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Response of the antioxidant systems of the nitrogen fixing cyanobacterium *Cylindrospermum* sp. to lead stress

■ ABHISHEK CHRIS

Author for Correspondence -
ABHISHEK CHRIS
Department of Botany, Wilson College, MUMBAI (M.S.) INDIA
Email : achris1@rediffmail.com

ABSTRACT - Cyanobacteria *Cylindrospermum* sp. as biofertilizer for rice cultivation has a beneficial effect on crop productivity and maintenance of soil fertility. A study was undertaken under controlled laboratory conditions, to study the influence of different concentrations of lead (0, 25, 50, 75, 100 and 125 µM) on the growth, lipid peroxidation (MDA), peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) and proline of cyanobacterium *Cylindrospermum* sp. Lead stress caused negative impact on growth (Dry weight) of *Cylindrospermum* sp. and the damaging effect was further increased by enhanced lipid peroxidation probably because of generation of reactive oxygen species. To explore the survival stretegies of cyanobacterium under Pb stress enzymatic antioxidants SOD, POD, CAT and non enzymatic antioxidant proline was studied. A general induction of SOD, POD and proline was observed. In contrast to this CAT activity was reduced after 50 µM Pb. This study may be helpful in biological indication of lead toxicity in cyanobacteria.

Key words - *Cylindrospermum* sp., Pb, MDA, POD, SOD

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Cyanobacteria are capable of both carbon assimilation and nitrogen fixation, thereby enhancing productivity in varieties of environments. Apart from fixing atmospheric nitrogen they secrete a number of biologically active substances (Muthukumar *et al*., 2007). Tropical conditions such as those in India provide favorable environment for the luxuriant growth of these organisms in the natural ecosystems such as different type of soil, freshwater bodies, oceans, saline backwaters, estuaries and also hyper saline saltpans (Srivastava and Odhwani, 1992; Thajuddin and Subramanian, 1992; Rajkumar, 2004).Since cyanobacteria are unique and cosmopolitan in distribution, they are therefore known to have survived a wide spectrum of environmental stresses like salinity, pesticide, temperature, cold, UV-B and heavy metals (Tandeau-De- Marsac and Houmard, 1993).Heavy metals play an important but dual role in plant metabolism. On one hand, some of them are essential micronutrients acting, for example, as cofactors of key metabolic enzymes. On the other hand, when exceeding their critical concentrations, the same metals become the most toxic pollutants in the soil (Stobrawa and Lorenc-Plucinska, 2007). Lead, a heavy metal is a potent environmental pollutant (Verma and Dubey, 2003). Pb contamination has resulted from mining, smelting activities, Pb containing paints, gasoline, explosives, as well as from the disposal of municipal sewage sludge enriched in lead (Nidelkoska and Doran, 2000). Although it is not essential for plants; it is absorbed and accumulated in different plant tissues (Van Assache and Clijsters, 1990). Elevated concentrations of heavy metals specially lead in the soil can lead to toxicity symptoms and growth inhibition in plants (Pourrut *et al.,* 2011). Toxicity may results from the binding of metals to sulphydryl groups in proteins, leading to inhibition of activity or disruption of structure or from displacement of an essential element, resulting in deficiency effects (Van Assache and Clijsters, 1990). In addition, a heavy metal excess may stimulate the formation of free radicals and

reactive oxygen species, perhaps resulting in oxidative stress (Dietz *et al.,* 1999). The lifetime of reactive oxygen species within the cellular environment is determined by the antioxidant system, which provides crucial protection against oxidative damage (Chris and Alexander, 2010). The antioxidative system comprises numerous enzymes like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) etc and compounds of low molecular weight compounds like proline, ascorbate etc. (Chris and Masih, 2012). SOD removes superoxide radicals (O_2^-) by catalyzing its dismutation, one O_2^- being reduced to H_2O_2 and another oxidized to O_2. It removes $O_2^-$and hence decreases the risk of hydroxyl radical (OH^-) formation via the metal catalyzed Haber-Weiss type reaction.H_2O_2 is still toxic and must be eliminated by the activity of catalase or peroxidase which directly dismutate H_2O_2 and O_2. Proline and ascorbate components also play important role in defense system against ROS (Gill *et al.,* 2012).

