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Effect of Cu and Cd on Oxidative Enzymes and Chlorophyll Content of Moss *Racomitrium crispulum*

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ABSTRACT: Present study was carried out to evaluate antioxidant, photosynthetic and productivity of moss *Racomitrium crispulum* (Hook. f. et Wils.) Hook. f. et Wils. under various phytotoxic concentration of metals Cu and Cd for different days. Exogenous supplied Cu and Cd to *R. crispulum* significantly give stress on oxidative enzymes as well as on photosynthesis. After 15 days of treatment, the maximum decrease in NRA were 27% and 47% at 0.2 M concentration both in Cu and Cd treated moss is concomitant with the decrease in percent nitrogen and protein of the respective samples. A significant degradation of chlorophyll was 28% at 0.1 M Cd after 15 days. Increase in carotenoid content was noticed at low concentration of metals (0.01 M) attributed to photoprotective role of them. Carbohydrate content decreases 54% and 57% of Cu and Cd treated moss after 15 days showed their deleterious effect only after prolong period of incubation. Total sugar content is in inverse of carbohydrate to compensate the moss physiology. Peroxidase activity increased with the increase in treatment concentration, while it decreases with respect to increase in exposure time. The maximum peroxidase activity was observed is 11% at 10 mM treatment in 6 days Cu treated sample. The increase in SOD activity was observed under higher concentration and also concomitant with respect to increase in incubation period under both metal (Cu and Cd). Our findings and those of other cited herein suggested that under mild to moderate metal (Cu and Cd) stress for prolong period give inductive effect and also on photosynthates of moss *R. crispulum*. This further suggests that under every possible alteration, cell maintains their integrity and homeostasis by shuffling their metabolic and physiological responses. Present finding recommends that *R. crispulum* of Kumaon hills (India) is tolerant to Cu and Cd metal pollution and can be use as bioaccumulators for these two metals.

KEY WORDS: Phytotoxic metals Cu and Cd, Moss *Racomitrium crispulum*, Oxidative enzymes, Photsynthates, Productivity response.

INTRODUCTION

Alteration in environmental condition can be assessed by change in physiological responses of community. Plants having tolerance potential can fight to maintain the cell integrity and homeostasis. Heavy metals constitute an environmental pollutant with toxicity to biota. Non biodegradable nature and high density of metals make them distinguished from other toxic pollutants to accumulate in living tissue. Since the industrialization and improper control measure of pollution make it serious threat to induct global warming. Certain metals at low concentrations act as an essential element for their metabolism and their high doses are toxic (Wu et al., 2009). The mechanism of metal action at the physiological and biochemical levels were reviewed and studied by several workers (Saxena and Kaur, 2005; Saxena and Arfeen, 2006a, b). Heavy metal like Cu and Cd can modify the rate of plant development (Saxena and Kaur, 2005).

Environmental stress such as metal shows direct response on Nitrate reductase (NR) activity, oxidative responding enzyme Super oxide dismutase (SOD) and Peroxidase (Munné-Bosch and Alegre, 2002). Never the less Nitrate reductase activity (NRA) is an indicator enzyme of nitrogen assimilation pathway and gives direct response of plant growth by giving inductive effect on nitrogen and protein synthesis (Ali et al., 2007). Chlorophyll and carotenoid degradation is the routinely observed response to stress or chiefly in elevated concentrations of various heavy metals (Chen and Djuric, 2001). Thus, changes in chlorophyll and carotenoid content and pigment ratios are important indicators of environmental stress and describes about the tolerance status of the species (Tuba et al., 1996).

The damage caused by reactive oxygen species (ROS) is known as oxidative stress. In response, plants have developed defense systems via non-enzymatic and enzymatic scavenging of cellular ROS to cope with oxidative stress (Okamoto et al., 2001; Pinto et al., 2003). These ROS can have significant role in damaging biomolecule of cell wall and plasma membrane. Since, plant have evolved detoxifying mechanisms (Chaoui et al., 1997) where by SOD, dismutate these superoxides and H_2O_2 and molecular oxygen; insignificantly by Fenton reaction it is further degraded into very reactive hydroxyl radical ($OH^{\cdot\cdot}$). These reactive oxygen species shows maximum reaction velocity (V_{max}) in hydrophobic atmosphere rather than in hydrophilic system. Peroxidase may, play a major role scavenging H_2O_2 to prevent ROS mediated damages.

There is a strong evidence for detection and investigation of environmental element contamination, cryptogams (mosses) respond more sensitive than other plant species (Glime and Saxena, 1991; Saxena et al., 2008; Sun et al., 2009). Only bryophytes have tendency to grow in any kind of habitat as they have evolutionary background to adapt environmental stress. However, very little is known about physiological responses and oxidative stress damages in bryophytes under metal pollution (Panda, 2003). In present study an attempt is made to correlate the different enzymatic responses of a moss *Racomitrium crispulum* (Hook. f. et Wils.) to Cu and Cd.

MATERIALS AND METHODS

Sample of moss *Racomitrium crispulum* (Hook. f. et Wils.) were collected in the month of December (winter season) from a uniform area of at least 50 cm^2 to avoid intraspecific variability from Mukteswar (Kumaon hill). Samples were carefully cleaned of all dead material and attached litter and finally washed with running tap water to remove soil and adhering dust particles and were then washed in distilled water. Only green or green-brown shoots were transferred to Petri plates containing various concentrations (0.01, 0.1, 0.2 M) of $CuSO_4$ and $CdCl_2$. The objective of taking higher doses of metals is that tolerant moss has capability to accumulate metals at very high level (Sun et al., 2009). The plates were transferred to Biological oxygen Demand (B.O.D.) incubator under continuous white light by two fluorescent lamps (Philips 20 W TLD. India) with a photon flux density of 52 μE m^{-2} S^{-1} and kept at 22 ± 2°C. Assay was carried out after 3, 6 and 15 days of incubation. Plant material was taken out for various physiological experiments. No nutrient medium was provided exogenously as bryophytes have unique property to intake directly from their surrounding air (Glime and Saxena, 1991).

