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RESEARCH ARTICLE

Induction of oxidative stress and antioxidant responses in *Azolla microphylla* by cadmium stress

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SUMMARY

In the present srudy, *Azolla microphylla* fronds were grown in with different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}) of cadmium chloride, in order to investigate the effects of cadmium on the growth (dry weight), lipid peroxidation, ascorbate peroxidase (APX), superoxide dismutase (SOD) and proline content. A concentration dependent reduced growth was observed in the *Azolla* fronds.MDA which is the measure of lipid peroxidation increased in plants under all the treatments of cadmium. Stimulation of antioxidant enzymes (SOD and APX) was observed due to Cd stress. Proline accumulation also showed significant increase at all the concentrations of cadmium. The results suggested that *Azolla microphylla* may have better protection against oxidative stress by increasing antioxidant activity exposed to cadmium stress.

Key Words : *Azolla microphylla*, Cd, MDA, SOD, Proline

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The *Azolla- Anabaena* symbiosis represents potentially an ideal biofertilizer for rice production due to its high nitrogen fixing ability and rapid growth (Peters and Meeks 1989). According to Watanabe *et al.* (1977) *Azolla* can fix atmospheric nitrogen at the rate of 1.1 kg N $ha^{-1}day^{-1}$. It is suitably called green "gold" because it is economically important as an animal feed, medicine, hydrogen fuel, biogas producer and weed controller (Wagner, 1997). Furthermore *Azolla* ferns have been also reported as an important biological component cleaning up contaminated surface waters with either inorganic or organic pollutants (Bennicelli *et al.* 2004; Rai, 2008).

Cadmium is a major heavy metal pollutant in the environment resulting from agricultural, mining and industrial activities as well as from automobiles emissions (Wagner, 1993). This non essential element with a long biological half life strongly inhibits the growth and development of plants and cause death, even at very low concentrations (Hernandez *et al.*, 1996). Cd is also highly toxic in humans and even trace amounts of it can result in neurological disorders and kidney damage (Lang *et al.,* 2005). In plants it affects many physiological and biochemical processes, such as, photosynthesis, respiration nitrogen metabolism, pigment degradation etc (Chaffei *et al.,* 2003; Dai *et al.,* 2006; Gharmash and Golovko, 2009; Chen *et al.,* 2011; Gill *et al.,* 2012).

Cadmium not only damages the biomolecules such as nucleic acids and proteins in living organisms but also triggers oxidative stress through the formation of reactive oxygen species (ROS), which in turn cause enhanced lipid peroxidation (Arora *et al.,* 2002; Xu *et al.,* 2009). Plants can reduce cadmium toxicity through a variety of mechanisms, including the production of ROS scavengers and cadmium binding factors and by excretion or compartmentization (Siripornadulsil *et al.,* 2002).The efficient destruction of ROS requires the action of several antioxidative enzymes, including superoxide dismutase (SOD) and catalase which can convert superoxide radicals into hydrogen peroxide, water and oxygen (Dinakar *et al.*, 2010). Plants can also synthesize non-enzymatic ROS scavenging molecules, such as proline which can detoxify oxygen free radicals directly (Chris *et al.,* 2006a).

The toxic effect of cadmium on *Azolla* in terms of growth (Masood and Abraham, 2003) and nitrogen metabolism (Dai *et al.,* 2009) studied earlier but the effect of Cd on oxidative

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stress and antioxidants has not been reported so far. Considering the above facts the present work was conducted with the aim to investigate the effect of cadmium on growth, lipid peroxidation and antioxidant machinery in *Azolla microphylla.*

MATERIALS AND METHODS

Plant material and cadmium treatment :

Azolla microphylla one of the dominant strains of the locality was collected from paddy fields of Allahabad. Plants were washed and cleaned of contaminating organisms. The plants were surface sterilized with a solution of mercuric chloride (0.1% for 30 min) and were dipped immediately into a large volume of sterile distilled water. Plants were then transferred into dishes containing combined-N free 2/5 strength sterile Hoagland's medium (Allen, 1968) and 0.04 mM ferrous ion as Fe-EDTA, pH 5.6.The cultures were grown at 26^0 C under a 16:8 (light: dark) photoperiod with light from a combination of incandescent and cool white light fluorescent lamps at a photon fluence rate of 95 ìmol $m^{-2}s^{-1}$. Log phase fronds were used for the experiment. For the experiments plants were kept in 100 ml of the medium containing different levels of Cd (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}). Parameters were analyzed after 96 h of exposure.

