Changes in nitrate reductase activity and oxidative stress response in the moss *Polytrichum commune* subjected to chromium, copper and zinc phytotoxicity

Sanjib Kumar Panda [1,2] and Shuvasish Choudhury [2*]

[1]Research Institute for Bioresources, Okayama University, Kurashiki-7100046, Japan; [2]Plant Biochemistry Laboratory, Department of Life Sciences, School of Life Sciences, Assam (Central)University, Silchar-788011, India; [*]Corresponding author: shuvasish_au@indiatimes.com
Received: 21/06/2004, Accepted: 16/05/2005

The main aim of this paper was to investigate the effect of chromium (Cr), copper (Cu) and zinc (Zn) on nitrate reductase (NR) activity and oxidative stress responses in the moss *Polytrichum commune*. Cr, Cu and Zn resulted in the inhibition of NR activity. A decline in total chlorophyll content was observed after 24 and 48 h of metal treatment. Accumulation of the metals showed a dose and time dependent increase. High accumulation of Cu, Cr and Zn were seen in moss shoots after 24 and 48 h of treatment. Treatment of Cr, Cu and Zn for 24 or 48 h resulted in the increase of malondialdehyde (MDA) content in moss shoots. The highest increase was observed in shoots under Cu treatment followed by Cr and Zn. The MDA content was significantly higher after 48h. Antioxidant enzymes viz., catalase (CAT), guaiacol peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) were affected by elevated concentrations of the three metals. Increase in the activity of CAT, GR and SOD was seen after 24 and 48 h of treatment. GPx activity declined under Cr treatment. However, under Cu and Zn, an increase in GPx was seen after 24 h and 48 h of treatment. For Zn, the antioxidant efficiency was less affected as compared to Cr and Cu. The response of *Polytrichum commune* to toxic concentrations of Cr, Cu and Zn appears to induce oxidative damage as observed by the increase in MDA content and antioxidant metabolism.
Key words: antioxidants, chromium, copper, malondialdehyde, nitrate reductase, oxidative stress, *Polytrichum commune*, zinc

Alterações na atividade da redutase do nitrato e respostas ao estresse oxidativo no musgo *Polytrichum commune* sujeito ao tratamento fitotóxico com cromo, cobre e zinco: O principal objetivo deste trabalho foi investigar o efeito do cromo (Cr), cobre (Cu) e zinco (Zn) sobre a atividade da redutase do nitrato (RN) e respostas ao estresse oxidativo no musgo *Polytrichum commune*. Cr, Cu e Zn causaram a inibição da atividade da RN. Uma diminuição na clorofila total foi observada após 24 e 48 h dos tratamentos com os metais. O acúmulo de metais mostrou ser dependente do aumento da dose e tempo. Observou-se grande acúmulo de Cu, Cr e Zn nos brotos depois de 24 e 48 h dos tratamentos. Aqueles com Cr, Cu e Zn por 24 ou 48 h resultou no aumento do conteúdo de malondialdeído (MDA) nos brotos. Verificou o maior aumento nos brotos tratados com Cu, seguidos por Cr e Zn. O conteúdo de MDA foi significamente maior após 48 h. Enzimas antioxidantes, como catalase (CAT), peroxidase do guaiacol (GPx), redutase da glutationa (GR) e dismutase do superóxido (SOD) foram afetadas por elevadas concentrações dos três metais. Aumentos nas atividades de CAT, GR e SOD foram observados após 24 e 48 h dos tratamentos. A atividade de GPx diminuiu com o tratamento de Cr, porém, com Cu e Zn, observou-se aumento de GPx após 24 h e 48 h do tratamento. Para o Zn, a eficiência antioxidante foi menos afetada quando comparada ao Cr e Cu. A resposta de *Polytrichum commune* a concentrações tóxicas de Cr, Cu e Zn parece ser induzida pelo dano oxidativo quando observado através do aumento do conteúdo de MDA e metabolismo antioxidante.
Palavras-chave: antioxidantes, estresse oxidativo, cobre, cromo, malondialdeído, redutase do nitrato, zinco.