Photosynthetic pigments were estimated according to the method of Arnon (1949) by extracting in 80 % acetone in liquid nitrogen. Guaiacol peroxidase and *in vivo* NR activity were estimated according to Putter (1974) and Srivastava (1975) respectively. Total nitrogen was estimated by Micro-Kjeldahl method (Jackson, 1962) while, protein was measured by Lowry et al., (1951) method. To determine the dry matter, the plants were dried in oven at 70 ⁰C for 2 days. The dried samples were then weighed to determine the plant dry matter with respect to 0.5 g of fresh weight. Total carbohydrate was estimated by the method of Hedge and Hofreiter (1962). The total soluble sugar was determined by the method of Loewus (1952) with modifications, Cerning and Beroard (1975). Proline content and SOD activity was measured by using the methods of Bates et al., (1973) and Beauchamp, (1971) respectively. All the statistical analysis was performed by using software Statistical Package for the Social Science (SPSS 14.0) (Illinois, USA) for windows program. All data represents mean of three separate experiments ± standard error (n = 3). The data were analyzed by student's t-test at $P \leq 0.05$ significance level.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the of moss *Racomitrium crispulum* revealed the presence of various antioxidative enzyme along with photosynthetic response were the most prominent and the result of physiological test has been summarized in the Figures one to four. The phytotoxicity of metals on moss *R. crispulum* is depend on both heavy metal concentration (0.01 M, 0.1 M and 0.2 M) and as well as the exposure period (i.e. 3, 6 and 15 days).

Nitrate reductase activity

Maximum decrease in NR activity (NRA) after 15 days was significantly ($p<0.05$) ranged from 27-47% in Cd and Cu treated moss at higher dose and prolong exposure, is evident in present finding (Fig. 1a). Decrease in NRA after prolong period of treatment was reported earlier by suppressing the synthesis of enzyme or by reducing the activity of existing enzyme molecules (Ali et al., 2007; Saxena and Arfeen, 2006b). In addition metal ions Cd and Cu can interfere with the plants metabolism by binding to essential sulfhydryl groups of enzymes, largely defining their phytotoxicity (Wang and Su, 2005). In present study, small dose (0.01 M) exhibited a small increase in NR activity (Fig. 1a), that small doses of such metals act as micro nutrient for their growth (Berg et al., 1995).

The specificity and accumulating capability of heavy metal specifically Cd in moss depend on the genotype for their metal tolerance and bioremediation (Tickoo et al., 2007). Furthermore it has been reported that, both Cd and Cu under high dose can inhibit photosystem II, ATP synthetase and various Calvin cycle enzymes (Mostowska, 1997). The heavy metal accumulation in leaves reduces net photosynthesis by altering the chlorophyll content (Rascio et al., 1993) as evident in present study (Figs. 2a-c).

There are reports on reduction in the chlorophyll content, triggers in reduction of NRA (Huffaker et al., 1970), as depicted in present finding (Figs. 1a and 2a-c). It is also noteworthy that Cd induces water stress (Costa and Morel, 1994) however among enzymes of nitrogen metabolism NR activity is perhaps the most responsive to water stress (Srivastava, 1980). The metal induced water stress is evident in induction of proline synthesis (Fig. 4c).