Growth estimation :

The dry weight of plants exposed to various doses of cadmium (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}) was measured after drying the samples for 24 h at 90^0C.

Estimation of lipid peroxidation and proline :

Lipid peroxidation was estimated by measuring the content of 2-thiobarbituric acid-reactive substances in leaf homogenate, prepared in 20 per cent TCA containing 0.5 per cent 2-thiobarbituric acid and heated at 95^0C for 25 min (Heath and Packer,1968).Melondialdehyde (MDA) content was determined spectrophotometrically at A_{532} and corrected for non specific turbidity at A_{600}.

Proline concentration in treated and untreated fronds was determined spectrophotometrically by the method of Bates *et al.* (1973).

Assay of enzymes :

For ascorbate peroxidase APX (EC 1.11.1.11) activity plants were homogenized in 50 mM potassium phosphate buffer (pH 7.8) at 4 ^{0}C, while for SOD (EC 1.15.1.1) estimation fronds were homogenized in 100 mM EDTA phosphate buffer (pH 7.8) at 4 ^{0}C.The homogenate was filtered and centrifuged at 10,000 x g for 15 min and the supernatant obtained was used for enzyme assay. APX and SOD were determined spectrophotometrically (Systronics India Ltd Model No 117) according to the methods of Aono *et al.* (1995) and Giannopolitis and Ries (1977), respectively.

Statistical analysis :

The different parameters were statistically analyzed using one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Fig. 1 shows the changing effect of different concentrations (0-3.0 mg l^{-1}) of Cd on growth of *Azolla microphylla*. Cd was found to be toxic at all the concentrations and the growth reduction was significant at all doses (0-3.0 mg l^{-1}) of Cd. A reduction of 12, 20, 30, 42, 55 and 70 per cent in the dry weight was recorded at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1} doses of Cd, respectively as compared to control. The negative effect of Cd on plant growth has been very well documented by Prasad (1995). Masood and Abraham (2003) also reported reduced growth due to cadmium stress in *Azolla pinnata* plants. Reduction in growth may be due to chromosomal aberration and abnormal cell divisions (Maria and Tadeusz, 2005). It may also be correlated with the metal induced inhibition of photosynthetic process, respiration and protein synthesis in plants (Iannelli *et al.,* 2002; Hatata and Aal, 2008; Gill *et al.,* 2012)

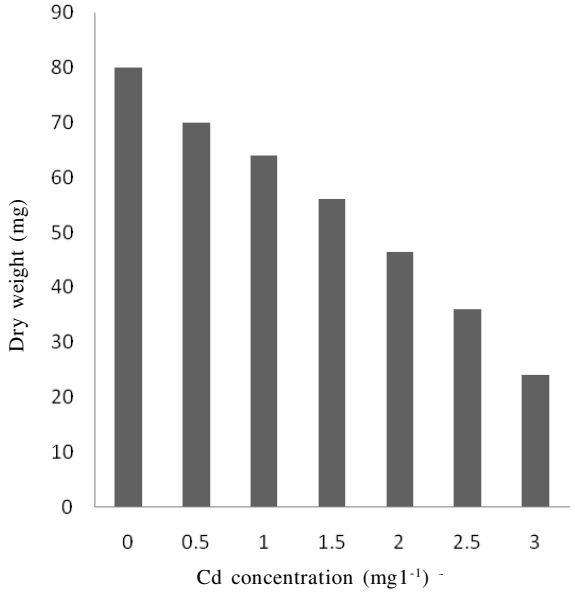

Fig. 1 : Effect of different doses of cadmium on growth (dry weight) of *Azolla microphylla*. Values are mean ±SE of three replicates. Values are significant at 0.05 (5%) level of significance (ANOVA)

The lipid peroxidation in treated and untreated *Azolla* fronds was estimated and data are presented in Table 1. *Azolla microphylla* showed an increase in the MDA level considerably and the increasing trend continued with rising concentrations of cadmium. Highest MDA content (325%) was measured at 3.0 mg l^{-1} Cd indicating a higher degree of

Table 1: Effect of cadmium on lipid peroxidation (MDA), superoxide dismutase (SOD), ascorbate peroxidase (APX) and proline content of *Azolla microphylla*

