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INDUCTION OF OXIDATIVE STRESS AND ANTIOXIDANT METABOLISM IN *CALAMUS TENUIS* LEAVES UNDER CHROMIUM AND ZINC TOXICITY

M.H. KHAN*

Plant Biochemistry Laboratory, School of Life Sciences, Assam University, Silchar-788 011, Assam, India

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SUMMARY

The possible role of Cr (VI) and Zn as catalytic inducers of free radicals and antioxidant metabolism in *Calamus tenuis* leaves was investigated. Total peroxide and lipid peroxidation measured in terms of thiobarbituric acid reactive substances showed uniform increase under metal treatment. The activity of the antioxidative enzymes superoxide dismutase decreased, whereas, catalase, peroxidase and glutathione reductase increased except higher Cr (VI) treatments decreased catalase activity. Non-enzymic antioxidants ascorbate and glutathione content increased uniformly. At higher concentration both Cr (VI) and Zn decreased the dry mass and the uptake of Cr and Zn increased with the increase in the concentrations of the heavy metals.

***Key words*: Antioxidant enzymes, *Calamus tenuis*, chromium, zinc.**

INTRODUCTION

Increasing concentrations of heavy metals in soil caused by industrial wastes result in a concomitant increase in their concentration in plants. Heavy metals when present at an elevated level in soil are taken up by the root system, accumulate in different parts of plants, reduce their growth and impair plant metabolism. Though some heavy metals are essential as micronutrients for plants, at higher concentrations they are toxic. Chromium in nature exists in the form of Cr (VI) and Cr (III) of which Cr (VI) is more toxic. Chromium is known to affect seed germination, seedling growth, pigment content, nutrient content and enzyme activities of various crop plants (Barcelo and Poschenreider 1997, Panda and Patra 2000, Cakmak 2000, Panda *et al*. 2003, Panda and Khan 2003, Panda 2004). Zinc as an essential micronutrient has a role in several metabolic processes of plant. Zinc deficiency leads to inhibition of plant growth and development (White and Zasoski 1999). However, excess zinc decreases growth and development, impair metabolism and an induction of oxidative stress in various plant species (Weckx and Clijsters 1997, Chaoui *et al*. 1997a, 1997b, Prasad *et al*. 1999, Fargasova 2000, Zeid 2001, Panda *et al*. 2003, Panda and Khan 2003, Panda 2004, Panda and Choudhury 2004). Any imbalance in the cellular redox homeostasis leads to oxidative stress. The successive reduction of molecular oxygen to H_2O_2 yields the intermediates $O_2^{\cdot}$, $HO^{\cdot}$, $^1O_2$, etc which are potentially toxic and may lead to the unspecific oxidation of proteins and membrane lipids or may cause DNA and RNA injury (Grant and Loake 2000).

Plants have developed a complex antioxidative defence system to alleviate the damage caused by reactive oxygen species (ROS) and the degree of damage depends on the balance between the formation of ROS and its removal by the antioxidative scavenging systems that defend against them. The antioxidative

*Present address: Department of Botany, M.H.C.M. Science College, Algapur, Hailakandi-788 150, Assam, India, E-mail: mhkbio@yahoo.co. in

system includes carotenoids, ascorbate, glutathione, a-tocopherols and enzymes such as superoxide dismutase, catalase, glutathione peroxidase, peroxidases and enzymes involved in ascorbate glutathione cycle (ASC-GSH Cycle), ascorbate peroxides, dehydroascorbate reductase, monodehydrosacorbate reductase and glutathione reductase (Noctor and Foyer 1998, Panda *et al*. 2003, Panda and Khan 2003, 2004, Choudhury and Panda 2004, Khan and Patra 2007).

Calamus tenuis (rattan) is a tropical climbing palm, grows under various habitats and has multiple economic uses. The effect of chromium and zinc toxicity on the physiology of oxidative stress and antioxidant metabolism in rattan are relatively less known. The present investigation was carried out to evaluate the oxidative stress, differential antioxidant responses to Cr (VI) and Zn in rattan leaves.