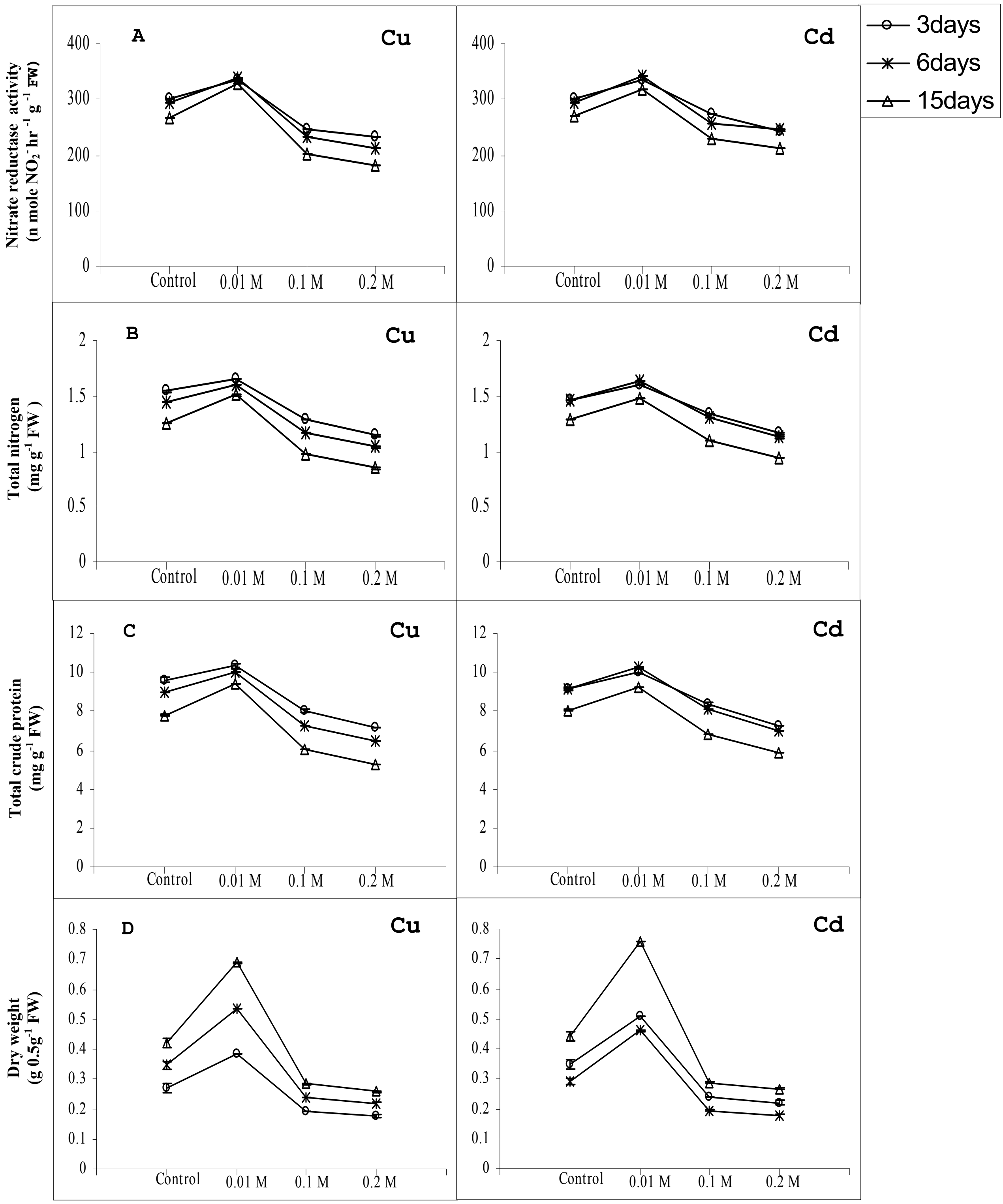

Fig. 1. Effect of metals (Cu and Cd) on A: Nitrate reductase activity. B: Total nitrogen. C: Total crude protein. D: Dry weight of moss *Racomitrium crispulum*. Each value is mean of 3 replicates ± S.E.

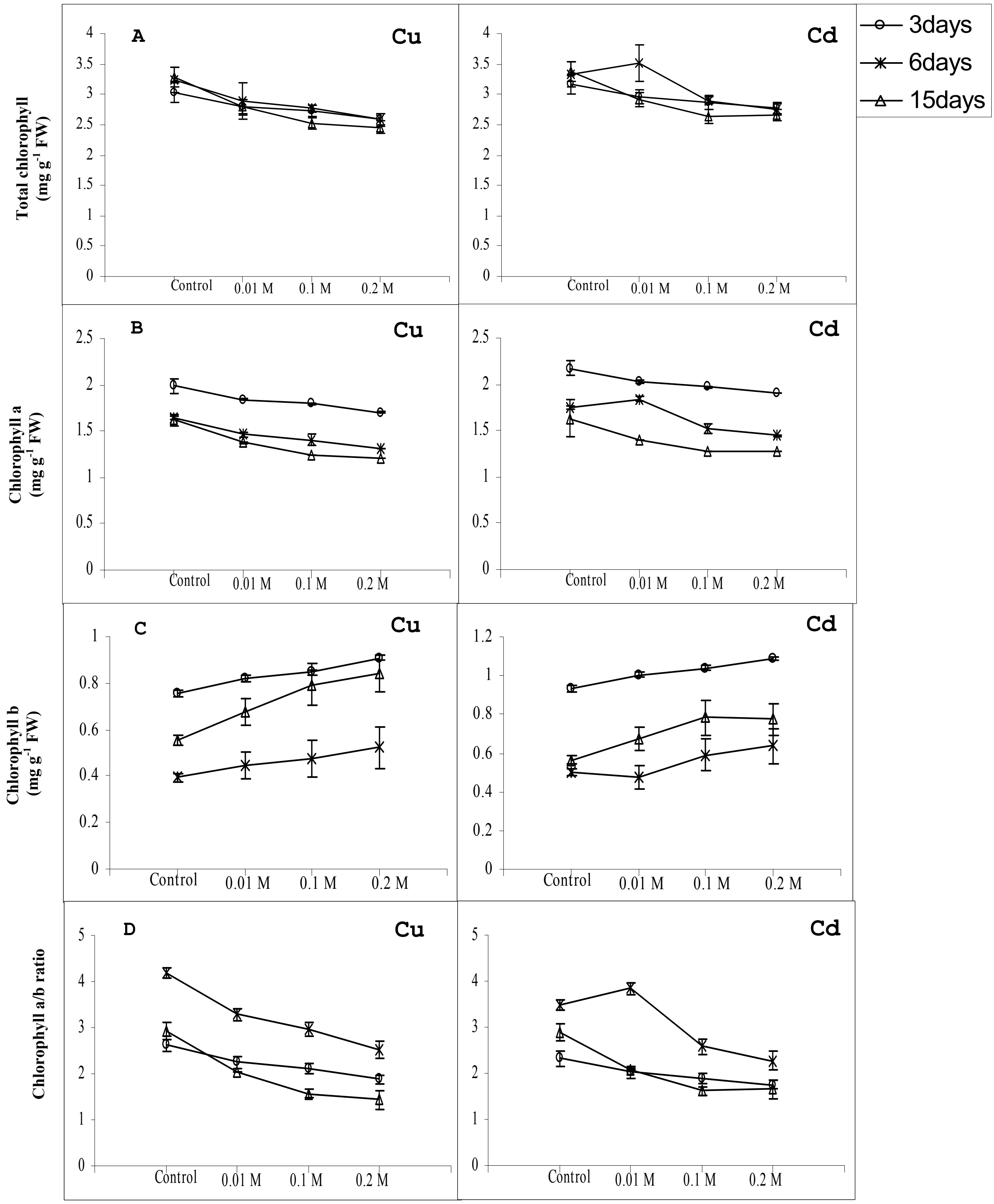

Fig. 2. Effect of metals (Cu and Cd) on A: Total chlorophyll. B: Chlorophyll a. C: Chlorophyll b. D: Chlorophyll a/b ratio of moss *Racomitrium crispulum*. Each value is mean of 3 replicates ± S.E.

Total nitrogen, crude protein and dry weight

An increase in both nitrogen and protein content of the moss plants was measured in 0.01M of Cu and Cd treatment (Figs. 1b and 1c), on the contrary Saxena and Saxena (2002) reported decline in both nitrogen and protein content in the peat moss *Sphagnum cuspidatum*, supplied with $NiSO_4$ in the range of 0.0001-0.1 M under *in vitro* conditions. The increase observed in total nitrogen may be due to the increase in amino acids under mild stress (Ali et al., 2007). Further result of NR activity is support for the increase in nitrogen and crude protein (Shaner and Boyer, 1976).

