SENRA Academic Publishers, Burnaby, British Columbia
Vol. 2, No. 3, pp. 537-545, 2008
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

ROLE OF ANTIOXIDATIVE DEFENCE SYSTEM IN *EICHHORNIA CRASSIPES* (MART.) SOLMS DURING PHYTOREMEDIATION OF MERCURY

Upma Narang[1], *AK Thukral[1], Renu Bhardwaj[1] and SK Garg[2]
[1]Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar- 143005
[2]Department of Molecular Biology & Biochemistry, Guru Nanak Dev University, Amritsar- 143005, India

ABSTRACT

Mercury is rated third after heavy metals like arsenic and lead in the top ten of the priority list as provided by the American Agency for Toxic Substances and Disease Registry. The clean up of Hg requires the removal of this metal. Since Hg is toxic at very low concentrations, its removal from water bodies is not possible with chemical treatment. Phytoremediation can prove to be most effective eco-friendly technique for removal of this heavy metal. *Eichhornia crassipes* (Mart.) Solms is being used successfully for the phytoremediation of trace elements in natural and constructed wetlands. The present investigation was undertaken to study the role of antioxidative system of *E. crassipes* cultured in solutions containing mercuric chloride. It was found from the results of present work that malondialdehyde content of the plants increased up to 3.7-fold thereby indicating a high level of Hg stress. It was observed that the plants counteracted this stress by stimulation of the antioxidative enzymes *viz*. superoxide dismutase (SOD), guaiacol peroxidase (GPX) and catalase (CAT) and molecular antioxidant vitamin E. The activities of these enzymes and antioxidant increased up to 0.8, 3.9, 24.6 and 3.7-fold respectively under Hg stress.

Keywords: *Eichhornia crassipes* (Mart.) solms, mercury, oxidative stress, MDA, superoxide dismutase, catalase, guaiacol peroxidase and vitamin E.

INTRODUCTION

The incessant increase in industrial, mining, agricultural and construction activities being undertaken by man have been responsible for the release of potentially hazardous and toxic chemicals in the environment in dangerous proportions. Heavy metals are non-degradable pollutants and undergo bioaccumulation along the food chain, thereby accentuating their toxic effects (Islam *et al*., 2007). Mercury is one of the most toxic heavy metals. It is a pollutant of primary importance because of its global dispersion and direct uptake by plants and animals (Mattingly *et al*., 2001; Ip *et al*., 2004; French *et al*., 1999).

Heavy metals are difficult to remove from the environment, and unlike many other pollutants cannot be chemically or biologically degraded (Mejare and Bulow, 2001). Conventional physico-chemical and electrochemical methods used for the removal of heavy metals from waste waters are not only cost intensive, but can not be used for natural ecosystems on account of there damaging effects on flora and fauna. This limitation of chemical control of pollution warrants the use of a new, easy to apply, cost-effective and eco-friendly green clean technology, phytoremediation (Solis-Dominguez *et al*., 2007). Aquatic plants in fresh water, marine and estuarine systems act as receptacles for several metals (Prasad and Freitas, 2003). The use of rooted plants in aquatic habitats is promising as they accumulate pollutants from water, sediments and soil (Sinha, 1999). *Eichhornia crassipes* is a well known aquatic phytoremediator. Its capacity to accumulate various heavy metals like Pb, Ni, Cr, Hg, Cd, Zn and As is well documented (Riddle *et al*., 2002; Lu *et al*., 2004; Jayaweera *et al*., 2007)

The use of higher plants in phytoextraction of heavy metals from polluted environment is based not only on their ability to uptake, translocate and accumulate metals, but also on their ability to alleviate toxicity. The toxic effects of heavy metals leading to the impairment of the metabolic functions related with energy metabolism are generally accompanied by the oxidative damage of the cells and tissues due to the accumulation of reactive oxygen species (ROS), superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^{\cdot}$) and singlet oxygen ($^1O_2$) (Stohs and Bagchi, 1995; Shaw and Rout, 1998). ROS possess strong oxidizing activities and can attack all types of biomolecules (Mithofer *et al*., 2004). Plants exposed to heavy metals evoke a vast array of defense mechanisms for stress avoidance such as, immobilization, exclusion, chelation and compart-mentalization of heavy metal ions, expression of stress proteins and activation of ethylene response to stress (Cobbett, 2000). Stress tolerance in plants is a result of the enhancement of antioxidative defense system (Arora

*Corresponding author email: akthukral@rediffmail.com

Table 1. MDA content (μmoles g^{-1} FW; Mean ± S.E) in roots, petioles and leaf laminae of *E. crassipes* with different concentrations of mercuric chloride.