INTRODUCTION

Plants need relatively small amounts of metals for their growth and soils harbor these metal ions either naturally or as a consequence of contamination. Soil contamination with heavy metals is now a worldwide problem, leading to agricultural losses and hazardous health effects as metals enter the food chain (Nellessen and Fletcher, 1993; Guo and Marschner, 1995; Salt et al., 1995). Copper, zinc and chromium are three broadline heavy metals that are phytotoxic above certain threshold levels (Neiborer and Richardson, 1980). Copper, which is relatively mild in character, is highly toxic to plants even at a micromolar range of exposure (Carbal, 2003). Copper can inhibit root elongation, block the photosynthetic electron transport chain and degrade chlorophyll (Quartacci et al., 2001; Patsikka et al., 2002). Copper can substitute co-factors of various enzymes and in turn degrade their activities (Nieboer and Richardson, 1980; Murphy et al., 1991; Quartacci et al., 2001). Copper can degrade the phospholipid structure and thereby alter the membrane structure and function (Quartacci et al., 2001). Chromium is an important polluting heavy metals that can induce severe damage to bacterial, plant and animal life. Zinc is one of the micronutrients essential for plant growth but is toxic to plants at higher concentrations and can retard plant growth and disrupt various essential physiological processes (Cakmak and Marschner, 1993; Chaney, 1993; Bhattacharjee and Mukherjee, 1994; Prasad et al., 1999; Panda et al., 2003). Toxic concentrations of zinc can retard growth and can initiate lipid peroxidation in plants (Prasad et al., 1999; Panda et al., 2003). In nature, chromium exists in two different forms, Cr (III) and Cr (VI) of which Cr (VI) is more toxic than Cr (III). Cr can induce phytotoxic symptoms in plants like morphological changes (Bassi et al., 1990), proline accumulation and alterations in antioxidant metabolism (Panda and Patra, 2000; Panda, 2003; Panda et al., 2003).

The presence of various reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), etc., generated as a result of heavy metals can cause severe oxidative damage to biomolecules like lipids, proteins and nucleic acids (Alia et al., 1995; Gille and Singler, 1995). The presence of high concentrations of these ROS can thus disrupt the normal physiological and cellular functions (Asada, 1994; Gille and Singler, 1995). The presence of heavy metals in toxic concentrations can result in the formation of ROS, which can be initiated directly or indirectly by heavy metals. To counter the deleterious effects of the ROS, plants have evolved various enzymic and non-enzymic antioxidant systems, which can protect the plants from the toxic action of various ROS. The activities of these antioxidants are induced as a response to adverse biotic and abiotic stresses that can be considered as a general response to adverse conditions (Foyer et al., 1994). Cr, Cu and Zn are known to induce ROS in cells. In comparison to Zn, Cr has much higher catalytic activity in the Fenton reaction system but lower than Cu (see Panda and Choudhury, 2005 and references therein). However, this phenomenon is not well understood for Cr (VI). As a common consequence of ROS formation antioxidant metabolism is affected. Cr, Cu and Zn can induce the activity of various antioxidant enzymes like CAT, GPx, SOD and GR and also non-enzymes like ascorbate and glutathione (Prasad et al., 1999; Panda, 2003; Panda et al., 2003; Choudhury and Panda, 2004a). Unlike Cu and Zn, Cr cannot induce phytochelatin (PC) (Sanita di Toppi et al., 2004) and the defense mechanisms adopted by lower plants like mosses are less understood. However, metallothioneins (MTs) have a possible role in Cr detoxification in plants (Sanker et al., 2004; Panda and Choudhury, 2005). MTs in relation to Cr detoxification in plants have been investigated in sorghum where MT-like proteins are expressed under Cr (VI) stress (Sanker et al., 2004). A role of PC in counteracting metal toxicity has been reported in lichens *Xanthoria parietina*, *Physcia adscedens* (Fr.) H Oliver and *Physconia grisea* (Lam.) Poelt. (Pawlik-Skoworonska et al., 2002).

Interactions between bryophytes and heavy metals have been studied in many previous investigations (Sidhu and Brown, 1996; Bassile et al., 2001; Saxena et al., 2003; Choudhury and Panda 2004a; Panda and Choudhury 2005). Bryophytes have been frequently used as biomonitors of heavy metal pollution in many field studies (Tyler, 1990; Bargagli, 1998). They are especially suitable for the accumulation of heavy metals in view of their high surface-to-volume ratio and limited cuticle development (Brown, 1984). Beside this, they have high countergradient mechanisms for the accumulation of heavy metals in their tissue (Carginale et al., 2004). The mechanism of oxidative damage induced by heavy metals has been thoroughly studied in higher plants compared to that in bryophytes. However, there are very few studies dealing with oxidative stress in bryophytes (Panda 2003; Choudhury and Panda, 2004). In the present investigation we investigate the effect of Cr, Cu and Zn on nitrate reducatse activity (NR), malondialdehyde (MDA) content and antioxidant response in moss *Polytrichum commune*. This investigation will highlight a better

understanding of the mechanisms adopted by this moss species in response to toxic concentration of heavy metals. The study could be useful in determining oxidative stress parameters as toxicity bioindicators in mosses.