Particularly increase in proline accumulation in response to heavy metal exposure in present study has been suggested to perform dual role during stress development. Firstly, it acts as a source of solute for intra-cellular osmotic adjustment (Delauney and Verma, 1993) and secondly it is hypothesized that proline provides a store of nitrogen and carbon for subsequent utilization as an energy source after period of stress (Sivaramakrishnan et al., 1988).

Similarly increase in crude protein content in comparison to untreated plants was also noticed in moss at low concentration doses (i.e. 0.01 M) for all treatment days of exposure period to Cu and Cd (Fig. 1c). A comparable crude protein relative to control or slight increase was reported by several workers after metal exposure in a range of plants (Srivastava, 2004; Gupta et al., 2003).

The decrease observed at high doses (0.1 and 0.2 M) in protein content in Cu and Cd treated moss may be due to the breakdown of soluble proteins or due to the increased activity of proteins or other catabolic enzymes which were activated and destroyed the proteins (Ali et al., 2007).

The 0.01 M treatment of Cu and Cd in moss showed marked difference in their dry weight in moss comparison to control (Fig. 1d) and therefore the study conducted on biomass, attributes that low concentration metal increases the plant productivity or plant is able to mitigate the toxicity of low metal treatment (González et al., 2009). However, the prolonged treatment data of 15 days gives significant decrease in dry weight at higher concentration i.e. 0.2 M.

Chlorophyll and Chl a/b ratio:

A statistically significant decrease ($p<0.05$) in chlorophyll content was observed in moss after application of different concentrations of Cu and Cd, resulted in toxicity at 0.2 M concentration which become pronounced at prolong period (Fig. 2). However, there are reports that in tolerant mosses the total chlorophyll content is apparently not influenced much under short exposure and low doses (Tuba et al., 1996; Saxena and Arfeen, 2006a). In present finding same trend was observed in undertaken moss *R. crispulum*. Dose of Copper (0.2 M) decreased the chlorophyll content after 6th days till study period of 15th day. Chettri et al. (1998) also described the same trend in lower plants.

Photosynthetic pigments

A mild increase in chlorophyll content was reported earlier by Wu et al., (2003) at 0.1 μM Cd concentration. The significant increase ($P<0.05$) in total chlorophyll on 0.01 M Cd treated moss was supported by few earlier findings (Wu and Zhang, 2002; Lu et al., 2004), that there is some potentially positive impact of Cd on plant growth. After 15 days of Cd treatment, its phytotoxicity was evident, as noticed by significant decrease ($P<0.05$) in chlorophyll content (28%) at 0.01M Cd (Fig 2a). These results are in general agreement with earlier findings on moss *Tortula ruralis* by Csintalan et al., (1991), who reported 40% reduction (0.1 M) in chlorophyll content after two weeks treatment.

Cadmium has no biological function and is extremely toxic, even at low concentrations and is easily assimilated by plants (Milone et al., 2003). In contrast reports on lower plants like moss execute efficient Cd detoxification mechanism, imparting low stress (Reichmann, 2002).

The ratio of chlorophyll a/b is more sensitive to changes than *chl* a+b (Chettri et al., 1998). The *Chl* a/b ratio was less affected in the tolerant species. Moss of 0.01 M Cd treated sample exhibited 9% increase in there a/b ratio. Nevertheless the experimental concentrations used in present study are too high, suggestive of enhanced tolerance mechanism in mosses compared to higher plants.

Carotenoid and Chlorophyll / Carotenoid ratio:

It is feasible that singlet oxygen produced due to metal stress might have caused co-oxidation of chlorophyll and other pigments, initiating their bleaching further suggesting that the defense system of the chloroplast is over changed (Chen and Djuric, 2001). This might perhaps explain the low carotenoid content in the Cu and Cd treated plants and its degradation after long term exposure (Fig. 3i). Due to this low quantity of carotenoids, ROS may also cause oxidative damage to chloroplast structure and chlorophyll in sensitive species (Thompson et al., 1987). In present study an increase in carotenoid content were noticed at low concentration of metals (0.01M), attributed to photoprotective role of them (Fig. 3a).

The increase in the chlorophyll and carotenoid ratio under long incubation period of metals signifies rapid degradation of chlorophyll pigment and significantly decrease in the carotenoid imply decrease in protection action of carotenoid (Saxena and Arfeen, 2006a).