Conc. of Hg	Roots	Petioles	Leaf laminae
Initial values	0.280 ± 0.048	0.826 ± 0.076	1.935 ± 0.137
After 7 days			
Control	0.323 ± 0.003	0.833 ± 0.002	1.935 ± 0.001
1 μg l^{-1}	0.497 ± 0.004	1.532 ± 0.003	2.117 ± 0.004
10 μg l^{-1}	0.713 ± 0.003	1.75 ± 0.004	2.801 ± 0.004
100 μg l^{-1}	0.899 ± 0.003	2.108 ± 0.003	3.356 ± 0.004
1000 μg l^{-1}	0.956 ± 0.003	2.411 ± 0.003	3.951 ± 0.001
F – Ratio (HSD)	6399.64* (0.015)	43293.31* (0.013)	76396.01* (0.014)
After 14 days			
Control	0.410 ± 0.005	0.833 ± 0.003	2.480 ± 0.004
1 μg l^{-1}	0.701 ± 0.003	1.997 ± 0.003	2.894 ± 0.001
10 μg l^{-1}	0.998 ± 0.003	2.402 ± 0.004	3.806 ± 0.002
100 μg l^{-1}	1.532 ± 0.003	3.265 ± 0.002	4.149 ± 0.003
1000 μg l^{-1}	1.844 ± 0.003	3.738 ± 0.003	4.344 ± 0.001
F – Ratio (HSD)	26424.53* (0.017)	138450.1* (0.014)	96964.79* (0.012)

Table 2. SOD activity (units mg^{-1} protein; Mean ± S.E) in roots, petioles and leaf laminae of *E. crassipes* with different concentrations of mercuric chloride.

Conc. of Hg	Roots	Petioles	Leaf laminae
Initial values	279.5 ± 5.397	176.7 ± 2.296	55.18 ± 2.503
After 7 days			
Control	248.5 ± 6.048	163.0 ± 8.684	51.13 ± 0.277
1 μg l^{-1}	293.9 ± 6.70	203.3 ± 4.73	81.19 ± 0.767
10 μg l^{-1}	309.6 ± 5.52	211.6 ± 9.16	80.55 ± 0.427
100 μg l^{-1}	326.3 ± 3.84	220.6 ± 5.39	84.05 ± 0.510
1000 μg l^{-1}	335.0 ± 3.11	213.8 ± 6.64	88.85 ± 0.456
F – Ratio (HSD)	42.78* (24.28)	10.27* (33.20)	932.27* (2.27)
After 14 days			
Control	319.2 ± 3.235	237.7 ± 0.350	54.64 ± 4.378
1 μg l^{-1}	389.8 ± 0.533	249.7 ± 1.493	88.82 ± 0.353
10 μg l^{-1}	401.6 ± 0.980	256.6 ± 4.279	92.05 ± 0.421
100 μg l^{-1}	416.8 ± 4.433	267.5 ± 6.274	99.88 ± 2.329
1000 μg l^{-1}	392.7 ± 6.277	257.6 ± 3.201	92.54 ± 1.345
F – Ratio (HSD)	100.56* (17.49)	8.93* (17.24)	59.09* (10.74)

et al., 2002) that comprises of enzymatic components like superoxide dismutase, catalase, peroxidases, glutathione reductase and nonenzymatic components like ascorbic acid, carotenoids, glutathione and tocopherols etc. The present study was planned to test the hypothesis that exposure of *E. crassipes* to Hg would stimulate their antioxidative defence system to counteract heavy metal stress.

MATERIALS AND METHODS

The plants of *E. crassipes* were treated with different concentrations of mercuric chloride (0, 1, 10, 100 and 1000 μg l^{-1}) and different plant parts (roots, petioles and leaf laminae) were harvested after 7 and 14 days of Hg treatment. Lipid peroxidation was measured in terms of malondialdehyde (MDA) content (Heath and Packer, 1968). For the estimation of superoxide dismutase, catalase and guaiacol peroxidase, 1 g of plant tissue was homogenized in pre-chilled mortar and pestle with 3 ml of 100 mM potassium phosphate buffer of (pH 7.0) under ice-cold conditions. The plant extract for vitamin E content estimation was prepared by homogenizing 1 g of fresh plant tissue in pre-chilled mortar and pestle under ice cold conditions in 3 ml of Tris buffer (50 mM, pH 10.0) containing 1 mM EDTA. The homogenate was centrifuged at 12,000 g for 15 minutes and the supernatant was used for the estimation of vitamin E. Superoxide

Table 3. GPX activity (units mg^{-1} protein; Mean ± S.E) in roots, petioles and leaf laminae of *E. crassipes* with different concentrations of mercuric chloride.

Conc. of Hg	Roots	Petioles	Leaf laminae
Initial values	0.145 ± 0.006	0.047 ± 0.005	0.027 ± 0.003
After 7days			
Control	0.044 ± 0.010	0.032 ± 0.003	0.029 ± 0.002
1 µg l^{-1}	0.059 ± 0.005	0.051 ± 0.007	0.027 ± 0.003
10 µg l^{-1}	0.136 ± 0.003	0.070 ± 0.006	0.059 ± 0.002
100 µg l^{-1}	0.195 ± 0.010	0.110 ± 0.002	0.072 ± 0.002
1000 µg l^{-1}	0.193 ± 0.002	0.086 ± 0.003	0.065 ± 0.001
F – Ratio (HSD)	112.75* (0.031)	43.85* (0.021)	90.56* (0.010)
After 14 days			
Control	0.063 ± 0.008	0.039 ± 0.004	0.041 ± 0.004
1 µg l^{-1}	0.084 ± 0.011	0.066 ± 0.009	0.053 ± 0.005
10 µg l^{-1}	0.220 ± 0.011	0.125 ± 0.005	0.106 ± 0.002
100 µg l^{-1}	0.312 ± 0.010	0.188 ± 0.005	0.128 ± 0.002
1000 µg l^{-1}	0.296 ± 0.008	0.154 ± 0.004	0.106 ± 0.002
F – Ratio (HSD)	139.96* (0.046)	125.33* (0.025)	133.09* (0.015)

Table 4. CAT activity (units mg^{-1} protein; Mean ± S.E) in roots, petioles and leaf laminae of *E. crassipes* with different concentrations of mercuric chloride.

