Plant Stress ©2008 Global Science Books

Mercury-Induced Lipid Peroxidation and Changes in Antioxidants in *Eichhornia crassipes* (Mart.) Solms

Upma Narang[1] • Renu Bhardwaj[1*] • A. K. Thukral[1] • S. K. Garg[2]

[1] Department of Botanical & Environmental Sciences, Guru Nanak Dev University, Amritsar- 143005, India
[2] Department of Molecular Biology & Biochemistry, Guru Nanak Dev University, Amritsar- 143005, India

Corresponding author: * **renu_bhardwaj@rediffmail.com**

ABSTRACT

Phytoremediators use their internal defence system during phytoremediation leading to environmental restoration. *Eichhornia crassipes* (Mart.) Solms is an efficient remediator of heavy metals and grows luxuriantly in wastewaters especially in tropical climates. The present study was undertaken to determine the oxidative stress caused by Hg in *E. crassipes* and corresponding variations in the antioxidants. We found that malondialdehyde content of the plants increased up to 3.7-fold, thereby indicating a high level of Hg stress. The plants counteracted this stress by stimulation of antioxidant production, viz. ascorbic acid, glutathione and vitamin E. The three antioxidants increased up to 7.9-, 1.14- and 4.2-fold more, respectively than the control.

Keywords: ascorbic acid, glutathione, heavy metals, MDA, mercury, oxidative stress, vitamin E

INTRODUCTION

Phytoremediation is a cost-effective, environment-friendly technology for the reclamation of habitats impregnated with heavy metals (Kramer and Chardonnens 2001). For aquatic systems, phytoremediation, i.e., the use of plants for pollution abatement has come out as a natural extension of the historical use of plants to treat wastewaters (Prasad 2004). Wetland plants are being successfully used for the phytoremediation of heavy metals in natural and constructed wetlands. *Eichhornia crassipes*, a free-floating aquatic macrophyte is a well established phytoremediator (Murugesan and Sukumaran 1997; Kelley *et al*. 2000). Its capacity to accumulate various heavy metals like Pb, Ni, Cr, Hg, Cd, Zn and As is well documented (Muramoto and Oki 1983; Tabbada *et al*. 1990; Riddle *et al*. 2002; Lu *et al*. 2004; Jayaweera *et al*. 2007).

For achieving the best results from this technique, the response of plants to pollution needs to be understood at physiological and biochemical levels in terms of factors that determine resistance and susceptibility of plants to such factors (Arora *et al*. 2002). Under stress conditions, the metabolism of plants is disrupted, with the system responding to altered conditions to minimize stress by activating the antistress machinery of the plants to achieve homeostasis (Hall 2002). When the toxic effects of heavy metals cause the impairment of the metabolic functions related with energy metabolism i.e., respiration and photosynthesis, then oxidative damage of cells and tissues might occur due to the accumulation of reactive oxygen species (Stohs and Bagchi 1995; Shaw and Rout 1998). The antistress mechanism of plants comprises of antioxidative enzymes and antioxidants. 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).

Our hypothesis was that accumulation of Hg in *E. crassipes* would cause oxidative stress and would stimulate the antioxidative defence system of the plant to manage this stress. Therefore, in the present study variations in malondialdehyde (MDA) level and corresponding changes in the contents of antioxidants, namely ascorbic acid (AA), glutathione (GSH) and vitamin E (vit E) were determined in *E. crassipes* during phytoremediation of Hg.