MATERIALS AND METHODS

Rattan (*Calamus tenuis* Roxb.) seeds were sown and germinated in a plastic tray containing sand moistened with tap water for four weeks. The germinated seeds one in each was transferred to plastic pots containing sand and grown with half strength nutrient Hoagland's solution in green house for 9 (nine) months. The environmental conditions in the green house containing the germinated plants were 28 ^{0}C / 24 ^{0}C (day / night) temperature, 80 % relative humidity and 7000 lux of light intensity with 18h photoperiod. The 9 months old plants were treated with different concentrations of chromium (VI) and zinc in the form of potassium dichromate ($K_2Cr_2O_7$) and zinc sulphate ($ZnSO_4.7H_2O$) (0, 0.1, 1 and 10 mmol L^{-1}) respectively for 5 (five) days. Seedling grown in soil with half strength Hoagland's nutrient solution only was used as control. On 6th day, the young leaves were used for various biochemical estimations. Leaf (0.5 g) tissue was homogenized in 5 % trichloroacetic acid (TCA) and the homogenate was used for the determination of hydrogen peroxide (H_2O_2) levels by the method of Sagisaka (1976). The level of lipid peroxidation in the root tissues was determined as thiobarbituric reactive substances (TBARS) as described by Heath and Packer (1968). For the extraction and estimation of ascorbate, the method of Oser (1979) was used. Glutathione was extracted and estimated as per the method of Griffith (1980). Leaf tissue (0.2 g) was homogenized at 4 ^{0}C in 5 ml chilled extraction buffer (0.1 M phosphate buffer, pH 6.8) with mortar and pestle, homogenate was then centrifuged at 15,000 g for 20 min and was used as crude extract for the assay of superoxide dismutase (SOD), as per the methods described by Giannopolitis and Reis (1977), catalase (CAT) and peroxidase (POX) by Chance and Maehly (1955) and glutathione reductase (GR) by Smith *et al*. (1988) respectively. Metal concentrations were estimated as per the method described by Chaoui et al (1997a). Leaves were washed twice with deionized water and oven dried at 70^{0}C for 48h and acid digested. Then, oven-dried plant material was wet-ashed with an acid mixture (HNO_3:$HClO_4$, 4:1) and analysed for Cr and Zn by atomic absorption spectrophotometer (Perkin Elmer 3110, USA). Values presented in the experiment are means of two independent experiments with three replicates each ± standard errors of mean (±SEM).

RESULTS AND DISCUSSION

Effects of Cr (VI) (0-10 mmol) and Zn (0-10 mmol) on total peroxide and thiobarbituric acid reacting substances (TBARS) is presented in figure 1. There is an increase in total peroxide content with the increase in the concentrations of both the metals and the amount of peroxide is greater in Cr (VI) treated leaves than in Zn treated leaves imposing a challenge for its detoxification (Fig. 1A). As an indicator of lipid peroxidation, TBARS content was measured. Increasing concentrations of Cr (VI) and Zn caused an enhancement of TBARS, an index of lipid peroxidation which is produced when unsaturated fatty acids in the membrane undergo oxidation by the accumulation of free radical and therefore oxidative stress. The effect of Cr (VI) is more than Zn as the amount of TBARS is greater in Cr (VI) treatment than in Zn treated leaves (Fig. 1B). The present biochemical and physiological knowledge of the mechanism controlling stress resistance of plants suggest that membranes are amongst the main cellular targets common to different stresses (Vranova 2002). Since membranes may have a central role in cellular response and tolerance to stresses, the extent of their damage is commonly used as a measure of tolerance to various stresses in plants. Lipid peroxidation is a process by which the functionality and integrity of