Conc. of Hg	Roots	Petioles	Leaf laminae
Initial values	146.8 ± 11.26	72.23 ± 6.851	33.64 ± 2.025
After 7 days			
Control	48.21 ± 9.229	12.75 ± 3.177	9.05 ± 0.700
1 µg l^{-1}	119.9 ± 8.613	133.9 ± 2.475	54.4 ± 0.910
10 µg l^{-1}	188.6 ± 1.811	130.1 ± 2.239	58.92 ± 1.088
100 µg l^{-1}	195.1 ± 4.428	137.9 ± 2.963	64.42 ± 2.643
1000 µg l^{-1}	267.6 ± 2.168	137.5 ± 1.333	84.56 ± 2.887
F – Ratio (HSD)	184.87* (28.43)	470.58* (11.72)	216.29* (8.78)
After 14 days			
Control	56.56 ± 8.163	17.30 ± 3.453	15.57 ± 2.247
1 µg l^{-1}	292.6 ± 7.59	211.5 ± 7.03	136.1 ± 3.44
10 µg l^{-1}	375.6 ± 9.33	235.6 ± 4.24	247.0 ± 2.18
100 µg l^{-1}	379.1 ± 7.06	205.2 ± 6.04	317.9 ± 2.36
1000 µg l^{-1}	376.1 ± 18.5	222.4 ± 4.87	398.9 ± 0.52
F – Ratio (HSD)	159.34* (51.11)	295.34* (24.57)	4139.95* (10.90)

dismutase (SOD, EC 1.15.1.1) was estimated according to the methodology of Kono (1978). Guaiacol peroxidase (GPX, EC 1.11.1.7) was estimated according to the method given by Putter (1974). Catalase (CAT, EC 1.11.1.6) activity was determined as per the method of Aebi (1983) and Vitamin E content was determined after Martinek (1964). The data was subjected to statistical analysis for mean, standard deviation, linear and curvilinear regression and correlation, one-way and two-way analysis of variance (ANOVA) and Tukey's multiple comparison tests.

RESULTS AND DISCUSSION

MDA content in roots, petioles and leaf laminae increased significantly with the increase in Hg concentration (Table 1). The MDA levels in roots, petioles and leaf laminae increased from the initial values of 0.280, 0.826 and 1.935 µmoles g^{-1} FW respectively to maximum values of 1.844, 3.738 and 4.344 µmoles g^{-1} FW respectively at a concentration of 1000 µg l^{-1} on 14th day. The observations made for SOD activity revealed that the enzyme activity increased with the increase in Hg concentration as well as

Table 5. Vitamin E content (mg g^{-1} FW; Mean ± S.E) in roots, petioles and leaf laminae of *E. crassipes* with different concentrations of mercuric chloride.

Conc. of Hg	Roots	Petioles	Leaf laminae
Initial values	0.025 ± 0.002	0.022 ± 0.001	0.042 ± 0.002
After 7 days			
Control	0.019 ± 0.004	0.017 ± 0.001	0.026 ± 0.001
1 µg l^{-1}	0.029 ± 0.003	0.020 ± 0.004	0.047 ± 0.006
10 µg l^{-1}	0.041 ± 0.003	0.026 ± 0.004	0.068 ± 0.004
100 µg l^{-1}	0.056 ± 0.001	0.033 ± 0.003	0.092 ± 0.003
1000 µg l^{-1}	0.053 ± 0.005	0.028 ± 0.002	0.080 ± 0.005
F – Ratio (HSD)	20.96* (0.016)	4.74* (0.014)	35.72* (0.020)
After 14 days			
Control	0.023 ± 0.002	0.016 ± 0.002	0.044 ± 0.003
1 µg l^{-1}	0.032 ± 0.003	0.022 ± 0.004	0.054 ± 0.003
10 µg l^{-1}	0.059 ± 0.004	0.038 ± 0.003	0.083 ± 0.003
100 µg l^{-1}	0.086 ± 0.007	0.059 ± 0.003	0.164 ± 0.007
1000 µg l^{-1}	0.077 ± 0.003	0.053 ± 0.004	0.155 ± 0.007
F – Ratio (HSD)	48.98* (0.018)	35.22* (0.014)	128.25* (0.023)

Table 6. Two-way ANOVA for effect of 7th and 14th days (treatment) and different concentrations (dose) in roots, petioles and laminae of *E. crassipes*.