MATERIALS AND METHODS

One-month old *E. crassipes* plants (at vegetative stage) collected from aquatic habitats of Amritsar (31º37′N, 74º52′E), India were cultured in 10% Hoagland's nutrient medium in the laboratory, then treated with different concentrations of mercuric acetate (MERCK, GR) containing 1 μg l^{-1}, 10 μg l^{-1}, 100 μg l^{-1} and 1000 μg l^{-1} of Hg. Roots, petioles and leaf laminae of the plants were harvested after 7 and 14 days of Hg treatment. Lipid peroxidation was measured in terms of MDA content (Heath and Packer 1968). The plant extracts for AA, GSH and vit E content estimation were prepared by homogenizing 1 g of fresh plant tissue in a 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 min and the supernatant was used for the antioxidant assays. AA content was determined following the method given by Roe and Kuether (1943). The GSH content was determined after Sedlak and Lindsay (1968) using Ellman's reagent. Vit E content was estimated by the method of Martinek (1964). All parameters were statistically analyzed for mean, standard deviation, standard error, regression (linear, logarithmic and exponential), and one-way and two-way analyses of variance. Significance of the differences was determined from the variance ratio (F values) of ANOVA tables. HSD values were calculated using Tukey's multiple comparison test at P = 0.05 with the help of Window's Excel.

RESULTS

The initial concentrations of MDA, AA, GSH and vit E are given in **Table 1**. There was a significant increase in MDA content in all plant parts over time and an increase in Hg concentration (**Fig. 1**). The MDA level increased from the initial values of 0.280, 0.826 and 1.935 μM g^{-1} FW to 1.943, 3.818 and 4.442 μM g^{-1} FW in roots, petioles and leaf laminae respectively for 1000 μg l^{-1} concentration on the 14th day. A higher increase in MDA content in roots (370%)

Received: 19 March, 2008. Accepted: 6 April, 2008.

Original Research Paper

Table 1 Initial contents of malondialdehyde (μmoles g^{-1} FW) and antioxidants (mg g^{-1} FW).

MDA/ Antioxidant	Roots	Petioles	Laminae
MDA	0.280	0.826	1.935
Ascorbic acid	0.030	0.058	0.129
Glutathione	0.508	0.582	0.608
Vitamin E	0.025	0.022	0.042

compared to petioles (350%) and leaf laminae (100%) indicates that toxicity is being caused by higher amounts of mercury accumulating in the roots. The initial AA content was observed to be 0.03, 0.058 and 0.129 mg g^{-1} FW in roots, petioles and leaf laminae, respectively. Maximum AA content was observed as 0.75 mg g^{-1} FW (roots), 0.828 mg g^{-1} FW (petioles) and 1.749 mg g^{-1} FW (leaf laminae) on the 14th day of treatment with 1000 μg l^{-1} of Hg (**Fig. 2**). The initial values of GSH content in roots, petioles and leaf laminae were 0.508, 0.582 and 0.608 mg g^{-1} FW, respectively. The maximum GSH values were observed for 1 μg l^{-1} of Hg on the 7th day as 2.300 mg g^{-1} FW, 1.916 mg g^{-1} FW and 2.378 mg g^{-1} FW in roots, petioles and leaf laminae, respectively, whereas the minimum content was observed to be 1.187 mg g^{-1} FW in roots, 1.385 mg g^{-1} FW in petioles and 1.445 mg g^{-1} FW in leaf laminae of plants treated with 1 μg l^{-1} of Hg on the 14th day of treatment (**Fig. 3**). The vit E content increased from initial values of 0.025, 0.022 and 0.042 mg g^{-1} FW to 0.095, 0.065 and 0.185 mg g^{-1} FW in roots, petioles and leaf laminae, respectively for 100 μg l^{-1} of Hg on 14th day of treatment (**Fig. 4**).

The results of one-way ANOVA are given in **Table 2**. The variations in MDA and antioxidant contents were found to be significant for all plant parts both on the 7th and 14th days. **Table 3** describes the interaction between days of treatment (7 and 14 days) vs mercury concentration (0, 1, 10, 100 and 1000 μg l^{-1}), and plant parts (roots, petioles and leaf laminae) vs mercury concentration. The two-way ANOVA for the effect of days × concentration revealed significant differences among all treatments except for roots for AA and petioles for vit E. The significance of interactions between mercury concentration and treatment period implies that the effect of Hg concentration is affected by duration of treatment and *vice versa*. Similarly, significant differences were observed for all parameters on the 7th and 14th days in different plant parts (plant parts × concentration) in all the treatments except for AA on 7th day and vit E on both days. In order to find a relation between the mercury accumulated by the plants and changes in the MDA and antioxidants, regression analyses were done for the data after 14 days of treatment. It was observed that MDA and ascorbic acid were positively correlated with the Hg content in the roots of plants cultured at concentrations up to 1000