Numerous investigations have indicated that upregulation of the antioxidant systems is an early response of cyanobacteria to heavy metal stress (Srivastava *et al.,* 2005; Kiran *et al.*, 2008; Chris, 2012; Singh *et al.,* 2012) but we do not have any report on antioxidative response of cyanobacteria to lead stress. So the present study has been undertaken to investigate the effect of lead stress on growth and antioxidants of cyanobacterium *Cylindrospermum* sp. Data obtained from this study may be useful for better understanding the mechanisms of Pb tolerance in cyanobacteria.

EXPERIMENTAL METHODOLOGY

Organism, growth conditions and nickel treatment:

The filamentous heterocystous cyanobacterium C*ylindrospermum* sp. was isolated from rice fields near Allahabad and was raised to axenic culture. The culture was axenically grown in nitrogen free BG-11 medium (Rippka, *et al.,* 1979) at 27 ± 2^0 C pH under 75µ mol $m^{-2}s^{-1}$ photon flux density (PFD) with a photoperiod of 14:10 h. Stock solution (500 µM) of Pb was prepared by using lead chloride salt. Various concentrations (0, 25, 50, 75, 100 and 125 µM) of Pb were prepared by diluting the stock with BG-11 medium and filtered through a Millipore membrane filter (0.45 mm).Mid logarithmic phase cultures, were used for experimentation.

Estimation of growth, lipid peroxidation and proline:

Growth was estimated by estimating the dry weight of cyanobacterium after four days of treatment.Melondialdehyde (MDA) level in test samples was determined according to the procedure of Heath and Packer (1968).Proline concentration in the cells of Pb treated and untreated cells suspensions was determined spectrophotometrically by the method of Bates *et al*. (1973).

Assay of enzymes:

Superoxide dismutase (SOD) activity was measured spectrophotometrically by following the method of Giannopolitis and Ries (1977) using a reaction mixture (3 ml) containing of riboflavin (1.3 mM), L- methionine (13 mM), Na_2CO_3 (0.05 mM), (pH 10.2), p- nitroblue tetrazolium chloride (63µM) and 0.1 ml of crude extract (isolated in 100 mM EDTA phosphate buffer, pH 7.8). Peroxidase (POD) activity was determined as per the method of Gahagen *et al.* (1968). A reaction mixture (3 ml) consisting of H_2O_2 (1 ml), 100 mM pyrogallol (1 ml) and crude extract (1 ml) was used for the assay. Catalase (CAT) activity was determined spectrophotometrically by the method of Aebi (1984).

Statistical analysis:

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

EXPERIMENTAL FINDINGS AND ANALYSIS

Fig. 1 shows the effect of different concentrations (0-125 µM) of Pb on growth of *Cylindrospermum* sp. Pb was found to be toxic at all the concentrations and the growth reduction was significant at all doses of Pb. A reduction of 10, 25, 38, 50, and 75 per cent in the dry weight was recorded at 25, 50, 75, 100 and 125 µM doses of Pb as compared to control. A considerable decrease in dry weight in plant is observed under Pb treatment. Lead toxicity lowers the protein content of cells and causes significance alterations in lipid composition (Stefanov *et al.,* 1995). Synthesis of DNA and RNA are also