MATERIAL AND METHODS

Fresh samples of the moss *Polytrichum commune* were collected during the month of July 2002 and brought to the laboratory in clean polythene bags. Samples were cleaned in running tap water followed by three rinses in sterile distilled water. Shoots were placed in petri dishes with different concentrations of Cr, Cu and Zn (0, 0.01, 0.1 and 1 mM). The dishes were kept inside a growth chamber under continuous white light (52 µmoles.s^{-1}.m^{-2}, PAR) at 22 $\pm$ 2^{0}C. Shoots were harvested after 24 or 48 h of metal treatment for analysis.

Nitrate reductase (NR) activity was measured according to Srivastava (1980). Total chlorophyll content was measured spectrometrically by the method of Arnon (1949). The total Cr, Cu and Zn content in the shoot was estimated by drying known amounts of the shoot for 48 h at 70^{0}C. The dried shoots were digested with 5 mL concentrated nitric acid (HNO_3) at 100^{0}C until the solution turned clear. The total sample volume was adjusted to 20 mL with distilled water. The amount of Cr, Cu and Zn was then measured using an atomic absorption spectrometer (Perkin-Elmer, 3110, Germany).

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) produced using the thiobarbituric (TBA) reaction as suggested by Heath and Packer (1968). 200 mg of the moss shoot was homogenized with 2 mL of 0.1 % trichloroacetic acid (TCA) and centrifuged at 10,000 g_n for 20 min. To 1 mL of the supernatant extract, 1 mL of 20 % TCA containing 0.5 % TBA and 0.001 mL butylated hydroxyl toluene (BHT) (a 4 % solution in ethanol) were added. The mixture was heated at 95^{0}C for 30 min and centrifuged at 10,000 g_n for 15 min. The absorbance was recorded at 532 nm and corrected by measurements at 600 nm.

The moss tissues were homogenized in 0.1 M phosphate buffer (pH 6.8) in a pre-chilled mortar and pestle. The extract was centrifuged at 4^{0}C at 12,000 g_n for 15 min. The supernatant was used for the assay of Catalase (CAT) [EC 1.11.1.6], guaiacol peroxidase (GPX) [EC 1.11.1.7], superoxide dismutase (SOD) [EC 1.15.1.1] and glutathione reductase (GR) [EC 1.6.4.2]. GPX activity was assayed according to Chance and Maehly (1955). The 3 mL reaction mixture of GPX contained 0.1 M phosphate buffer (pH 6.8), guaiacol (30 mM), H_2O_2 (30 mM) and 0.3 ml of the enzyme extract. The rate of absorbance change was measured at 420 nm. The assay of SOD was carried out according to Giannopoltis and Ries (1977). The 3 mL assay mixture comprised of 79.2 mM EDTA, 10.8 mM tetraethylene diamine, 0.0033 % bovine serine albumin, 6 mM nitroblue tetrazolium (NBT), 600 µM riboflavin and 0.2 mL enzyme extract. The reaction was initiated by placing the glass tubes between fluorescent bulbs (Philips 20W). By switching the light on and off, the reaction was started and terminated respectively. The increase in absorbance due to formazan formation was read at 560 nm. GR was estimated according to Smith et al. (1988). The reaction mixture contained 0.2 M phosphate buffer (pH 7.5) together with 1 mM EDTA, 3 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) in 0.01 M potassium phosphate buffer (pH 7.5), 2 mM NADPH, 1 mL enzyme extract and distilled water to a final volume of 2.9 mL. The reaction was initiated by adding 2 mM oxidized glutathione or glutathione disulphide (GSSG). The increase in absorbance was measured at 412 nm at 25^{0}C.

The data presented are the mean of three separate experiments $\pm$ SEM.

RESULTS

Figure 1 shows the effect of Cr, Cu and Zn on total chlorophyll content and NR activity in shoots of *P. commune* after 24 or 48 h of treatment. NR activity was significantly reduced under treatment with Cu, Cr and Zn. After 24 h, NR activity was reduced by 17.31 %, 30.72 % and 45 % compared to the control at 1, 10 and 100 mM of Cr. Under Cu treatment, the inhibition was more pronounced. At 1, 10 and 100 mM the NR activity reduced by 21.5 %, 36 % and 46 % respectively with respect to the controls. Under Zn treatment NR activity was reduced by 11 %, 19 % and 21 % with respect to controls at 1, 10 and 100 mM respectively. The inhibitory effect was significantly higher after 48 h. The highest inhibition of NR activity was observed for Cu followed by Cr and Zn. The total chlorophyll content was significantly affected by metal treatment. A decline in chlorophyll content was highest for Cu, followed by Cr and Zn. The effect of both Cu and Cr on the total chlorophyll content was pronounced after 24 h. However, moss shoots under Zn treatment did not shown any significant decline at 1 and 10 mM after 24 h. After 48 h of treatment, a significant decline in total chlorophyll content was observed in moss shoots under Cu, Cr and Zn treatment. Treatment with Zn for 48 h showed the least effect on the total chlorophyll content.