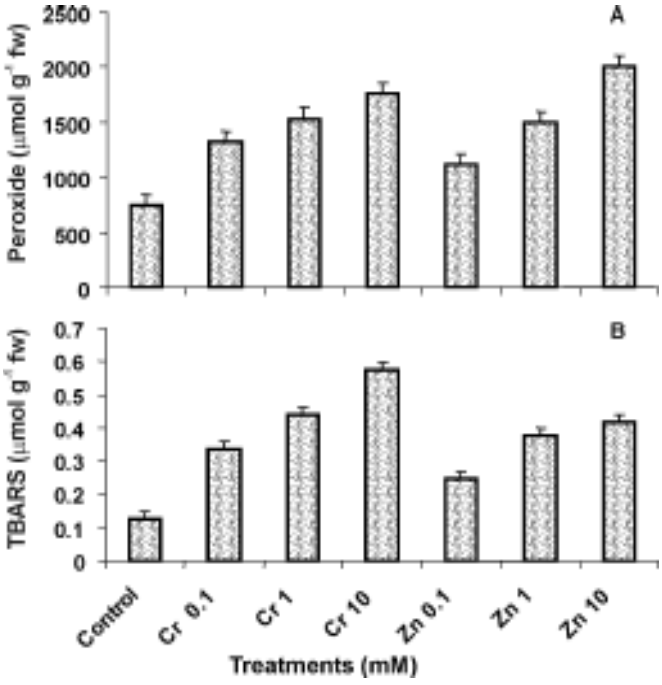

Fig. 1. Changes in (A) peroxide and (B) thiobarbituric acid reactive substance (TBARS) content subjected to Cr (VI) and Zn treatments in *Calamus tenuis* leaves. Data presented means ± SE.

the membrane is affected and can produce irreversible damage to cell function. Lipid peroxidation gets initiated by ROS such as $O_2^{\cdot}$, H_2O_2, $^{\cdot}OH$ or by lipooxygenases. Both Cr (VI) and Zn enhanced the peroxide content and TBARS level in rattan leaves, an index of lipid peroxidation and oxidative stress as observed for various plants under metal toxicity (Gallego *et al*. 1996, Weckx and Clijsters 1997, Mazhoudi *et al*. 1997, Dietz *et al*. 1999, Panda and Patra 2000, Panda *et al*. 2003, Panda and Khan 2003, 2004, Choudhury and Panda 2004, Khan and Patra 2007). Oxidative stress induced by chromium initiates the degradation of photosynthetic pigments and disturbing the chloroplast ultrastructure thereby disturbing the photosynthetic process. Like copper and iron, chromium is also a redox metal and its redox behaviour exceeds that of other metals like Co, Fe, Zn, Ni, etc. The redox behaviour can thus be attributed to the direct involvement of chromium in inducing oxidative stress in plants (Shi and Dalal 1989, Panda and Choudhury 2005).

The non-enzymic antioxidants ascorbate and glutathione content increased significantly in the Cr (VI) as well as Zn treated rattan leaves with increasing concentrations compared to control values, however, glutathione content declined at higher concentrations of Cr (VI) and Zn (Fig. 2A, B). Ascorbate react directly with the ROS in photosynthetic tissues, recycles a tocopherol and protect enzymes with prosthetic metals ions and is utilized as a substrate for ascorbate peroxidase which catalyzes H_2O_2 detoxification. Glutathione is a measure of non-protein thiol in plants and is involved in the detoxification of heavy metals and xenobiotic compounds that plays an important role in gene activation for protection against oxidative stress and seems to be an important signal molecule by acting as a direct link between environmental stress and key adaptive responses (Noctor and Foyer 1998). Ascorbate and glutathione contents increased in all concentrations of Cr (VI) and Zn suggesting their participation in detoxification of ROS and therefore might restrict heavy metal-induced lipid peroxidation and oxidative stress (Mazhoudi *et al*. 1997, Dietz *et al*. 1999, Panda *et al*. 2003, Panda and Khan 2003, Prasad *et al*. 1999, Choudhury and Panda 2004). During oxidative stress, induced by heavy metals, there is inhibition of catalase activity, whereas,