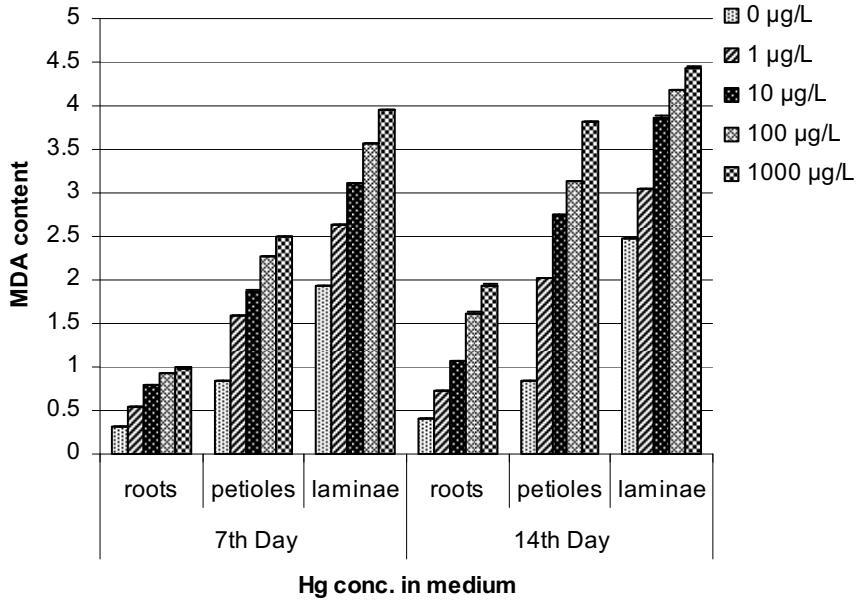

Fig. 1 MDA content (μmoles g^{-1} FW) in different plant parts of *E. crassipes* on treatment with mercuric acetate (μg/L).

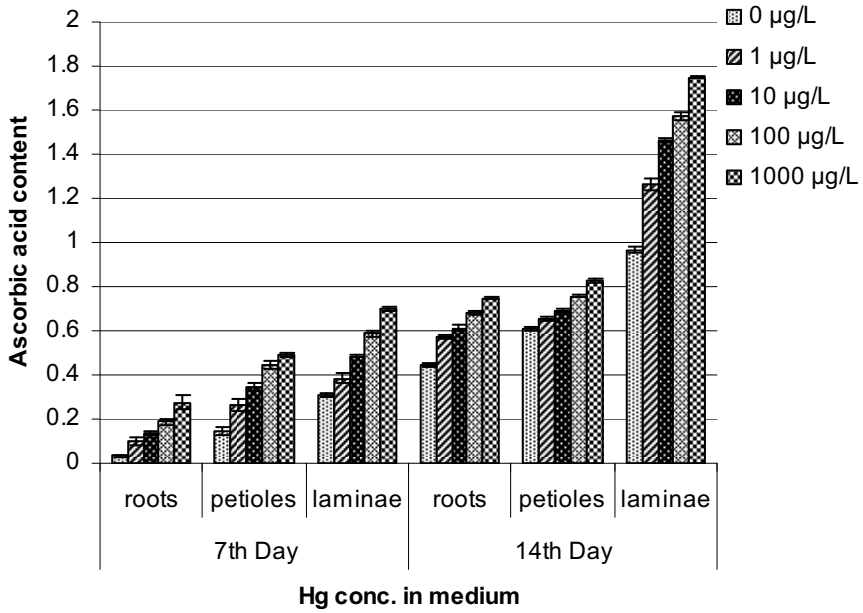

Fig. 2 Ascorbic acid content (mg g^{-1} FW) in different plant parts of *E. crassipes* on treatment with mercuric acetate (μg/L).