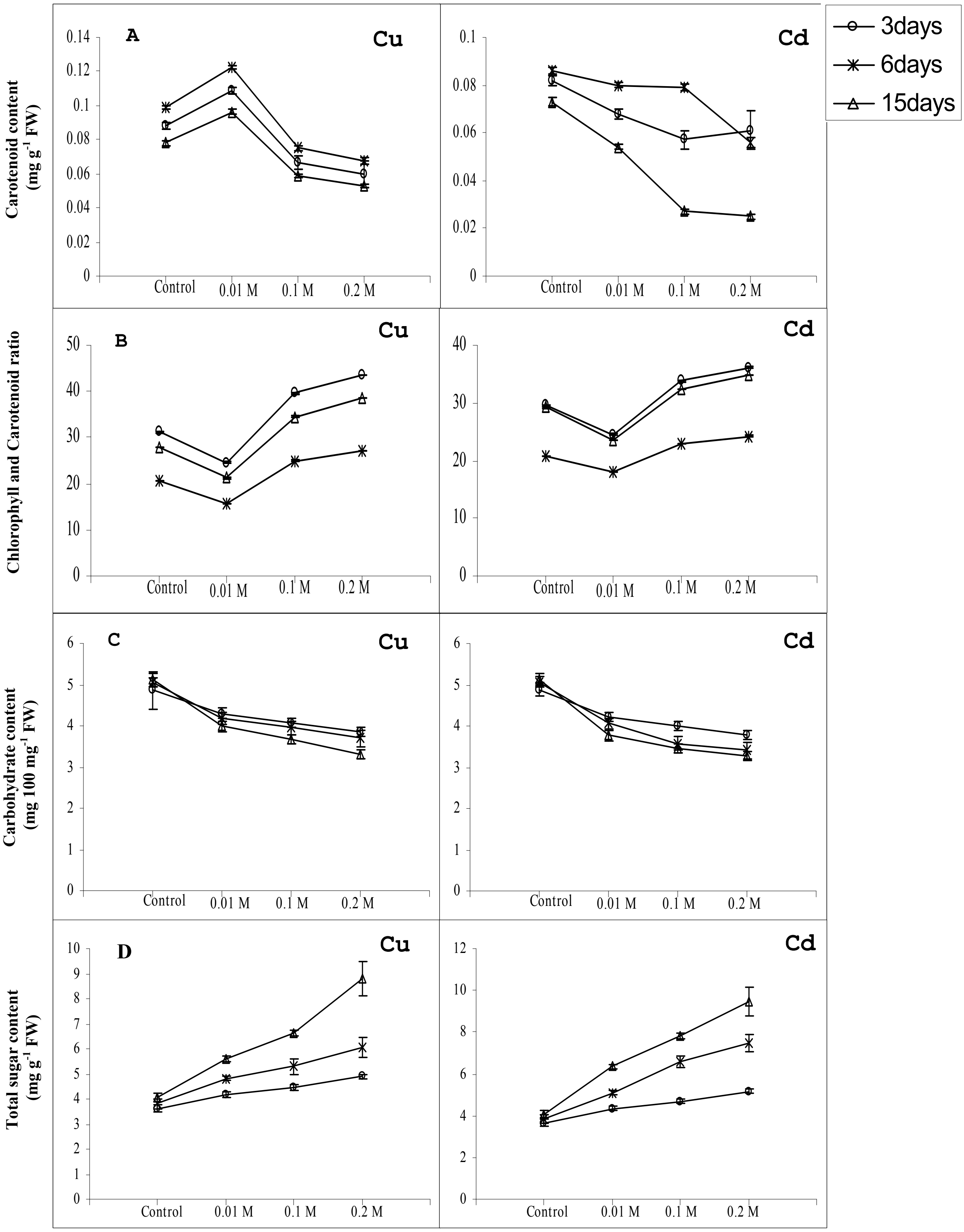

Fig. 3. Effect of metals (Cu and Cd) on A: Carotenoid. B: Chlorophyll-Carotenoid ratio. C: Carbohydrate. D: Total sugar content of moss *Racomitrium crispulum*. Each value is mean of 3 replicates ± S.E.

