RESEARCH PAPER

Effect of nickel stress on growth and antioxidants in cyanobacterium *Cylindrospermum* sp.

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Paddy field cyanocterium *Cylindrospermum* sp. grown in BG-11 medium containing various concentrations (0, 25, 50, 75 and 100 µM) of Ni, showed a dose dependent decreases in growth (Chlorophyll-a). Nickel treated cells exhibited increased rates of MDA, demonstrating enhanced lipid peroxidation. Antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD) activity increased with the increase in nickel concentrations. Proline contents also proportionately increased with the elevated Ni concentration in cyanobacterium. Study shows that the antioxidant (enzymatic and non enzymatic) activities might play a central role in cellular protection against the Ni induced oxidative stress.

Key words **:** *Cylindrospermum* sp., Ni, MDA, POD, SOD

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INTRODUCTION

Heavy metals can be included in the main category of pollutants. Nickel is a heavy metal used extensively and can contaminate the soil mainly through sewage sludge, industrial compost and atmospheric fallout, especially near processing plants (Poulik, 1997).Nickel is an essential micronutrient for plant growth and it is also a component of enzyme urease which is required for nitrogen metabolism in higher plants (Dixon *et al.,* 2004). However, excess nickel is known to be toxic and many studies have been conducted concerning Ni toxicity in various plant species (Pandolfini *et al.,* 1992; Gajewska and Skodowska, 2008; Ahmad, *et al*., 2009; Khan and Khan, 2010; Gajewska and Skodowska, 2010; Singh and Pandey, 2011). The most common symptoms of nickel toxicity in plants are growth inhibition, photosynthesis, mineral nutrition, sugar transport and water relations (Seregin and Kozhevnikova, 2006).Over production of reactive oxygen species (ROS) is a common response of plants to heavy metal stress especially nickel stress (Blokhina *et al.,* 2003). It is well established that the overproduction of ROS induces oxidative damage to various cellular constituents, such as lipid, proteins and nucleic acids (Shah *et al.,* 2001). One of the most damaging oxidative effects is the peroxidation of membrane lipids, which results in concomitant production of melondialdehyde (MDA) (Hodges *et al.,* 1999). Plants have evolved antioxidative mechanism to detoxify and eliminate these harmful ROS (Chri, *et al*., 2008). The antioxidative defense system includes antioxidant molecules like proline and antioxidant enzymes such as superoxide dismutase (SOD, EC 1.15.1.1) peroxidase (POD, EC 1.11.1.7) as well as catatlase (CAT, EC 1.11.1.6). SOD is the first enzyme in detoxifying process, converts O^-_2 to H_2O_2 and POD and CAT catalyse the breakdown of H_2O_2 (Asada, 1992, 1999). Proline also acts as an effective ROS quencher and accumulates heavily in plants under metal stress (Alia and Pardha Saradhi, 1991).

The role of nitrogen fixing cyanobacteria in enhancing soil fertility has been long known and is well documented (De, 1939; Venkataraman, 1981; Sinha and Hader, 1996). Cyanobacteria contributes to overall soil health not only by its ability to perform biological nitrogen fixation but also because of its ability to produce polysaccharides and other bioactive compounds which has a growth stimulating effect on plants, as well as ensuring maintenance of soil quality and preventing erosion (Singh, 1950).

The toxic effect of nickel on cyanobacteria especially on their growth, carbon fixation, nitrogen metabolism (Rai and Raizada, 1985, 1986), phosphorus metabolism (Asthana *et al*., 1992) and bioremediation (Shukla *et al.,* 2009) are studied earlier

but any report on effect of nickel on oxidative stress and antioxidants in cyanobacteria is lacking. So the present study has been undertaken to investigate the effect of nickel on growth and antioxidants of cyanobacterium *Cylindrospermum* sp.

RESEARCH 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 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 Ni was prepared by using nickel chloride salt. Various concentrations (0, 25, 50, 75 and 100 µM) of Ni 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.

Growth estimation:

Growth was estimated by estimating the Chlorophyll content. Chl-a was extracted in 80 per cent acetone and measured according to the method of Myres and Kratz (1955).

Estimation of lipid peroxidation and proline:

Melondialdehyde (MDA) level in test samples was determined according to the procedure of Heath and Packer (1968). Proline concentration in the cells of Ni 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.

Statistical analysis:

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

RESEARCH FINDINGS AND ANALYSIS

In this paper, author has investigated oxidative damage and capacities of antioxidants involved in oxidative stress detoxification in cells of *Cylindrospermum* sp. All the nickel treatments reduced the growth of the cyanobacterium as evident from the reduced chlorophyll content. The amount of Chl-a content in *Cylindrospermum* sp. was reduced by 7, 15, 25 and 38 per cent at 25, 50, 75 and 100 µM nickel concentrations, respectively. The result shows that the nickel treatment caused a reduction of chlorophyll pigment which could be due to inhibition of Chl biosynthesis by inhibiting ä-aminolevulinic acid dehydrogenase and protochlorophyllide reductase activities and breakdown of pigments or their precursors (Ouzounidou, 1995; Gajewska, *et al.,* 2006).