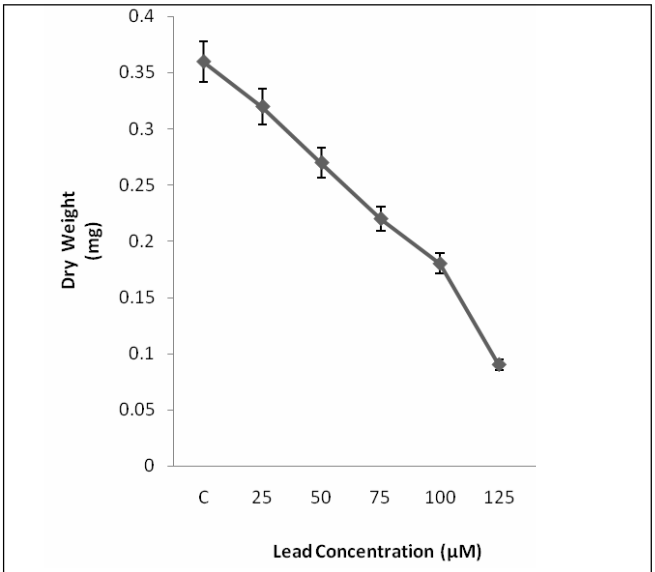

Fig. 1 : Effect of different concentrations of lead on growth (dry weight) of *Cylindrospermum* sp. Values are mean ±SE of three replicates. Values are significant at 0.05 (5%) level of significance (ANOVA)

greatly reduced in the cells as reported by Mitra and Mukherji (1977). At the same time Pb stress also disturb many plant species including photosynthsis, respiration, mineral nutrition, membrane structure etc. (Zhao *et al.,* 2011).

The activity of lipid peroxidation measured in terms of melondialdehyde (MDA). 25 µM Pb led to 30 per cent increase in the cells and the highest increment recorded at 125 µM *i.e.* 145 per cent (Fig. 2). MDA is a final product of peroxidation of membrane lipid and accumulates when the plants are subjected to oxidative stress (Chris, 2012). Therefore MDA level is routinely used as an index of lipid peroxidation under stress conditions. Results show an increase in the level of lipid peroxides with increasing concentrations of Pb, indicating that Pb induces oxidative stress in *Cylindrospermum* sp.These results are in conformity with the observations of Malecka *et al.* (2001) and Shu *et al.* (2012).

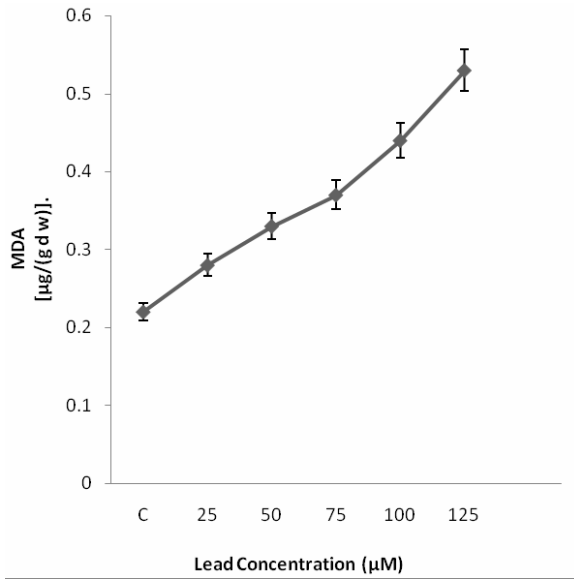

Fig. 2 : Effect of different concentrations of lead on lipid peroxidation (MDA) of *Cylindrospermum* sp.Values are mean ±SE of three replicates. Values are significant at 0.05 (5%) level of significance (ANOVA)

The enzymatic components associated with defense against ROS include SOD, CAT, POD and non enzymatic antioxidants proline. Fig. 3 shows the impact of lead stress on superoxide dismutase (SOD) activity. The SOD activity was increased gradually from 25 µM to 100 µM. A slight decreased was also observed at highest dose *i.e.* 125 µM. SOD, CAT and POD have been identified as enzymatic protectors against peroxidation reactions. SOD is an essential component of antioxidative defense system in plants and it dismutates two superoxide radicals (O^-_2) to water and O_2. Results show increased activity of SOD in *Cylindrospermum* sp. growing under toxic level of Pb. The increase in the activity of SOD in response to Pb appears to be due to *de novo* synthesis of enzymatic protein (Lozano *et al.,* 1996).SOD activity has been reported to increase under UV-B (Chris *et al.,* 2008), NaCl (Chris and Masih, 2012), Nickel (Chris, 2012) and cadmium (Singh *et al.,* 2012) in cyanobacteria.

