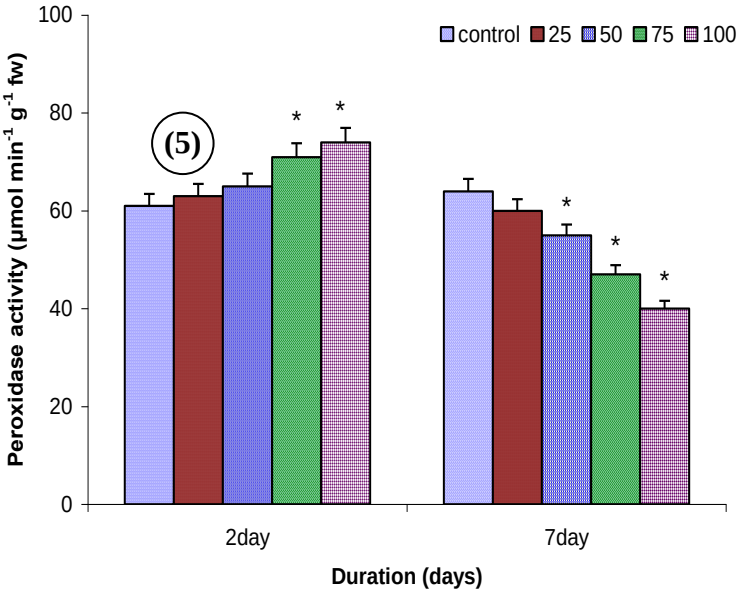


Figure 4. **Effect of various concentrations of tannery effluent on catalase activity in *E. crassipes* at different durations (days). Values are mean of three replicates ± SD. *LSD significant at p< 0.05.**

Figure 5. **Effect of various concentrations of tannery effluent on peroxidase activity in *E. crassipes* at different durations (days). Values are mean of three replicates ± SD. *LSD significant at p< 0.05.**

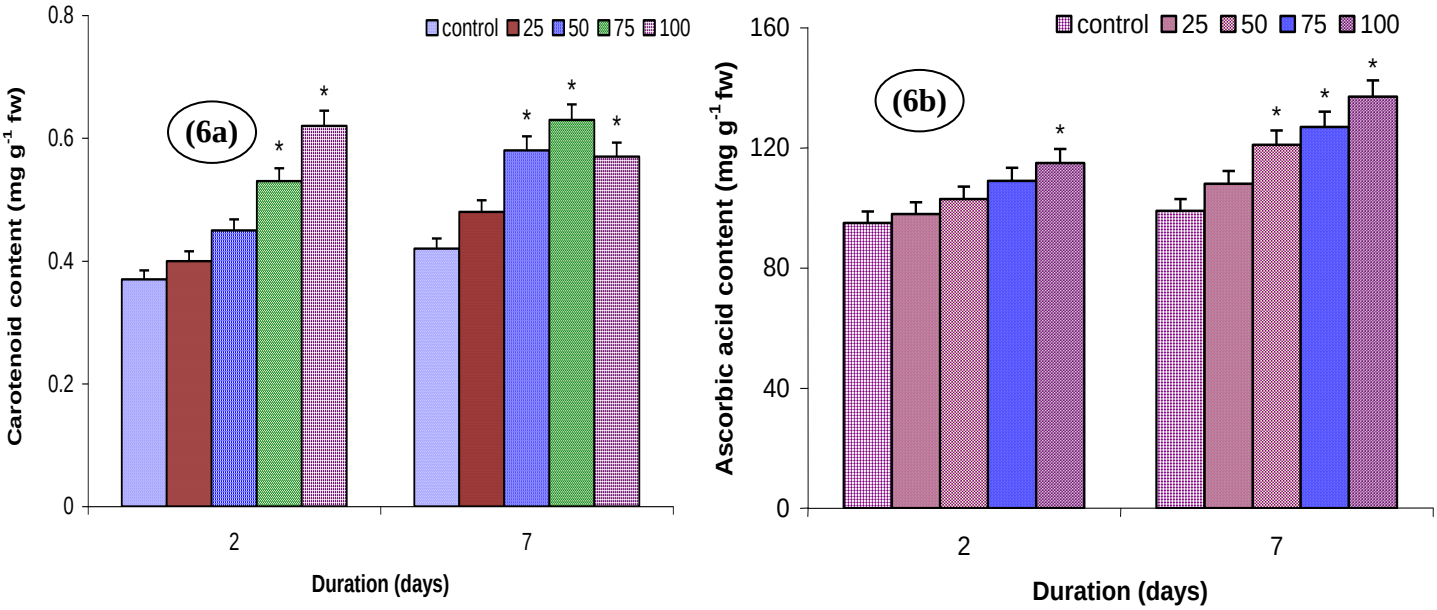


Figure 6a. **Effect of various concentrations of tannery effluent on carotenoid content in *E. crassipes* at different durations (days). Values are mean of three replicates ± SD. *LSD significant at p< 0.05.**

Figure 6b. **Effect of various concentrations of tannery effluent on ascorbic acid content in *E. crassipes* at different durations (days). Values are mean of three replicates ± SD. *LSD significant at p< 0.05.**

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

Parameters	Treated
Colour	Grey
pH	8.5±0.39
Odour	Slight pungent
Temperature (^oC)	25±2.2
EC (dSm^{-1})	12.05±0.33
TDS	4156±61.4
TS	4523±15.6
TSS	367±8.6
BOD	494±12.1
COD	1382±19.3
Nitrate	53.2±7.2
Na	78.4±1.3
K	212±8.2
Cr	2.32±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.