Treatments	MDA [μ mol (g FW)$^{-1}$]	SOD [Unit (g FW)$^{-1}$]	APX [Unit (g FW^{-1}.min^{-1})]	Proline [μg (g FW)$^{-1}$]
Control	1.60	20.00	580.00	11.00
0.5 mg l^{-1}	1.92 (+20)	28.00 (+40)	667.00 (+15)	12.90 (+18)
1.0 mg l^{-1}	2.40 (+50)	31.40 (+57)	725.00 (+25)	14.85 (+35)
1.5 mg l^{-1}	2.72 (+70)	34.00 (+70)	522.00 (-10)	16.50 (+50)
2.0 mg l^{-1}	3.20 (+100)	26.00 (+30)	435.00 (-25)	18.15 (+65)
2.5 mg l^{-1}	3.92 (+145)	23.00 (+15)	290.00 (-50)	15.40 (+40)
3.0 mg l^{-1}	5.20 (+225)	18.00 (-10)	116.00 (-80)	12.65 (+15)

[Mean+SE, values in parenthesis are percent decrease or increase with reference to respective control. All treatments are significantly different (0.05) from control (ANOVA)]

lipid peroxidation under metal stress condition while it was minimum at 0.5 mg l^{-1} (120%). A variety of abiotic stresses including heavy metals may cause molecular damage to plant cells either directly or indirectly through the formation of ROS (Hatata and Aal, 2008). Enhancement of O^-_2 can produce the hydroperoxyl radical ($^{\cdot}OH$, H_2O_2), which in turn convert fatty acids to toxic lipid peroxides, destroying biological membranes (Kishor *et al.,* 2010; Anjum *et al.,* 2011). Measurement of MDA level is used as an index of lipid peroxidation under stress conditions. Increased MDA under Cd stress in *Azolla* plants suggests that higher cadmium levels stimulate lipid peroxidation, resulting in damage to tissue development and function.

Data related to the effect of cadmium stress on SOD and APX enzymes are compiled in Table1. 0.5, 1.0, 1.5 and 2.0 mg l^{-1} treatment increased the SOD by 40, 57, 70 and 30 per cent, respectively as compared to control. A decline of 10 per cent was also noticed in SOD activity at highest dose *i.e.* 3.0 mg l^{-1}.Similarly cadmium 0.5 and 1.0 mg l^{-1} doses also enhanced the APX activity in *Azolla microphylla* (Table 1). The APX enzyme was maximum in 1.0 in mg l^{-1} (725 Unit g^{-1} f w min^{-1}). However, it reduced to 435, 290 and 116 Unit g^{-1} f w min^{-1} at 2.0, 2.5 and 3.0 mg l^{-1} doses. Antioxidative enzymes play an important role in adaptation and ultimate survival of plants under stress condition (Chris *et al.,* 2008). Gajewska and Skiodowska (2010) also reported that activities of antioxidative enzymes were inducible by oxidative stress, which reflected a general strategy require to minimize/overcome stress.SOD is one of the stress- resistant enzymes and can catalyze the disproportination of O^-_2 radicals to H_2O_2 and O_2. H_2O_2 is also toxic to plant cells and APX could eliminate H_2O_2 by breaking it down directly to form water and oxygen. Therefore, the combination of SOD and APX plays an important role in the resistant of a plant to environmental stress (Dinakar *et al.,* 2010). Recent researches has demonstrated that reactive oxygen species (ROS) are involved in cellular signaling processes as secondary messengers to induce antioxidative enzymes like SOD and APX (Mahalingam and Fedoroff, 2003).Thus, the increased level of ROS triggered the activity of antioxidative enzymes *i.e.* SOD and APX in *Azolla microphylla*.

The stress indicator amino acid proline increased with the increase in concentration of cadmium. Proline exhibited an increase of 1.35, 1.50 and 1.65 fold after the treatment of 1.0, 1.5 and 2.0 mg l^{-1} treatments, respectively as compared to untreated control and a slight decrease was also found at the highest dose (3.0 mg l^{-1}) but it was higher over to control. Proline accumulation in higher plants under heavy metal stresss has been reported by many workers (Shah and Dubey, 1998; Ashraf and Harris, 2004; Mansour *et al.,* 2005). In present study proline content was always higher under over control, which might protected the plant cells against cadmium stress because it functions as a stabilizer (Shah and Dubey, 1998), a metal chelator (Sun *et al.,* 2007), an inhibitor of lipid peroxidation (Chris *et al.,* 2006b), a hydroxyl and singlet oxygen scavenger (Alia *et al.,* 1997).