Source of variation	Roots		Petioles		Leaf laminae	
	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD
MDA Content						
Treatment	36209.22*	0.018	146880.0*	0.016	153499.6*	0.013
Dose	29887.63*		170421.2*		166617.6*	
Treatment x Dose	4530.48*		16229.6*		3504.4*	
SOD Activity						
Treatment	800.89*	22.77	202.95*	28.56	63.78*	8.36
Dose	118.81*		17.21*		188.07*	
Treatment x Dose	6.55*		2.64		4.99	
CAT Activity						
Treatment	551.67*	44.58	675.92*	20.71	15654.7*	10.69
Dose	293.77*		614.89*		3450.0*	
Treatment x Dose	36.70*		42.46*		1720.3*	
GPX Activity						
Treatment	169.87*	0.041	196.37*	0.025	424.85*	0.014
Dose	248.32*		164.36*		219.76*	
Treatment x Dose	14.17*		19.87*		20.08*	
Vitamin E Content						
Treatment	44.53*	0.018	43.57*	0.015	159.35*	0.023
Dose	68.44*		33.30*		150.37*	
Treatment x Dose	5.29*		7.95*		25.26*	

time (Table 2). SOD activity was observed to increase from 279.5, 176.7 and 55.18 units mg^{-1} protein in roots, petioles and leaf laminae respectively on zero day, to 416.8, 267.5 and 99.88 units mg^{-1} protein in roots, petioles and leaf laminae respectively for 100 µg l^{-1} concentration on the 14th day. Table 3 shows the results obtained for GPX activity in different plant parts. The maximum GPX activity was observed for 100 µg l^{-1} on 14th day of treatment as 0.312, 0.188 and 0.128 units mg^{-1} protein in roots, petioles and leaf laminae respectively, whereas the minimum activity was observed on 7th day as 0.059, 0.051 and 0.027 units mg^{-1} protein respectively for 1 µg l^{-1} concentration. CAT activity of control plants decreased significantly from zero days (146.8, 72.23 and

Table 7. Two-way ANOVA for effect of plant parts (treatment) and different concentrations (dose) for 7th and 14th days of treatment.

Source of variation	7th Day		14th Day	
	F-ratio	HSD	F-ratio	HSD
MDA content				
Treatment	602981.2*	0.016	766625.6*	0.016
Dose	98721.6*		214881.5*	
Treatment x Dose	10166.4*		10270.8*	
SOD activity				
Treatment	2702.2*	25.315	10001.8*	17.428
Dose	70.79*		128.9*	
Treatment x Dose	4.92		18.64*	
GPX activity				
Treatment	317.26*	0.025	343.81*	0.030
Dose	206.37*		333.25*	
Treatment x Dose	33.63*		37.72*	
CAT activity				
Treatment	951.6*	20.696	342.4*	37.377
Dose	529.6*		859.2*	
Treatment x Dose	73.34*		58.73*	
Vitamin E content				
Treatment	136.5*	0.019	310.9*	0.021
Dose	58.26*		206.9*	
Treatment x Dose	7.44*		24.72*	

* Significant at 5%.

33.64 units mg^{-1} protein in roots, petioles and leaf laminae respectively) to 14th day (56.56, 17.3 and 15.57 units mg^{-1} protein in roots, petioles and leaf laminae respectively). For Hg exposed plants, the maximum activity in roots (379.1 units mg^{-1} protein) was observed for 100 μg l^{-1} concentration on 14th day. The petioles and leaf laminae achieved the maximum activities of 235.6 units mg^{-1} protein for 10 μg l^{-1} and 398.9 units mg^{-1} protein for 1000 μg l^{-1} on 14th day (Table 4). The vitamin E content in roots was observed to increase from an initial value of 0.025 mg g^{-1} FW to 0.086 mg g^{-1} FW (100 μg l^{-1}) over the 14 days of culturing. In petioles and leaf laminae also, the vitamin E content increased from 0.022 and 0.042 mg g^{-1} FW to 0.059 and 0.164 mg g^{-1} FW respectively on 14th day of treatment with 100 μg l^{-1} concentration. The two-way ANOVA for comparing MDA content, SOD activity, CAT activity, GPX activity and vitamin E content on 7th and 14th days of treatment (days x concentration) revealed significant differences for all treatments (Table 6). Similarly significant differences were observed in the contents/ activities on 7th and 14th days in different plant parts (plant parts x concentration) in all the treatments (Table 7). The treatment X dose interactions in the two-way ANOVA's clearly indicate dependence of Hg uptake on plant parts and time.

Metal toxicity, might result from the chemical inactivation of enzymes or disruption of structure due to binding of metals to sulphydryl groups in proteins, or from the displacing of an essential element resulting in deficiency effects. In addition, heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, resulting in oxidative stress (Hall, 2002). Heavy metal stress in plants results in the production of reactive oxygen species (ROS) e.g., $^{\cdot}O_2^-$, H_2O_2 and $^{\cdot}OH$, destructively affecting cell structure and metabolism (Weckx and Clijsters, 1997). Production of ROS by autoxidation and Fenton reaction is typical for transition metals such as iron and copper. But exposure of plants to non-redox reactive metals like cadmium and mercury also results in oxidative stress as indicated by lipid peroxidation, H_2O_2 accumulation, and an oxidative burst (Schutzendubel and Polle, 2002). Levels of MDA, a secondary product of lipid peroxidation, indicate the degree of plant oxidative stress (Arora *et al*., 2002). Increase in MDA content due to different stress factors in various plants has been observed by different workers. Singh and Tewari (2003) showed an increase in MDA content of leaf tissue of *B. juncea* with the increase in concentration of cadmium. The roots and leaves of *Sesbania cannabina* plants showing accumulation of heavy metals like Fe, Cu, Mn, Zn, Pb and Ni because of