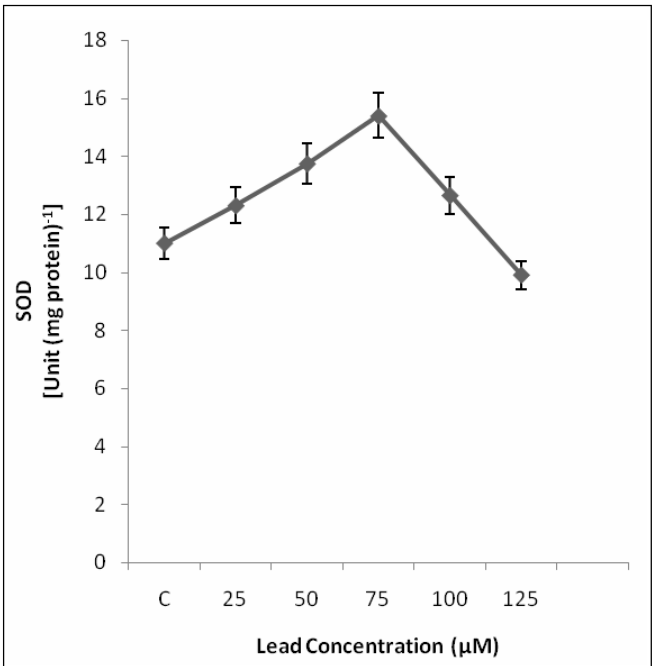

Fig. 3 : Effect of different concentrations of lead on superoxide dismutase (SOD) activity of *Cylindrospermum* sp. Values are mean ±SE of three replicates. Values are significant at 0.05 (5%) level of significance (ANOVA)

Data related to the effect of lead stress on peroxidase (POD) and catalase (CAT) enzymes are compiled in Fig 4 and 5, respectively 25, 50, 75 and 100 µM treatment increased the POD activity by 10, 20, 40 and 25 per cent, respectively as compared to control. A decline was also noticed at 125 µM in POD the, highest dose of Pb but, it was higher than the control value. The activity of catalase increased till 50 µM but it was declined thereafter and even 40 per cent decline was observed at 125 µM treatment of Pb. Lead has been reported to induce peroxidase activity in soybean, rice seedlings etc. (Lee *et al.,* 1976; Verma and Dubey, 2003). The role of peroxidase as a stress enzyme in plants has been widely accepted (Gasper, 1982;Subhashini and Reddy,1990). Increased peroxidase activity with Pb treatment can be correlated with the release of peroxidase localized in the cell (Gasper, 1982).Among the antioxidative enzymes catalase decomposes H_2O_2 to water and molecular oxygen. A decline in the activity of catalase has been observed in Pb-stressed plants (Verma and Dubey, 2003). Such a decrease appears to be due to decline in enzyme synthesis or a change in the assembly of enzyme subunits

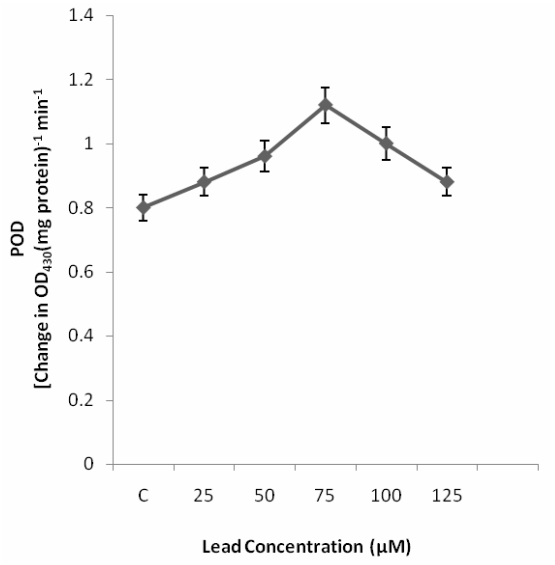

Fig. 4 : Effect of different concentrations of lead on peroxidase of *Cylindrospermum* sp. Values are mean ±SE of three replicates. Values are significant at 0.05 (5%) level of significance (ANOVA)