In conclusion present findings suggest that Cd toxicity negatively affected the growth of *Azolla microphylla*. Cadmium stress led to an increased lipid peroxidation, probably due to heavy accumulation of reactive oxygen species and declined ascorbate peroxidase activity. There was stimulation of superoxide dismutase and proline content under Cd stress to cope up the oxidative stress generated by reactive oxygen species. Present results support the fact that the growth and antioxidant responses in *Azolla microphylla* depend on the cadmium concentrations.

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REFERENCES

Alia , Pardha, Saradhi, P. and Mohanty, P.(1997). Involvement of proline in protecting thylakoid membranes against free radical-induced photodamage. *J.Photochem.Photobiol. B: Biol.,* **38**:253–257.

Allen, M.M. (1968). Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol.,* **4**:1-4.

Aono, M., Saji, H., Fujiyama, K., Sugita, M., Kondo, N. and Tanaka, K. (1995). Decrease in activity of glutathione reductase enhances paraquate sensitivity in transgenic *Nicotiana tabaccum*. *Plant. Physiol.,* **107**:645-648.

Anjum, N.A., Umar, S., Iqbal, M. and Khan, N.A. (2011). Cadmium causes oxidative stress in mung bean by affecting the antioxidant enzyme system and ascorbate- glutathione cycle metabolism. *Rus. J. Plant. Physiol.,* **58**:92-99.

Arora, A., Sairam, R.K.and Srivastava, G. C. (2002).Oxidative stress and oxidative system in plants.*Curr., Sci.,* **82**:1227-1238.

Ashraf, M. and Harris, P.J.C. (2004).Potential biochemical indicators of sainity tolerance in plants. *Plant Sci.,* **166**:3-16.

Bates, L. S., Waldren, R. P., Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil.,***39**: 205–207.

Bennicelli, R., Stêpniewska, Z., Banach, A., Szajnocha, K.and Ostrowski, J. (2004). The ability of *Azolla caroliana* to remove heavy metals (Hg(II), Cr(III), Cr(VI)) from municipal waste water. *Chemo*., **55**:141-146.

Chaffei, C.,Gouia, H. and Ghorbel, M.H. (2003). Nitrogen metabolism of tomato under cadmium stress conditions. *J Plant Nutr.,* **26**:1617-1634.

Chen, X., Wang, J., Shi, Y., Zhao, M.Q.and Chi, G.Y. (2011). Effects of cadmium on growth and photosynthetic activities in pakchoi and mustard. *Bot. Stud.,* **52**: 41-46

Chris, A., Zeeshan, M., Abraham, G. and Prasad, S.M. (2006a). Proline accumulation in *Cylindrospermum* sp. *Env. Exp. Bot*., **57**:154-159.

Chris, A., Zeeshan, M. and Abraham, G. (2006b). Effect of exogenous supply of antioxidants on UV-B induced oxidative stress in a cyanobacterium *Cylindrospermum* sp. *Algo. Stud.,* **121**:107-118.

Chris, A., Zeeshan, M. and Masih, J. (2008). UV-B induced oxidative stress and oxidative defences in cyanobacterium *Cylindrospermum* sp. *Biosci. Res.,* **5**: 1-8.

Dai, L.P., Xiong, Z. T., Huang, Y. and Li, M. Z. (2006). Cadmium induced changes in pigments, total phenolics and phenylalanine ammonia-lyase activity in fronds of *Azolla imbricata*. *Env.Toxicol.,* **21**:505-512.

Dai, L.P., Xiong, Z.T. and Ma, H.H. (2009). Effect of cadmium on nitrogen metabolism in *Azolla imbricata- Anabaena azollae* symbiosis. *Acta. Ecol. Sinica.,* **29**:1629-1635.

Dinakar, C., Vishwakarma, A.P., Yearla, S.R., Raghvendra, A.S. and Padmasree, K. (2010). Importance of ROS and antioxidant system during the beneficial interactions of mitochondrial metabolism with photosynthetic carbon assimilation. *Planta.,* **231**:461-474.

Gajewska, E. and Sklodowska, M. (2010). Differential effect of equal copper, cadmium and nickel concentration on biochemical reactions in wheat seedlings. *Ecotox. Environ. Safe,* **73** : 996-1003.

Gharmash, E. V. and Golovko, T.K. (2009). Effect of cadmium on growth and respiration of barley plants grown under two temperature regimes. *Rus. J. Plant. Physiol.,* **56**: 343-347.