Cr, Cu and Zn accumulation in moss shoots after 24 or 48 h is shown in figure 2. Highest concentrations in Cr, Cu

and Zn were observed at the 10 and 100 mM treatments after 48 h. The difference in the accumulation pattern for the three metals reflected the metal specificity of the moss. Unlike Cu and Zn, no Cr could be detected in control moss shoots.

The variation in MDA content under Cr, Cu and Zn treatment for 24 or 48 h in the moss shoot are shown in figure 3. MDA concentration was significantly higher under Cu. After 24 h, the MDA content increased by 227 %, 323 % and 518 % respectively at 1, 10 and 100 mM with respect to controls. Much higher levels of MDA were observed after 48 h, where about 328 %, 500 % and 698 % increases were observed respectively at 1, 10 and 100 mM of Cr as compared to the controls. For Cu the MDA concentration was significantly higher and increased with concentration and treatment duration. However, under Zn treatment, the MDA content increased by 102 %, 288 % and 302 % respectively at 1, 10 and 100 mM with respect to the controls. The increment in MDA content was much higher for Cr and Cu-treated moss shoots. The increase in MDA content under the metal treatment can be correlated with an increase in treatment concentration and duration of treatment of the metals.

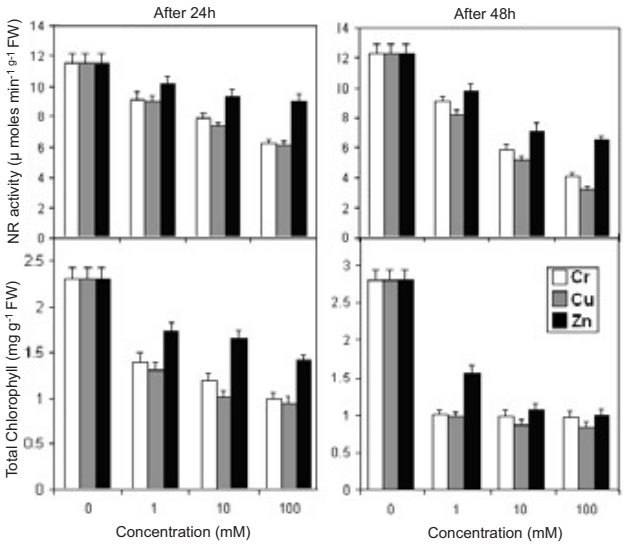

Figure 1. Effect of Cr, Cu and Zn on nitrate reductase activity and total chlorophyll in shoots of the moss *Polytrichum commune* after 24 and 48 h of treatment. The data represent the mean of three separate experiments, ± SE.

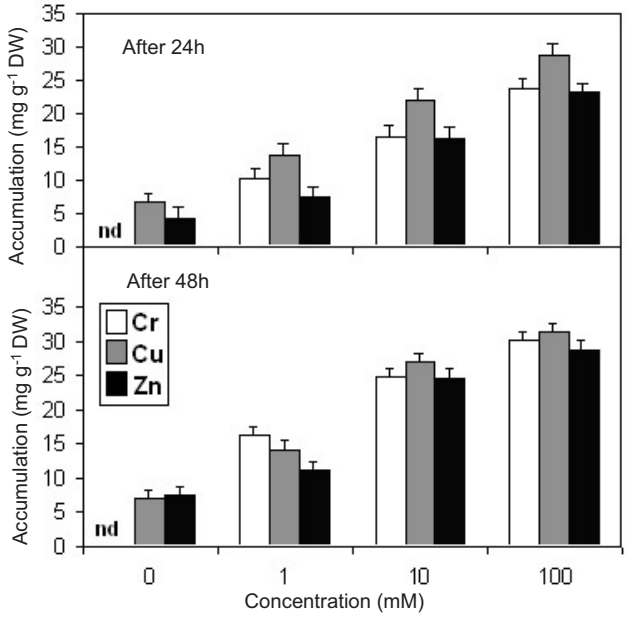

Figure 2. Accumulation of Cr, Cu and Zn in shoots of the moss *Polytrichum commune* after 24 and 48 h of treatment. Legend as in figure 1. (nd: not detectable).