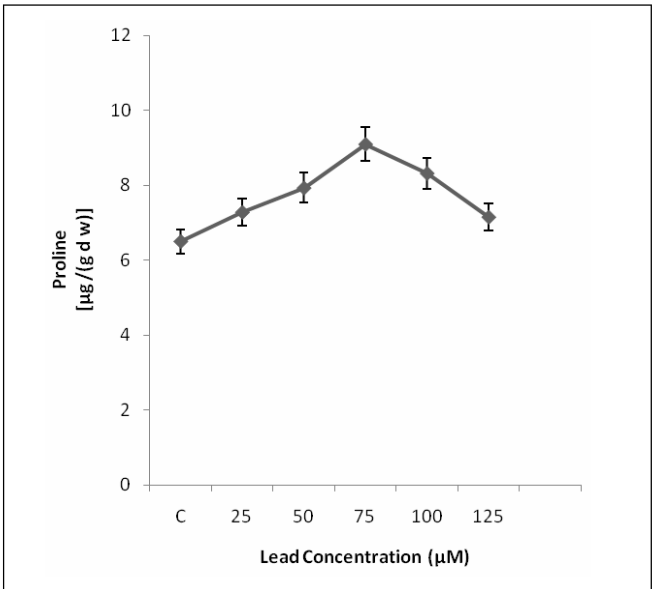

Fig. 6 : Effect of different concentrations of lead on proline content of *Cylindrospermum* sp.Values are mean ±SE of three replicates. Values are significant at 0.05 (5%) level of significance (ANOVA)

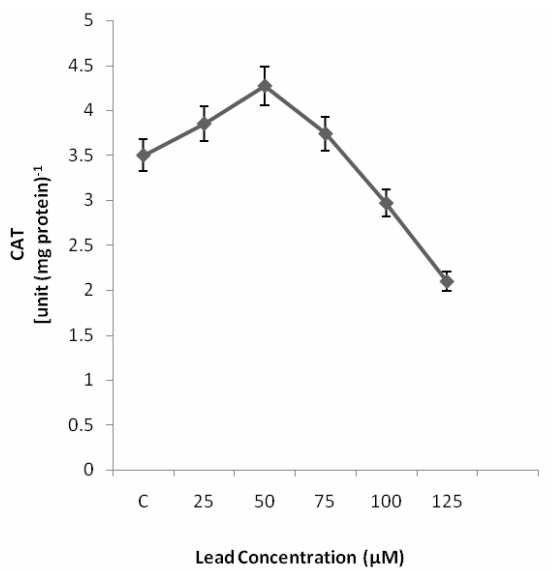

Fig. 5 : Effect of different concentrations of lead on catalase of *Cylindrospermum* sp. Values are mean ±SE of three replicates. Values are significant at 0.05 (5%) level of significance (ANOVA)

(Hertwig *et al.,* 1992).

Fig. 6 compiles data on the effect of different concentration of lead on proline an antioxidant metabolite. The levels of proline in cells of *Cylindrospermum* sp. increased with the increase in lead concentration. Proline exhibited an increase of 1.10, 1.22 and 1.40 fold after the treatment of 25, 50 and 75 µM, respectively as compared to control. A little decrease was found at the highest dose but it was always higher than the control value. Several non specific defense systems are also activated when plants are exposed to Pb; synthesis of proline is also one of them. There are different opinions regarding mechanism by which proline alleviate metal toxicity effects within the cell. It has been shown that free proline acts as an osmoprotectant (Paleg *et al.,* 1984), protein stabilizer (Sharma and Dubey, 2004), metal chelator (Farago and Mullen, 1979), inhibitor of lipid peroxidation (Mehta and Gaur, 1999), free radical scavenger (Chris *et al.*, 2006) etc.

Results suggest that lead toxicity causes oxidative stress and bring reduction in growth in *Cylindrospermum* sp. The antioxidants like SOD, POD, CAT and proline play a pivotal role in combating the oxidative stress in the cyanobacterium. Pb is not a oxido- reducing metal, the oxidative stress induced by Pb in growing cells of *Cylindrospermum* sp. appears to be an indirect effect of Pb toxicity leading to production of ROS with a simultaneous increase cellular level of SOD, POD, CAT and proline.

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