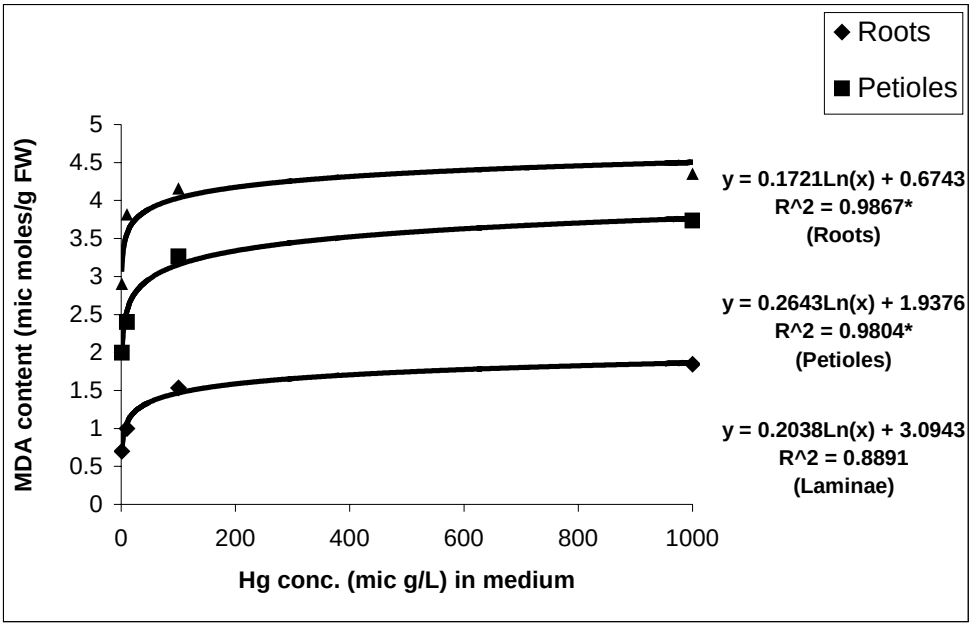

Fig. 1. MDA content (µmoles g^{-1} fresh wt) of different parts of *E. crassipes* plants after 14 days of treatment with mercuric chloride.

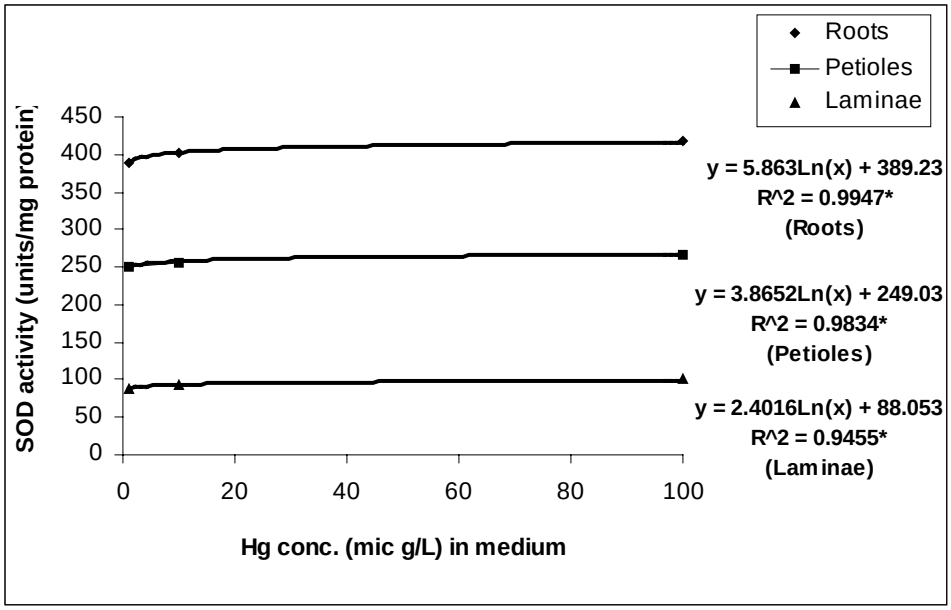

Fig. 2. SOD activity (units mg^{-1} protein) of different parts of *E. crassipes* plants after 14 days of treatment with mercuric chloride.

being grown on fly ash were found to contain enhanced levels of MDA (Sinha and Gupta, 2005). Gajewska and Sklodowska (2007) reported an increase in MDA content in wheat shoots as a result of nickel stress (50 and 100 µM). During present investigation, the MDA content doubled in the leaf laminae at 1000 µg l^{-1}, whereas in petioles and roots the levels were found to be 4.5 to 4.7 fold higher than control values indicating the occurrence of oxidative stress in *E. crassipes* due to Hg treatment (Fig. 1).