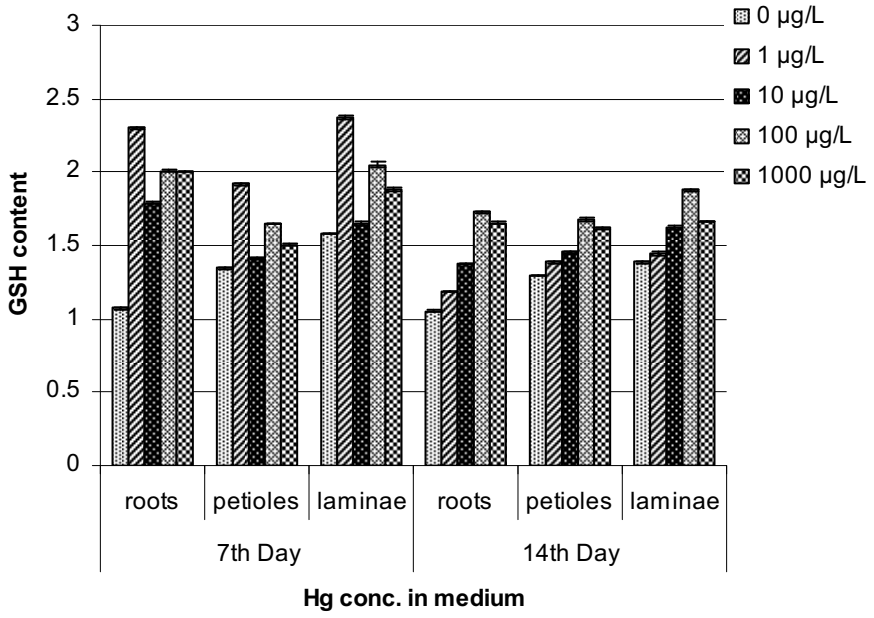

Fig. 3 Glutathione content (mg g^{-1} FW) in different plant parts of *E. crassipes* on treatment with mercuric acetate (µg/L).

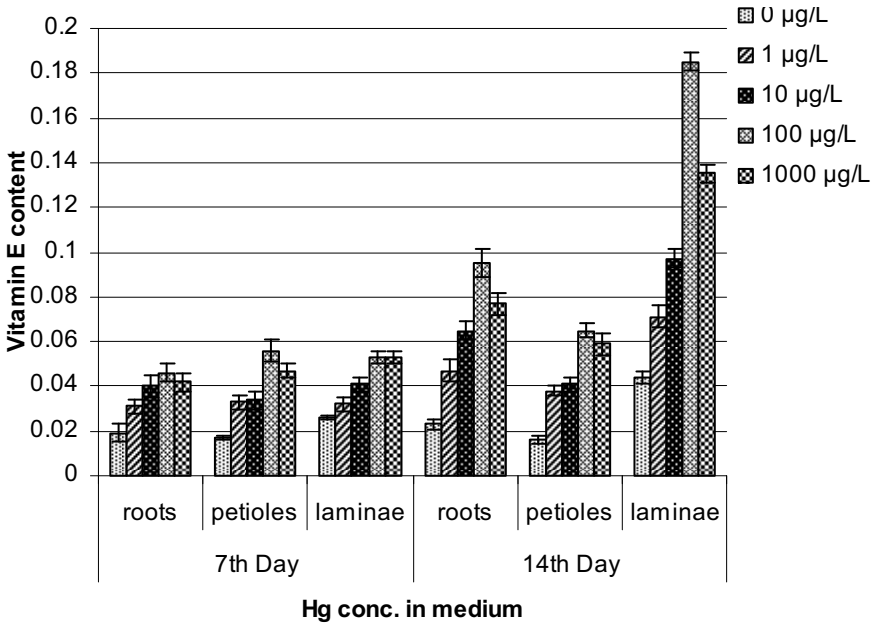

Fig. 4 Vitamin E content (mg g^{-1} FW) in different plant parts of *E. crassipes* on treatment with mercuric acetate (µg/L).