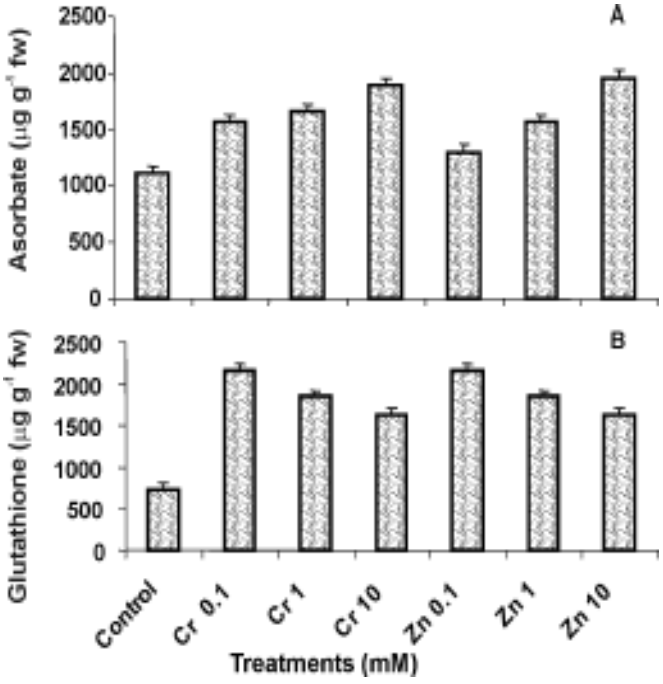

Fig. 2. Changes in (A) ascorbate and (B) glutathione content subjected to Cr (VI) and Zn treatments in *Calamus tenuis* leaves. Data presented means ± SE.

glutathione accumulates (Chamnongpol *et al*. 1996, Willekens *et al*. 1997, Choudhury and Panda 2004). High cellular glutathione levels are associated with resistance to heavy metals in tomato cells (Noctor *et al*. 1998), while heavy metal exposure has been shown to lead to accelerated glutathione synthesis in roots and cultured cells. These correlative studies not only implicated glutathione in protection against various forms of stress but also drew attention to the regulatory factors mediating the metabolic signaling for modified rates of glutathione synthesis and accumulation, the results found in this present investigation with improved capacities for glutathione synthesis and its accumulation displayed higher Cr and Zn tolerance (Schneider and Bergmann 1995, Schickler and Caspi 1999).

The illustrations in the Fig. 3 and 4 depict the effects of Cr (VI) and Zn on the enzymic antioxidants in rattan leaves. There is a significant decrease in SOD activity in all the treatments, thereby, lowering the dismutation of $O_2^{\cdot}$ and the plants are unable to resist the potential oxidative damage caused by the Cr (VI) and Zn exposure (Fig. 3A). An increased CAT activity was observed with the increase in the concentrations of both Cr (VI) and treated leaves, however, higher Cr (VI) and Zn concentrations decreased CAT activity (Fig. 3B). Superoxide dismutase (SOD) catalyzes the dismutation of $O_2^{\cdot}$ to H_2O_2 and O_2. SOD is key enzyme in protecting cells from oxidative stress. Similar results of reduced SOD activity have been observed in various plants after treatment with Cr (VI) and Zn (Panda *et al*. 2003, Panda and Khan 2003, 2004, Khan and Patra 2007). Catalase (CAT) removes peroxide (H_2O_2) by catalyzing H_2O_2 to H_2O. Rattan leaves showed increased CAT activity in lower Cr (VI) and Zn concentrations, therefore, showing better detoxification of H_2O_2 produced during the stress, however, under higher concentrations of Cr (VI) and Zn, CAT activity declined in the rattan leaves, the cell is not fully competent to remove the H_2O_2 and would accumulate to toxic levels (Panda and Patra 2000, Panda *et al*. 2003, Panda and Khan 2003, 2004, Khan and Patra 2007).