An oxidative burst is an intrinsic feature of the plant's response to biotic and abiotic stresses. Adaptations to oxidative stress involve the regulation of the synthesis and repair of proteins and enhance antioxidant protection. Superoxide dismutase catalyses the disproportionation of superoxide ($\cdot O_2^-$) and hydroxyl ($\cdot OH$) radicals to molecular oxygen and H_2O_2. H_2O_2 is degraded into water and molecular oxygen by catalase and peroxidases (De Vos *et al*., 1992). The difference between these two is in their functioning, *i.e.,* whereas catalase operates alone,

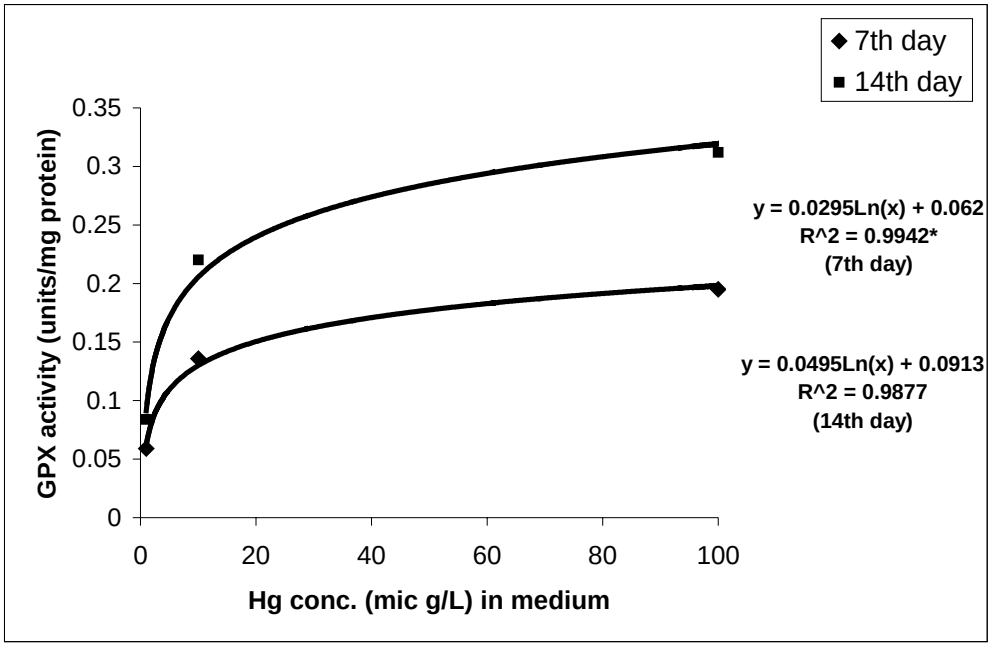

Fig. 3. GPX activity (units mg^{-1} protein) of roots of *E. crassipes* plants treated with mercuric chloride.

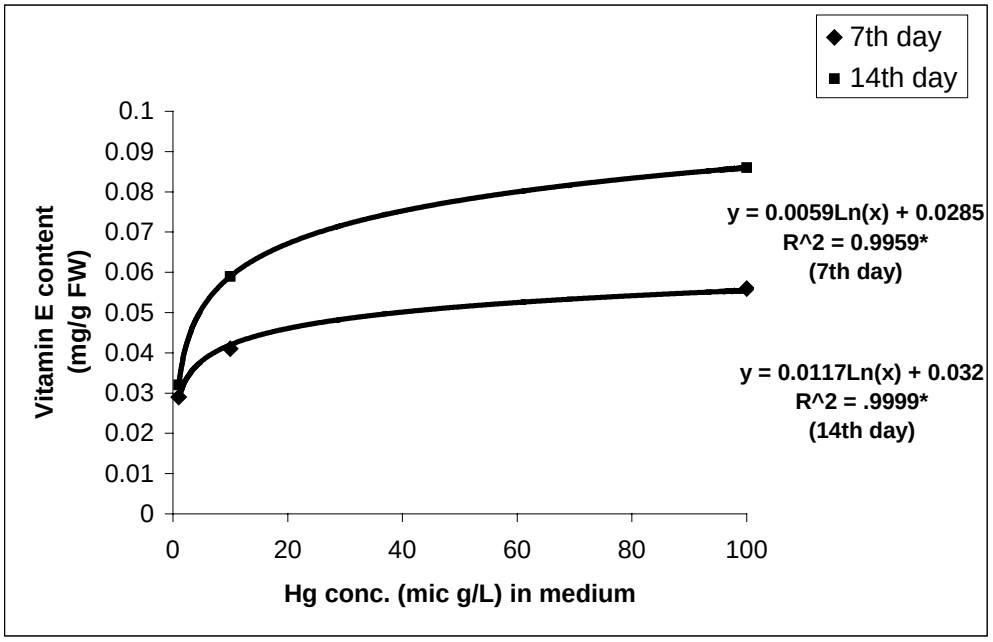

Fig. 4. Vitamin E content (mg g^{-1} fresh wt) of roots of *E. crassipes* plants treated with mercuric chloride.

has low substrate affinity and needs two molecules of H_2O_2 per cycle; peroxidases have a much higher affinity to H_2O_2 and require a reductant like guaiacol. Vitamin E (tocopherols), the membrane associated antioxidant, scavenges singlet oxygen and lipid peroxides. α-tocopherol and ascorbic acid are relatively poor electron donors and act primarily by transfer of single hydrogen atoms and hence, are extremely effective antioxidants (Arora *et al*., 2002).