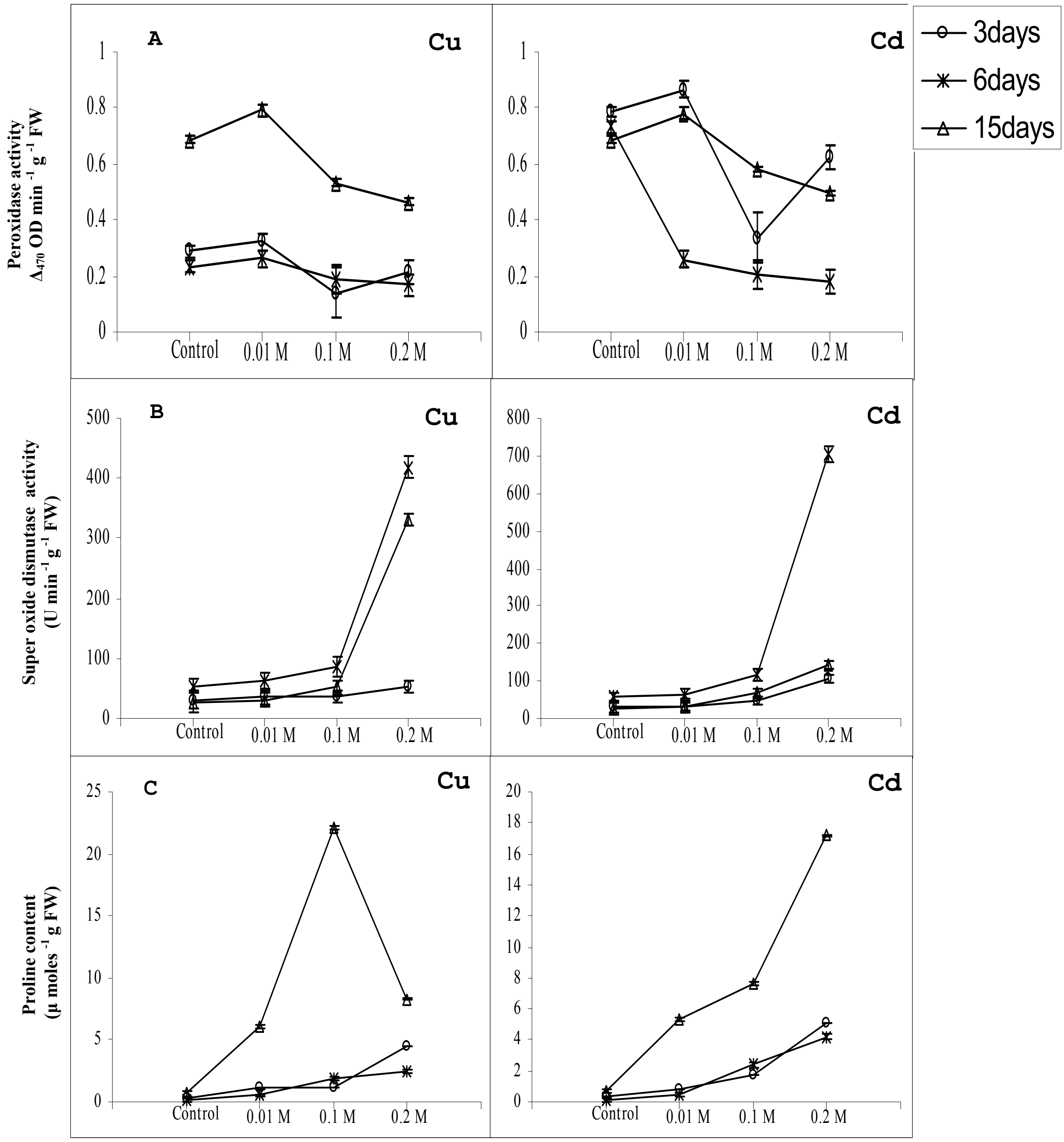

Fig. 4. Effect of metals (Cu and Cd) on A: Peroxidase. B: Superoxide dismutase. C: Proline content of moss *Racomitrium crispulum*. Each value is mean of 3 replicates ± S.E.

Carbohydrate and Sugar

The deleterious effect of Cu and Cd was most apparent after 15 days of treatment, where the decrease in carbohydrate content is 54% and 57% respectively. The metal stress limits the photosynthetic capacity of plants (Reichmann, 2002) and this was consequently reflected on the carbohydrate content. Carbohydrate metabolism seems to be associated with stress responses in various plant systems. It is probable that the metals might have caused a decrease in carbohydrate synthesis.

Decrease in carbohydrate is in inverse with increase in sugar content (Figs. 3c and d). Present result is also consonance with earlier findings (Subbaiah and Sachs, 2003) that at higher doses of metals (Cu and Cd), carbohydrate content decreases and sugar content increases. Seemingly the starch reserves serve as a buffer to compensate the reduced production of photosynthates (Kumar and Rajam, 2002).

Anti-oxidant enzymes

Peroxidase (POX):

The Cd and Cu stress elevated the level of Guaiacol Peroxidase (GPOX) which varied with the intensity and duration of exposure (Fig. 4a). It is well documented that in plants subjected to stress the activity of peroxidase increases (Gaspar et al., 1991) and in present study there is 11% increase ($p<0.05$) in peroxidase activity recorded in 0.01 M Cu treated moss for 6 days. The increase in Guaiacol Peroxidase (GPOX) activity in moss treated to Cu and Cd is probably related to oxidative reactions corresponding to an increase in peroxides and free radicals in the plant cells. This increase in enzyme activity could represent an appropriate protection against overproduction of peroxides radicals under low concentration (0.01 M) of heavy metals (Munné-Bosch and Alegre, 2002).

From present study it can be safely stated that high POX activity observed in the plants is indicative of oxidative stresses (Okamoto et al., 2001a). It probably suggests higher capability of moss *Racomitrium* species to adapt to oxidative stress by eliminating reactive H_2O_2 species under tolerable limit i.e. 0.01M.

Superoxide dismutase (SOD):

Moss plants exposed to different levels of Cu and Cd for 3, 6 and 15 days showed an induced SOD-specific activity initially, indicating that this moss plant have the greater capacity to adapt to heavy metal stress by developing antioxidant system (Saxena and Arfeen, 2006b; Pinto et al., 2003). Interestingly, an increase CdSOD caused by Cd exposure after 6 days, where the activity increased to 92% at $p<0.05$, well above the treated plants. The enzyme SOD dismutates O_2^- to H_2O_2 and oxygen. The transient increase in SOD during initial periods of metal stress might protect plants from oxidative injury. Cu and Cd has been shown to elevate lipid peroxidation under low doses (Chaoui et al., 1997; WU et al., 2009), resulting in ROS formation.

Proline:

Increase in proline content in plants is either due to the inhibition of proline oxidation or to the more rapid biosynthesis of proline from its precursors (Claussen, 2005). Proline accumulation in plant is accompanied bydecrease in osmotic potential. Osmotic adjustment or osmoregulation enables plant to maintain growth as plant water potential decrease (Mohammed et al., 1998). The value of proline content and NR activity are inversely proportional as evident in present study (Figs. 1a and 4c).

Proline accumulation under 15 days treatments of Cd showed protective response, not only due to the osmo-protectent role of proline that prevent a metal induced water deficit stress, but also for the radical scavenger and protein stabilization properties (Fig. 4c). Further, the result of total nitrogen and protein content are also down the line of proline, justify the role of proline to ignite the store nitrogen and protein to satisfy the energy gap due to heavy metal stress (Sivaramakrishnan et al., 1988).

This finding recommends that *Racomitrium crispulum* (Hook. f. et Wils.) of Kumaon hill is more over tolerant to Cu and Cd metal pollution and can be use as bioaccumulator. Which further supported by metal accumulated under different treatment concentration. Our earlier finding on metal biomonitoring value by *R. crispulum* is validated from the present study (Saxena and Arfeen, 2006c).

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LITERATURE CITED

Ali, A., S. Sivakami and N. Raghuram. 2007. Effect of nitrate, nitrite, ammonium, glutamate, glutamine and 2-oxoglutarate on the RNA levels and enzyme activities of nitrate reductase and nitrite reductase in rice. Physiol. Mol. Biol. Plants **13**: 17-25.

Arnon, D. L. 1949. A copper enzyme is isolated chloroplast polyphenol oxidase in *Beta vulgaries*. Plant Physiol. **24**: 1-15.

Bate, L. S., R. R. Waldeen and I. D. Teare. 1973. Estimation of proline under physiological stress condition. Plant Soil **39:** 205.

Beauchamp, C. and I. Fridovich. 1971. Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. Anal. Biochem. **44**: 276-287.

Berg, T., O. Royset, E. Steinnes and M. Vadset. 1995. Atmospheric trace-element deposition - Principal component analysis of ICP-MS data from moss samples. Environ. Pollut. **88**: 67-77.

Cerning-Beroard, J. 1975. A note on sugar determination by the anthrone method. Cereal Chem. **52**: 857-860.