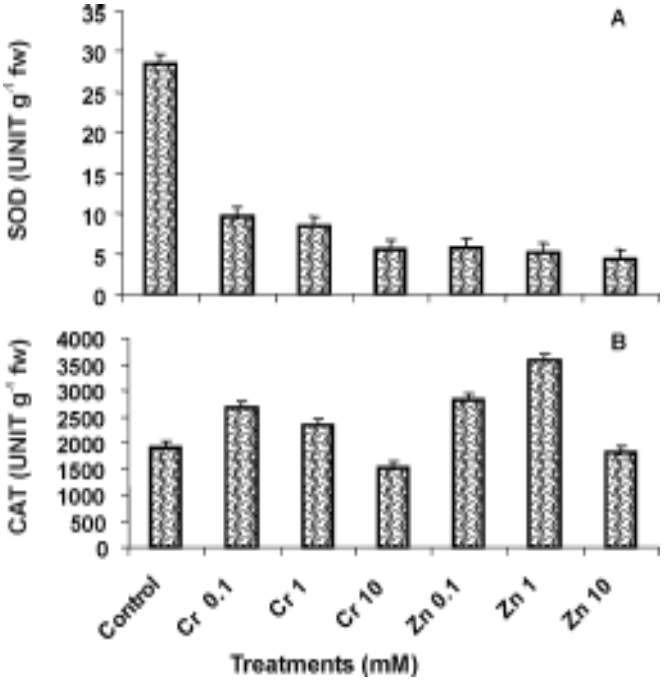

Fig. 3. Changes in (A) superoxide dismutase (SOD) and (B) catalase (CAT) activities subjected to Cr (VI) and Zn treatments in *Calamus tenuis* leaves. Data presented means ± SE.

There is an increase in the POX activity at lower (0.1 mM) concentrations of Cr (VI), followed by a significant decline in higher concentrations (Fig. 4A), showing better detoxification of H_2O_2 produced during oxidative stress at 0.1 mmol Cr (VI) treated leaves, whereas, Zn treated leaves showed an increased POX activity with the increase in the concentrations compared to control values. Glutathione reductase (GR) a key enzyme of the ascorbate-glutathione cycle. Except Higher Cr (10 mmol) and lower Zn (0.1 mmol) concentrations, GR activity decreased significantly (Fig. 4B). Peroxidase (POX) scavenges H-$_2O_2$ and is produced through the dismutation of $O_2^{\cdot}$ catalayzed by SOD. The results showed increased POX activity in lower Cr (VI) and in all Zn concentrations (Fig. 4A), showing better detoxification of H_2O_2 produced during oxidative stress in Cr (VI) and Zn treated leaves. Increased and decreased POX activity has been reported in various plants treated with Cr (VI) and Zn (Panda *et al*. 2003, Panda and Khan 2003, 2004, Khan and Patra 2007). GR plays a key role in oxidative stress by converting the oxidized glutathione (GSSG) to reduced glutathione (GSH) and maintaining a high GSH/GSSG ratio. Increased GR activity in higher Cr (VI) and lower Zn concentrations treated leaves may be attributed in maintaining high GSH/GSSG ratio (Sandalio *et al*. 2001,

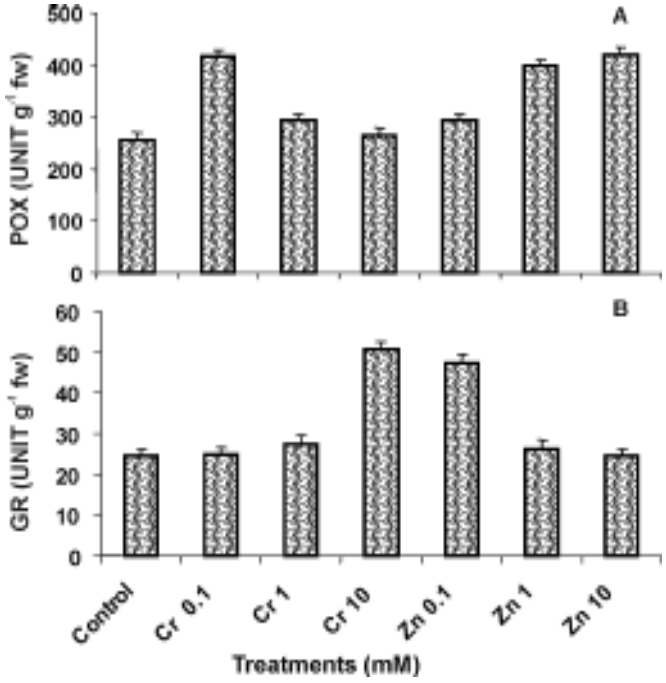

Fig. 4. Changes in (A) peroxidase (POX) and (B) glutathione reductase (GR) activities subjected to Cr (VI) and Zn treatments in *Calamus tenuis* leaves. Data presented means ± SE.