The changes in the antioxidant enzymes catalase (CAT), guaiacol peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) after 24 or 48 h of treatment is shown in figure 4. The CAT activity increased upon metal treatment. CAT is an important enzyme involved in hydrogen peroxide (H_2O_2) detoxification in plants. The increase in CAT activity was consistent both after 24 and 48 h of Cr treatment. Under Cu and Zn a similar trend was observed in CAT activity. There were differences in GPx activity in moss shoots under metal treatment. For Cr, GPx showed increased activity after 24h, but declined after 48 h of treatment. This

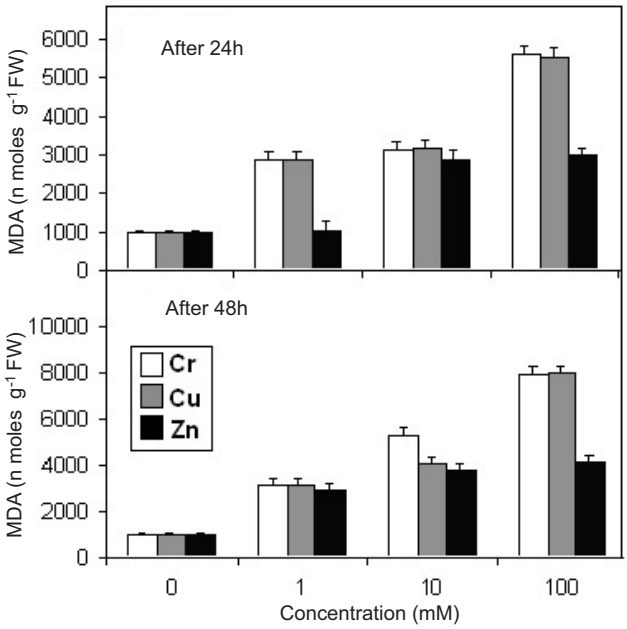

Figure 3. Changes in malondialdehyde (MDA) content in shoots of the moss Figure 3. *Polytrichum commune* after 24 h and 48 h of Cr, Cu and Zn treatment. Legend as in figure 1.

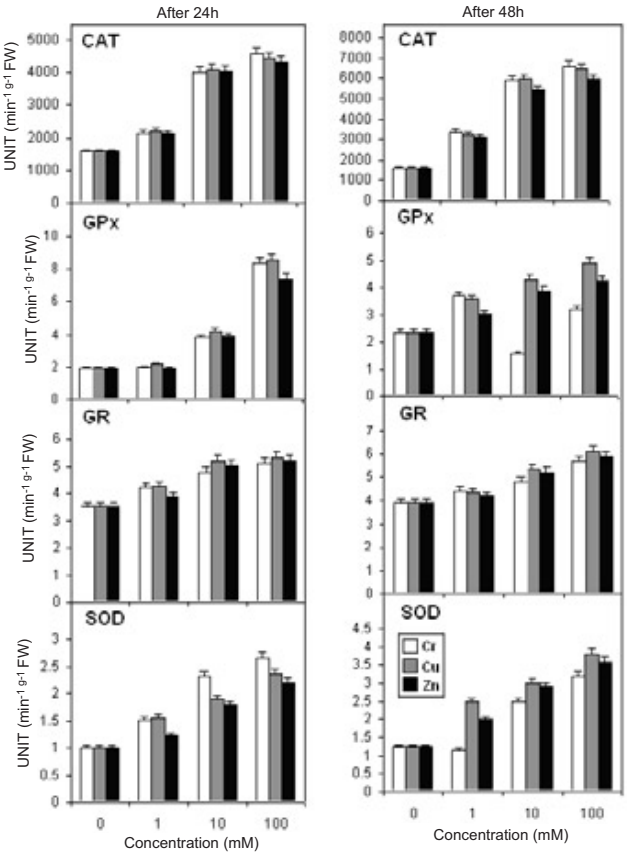

Figure 4. Changes in the activities of antioxidant enzymes CAT, GPx, GR and SOD in shoots of the moss *Polytrichum commune* after 24 and 48 h of Cr, Cu and Zn treatment Legend as in figure 1.

was completely different for Cu and Zn-treated moss shoots, where an increase in GPx activity was recorded after 24 and 48 h of treatment. Increase in GR and SOD activity was seen in moss shoots after 24 and 48 h of treatment.

DISCUSSION

We observed that elevated concentrations of Cr, Cu and Zn can induce oxidative stress in moss *P. commune*. The effect of the metals on NR activity was inhibitory. The inhibition of NR activity by Cr, Cu and Zn affected the ammonia assimilation pathway. Panda and Patra (1998) reported increases in NR activity under Cr treatment in wheat seedlings. However, this was only possible in wheat seedlings when nitrate and ammonium ions were supplemented in the growth media containing the lower (0.001 mM) concentration of Cr. The lower Cr concentration containing nitrogen supplement in the medium as reported by Panda and Patra (1998) in wheat might have helped in *de novo* synthesis of NR isoenzymes. For high concentrations of metals like Cr, Cu and Zn the SH-group of the NR enzyme is affected resulting in a decline in its activity. Inhibition of NR activity has been reported for higher plants under Zn and Pb toxicity (Mathys, 1975; Jain and Garde, 1997; Luna et al., 2000).