Giannopolitis,C.N.and Ries, S.K. (1977).Superoxide dismutases: I.Occurrence in higher plants. *Plant. Physiol.,* **59**: 309-314.

Gill, S.S., Khana, N.A. and Tuteja, N. (2012). Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress [*Lepidium sativum* (L.)]. *Plant.Sci.,* **182**:112–120.

Hatata, M.H. and Aal, E.A.A. (2008). Oxidative stress and antioxidant defense mechanism in response to cadmium treatments. *Ame-Eure. J. Agri. Env. Sci.,* **4**: 655-669.

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

Hernandez, L.E., Carpena-Ruiz, R., Garate, A. (1996). Alterations in the mineral nutrition of pea seedlings exposed to cadmium. *J. Plant. Nutr.,* **19**:1581-1598.

Iannelli, M.A., Pietrini, F., Fiore, L., Petrilli, L. and Massacci, A. (2002). Antioxidant response to cadmium in *Phragmites australis* plants. *Plant. Physiol. Biochem.,* **40**: 977-982.

Kishor, G., Sarma, A.P.and Murthy, S.D.S.(2010). Free radical mediated damage in the thylakoid photo functions of barley thylakoid membranes under Uv-B radiation: Analysis of possible protection mechanisms. *Ame-Eure. J. Agri. Env. Sci.,* **5**: 1-4.

Lang, M.L., Zhang, Y. X. and Chai, T. Y. (2005). Identifications of genes upregulated in response to cadmium exposure in *Brassica juncea* L.*Gene.,* **363**:151-158.

Mahalingam, R. and Fedoroff, N. (2003). Stress response cell death and signaling:The many faces of reactive oxygen species. *Physiol. Plant.,* **119**:56-68.

Mansour, M.M.F., Salama, K.H.A., Ali, F.Z.M. and Abou hadiaa, A. F. (2005). Cell and plant response to NaCl in *Zea mays* L. Cultivars differing in salt tolerance. *Gen. Appl. Plant. Physiol.,* **31**:29-41.

Maria, D. and Tadeusz, B.(2005).Growth parameters and photosynthetic pigments in leaf segments of *Zea mays* exposed to cadmium, as related to protection mechanisms. *Plant. Physiol*., **162**: 1013-1021.

Masood, A. and Abraham, G. (2003).Growth behaviour of *Azolla pinnata* to cadmium toxicity. *Plant. Arch*., **3**: 227-230.

Peters, G. A. and Meeks, J.C. (1989). The *Azolla – Anabaena* symbiosis: basic biology. *Ann. Rev. Plant. Physiol. Plant. Mol. Biol.,* **40**:193-210.

Prasad, M. N. V. (1995). Cadmium toxicity and tolerance in vascular plants. *Env. Exp. Bot.*, **35**:525-545.

Rai, P.K. (2008). Phytoremediation of Hg and Cd from industrial effluents using an aquatic free floating macrophyte *Azolla pinnata*. *Internat. J. Phtorem.,* **10**:430-439.

Shah, K. and Dubey, R.S. (1998). Effect of cadmium on proline a cumulation and ribonuclease activity in rice seedlings: role of proline as a possible enzyme protectant. *Biol. Plant*. **40**:121-130.

Siripornadulsil, S., Traina, S., Verma, D.P.S. and Sayre, R. T. (2002). Molecular mechanisms of proline mediated tolerance to toxic heavy metals in transgenic microalgae. *Plant. Cell*., **14**:2837-2847.

Sun, R.L., Zhou, Q.X., Sun, F.H. and Jin, C. X. (2007). Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyper accumulator, *Solanum nigrum* L. *Env. Exp.Bot.,* **60**: 468-476.

Wagner, G. J. (1993). Accumulation of cadmium in crop plants and its consequences to human health. *Adv. Agron.,* **51**:173-213.

Wagner, G. M. (1997). *Azolla*: a review of its biology and utilization. *Bot. Rev.,* **63**: 1-21.

Watanabe, I., Espianase, C. R., Berja, N. S. and Alimango, B. A. (1977). Utilization of *Azolla- Anabaena* complex as a nitrogen fertilizer for rice. *IRRI, Res.Paper Series,* **11**: 1-15.

Xu, J., Wang, W. Y., Yin, H. X., Sun, H. and Mi, Q. (2009). Exogenous nitric oxide improves antioxidative capacity and reduces auxin degradation in roots of *Medicago truncatula* seedlings under cadmium stress. *Plant. Soil.,* **326**:321-330.

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