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

Chen, G. and Z. Djuric. 2001. Carotenoids are degraded by free radicals but do not affect lipid peroxidation in unilamellar liposomes under different oxygen tensions. FEBS Letters **505**: 151-154.

Chettri, M. K., C. M. Cook, E. Vardaka, T. Sawidis and T. Lanaras. 1998. The effect of Cu, Zn and Pb on the chlorophyll content of lichens *Cladonia convolute* and *Cladonia rangiformis*. Environ. Exp. Bot. **39**: 1-10.

Claussen, W. 2005. Proline as a measure of stress in tomato plants. Plant Sci. **168:** 241-248.

Costa, G. and J. L. Morel. 1994. Water relations, gas exchange and amino acid content in Cd-treated lettuce. Plant Physiol. Biochem. **32**: 561-570.

Csintalan, Zs., J. L. D. Meenks and Z. Tuba. 1991. Ecophysiological responses of *Tortula ruralis* upon cadmium and lead treatments. In: Proceedings of the international symposium, Debrecen, Hungary. pp. 31-44.

Delauney, A. J. and D. P. S. Verma. 1993. Proline biosynthesis and osmoregulation in plants. The Plant J. **4**: 215-223.

Gaspar,Th., C. Penel, D. Hagège and H. Greppin. 1991. Peroxidases in plant growth, differentiation, and development processes. In: J Lobarzewski, H Greppin, C Penel, Th Gaspar, eds, Biochemical, Molecular, and Physiological Aspects of Plant peroxidases. Univ M. Curie-Sklodowska, Lublin, Poland and Univ Geneva, Switzerland. pp. 249-280.

Glime, M. J. and D. K. Saxena. 1991. *'Uses of Bryophytes'*. Today & Tomorrow's Printers & Publisher, N. Delhi, India. pp. 1-100.

González1 J. A., M. Gallardo, M. Hilal, M. Rosa and F. E. Prado. 2009. Physiological responses of quinoa (*Chenopodium quinoa* Willd.) to drought and waterlogging stresses: dry matter partitioning. Bot. Studies **50**: 35-42.

Gupta, D. K., U. N. Rai, A. Singh and M. Inouhe. 2003. Cadmium accumulation and toxicity in *Cicer arietinum* L. Poll. Res. **22**: 457-463.

Hedge, J. E. and B. T. Hofreiter. 1962. In: Whistler, R. L. and J. N. Be Miller (eds.), Carbohydrate Chemistry. Academic Press, New York, USA. pp. 17-22.

Huffaker, R. C., T. Radian, G. E. Kleinkoptand and E. L. Cox. 1970. Effect of mild water stress on enzyme of nitrate assimilation and of the carboxylative phase of photosynthesis of barley. Crop Sci. **10**: 471-474.

Jackson, M. L. 1962. *Soil Chemical Analysis*, Englewood Cliffs, N. J. Prentice-Hall, Inc. England. pp. 57-67.

Kumar, S. V. and M. V. Rajam. 2002. Metabolic engineering of carbohydrates for abiotic stress tolerance. In: Nandi, S. K., Palni, L. M. S. and Kumar, A. (eds.), Role of Plant tissue culture in Biodiversity conservation and economic development. Gyanodaya Prakashan, Nainital, India. pp. 479-489.

Loewus, F. A. 1952. Improvement in anthrone method for the determination of carbohydrates. Anal. Chem. **24**: 219-223.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265-275.

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

Milone, M. T., S. Cristina, H. Clijsters and F. Navari-Izzo. 2003. Antioxidative responses of wheat treated with realistic concentration of cadmium. Environ. Exp. Bot. **50**: 265-276.

Mohammed, S., P. K. Kasera, D. N. Sen and D. D. Chawan. 1998. Osmotic potential in the leaf sap of halophytes in Indian arid zone. J. Indian Bot. Soc. **77**: 179-184.

Mostowska, A. 1997. Environmental factors affecting chloroplasts. In: Pessarakli M (ed.), *Handbook of Photosynthesis*, Dekker, New York, USA. pp. 407-426.

Munné-Bosch, S. and L. Alegre. 2002. The function of tocopherols and tocotrienols in plants. Cri. Rev. Plant Sci. **21:** 31-57.

Okamoto, O. K., D. L. Robertson, T. F. Fagan, J. W. Hastings and P. Colepicolo. 2001. Different regulatory mechanisms modulate the expression of a dinoflagellate iron-superoxide dismutase. J. Biol. Chem. **276:** 19989-19993.

Panda, S. K. 2003. Heavy-metal phytotoxicity induces oxidative stress in a moss,*Taxithellium* sp. Curr. Sci. **84**: 631-633.

Pinto, E., T. S. C. Sigaud-Kutner, M. A. S. Leitao, O. K. Okamoto, D. Morse and P. Colepicolo. 2003. Heavy metal-induced oxidative stress in algae. J. Phycol. **39:** 1008-1018.

Putter, J. 1974. In: *Methods of enzymatic analysis*, Bergmeyer, H. U. (eds.), Vol. **II**. New York: Academic Press, USA. pp. 685-690.

Rascio, N., F. Dallavecchia, M. Ferretti, L. Merlo and R. Ghisi. 1993. Some effects of cadmium on maize plants. Arch. of Env. Contam. and Toxicol. **25**: 244-249.

Reichmann, S. M. 2002. The Responses of Plants to Metal Toxicity: A review focusing on Copper, Manganese and Zinc. Published by the *Australian Minerals & Energy Environment Foundation*. No. 14.

Saxena, D. K and A. Saxena. 2002. Bioaccumulation of nickel and its inactivation at cellular level in *Sphagnum cuspidatum* Hoffm. In: Nandi, S. K., L. M. S. Palni and A. Kumar (eds.), Role of plant tissue culture in biodiversity conservation and economic development. Gyanodaya Prakashan, Nainital, India. pp. 605-621.