As shown in figure 1, the total chlorophyll content declined under metal treatment. A decline in total chlorophyll content has been reported in many plants under heavy metal stress (Panda and Patra, 1998; Panda et al., 2003; Choudhury and Panda, 2004a). Chlorophyll pigments are one of the main sites of heavy metal injury in plants. A significant decline as observed for Cr and Cu reflects the inhibitory effect of these metals on pigment biosynthesis which might be a metal specific action. Zn was found to be the least effective in degrading chlorophyll biosynthesis. Degradation of chlorophyll content has been previously reported in the case of Cr, Cu and Zn (McGrath, 1982; Panda and Patra, 1998; Vajpayee et al., 2000; Panda et al., 2003).

It is well established that bryophytes are hyperaccu-mulators of heavy metals (Tyler, 1991; Bargagli, 1998). Increases in accumulation of Cr, Cu and Zn were seen in moss shoots after 24 and 48 h of treatment. The accumulation of these metals in shoots occurred in a time and dose dependent manner. Mosses have high countergradient mechanisms by which they accumulate significant concentrations of metals in their tissues (Cargainale et al., 2004). The high metal accumulating capacity is also attributed to a high surface-to-volume ratio (Brown, 1984). Similar accumulation of heavy metals has been reported for other mosses and liverworts (Brown, 1984; Sidhu and Brown, 1996; Choudhury and Panda, 2004a).

The elevation in MDA content in moss shoot clearly reflects cell wall damage. MDA is a product of lipid peroxidation induced by ROS. Lipid peroxidation induced by ROS has been reported in plants under heavy metal stress. Participation of Cr in ROS generation, unlike Cu, is well understood. It is believed that the Cr ion in its trivalent and divalent oxidation states can enter the Fenton reaction (Strile et al., 2003; Panda and Choudhury, 2005). However, for the hexavalent form of Cr the mechanism of ROS generation is not well understood. The increase in MDA content reflects oxidative stress in the moss. Similar observations were reported in plants under heavy metals like Cr, Cu, Zn, Pb, As and Cd (Panda et al., 2003; Choudhury and Panda, 2004a,b; Cuny et al., 2004; Panda, 2003; Sanita di Toppi et al., 2004)

The response of the antioxidant enzymes viz. CAT, GPx, GR and SOD in moss shoot under Cr, Cu and Zn treatment followed a similar pattern. CAT, GR and SOD activity in-

creased upon metal treatment while GPx activity increased after 24 h of Cr treatment but declined after 48h. However, for Cu and Zn, an increase in GPx activity was seen after 24 or 48 h of treatment. CAT and GPx are important enzymes involved in the detoxification of hydrogen peroxide (Scandalios, 1993). The variation in the activity of GPx under Cr, Cu and Zn revealed a metal specific response of the enzyme in moss shoot. GR is involved in detoxification of hydrogen peroxide in the chloroplast and mitochondria (Foyer and Halliwell, 1976). Increase in GR activity along with CAT and GPx in the moss shoot reflected the constant detoxification of hydrogen peroxide. Such a response was reported for *Oryza sativa*, *Brassica juncea* under Zn treatment (Prasad et al., 1999). However, Panda (2003) and Choudhury and Panda (2004a,b) reported inhibition of CAT, GPx and GR activities in the moss *Taxithelium nepalense* (Schwaegr.) Broth. and in *O. sativa* L. roots under Cr, Zn, Pb, As, Cd, Cr and Cu toxicity. The SOD activity was higher in metal-treated shoots. SOD is an important enzyme involved in scavenging the superoxide radical (Panda, 2002). The increase in SOD activity under metal treatments indicates the constant detoxification of the superoxide radical that might have been generated. Increase in SOD activity was reported earlier in the moss *T. nepalense* under Pb, Cr, Zn, Cu and As toxicity and also in the lichen *Diploschistes muscorum* (Scop.) R. Sant. under Zn, Pb and Cd toxicity (Cuny et al., 2004). However, these antioxidant enzymes have been little studied in lower plants like mosses and lichens (Panda, 2003; Choudhury and Panda, 2004; Cuny et al., 2004; Sanita do Toppi et al., 2004). The antioxidant metabolism in moss shoots indicated constant detoxification of ROS under Cr, Cu and Zn stress.

In conclusion, the investigation highlighted the involvement of Cr, Cu and Zn in inducing oxidative stress in the moss *P. commune* reflected by the physiological disturbances induced by the heavy metals. The physiological disturbances like increase in MDA content, decline in total chlorophyll content and reduction in NR activity clearly indicated the toxic effects of the metal. However, activation of the antioxidant metabolism under metal stress reflected constant detoxification of ROS in moss cells. Thus, oxidative stress parameters can be considered as useful biomarkers of environmental pollution.