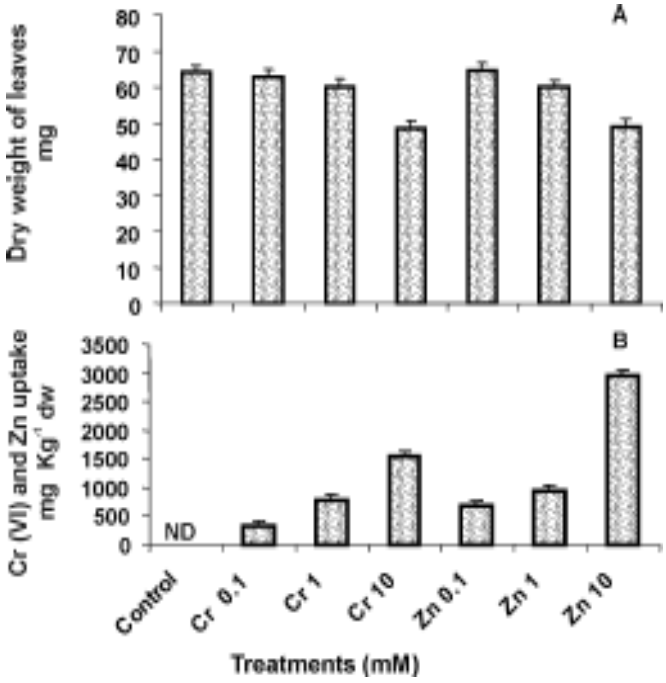

Fig. 5. Changes in (A) dry weight and (B) Cr (VI) and Zn uptake subjected to Cr (VI) and Zn treatments in *Calamus tenuis* leaves. Data presented means ± SE.

Khan and Patra 2007). Lower GR activity at higher concentrations of Zn could be due to tendency of the plant to acclimate or inactivation of the enzyme losing its ability to dismutase the superoxide anion as observed in various heavy metals (Sandalio *et al.* 2001, Choudhury and Panda 2004). Schickler and Capsi (1999) reported increased GR activity at low levels of Cd in *Alyssum argenteum*, considered as a metal-hyperaccumulator plant while the GR activity was reduced at higher Cd concentrations.

The growth of the plants measured in terms of dry weight showed no significant change in lower Cr (VI) and Zn concentrations but decreased in higher concentrations of Cr (VI) and Zn (10 mmol) (Fig. 5A). Though at lower concentration Cr (VI) uptake was low, higher concentrations showed significant uptake (Fig. 5B). The accumulation of Zn was found to increase significantly with the increase in Zn concentrations and the accumulation was 3 fold high in 10 mmol Zn compared to 0.1 mmol Zn (Fig. 5B). The decrease in dry biomass at higher Cr (VI) and Zn concentration (10 mmol) were similar with that of other plants, which may be due to degradation of chlorophyll resulting in inhibition of photosynthesis (Salt *et al*. 1995, Bargagli 1998). Plants are known to hyperaccumulate various metals and this helps in bioremediation of the environment (Salt *et al*. 1995, Jiang *et al*. 2000, Carginale *et al*. 2004). Though at lower Cr (VI) and Zn concentrations, accumulation was low at higher concentrations, both Cr and Zn showed significantly higher accumulation as seen for other plants suggesting the rattan plants having an efficient bioconcentration mechanism, can act as pollution indicator and bioremediation (Jiang *et al.* 2000, Sandalio *et al*. 2001, Choudhury Panda 2004, Carginale *et al*. 2004, Khan and Patra 2007).