µg l^{-1}, whereas GSH and vit E were positively correlated with the Hg content in the roots of plants cultured in concentrations up to 100 µg l^{-1} (**Table 4**).

DISCUSSION

Levels of MDA, a secondary product of lipid peroxidation, indicate the degree of plant oxidative stress (Arora *et al*. 2002), for example as observed by Gajewska and Sklodowska (2007), who reported an increase in MDA content in wheat shoots as a result of nickel stress (50 and 100 µM). MDA content was also found to be higher in *Spirodela polyrrhiza* and *Lemna minor* plants under copper stress (Tu *et al*. 2006). Lipid peroxidation has been linked to effects such as increased ion permeability, loss of fluidity, cross-linking of amino lipids and polypeptides, inactivation of membrane enzymes and receptors resulting in a decrease in photosynthesis (Sankhalkar and Sharma 2002). The plasma

Table 2 One-way ANOVA analysis.

Antioxidant content	MDA/Antioxidant concentration (mg g^{-1} FW)					
	Roots		Petioles		Leaf laminae	
	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD
7th day						
MDA	8835.30*	0.014	94861.17*	0.009	205555.2*	0.008
AA	24.52*	0.086	58.53*	0.083	143.36*	0.060
GSH	8861.12*	0.023	2133.51*	0.023	828.87*	0.052
Vit E	6.42*	0.019	18.86*	0.016	17.85*	0.013
14th day						
MDA	46492.43*	0.013	270134.1*	0.010	117424.3*	0.011
AA	137.84*	0.045	141.07*	0.036	325.31*	0.077
GSH	7618.81*	0.015	263.95*	0.045	648.52*	0.035
Vit E	36.44*	0.021	32.89*	0.015	129.06*	0.022

*Significant at 5%.

Table 3 Treatment × Dose interactions using two-way ANOVA.

Antioxidant content	**Days (treatment) × Hg concentration (dose)**					
	Roots		**Petioles**		**Leaf laminae**	
	F-ratio	**HSD**	**F-ratio**	**HSD**	**F-ratio**	**HSD**
MDA/Antioxidant concentration (mg g^{-1} FW)						
MDA	8069.9*	0.016	26487.1*	0.011	2074.0*	0.010
AA	2.27 (ns)	0.071	9.11*	0.065	52.46*	0.077
GSH	4733.5*	0.021	560.8*	0.041	691.6*	0.050
Vit E	7.81*	0.022	0.995 (ns)	0.017	81.62*	0.017
	Plant parts (treatment) × Hg concentration (dose)					
	7^{th} day			**14^{th} day**		
	F-ratio	**HSD**		**F-ratio**	**HSD**	
MDA/Antioxidant concentration (mg g^{-1} FW)						
MDA	13589.1*	0.012		13850.9*	0.013	
AA	4.99 (ns)	0.085		99.76*	0.060	
GSH	484.7*	0.043		111.0*	0.043	
Vit E	1.15 (ns)	0.018		1.15 (ns)	0.021	

*Significant at 5%, ns = not significant at $p \leq 0.05$.

Table 4 Regression between Hg content (μg g^{-1} dry wt., x) with antioxidant contents (mg g^{-1} FW, y) in roots of *E. crassipes* after 14 days of treatment.