REFERENCES

Alia, Prasad KVSK, Pardha Saradhi P (1995) Effect of zinc on free-radicals and proline in *Brassica juncea* and *Cajanus cajan*. Phytochemistry 39:45-47.

Arnon DI (1949) Copper enzyme in isolated chloroplast, polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.

Asada K (1994) Production and action of active oxygen species in photosynthetic tissue. In: Foyer CH, Mullmeaux PM (eds), Causes of Photooxidative Stress and Amelioration of Defense System in Plants, pp.77-107. CRC Press, Boca Raton, FL.

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

Basile A, Cogoni AE, Bassi P, Fabriz E, Sorbo S, Giordano S, Castaldo Cobianchi R (2001) Accumulation of Pb and Zn in gametophyte and sporophyte growth of the moss *Funaria hygrometrica* (Funariales). Ann. Bot. 87:537-543.

Bassi M, Grazia Corradi M, Realini M (1990) Effect of Cr (VI) on two fresh water plants *Lemna minor* and *Pistia stratores*, 1. Morphological observation. Cytobios 62:27-38.

Bhattacharjee S, Mukherjee AK (1994) Influence of cadmium and lead on physiological and biochemical response of *Vigna unguiculata* (L.) Walp. Seedlings. I. Germination behaviour, total protein, proline and protease activity. Pollut. Res. 13:269-277.

Brown DH (1984) Uptake of mineral elements and their use in pollution monitoring. In: Dyer AF, Duckett JG (eds), The Experimental Biology of Bryophytes, pp.229-255. Academic Press, NY.

Cabral JP (2003) Copper toxicity in five *Parmelia* lichens in vitro. Environ. Exp. Bot. 49:237-250.

Cakmak I, Marschner H (1993) Effect of zinc nutritional status on superoxide radical and hydrogen peroxide scavenging enzymes in bean leaves. In: Barrow NJ (ed), Plant Nutrition - from Genetic Engineering to Field Practice, pp.133-137. Kluwer Academic Publishers, Dordercht.

Carginale V, Sorbo S, Capasso C, Trinchella F, Cafiero G, Basile A (2004) Accumulation, localization and toxic effects of cadmium in the liverwort *Lunularia cruciata*. Protoplasma 223:53-61.

Chance B, Maehly AC (1955) Assay of catalase and peroxidase. Meth. Enzymol. 2:746-778.

Chaney RL (1993) Zinc phytotoxicity. In: Robson AD (ed), Zinc in Soil and Plants, pp.135-150. Kluwer Academic Publishers, Dordercht.

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

Choudhury S, Panda SK (2004b) Membrane deterioration and biochemical lesions in *Oryza sativa* L. under cadmium toxicity. In: Borah RC, Talukdar A, Kataky JCS, Unni BG, Modi MK, Deka PC (eds), Bioprospecting of Commercially Important Plants (Proceedings National Symposium, Indian Society of Agricultural Biochemists, Jorhat Chapter, 2003), pp.224-229. Assam Agricultural University and Indian Society od Agricultural Biochemists, Jorhat, India.

Cuny D, Van Haluwyn C, Shirali P, Zerimech F, Jerome L, Haguenoer JM (2004) Cellular impact of metal trace

elements in terricolous lichen *Diploschistes muscorum* (Scop.) R. Sant.- Identification of oxidative stress biomarker. Water Air Soil Pollut. 152:55-69.

Foyer C, Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplast: a proposed system for ascorbic metabolism. Planta 133:21-25.

Foyer CH, Lelandies M, Kunert KJ (1994) Photooxidative stress in plants. Physiol. Plant. 92:696-717.

Giannopololitis CN, Ries SK (1977) Superoxide dismutase. I. Occurrence in higher plants. Plant Physiol. 59:309-314.

Gille G, Sigler K (1995) Oxidative stress and living cells. Folia Microbiol. 40:131-152.

Guo Y, Marschner, H (1995) Uptake, distribution and binding of cadmium and nickel in different plant species. J. Plant Nutr. 18:2691-2706.

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplast, I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125:189-198.

Jain M, Gadre RP (1997) Inhibition of nitrate reductase activity by lead in greening bean leaf segments, a mechanistic approach. Ind. J. Plant Physiol. 2:5-9.

Luna CM, Casano LM, Trippi VS (2000) Nitrate reductase is inhibited in leaves of *Triticum aestivum* treated with high level of copper. Physiol. Plant. 101:103-108.