In conclusion rattan leaves treated with Cr (VI) and Zn induced a concentration-dependent oxidative stress, characterized by an increased uptake of Cr and Zn, accumulation of peroxides with increased lipid peroxidation levels, decreased superoxide dismutase activity, increased catalase, peroxidase and glutathione reductase activities, increased ascorbate and glutathione

content displaying better antioxidant response and detoxification of the oxidative damage and suggesting higher Cr (VI) and Zn tolerance.

REFERENCES

Barcelo, J. and Poschenreider, C. (1997). Chromium in plants. In: S. Carati, F. Tottarelli and P. Segmi (eds.), Chromium Environmental Issues, pp 101-129. Francotangati Press, Milan.

Bargagli, R. (1998). Mosses as passive and active biomonitors of trace elements. In: R. Bargagli (ed.), Trace Elements in Terrestrial Plants, pp. 207-236. Springer-Verlag, Berlin.

Cakmak, I. (2000). Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol*. **146:** 185-205.

Carginale, V., Sorbo, S., Capasso, C., Trincella, F., Cafiero, G. and Basile, A. (2004). Accumulation, localization and toxic effects of cadmium in the liverworts *Lunualria cruciata*. *Protoplasma* **223:** 53-56.

Chamnongpol, S., Willekens, H., Langebartels, C., Van Montagu, M., Inze, D. and Van Camp, W. (1996). Transgenic tobacco with a reduced catalase activity develops necrotic lesions and induces pathogenesis-related expression under high light. *Plant J*. **10:** 491-503.

Chance, B. and Maehly, A.C. (1955). Assay of catalase and peroxidase. *Methods Enzymol*. **2:** 764-775.

Chaoui, A., Ghorbal, M.H., Ferjani, E.I. (1997a). Effects of cadmium–zinc interaction on hydroponically grown bean (*Phaseolus vulgaris*). *Plant Sci*. **126:** 21-28.

Chaoui, A., Mazhoudi, S., Ghorbal, M.H. and Ferjani, E.I. (1997b). Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzymes activities in bean (*Phaseolus vulgaris* L.). *Plant Sci*. **127:** 139-147.

Choudhury, S. and Panda, S.K. (2004). Induction of oxidative stress and ultrastructural changes in moss *Taxithelium nepalense* (Schwaegr.) Broth. under lead and arsenic phytotoxicity. *Curr. Sci*. **87:** 342-348.

Dietz, K.J., Baier, M. and Kramer, U. (1999). Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. In: M.N.V. Prasad and J. Hagemeyer (eds.) Heavy Metal Stress in Plants: From Molecules to Ecosystem, pp. 73-99. Springer-Verlag Berlin.

Fargasova, A. (2001). Phytotoxic effects of Cd, Zn, Pb, Cu and Fe on *Sinapsis alba* L. seedlings and their accumulation in roots and shoots. *Biol. Plant* **44:** 471-473.

Gallego, S.M., Benavioes, M.P. and Tomaro, M.L. (1996). Effect of heavy metal ions on sunflower leaves – Evidence for involvement of oxidative stress. *Plant Sci*. **121:** 151-159.

Giannopolitis, C.N. and Ries, S.K. (1979). Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol*. **59:** 309-314.

Grant, J.J. and Loake, G.J. (2000). Role of reactive oxygen intermediate and cognate redox signaling in disease resistance. *Plant Physiol*. **24:** 21-29.

Griffith, O.W. (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2 – vinylpyridine. *Anal. Biochem*. **106:** 207-221.

Heath, R.L. and Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetic and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys*. **125:** 189-198.

Jiang, W., Lin, D. and Hon, W. (2000). Hyperaccumulation of lead by roots, hypocotyls and shoots of *Brassica juncea*. *Biol. Plant* **43:** 603-606.

Khan, M.H. and Patra, H.K. (2007). Sodium chloride and cadmium induced oxidative stress and antioxidant responses in *Calamus tenuis* leaves. *Indian J. Plant Physiol*. **12:** 34-40.

Mazhoudi, S., Chaoui, A., Ghorsab, M.W. and Ferjani, E.E. (1997). Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum* Mill.). *Plant Sci*. **127:** 129-137.