Discussion

It is well known that bioaccumulation of heavy metals in plants is often accompanied by induction of a variety of intracellular changes, some of which directly contribute to the metal tolerance capacity of plants (Hall, 2002). In the present study, Cr accumulation in the root and leaves of *E. crassipes* increased in a dose-duration dependent manner. Regardless of the treatment, the Cr accumulation was higher in roots than in leaves, implying that a considerable amount of Cr was retained in roots leading to less translocation to the aerial parts (Pratap et al., 2006). Roots act as a barrier against heavy metal translocation and this may be a potential tolerance mechanism operating in the roots (Singh et al, 2006). Poor translocation of metals to the shoots could be due to sequestration of metals in the vacuoles of the root cells to render it nontoxic to plants (8). Cr accumulation in *E. crassipes* in the present study is in agreement with earlier reports on aquatic plants (Piotrowska et al, 2009; Aslan et al, 2003).

Heavy metals induce oxidative stress by generating free radical and toxic reactive oxygen species (ROS) (Gupta, et al, 2011). Membrane lipids are proteins which are especially prone to attack by free radicals. Lipid peroxidation affects the function and integrity of the membrane and can produce irreversible damage to the cellular machinery (Gupta et al, 2011; Choudhary et al, 2007). In the present study a significant increase in MDA level was observed in stress plants as compared to unstressed plants.

Among various enzymatic enzymes involved in the abolishment of ROS, SOD plays a pivotal role, as it is a first line of defense against ROS. It reduces the oxidative stress by dismutation of two superoxide radicals to H_2O_2 and O_2 (I. Cakmak, 2000). An increase in SOD activity in response to stress may be due to the induction of genes of SOD by super oxides mediated signal transductions which causes synthesis of enzymes proteins (S. Verma and R.S Dubey, 2003). However, the reduction of SOD activity at higher Cr concentrations is probably due to enhanced level of H_2O_2 and its derivatives ROS (Mishra et al, 2006).

CAT present in peroxisomes and mitochondria decomposes H_2O_2 to water and oxygen. In the present study CAT did not participate in active H_2O_2 reduction as it reduced at lower concentration of 1 $\mu g\ ml^{-1}$ Cr solution after 24 h which has been reported previously by several workers (Mishra et al, 2006; Shankar et al, 2005). This decline in CAT activity might be due to inactivation of enzyme by free radicals, decrease in synthesis of enzyme or change in its subunits.

POD consumes H_2O_2 to generate phenoxy compounds that are polymerized to produce cell wall components such as lignin (Singh et al, 2006; Gupta et al, 2011). Decrease in POD activity at higher concentration as reported by several authors alongwith decreased activity of CAT may be due to accumulation of H_2O_2 to toxic levels causing oxidative stress in plants (Mishra et al, 2006;).

Among the non-enzymatic antioxidants, low molecular mass scavengers include carotenoids, ascorbic acid glutathione that efficiently remove these highly reactive molecules. Increased carotenoid content as observed in the present study is probably a part of strategy adopted by the plants to counteract the toxic effect of free radicals generated under stress conditions (Aslan, et al, 2003).

Stimulation in the ascorbic acid level in response to heavy metals suggests its role in ROS detoxification generated by stress. Ascorbic acid is known to operate as an antioxidant

either in direct chemical interaction with free oxyradicals or during the reaction catalyzed by ascorbate peroxidase (Piotrowska et al, 2009). Ascorbic acid induces resistance by protecting labile macromolecules against attack by ROS (Galli et al., 1996).

Summarizing our results, it is observed that enhanced antioxidant level especially non-enzymatic antioxidants in stressed plants of *E. crassipes* may account for its better survival in moderately Cr contaminated water bodies while high doses of tannery effluent decreased antioxidant activities dramatically showing reduced metal tolerance capacity and overall inhibition of plant growth.

Conclusion

From the present study it can be concluded that Cr accumulation in *E. crassipes* caused oxidative stress as evidenced by increased TBARS level. In order to cope with tannery effluent toxicity, *E. crassipes* develops a cellular defense mechanisms comprising of antioxidants that are activated in due course and can be regarded as stress biomarker. These biomarkers represent the overall health of the organism in toxic nature of the medium. However, this increase in antioxidant activities was not strong enough to eliminate deleterious effects caused by high level of tannery effluent. Therefore, *E. crassipes* can be utilized as a bioassay for phytotoxicity as well as bioindicator of polluted water bodies.

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