Mathys TM (1975) Enzymes of heavy metals resistant and non-resistant populations of *Silene cucubalus* and their interaction with some heavy metals *in vitro* and *in vivo*. Physiol. Plant. 33:161-165.

McGrath SP (1982) The uptake and translocation of trivalent and hexavalent chromium and effects on the growth of oat in flowering nutrient solution and in soil. New Phytol. 92: 381-390.

Murphy AS, Eisingter WR, Shaff JE, Kochian LV, Taiz L (1999) Early copper induced leakage from *Arabidopsis* seedlings is mediated by iron channels and coupled to citrate efflux. Plant Physiol. 121:1375-1382.

Nellessen H, Fletcher JS (1993) Assessment of published literature on uptake, accumulation and translocation of heavy metals in vascular plants. Chemosphere 9:1669-1680.

Nieboer E, Richardson DHS (1980) The replacement of non-descript term "heavy metals" by a biologically and chemically significant classification of metal ions. Environ. Pollut. 1:3-26.

Panda SK, Choudhury S (2005) Chromium stress in plants. Braz. J. Plant Physiol. 17:131-136.

Panda SK (2003) Heavy metal phytotoxicity induces oxidative stress in a moss, *Taxithelium* sp. Curr. Sci. 84:631-663.

Panda SK, Chaudhury I, Khan MH (2003) Heavy metals induce lipid peroxidation and affect antioxidants in wheat leaves. Biol. Plant. 46:289-294.

Panda SK (2002) The biology of oxidative stress in green cells: a review. In: Panda SK (ed), Advances in Stress Physiology of Plants, pp.1-13. Scientific Publishers, India.

Panda SK, Patra HK (1998) Role of nitrate and ammonium ions on chromium toxicity in developing wheat seedlings. Proc. Nat. Acad. Sci. India 70:75-80.

Panda SK, Patra HK (2000) Does chromium (III) produce oxidative damage in excised wheat leaves. J. Plant Biol. 27:105-110.

Patsikka E, Kairavuo M, Sersen F, Aro E-M, Tyystjarvi E (2002) Excess copper predisposes photosystem II to photoinhibition in vivo by outcompeting iron and causing decrease in leaf chlorophyll. Plant Physiol. 129:1359-1367.

Pawlik-Skowronska B, Sanita di Toppi L, Favali MA, Fossati F, Pirszel J, Skowronski T (2002) Lichen respond to heavy metals by phytochelatin synthesis. New Phytol. 156:95-102.

Prasad KVSK, Pardha Saradhi P, Sharmila P (1999) Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. Environ. Exp. Bot. 42: 1-10.

Quartacci MF, Cosi E, Navari-Izzo F (2001) Lipids and NADPH dependent superoxide production in plasma membrane vesicle from roots of wheat grown under copper deficiency or excess. J. Exp. Bot. 52:77-84.

Salt DE, Smith RD, Raskin I (1995) Phytoremedation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:643-668.

Sanita di Toppi L, Musetti R, Marabottini R, Corradi MG, Vattuone Z, Augusta M, Badiani M (2004) Responses of *Xanthoria parietina* thalli to environmentally relevant concentrations of hexavalent chromium. Func. Plant Biol. 31:329-338.

Saxena A, Saxena DK, Srivastava HS (2003) The influence of glutathione on physiological effects of lead and its accumulation in moss *Sphagnum squarrosum*. Water Air Soil Pollut. 143:351-361.

Scandalios JG (1993) Oxygen stress and superoxide dismutase. Plant Physiol. 101:7-12.

Shanker AK, Djanaguiraman M, Sudhagar R, Jayram K, Pathmanabhan G (2004) Expression of metallothioneins 3-like protein mRNA in sorghum cultivars under chromium (VI) stress. Curr. Sci. 86:901-902.

Sidhu M, Brown DH (1996) A new laboratory technique for studying the effect of heavy metals on bryophyte growth. Ann. Bot. 78:711-717.

Smith IK, Vierhgeller TL, Throne CA (1988) Assay of glutathione reductase in crude tissue homogenate using 5, 5'-dithiobis (2-nitrobenzoic) acid. Anal. Biochem. 175: 408-413.

Srivastava HS (1980) Regulation of nitrate reductase activity in higher plants. Phytochem. 19:725-733.

Strile M, Kolar J, Selih VS, Kocar D, Pihlar B (2003) A comparative study of several transition metals in Fenton like reaction system at circum-neutral pH. Acta Chim. Slov. 50:619-632.

Tyler G (1990) Bryophytes and heavy metals: a literature review. Bot. J. Linn. Soc. 104:231-253.

Vajpayee P, Tripati RD, Rai UN, Ali MB, Singh SN (2000) Chromium accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content of *Nymphaea alba*. Chemosphere 41:1075-1082.