In the present piece of work, the activities of SOD, CAT and GPX were studied along with the non enzymatic antioxidant vitamin E to evaluate the defense response of plant to Hg stress. Increase in SOD and GPX activity with increase in Hg concentration was observed up to 100 µg l^{-1} concentration beyond which the activity decreased (Figs. 2 and 3). SOD activity increased by 83% in leaf laminae, 38% in roots and 43% in petioles. The GPX activity increased to a maximum by 395, 382 and 212% in roots,

petioles and leaf laminae respectively on 14th day. Increase in SOD activity in leaf laminae is indicative of the enhanced production of superoxide radicals due to Hg exposure. H_2O_2 produced by the disproportionation of superoxide radicals is scavenged by catalase or peroxidase. Regarding the catalase activity, the roots and petioles showed sharp increase in the enzyme activity at low concentrations (up to 10 µg l^{-1}) beyond which the activity remained more or less constant, whereas in leaf laminae the enzyme activity exhibited dose and time dependent enhancement. The activity increased by a maximum of 25.6 times in leaf laminae that was quite high compared to that of petioles (14.6 times) and roots (7.7 times). The enhanced activity of GPX and CAT is indicative of the efficient system for peroxide scavenging inside the plant cells. These observations are in accordance with the results of earlier works done in this direction as Cho and Park (2000) reported 40% enhancement in CAT activity and 20 to 50% increase in activity of SOD due to Hg exposure in tomato roots. Treatment with Zn in *Brassica juncea* (Prasad *et al*., 1999) and *Cajanus cajan* (Rao and Sresty, 2000) has also been found to stimulate SOD and CAT. The enzyme activities have also been reported to increase due to Cd exposure in in *Bacopa monnieri* (Mishra *et al*., 2006), *B. juncea* (Singh and Tewari, 2003) and *Pisum sativum* (Metwally *et al*., 2005). Similarly, Cu toxicity in *Spirodela polyrrhiza* and *Lemna minor* (Tu *et al*., 2006) and *Zea mays* (Tanyolac *et al*., 2007) caused stimulation of various enzymes.

Vitamin E (α-tocopherol) is a membrane-associated antioxidant and scavenges singlet oxygen and lipid peroxides. It can also scavenge the hydroxyl radicals and superoxide radicals. During the 14 days of culturing of plants in Hg, vitamin E exhibited a concentration dependent (up to 100 µg l^{-1}) and time dependent stimulation (Fig. 4) showing a maximum of 3.7 fold enhancement. The results find support from the study of Munne-Bosch *et al*. (1999) who reported 15-fold increase in vitamin E content in response to drought in rosemary leaves. In wheat leaves, 2.4-fold increase in content of α - tocopherol due to drought was observed by Bartoli *et al*. (1999). Dose and time dependent increase in vitamin E content has been reported by Jiang and Zhang (2001) in maize leaves due to ABA treatment. The increase in vitamin E content in the plant cells is indirect evidence of oxidative stress in the plants as it is a potent antioxidant.

It can thus be concluded from the present study that culture of *E. crassipes* in solutions containing Hg results in increase of MDA content in plants causing oxidative stress. This stimulates antioxidative defense system of *E. crassipes* in terms of increased levels of superoxide dismutase, catalase, guaiacol peroxidase and vitamin E to equip the plant for stress tolerance. Controlling the expression of the negative genes and enhancing the antioxidant system by manipulation of the regulatory processes controlling their expression may provide improvement in the bioaccumulation capacity of *E. crassipes*.

REFERENCES

Aebi, HE. 1983. Catalase. In: Methods of Enzymatic Analysis. Eds. Bergmeyer, HU. Verlag Chemie, Weinham. 3:273-282.

Arora, A., Sairam, RK. and Srivastava, GC. 2002. Oxidative stress and antioxidative system in plants. Current Science. 82:1227-1238.

Bartoli, CG., Simontachhi, M., Tambussi, E., Beltrano, J., Montaldi, E. and Puntarulo, S. 1999. Drought and water dependent oxidative effect on antioxidant content in *Triticum aestivum* L. leaves. Journal of Experimental Botany. 50: 375-383.

Cho, UH. and Park, JO. 2000. Hg induced oxidative stress in tomato seedlings. Plant Science. 156:1-9.

Cobbett, CS. 2000. Phytochelatins and their roles in heavy metal detoxification. Plant Physiology. 123:825-832.

De Vos, CHR., Vonk, MJ., Vooijs, R. and Schat, H. 1992. Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus.* Plant Physiology. 98:853-858.

French, KJ., Scruton, DA., Anderson, MR. and Schneider, DC. 1999. Influence of physical and chemical characteristics on mercury in aquatic sediments. Water, Air, Soil Pollut. 110:347-362.

Gajewska, E. and Sklodowska, M. 2007. Relations between tocopherol, chlorophyll and lipid peroxides contents in shoots of Ni- treated wheat. Journal of Plant Physiology. 164:364-366.

Hall, JL. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany. 53:1-11.

Heath, RL. and Packer, L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 125:189-198.