MDA/ Antioxidant	**Regression equation**	**R^2**
MDA	$y = 0.1838\text{Ln}(x) + 0.7028$	0.9882*
AA	$y = 0.0263\text{Ln}(x) + 0.5626$	0.9874*
GSH	$y = 1.1714x^{0.0814}$	0.9853*$
Vit E	$y = 0.0466x^{0.1528}$	0.9979*$

*Significant at 5%

$ Regression equation computed for mercury concentration up to 100 μg l^{-1} solution. For other equations highest mercury concentration was 1000 μg l^{-1}.

membrane plays an important role in the regulation of the entry of metal ions into the cell (Hall 2002). Hence, metal-induced alteration of membrane lipids results in the disruption of several cellular processes (Cooke and Burden 1990). Ascorbate is the most important antioxidant in plants, with a fundamental role in scavenging of hydrogen peroxide. In this study, AA content increased to 7.9-fold in roots, 3.4-fold in petioles and 2.3-fold in leaf laminae of plants treated with 1000 μg l^{-1} of Hg on the 7^{th} day, and with limited stimulation (24 to 81%) on the 14^{th} day. This indicates utilization of AA in the scavenging of free radicals and hydrogen peroxide. An increase in ascorbate content with an increase in metal concentration was also been reported in earlier studies using Zn- and Cr-stressed (0 to 100 mM for 8 days) wheat (*Triticum aestivum*) leaves (Panda *et al*. 2003), and in roots and leaves of *Brassica juncea* plants that were found to be effective accumulators of Cr, Fe, Zn and Mn under tannery sludge treatment (10%, 25%, 35%, 50%, 75%, 100% after 30, 60, and 90 days of sowing) (Singh and Sinha 2005). GSH content in this study exhibited a maximum increase of 114% in roots, 43% in petioles and 50% in leaf laminae for 1 μg l^{-1} of Hg after 7 days of culture. For the remaining treatments, GSH content remained at a comparatively lower level (than observed for 1 μg l^{-1} of Hg on the 7^{th} day) despite the fact that it showed a concentration-dependent increase on the 14^{th} day in all plant parts. This signifies the active stimulation of GSH content in response to moderate stress caused by the lowest concentration of mercury. Among the different plant parts, leaf laminae exhibited a higher GSH content than the roots or petioles. An increase in GSH content due to Cd exposure (50 μM) was also reported in *Phragmites australis*, which was subsequently observed to decrease at high Cd (100 μM) treatment (Pietrini *et al*. 2003). GSH content was also found to increase initially followed by a decrease as the metal concentration increased over time in wheat leaves in response to Zn and Cr (0 to 100 mM for 8 days) stress (Panda *et al*. 2003). GSH is an important cellular antioxidant and is a substrate for phytochelatins. Hence the maintenance of lowered level of GSH at a higher concentration and prolonged exposure to Hg could be attributed to its being simultaneously utilized either in production of phytochelatins or in recovery of ascorbate. Vit E (α-tocopherol) is a membrane-associated antioxidant and scavenges singlet oxygen and lipid peroxides. It can also scavenge hydroxyl radicals and superoxide radicals. During the 14-day culturing period, the roots, petioles and leaf laminae of plants exposed to mercuric acetate showed a maximum increase of up to 4.0 to 4.2-fold for 100 μg l^{-1} of Hg. Reports in the literature also reveal similar results. A 15-fold increase in vit E content in response to drought was reported in rosemary (*Rosmarinus officinalis*) leaves (Munne-Bosch *et al*. 1999). A dose- and time-dependent increase in vit E content was reported by Jiang and Zhang (2001) in maize leaves following ABA (abscisic acid, at 10 to 100 μM) treatment. The increase in vit E content in plant cells is indirect evidence of oxidative stress in the plants as it is a potent antioxidant.