Exposure of cyanobacterium cells to nickel treatment increased the MDA content significantly. It was maximum in cells exposed to 100 µM nickel (168%) followed by 75 (145%), 50 (126%) and 25 µM nickel (112%) in comparison to control (Table 1). MDA is a product of lipid peroxidation and is usually used as an indicator of the degree of oxidative stress.Excessive production of ROS has been shown to cause lipid peroxidation

Table 1 : Effect of nickel on lipid peroxidation (MDA), superoxide dismutase (SOD), peroxidase (POD), and proline content of *Cylindrospermum* sp. Mean+SE. Values in parenthesis are percent increase with reference to respective control. All treatments are significantly different (0.05) from control (ANOVA)

Treatments	MDA [µ mol (g DW) $^{-1}$]	SOD [Unit (mg protein) $^{-1}$]	POD [Change in OD_{430} (mg protein)$^{-1}$ min^{-1}]	Proline [µg (g DW) $^{-1}$]
Control	0.22±0.003	11.00±0.3	0.80±0.004	6.5±0.2
	(0.00)	(0.00)	(0.00)	(0.00)
$Ni_{25µM}$	0.24±0.003	12.1±0.3	0.92±0.004	7.67±0.2
	(+12)	(+10)	(+15)	(+18)
$Ni_{50µM}$	0.27±0.004	13.2±0.4	1.0±0.005	8.45±0.3
	(+26)	(+20)	(+25)	(+30)
$Ni_{75µM}$	0.31±0.005	15.1±0.4	1.12±0.005	9.75±0.3
	(+45)	(+38)	(+41)	(+50)
$Ni_{100µM}$	0.36±0.005	17.0±0.5	1.28±0.006	11.18±0.4
	(+68)	(+55)	(+60)	(+72)

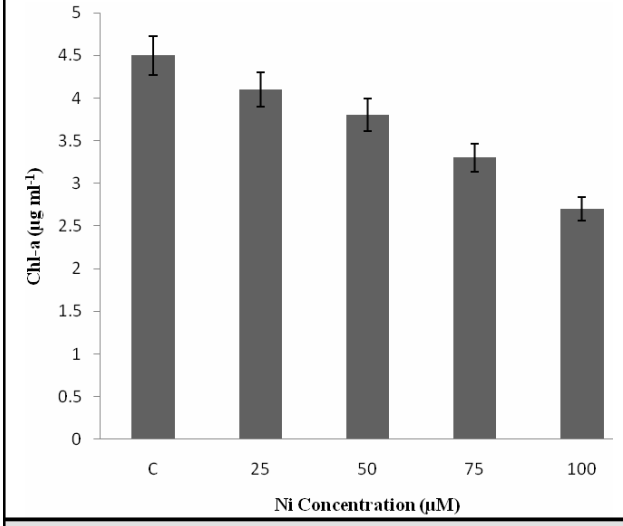

Fig. 1 : Effect of different concentrations of nickel on growth (chlorophyll-a) of *Cylindrospermum* sp. Values are mean ±SE of three replicates. Values are significant at 0.05 (5%) level of significance (ANOVA)

and oxidation of protein thiol groups (Dat *et al.,* 2000).The increase in lipid peroxide content with increasing Ni levels suggests that Ni induces oxidative stress in cyanobacterium cells.Increased lipid peroxidation and elevated ROS levels have been reported in many plant species exposed to toxic levels of Ni (Pandolfini *et al*., 1992; Prasad *et al*., 2005; Gajewska and Skiodowska, 2010).

Superoxide dismutase (SOD) was increased as the nickel concentration was increased; the maximum increment was observed at 100 µM nickel *i.e.* 55% (Table 1). At 25, 50 and 75 µM concentration it was 10, 20 and 38%, respectively. Environmental stresses can lead to enhanced production of ROS within plant tissues and induced SOD to detoxify these harmful ROS (Mittler, 2002). Induction in SOD activity in cyanobacterium cells can be correlated with development of increased tolerance to variety of chemical compounds and physical stresses (Prasad *et al.,* 2005; Chris *et al*., 2006). Increased SOD activity as observed in our study was either due to increased production of ROS or could be a protective measure adopted by *Cylindrospermum* sp. against oxidative damage.

A similar trend was noticed when metal (25 – 100 µM nickel) exposed cells were analyzed for peroxidase (POD) enzyme. The POD increased with the increase in metal concentration. Increase was found to be 15, 25, 41 and 60% at 25, 50, 75 and 100 µM nickel concentration. The activity of peroxidase has been reported to increase in response to various stress factors, including excess concentrations of heavy metals (Diaz *et al*., 2001). The enhanced activity of POD in excess nickel treated cells might result either in peroxidative damage of the thylakoid membrane or lower auxin and protein content in tissues (Sandman and Boger, 1980; Pandolfini *et al.*, 1992).

The exposure of the cyanobacterium to 25 and 50 µM nickel treatment increased the proline activity from 6.5 [µg (g FW)$^{-1}$] to 7.67 and 8.45. At 75 and 100 µM concentration the proline accumulation was 9.75 and 11.18 [ìg (g FW)$^{-1}$], respectively (Table 1). Accumulation of proline in plants subject to Ni stress has been well documentaed (Prasad *et al.,* 2005; Gajewska and Skiodowska, 2008; Singh and Pandey, 2011). Enhanced production of proline in cells of cyanobacterium could be linked with detoxification against Ni induced oxidative stress. Gajewska and Skiodowska (2008) suggested that it may be involved in the mechanisms of osmoregulation. Recently author has reported NaCl induced proline accumulation in cyanobacerium *Cylindrospermum* sp. (Chris and Masih, 2012).

In conclusion Ni stress caused significant reduction in chlorophyll content of *Cylindrospermum* sp. Heavy accumulation of ROS due to inhibited chlorophyll-a pigment and photosynthesis led to enhanced lipid peroxidation. SOD, POD and proline seem to play a prime role in regulation of ROS level upon excessive nickel. Further investigation on a cellular or molecular level is necessary to understand the mechanism of these antioxidants.

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