Noctor, G. and Foyer, C. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physol. Plant Mol. Biol*. **49:** 249-279.

Noctor, G., Arisi, A.M., Jouanin, L., Kunert, K.J., Rennenberg, H. and Foyer, C.H. (1998). Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. *J. Exp. Bot*. **49:** 623-647.

Oser, B.L. (1979). Hawks Physiological Chemistry. McGraw Hill. N.Y. USA.

Panda, S.K. and Khan, M.H. (2003). Antioxidant efficiency in rice (*Oryza sativa* L.) leaves under heavy metal toxicity. *J. Plant Biol*. **30:** 23-29.

Panda, S.K. and Khan, M.H. (2004). Lipid peroxidation and oxidative damage in aquatic duckweed (*Lemna minor* L.) in response to aluminium toxicity. *Indian J. Plant Physiol*. **9:** 176-180.

Panda, S.K. and Patra, H.K. (2000). Does chromium(III) produce oxidative damage in excised wheat leaves? *J. Plant Biol*. **27:** 105-110.

Panda, S.K., Chaudhury, I. and Khan, M.H. (2003). Heavy metal induce lipid peroxidation and affect antioxidants in wheat leaves. *Biol. Plant* **46:** 289-294.

Panda, S.K. (2004) Heavy metal phytotoxicity induces oxidative stress in a moss *Taxithellium sp. Curr. Sci*. **84:** 631-633.

Prasad, K.V.S., Pardha Saradhi, P. and Sharmila, P. (1999), Concerted action of antioxidant enzyme and curtailed growth under zinc toxicity in *Brassica juncea* L. *Env. Expt. Bot*. **42:** 1-10.

Sagisaka, S. (1976) The occurrence of peroxide in a perennial plant *Populas gelrica. Plant Physiol*. **57:** 308-309.

Salt, D.E., Blaylock, M., Kumar, P.B.A.N., Dushenkov, E., Ensley, B.B., Chet, I and Raskin, I. (1995). Phytoremidation: A novel strategy for removal of toxic metals from environment using plants. *Biotechnol*. **13:** 468-474.

Sandalio, L.M., Dalurzo, H.C., Gomez, M., Romero-Puertas, M.C. and del Rio, L.A. (2001). Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot*. **52:** 2115-2126.

Schickler, H. and Caspi, H. (1999). Response of antioxidative enzymess to nickel and cadmium stress in hyperacccumulator plants of the genus *Alyssum*. *Physiol. Plant*. **105:** 39-44.

Schneider, S. and Bergmann, L. (1995). Regulation of glutathione synthesis in suspension cultures of parsley and tobacco. *Bot. Acta*. **108:** 34-40.

Shi, X. and Dalal, N. S. (1989). Chromium (VI) and hydroxyl radical formation during the glutathione reductase-catalyzed reduction of chromium (VI). *Biochem. Biophys. Res.* **163:** 627-634.

Smith, I.K., Vierheller, T.L. and Thorne, C.A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5, 5/ - dithiobis (2-nitrobenzoic acid). *Anal. Biochem*. **175:** 408-413.

Vranova, E., Inze, D. and Van Breusegem, F. (2002). Signal transduction during oxidative stress. *J. Exp. Bot*. **53:** 1227-1236.

Weckx, J.E.J. and Clijsters, H.M.M. (1997). Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. *Plant Physiol. Biochem*. **35:** 405-410.

White, J.G. and Zasoski, R.H. (1999). Mapping soil micronutrients. *Field Crops Res*. **60:** 11-26.

Willekens, H., Chamnongpol, S., Davey, M., Schaudner, M., Langebartels, C., Van Montagu, M, Inze, D. and Van Camp, W. (1997). Catalase is a sink for H_2O_2 and is indespensible for stress defence in C_3 plants. *EMBO Journal* **16:** 4806-4816.

Zeid, L.M. (2001). Responses of *Phaseolus vulgaris* to chromium and cobalt treatments. *Biol. Plant* **44:** 111-115.