CONCLUSIONS

Oxidative stress is a regulated process, and the equilibrium between the oxidative and antioxidative capabilities determine the fate of the plant. There was an increase in the MDA content in different plant parts of *E. crassipes* cultured in solutions of mercuric acetate, with the maximum increase being observed in roots. This was accompanied by an increase in the contents of ascorbate, GSH and vit E in all plant parts. There was a significant positive correlation between the amount of Hg accumulated by roots and antioxidants. The significance of dose × treatment interactions (concentration of Hg × treatment period, and concentration of Hg × plant parts) implies that the effect of Hg concentration is affected by the duration of treatment as well as plant parts and *vice versa*. It is clear from the present results that the elevated levels of antioxidants in *E. crassipes* plants due to Hg exposure is correlated with increased stress tolerance. This property results in the enhanced capacity of *E. crassipes* for Hg accumulation that can in turn be utilized in reclamation of polluted aquatic habitats by periodic removal of the spent plants.

REFERENCES

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

Cooke DT, Burden R (1990) Lipid modulation of plasma membrane bound ATPases. *Physiologia Plantarum* **78**, 153-159

Gajewska E, 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, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* **125**, 189-198

Jayaweera MW, Kasturiarachchi JC, Kularatne RK, Wijeyekoon SL (2008) Contribution of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) grown under different nutrient conditions to Fe-removal mechanisms in constructed wetlands. *Journal of Environmental Management* **87**, 450-460

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

Kelley C, Curtis AJ, Uno JK, Berman CL (2000) Ultrascopic studies of the interaction of Eu(III) with the roots of water hyacinth. *Water, Air and Soil Pollution* **119**, 171-176

Kramer U, Chardonnens AN (2001) The use of transgenic plants in the bioremediation of soils contaminated with trace elements. *Applied Microbiology and Biotechnology* **55**, 661-672

Lu X, Kruatrachue M, Pokethitiyook P, 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

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

Muramoto S, Oki Y (1983) Removal of some heavy metals from polluted water by water hyacinth (*Eichhornia crassipes*). *Bulletin of Environmental Contamination and Toxicology* **30**, 170-177

Murugesan AG, Sukumaran N (1997) Perspectives of utilizing aquatic macrophytes for wastewater treatment. *Indian Journal of Environmental Botany* **17**, 502-507

Panda SK, Chaudhary I, Khan MH (2003) Heavy metals induce lipid peroxidation and affect antioxidants in wheat leaves. *Biologia Plantarum* **46**, 289-294

Pietrini F, Iannelli MA, Pasqualini S, Massacci A (2003) Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin. ex Steudel. *Plant Physiology* **133**, 829-837

Prasad MNV (2004) *Heavy Metal Stress in Plants – From Biomolecules to Ecosystems*, Narosa Publishing House, New Delhi, 462 pp

Riddle SG, Tran HH, Dewitt JG, Andrews JC (2002) Field, laboratory and X-ray absorption spectroscopic studies of mercury accumulation by water hyacinth. *Environmental Science and Technology* **36**, 1965-1970

Roe JH, Kuether CA (1943) The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenyl hydrazine derivative of dehydroascorbic acid. *Journal of Biological Chemistry* **147**, 399-407

Sankhalkar S, Sharma PK (2002) Protection against photooxidative damage provided by enzymatic and non enzymatic antioxidant system in *Sorghum* seedlings. *Indian Journal of Experimental Botany* **40**, 1260-1268

Sedlak J, Lindsay RH (1968) Estimation of total, protein bound and non-protein sulphydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry* **25**, 192-205

Shaw BP, Rout NP (1998) Age-dependent responses of *Phaseolus aureus* Roxb. to inorganic salts of mercury and cadmium. *Acta Physiologiae Plantarum* **20**, 85-90

Singh S, Sinha S (2005) Accumulation of metals and its effects in *Brassica juncea* (L.) Czern. (cv Rohini) grown on various amendments of tannery waste. *Ecotoxicology and Environmental Safety* **62**, 118-127

Stohs SJ, Bagchi D (1995) Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology and Medicine* **18**, 321-336

Tabbada RA, Florendo PE, Santiago AE (1990) Uptake and some physiological effects of mercury on water hyacinth, *Eichhornia crassipes* (Mart.) Solms. *BIOTROP, Bogor* **3**, 83-91

Tu J, Wang X, Liu D, 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