Ip, P., Wong, V., Ho, M., Lee, J. and Wong, W. 2004. Environmental Hg exposure in children: South China's experience. Pediatr Int. 46:715-721.

Islam, E., Yang, XE., He, ZL. and Mahmood, Q. 2007. Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. J Zhejiang Univ Sci B. 8:1-13.

Jayaweera, MW., Kasturiarachchi, JC., Kularatne, RK. and Wijeyekoon, SL. 2007. Contribution of water

hyacinth (*Eichhornia crassipes* (Mart.) Solms) grown under different nutrient conditions to Fe-removal mechanisms in constructed wetlands. J Environ Manage. Mar. 23, [Epub. Ahead of print].

Jiang, M. and Zhang, J. 2001. Effect of abscissic acid on active oxygen species, antioxidative defense system and oxidative damage in leaves of maize seedlings. Plant Cell Physiology. 42:1265-1273.

Kono, Y. 1978. Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. Archives of Biochemistry and Biophysics. 186:189-195.

Lu, X., Kruatrachue, M., Pokethitiyook, P. and Homyok, K. 2004. Removal of cadmium and zinc by water hyacinth, *Eichhornia crassipes*. Science Asia. 30:93-103.

Martinek, RG. 1964. Method for the determination of vitamin E (total tocopherols) in serum. Clinical Chemistry. 10: 1078-1086.

Mattingly, RR., Felczak, A., Chen, CC., McCabe, MJ. Jr. and Rosenspire, AJ. 2001. Low concentrations of inorganic mercury inhibit Ras activation during T cell receptor-mediated signal transduction. Toxicol Appl Pharmacol. 176:162-168.

Mejare, M. and Bulow, L. 2001. Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Trends in Biotechnology. 19:67-73.

Metwally, A., Safronova, VI., Belimov, AA. and Dietz, KJ. 2005. Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. Journal of Experimental Botany. 56:167-178.

Mishra, S., Srivastava, S., Tripathi, RD., Govindarajan, R., Kuriakose, SV. and Prasad, MN. 2006. Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiology and Biochemistry. 44:25-37.

Mithofer, A., Schulze, B. and Boland, W. 2004. Biotic and heavy metal stress response in plants: evidence for common signals. FEBS Letters. 566:1-5.

Munne-Bosch, S., Schwarz, K. and Alegre, L. 1999. Enhanced formation of α-tocopherol and highly oxidized abietane diterpenes in water-stressed rosemary plants. Plant Physiology. 121:1047-1052.

Prasad, KVSK., Paradha Saradhi, P. and Sharmila, P. 1999. Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. Environmental and Experimental Botany. 42:1-10.

Prasad, MNV. and Freitas, HM. 2003. Metal hyperaccumulation in plants – Biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology. 6:1-27.

Putter, J. 1974. Peroxidases. In: Methods of Enzymatic Analysis. Eds. Bergmeyer, HU. 2Verlag Chemie, Weinham. 685-690.

Rao, KVM. and Sresty, TVS. 2000. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to zinc and nickel stresses. Plant Science. 157:113-128.

Riddle, SG., Tran, HH., Dewitt, JG. and Andrews, JC. 2002. Field, laboratory and X-ray absorption spectroscopic studies of mercury accumulation by water hyacinth. Environ. Sci. Technol. 36:1965-1970.

Schutzendubel, A. and Polle, A. 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. Journal of Experimental Botany. 53:1351-1365.

Shaw, BP. and Rout, NP. 1998. Age-dependent responses of *Phaseolus aureus* Roxb. to inorganic salts of mercury and cadmium. Acta Physiol Plant. 20:85-90.

Singh, PK. and Tewari, RK. 2003. Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. Journal of Experimental Biology. 24:107-112.

Sinha, S. 1999. Accumulation of Cu, Cd, Cr, Mn and Pb from artificially contaminated soil by *Bacopa monnieri*. Environment Monitoring and Assessment. 57:253-264.

Sinha, S. and Gupta, AK. 2005. Translocation of metals from fly ash amended soil in the plant *Sesbania cannabina* L. Ritz. effect on antioxidants. Chemosphere. 61:1204-1214.

Solis-Dominguez, FA., Gonzalez-Chavez, MC., Carrillo-Gonzalez, R. and Rodriguez-Vazquez, R. 2007. Accumulation and localization of cadmium in *Echinochloa polystachya* grown within a hydroponic system. J Hazard Mater. 141:630-636.

Stohs, SJ. and Bagchi, D. 1995. Oxidative mechanisms in the toxicity of metal ions. Free Rad Biol Med. 18:321-336.

Tanyolac, D., Ekmekci, Y. and Unalan, S. 2007. Changes in photochemical and antioxidant enzyme activities in maize (*Zea mays* L.) leaves exposed to excess copper. Chemosphere. 67:89-98.

Tu, J., Wang, X., Liu, D. and Li, Z. 2006. Effect of different concentration copper on pigment content and antioxidase system of *Spirodela polyrrhiza* and *Lemna minor*. Ying Yong Sheng Tai Xue Bao. 17:502-506.

Weckx, JEJ. and Clijsters, HMM. 1997. Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. Plant Physiology and Biochemistry. 35:405-410.