Saxena, D. K. and Md. Saiful-Arfeen. 2006a. Screening of Pb tolerance in *Bryum cellulare* Hook. and *Plagiochasma appendiculatum* L. et. L. under growth response. J. Phytol. Res. **19**: 83-87.

Saxena, D. K. and H. Kaur. 2005. Effect of cadmium and nickel toxicity in the peroxidase activity and carotenoids content in moss *Thuidium cymbifolium.* Indian J. Plant Physiol. **10**: 397-399.

Saxena, D. K. and Md. Saiful-Arfeen. 2006b. Response of nitrate reductase activity and antioxidative defense system in moss *Racomitrium crispulum* (Hook. f. et Wils.) Hook. f. et Wils. to lead and zinc toxicity. Physiol. Mol. Biol. Plants. **12**: 303-306.

Saxena, D. K. and Md. Saiful-Arfeen. 2006c. Biomonitoring and inter species comparison of metal precipitation through bryophytes at petrol pump on Kumaon hill. Environmental Conservation Journal **7**: 69-77.

Saxena, D. K., S. Singh and K. Srivastava. 2008. Atmospheric Heavy Metal Deposition in Garhwal Hill Area (India): Estimation Based on Native Moss Analysis. Aerosol and Air Quality Research. **8**: 94-111.

Shaner, D. L. and J. S. Boyer. 1976. Nitrate reductase activity in maize (*Zea mays* L.) leaves. I. Regulation by nitrate flux. Plant Physiol. **58**: 499-504.

Sivaramakrishnan, S., V. Z., Patell, D. J. Flower and J. M. Peacock. 1988. Proline accumulation and nitrate reductase activity in contrasting sorghum lines during mid-season drought stress. Physiol. Plant. **74**: 418-426.

Srivastava, H. S. 1975. Distribution of nitrate reductase ageing bean seedlings. Plants Cell Physiol. **16:** 995-999.

Srivastava, H. S. 1980. Regulation of nitrate reductase activity in higher plants. Phytochem. **19**: 725-733.

Srivastava, H. S. 2004. Plant physiology: A text book for university students. Rastogi publications. India. pp. 167-181.

Subbaiah, C. C. and M. M. Sachs. 2003. Molecular and cellular adaptations of maize to flooding stress. Ann. Bot. **91:** 119-127.

Sun, S. Q., D. Y. Wang, He M and C. Zhang. 2009. Monitoring of atmospheric heavy metal deposition in Chongqing, China-based on moss bag technique. Environ. Monit. Assess. **148**: 1-9.

Thompson, J. E., R. L. Legge and R. L. Barber. 1987. The role of free radicals in senescence and wounding. New Phytol. **105**: 317-334.

Tickoo, S., V. K. Sindhu and H. B. Singh. 2007. Screening of Indian mustard (*Brassica juncea* L. Czern & Coss.) genotypes for its cadmium accumulation and tolerance. Physiol. Mol. Biol. Plants **13**: 37-46.

Tuba, Z., Zs., Csintalan and C. F. M. Proctor. 1996. Photosynthetic responses of a moss, *Tortula ruralis*, ssp. *ruralis*, and the lichens *Cladonia convolute* and *C. furcata* to water deficit and short period of desiccation, and their ecophysiological significance: a baseline study at present-day CO_2 concentration. **133**: 353-361.

Wang, J.-Q. and D.-C. Su. 2005. Distribution of Cd in oil seed rape and Indian mustard grown in Cadmium contaminated soil. J. Env. Sci. **17**: 572-575.

Wu, F.-B. and G. Zhang. 2002. Genotypic differences in effect of Cd on growth and mineral concentrations in barley seedling. Bull. Environ. Contam. Toxicol. **69**: 219-227.

Wu, F.-B., G. Zhang and P. Dominy. 2003. Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. Environ. Exp. Bot. **50**: 67-78.

Wu, T.-M., Y-T. Hsu and T.-M. Lee. 2009. Effects of cadmium on the regulation of antioxidant enzyme activity, gene expression, and antioxidant defenses in the marine macroalga *Ulva fasciata.* Bot. Studies. **50**: 25-34.

銅及鎘對蘚類植物 *Racomitrim crispulum* 氧化酵素及葉綠素含量的影響

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摘要：本報告主要是使用不同濃度的銅及鎘處理蘚類植物 *Racomitrim crispulum*，經一段時間後評估其對植物抗氧化劑，光合能力及產量的影響。*R. crispulum* 處理銅及鎘後對其氧化酵素及光合作用都有顯著的抑制作用。分別使用 0.2 M 的銅及鎘處理 15 天後，硝酸還原酵素活性分別減少 27% 及 47%，氮及蛋白質含量同時也減少。使用 0.1 M 鎘處理 15 天後，葉綠素明顯分解而減少，但是低濃度的金屬(0.01 M)處理，具有光保護功能的類胡蘿蔔素含量顯者增加。分別使用銅及鎘處理植物 15 天後，碳水化合物含量分別減少 54% 及 57%，此結果表示長時間的處理之後，金屬對植物的害處才能顯現出來。總糖量與碳水化合物含量呈反比關係，這是蘚類植物的補償生理作用。過氧化氫酶活性隨着處理金屬濃度提高而增加，但是隨着處理時間的延長而減少。使用 10 mM 銅處理植物 6 天其過氧化氫酶活性可達到最高。超氧物歧酶 (SOD) 活性隨着處理金屬濃度的提高及處理時間的延長而增加。本研究及參考文献都發現長時間的低等程度及中等程度的金屬逆境，對 *R. crispulum* 植物的氧化酵素及光合產物都有誘導作用。此結果顯示，植物處於每一種處理交替作用情況下，能够調整它們的新陳代謝作角及生理反應，以維持細胞的完整性及恆定性。綜合本研究結論是 *R. crispulum* 對銅及鎘的金屬汙染具有耐性，因此能够作為此兩種金屬的累積植物。

關鍵詞：植物毒害金屬銅及鎘、蘚類植物 *Racomitrim crispulum*、氧化酵素、光合產物、產量回應。