DOI: 10.1007/s11099-015-0096-3 PHOTOSYNTHETICA 53 (2): 223-230, 2015

Differential response of photosynthetic apparatus of cyanobacterium *Nostoc muscorum* to Pb and Cd toxicity

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Abstract

Nostoc muscorum cells showed metal-induced decrease in the relative growth, pigment contents, O_2 evolution, and Hill activity in response to lead (Pb^{2+}) and cadmium (Cd^{2+}) treatment, which was further accentuated with increase in metal exposure time and metal concentration. I_{50} concentrations (50% growth inhibitory concentrations) of Pb^{2+} and Cd^{2+} for growth of *N. muscorum* were 55 and 21 μg mL^{-1}, respectively. These results indicated that the cells of *N. muscorum* were more susceptible to Cd^{2+} in comparison to Pb^{2+}. The O_2 production was relatively more sensitive to both heavy metals (I_{50}: 16 and 10 μg mL^{-1} of Pb^{2+} and Cd^{2+}, respectively) than the Hill activity (I_{50}: 61 and 39 μg mL^{-1} of Pb^{2+} and Cd^{2+}, respectively). Further, measurement of Hill activity in the presence of metals and electron donors showed that inhibition sites of both Pb^{2+} and Cd^{2+} were located on the oxidizing site of PSII. The chlorophyll *a* (Chl *a*) and phycobilisome (PB) fluorescence emission spectra showed that energy transfer from Chl *a* and PB to PSII reaction center was more susceptible to Cd^{2+} than Pb^{2+}.

Additional key words: chlorophyll fluorescence; electrons donors; heavy metals; Hill activity; oxygen evolution.

Introduction

Many industries discharge waste water containing toxic heavy metals which poison the ecosystem even at very low concentrations. The International Agency for Research on Cancer (IARC) classified both Cd and Pb as highly toxic metals for human health causing damage to the human vital organs (Liping *et al.* 2008). Anthropogenic factors such as industrial activities are mainly held responsible for excessive discharge of metals into the freshwater ecosystems, leading to death of primary producers including cyanobacteria (Cavet *et al.* 2003).

Known sensitivity of individual algal strains to heavy metals may also provide a clue to select phycoremediating agents. Various studies have been carried out to find effects of heavy metals on growth (Fathi and E1-Shahed 2000, Giusti 2001, Lamai *et al.* 2005, Zhou *et al.* 2006, Thapar *et al.* 2008, Afkar *et al.* 2010, Deniz *et al.* 2011, Dudkowiak *et al.* 2011) and photosynthesis of algal species. Some of the studies related to influence of heavy metals on photosynthetic activity and pigments (Rai *et al.* 1994, Horne *et al.* 1998, Danilov and Ekelund 2001, Zaccaro *et al.* 2001) revealed that photosynthetic machinery of these photoautotrophs is relatively more sensitive to metal toxicity than the respiration and other metabolic activities.

Effects of heavy metals differed in various micro-organisms including cyanobacteria (Sandau *et al.* 1996). A critical survey of the literature revealed heavy metal-induced changes in growth, and physiological and biochemical processes in cyanobacteria (Bhargava *et al.* 2005, Afkar *et al.* 2010, Dudkowiak *et al.* 2011). The present study attempted to characterize the mode of Pb^{2+} and Cd^{2+} ion action on the growth, pigments, and photosynthetic electron transport in the nitrogen-fixing cyanobacterium, *N. muscorum.*

Received 25 April 2013, *accepted* 30 May 2014.
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Abbreviations: Chl *a* – chlorophyll *a*; DCPIP – 2,6-dichlorophenol-1-indophenol; DPC – 1,5-diphenyl carbazide; I_{50} – 50% inhibitory concentration; PB(s) – phycobilisome(s); PC – phycocyanin.
Acknowledgments: Authors are grateful to the reviewers and editors for their constructive comments on an early draft of the manuscript. The authors are also thankful to Head of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, for providing laboratory facilities.

Materials and methods

Chemicals: To examine the effect of heavy metals on *N. muscorum* cells, the stock solutions of Pb^{2+} and Cd^{2+} were prepared by dissolving nitrate salt of these metals in milli-Q water as needed. The resulting stock solution was further diluted to get desired concentrations. All other chemicals used in the present course of study were of analytical reagent grade.

Organism and growth conditions: The culture of cyanobacterium (*N. muscorum*) was obtained from the National Facility for Blue Green Algae, Indian Agricultural Research Institute, New Delhi. The cells were grown in modified Chu-10 medium (Gerloff *et al.* 1950) at 25 ± 1ºC under illumination of 10 W m^{-2} provided by fluorescent light with 16 h light/8 h dark cycle. The exponentially growing cells were harvested and used for different treatments.

Effect of different concentrations of Pb^{2+} and Cd^{2+} (5, 10, 20, 40, and 60 μg mL^{-1}) on the growth of the cells was recorded by measuring the absorbance at 750 nm in spectrophotometer (*UV-Visible 1601*, *Shimadzu,* Japan) over a period of time (0–5 d). Protein content of the cell suspension was estimated by the method of Lowry *et al.* (1951) modified by Herbert *et al.* (1971). The chlorophyll (Chl) *a* was extracted by the method given by McKinney (1941).

Measurement of photosynthetic pigments by whole cell scan: The homogenous algal cell suspension withdrawn after 72 h of Pb^{2+} and Cd^{2+} treatment (5, 10, 20, 40, and 60 μg mL^{-1}) was scanned (400–700 nm) in a double beam spectrophotometer (*UV-Visible–1601*, *Shimadzu,* Japan) by using a light path of 1.0 cm. The optical density of cells was 0.7 at 665 nm. The quantification of photosynthetic pigments was done by using the formula (Astier *et al.* 1979) as mentioned below:

$$\text{Phycocyanin (PC)} = (0.16\, A_{622} - 0.06\, A_{678}) \times 10^{-3} \quad [\mu g\, mL^{-1}] \quad (1)$$

$$\text{Carotenoids (Car)} = (7.60\, A_{480} - 3.60\, A_{510}) \times 10^{-3} \quad [\mu g\, mL^{-1}] \quad (2)$$

Oxygen evolution: Effect of Pb^{2+} and Cd^{2+} on the rate of photosynthetic oxygen evolution in *N. muscorum* was recorded by using a *Clark*-type liquid phase oxygen electrode (*Hansatech*, USA) fitted with circulating water jacket. The zero oxygen concentration was obtained by using solid sodium dithionite as described elsewhere (Singh and Kshatriya 2002). The cell suspension (optical density of 0.7 at 665 nm) was incubated in light in the presence of different concentrations of Pb^{2+} and Cd^{2+} (10, 20, 40, 60, 80, and 100 μg mL^{-1}) for 90 min before using it for measurement of O_2 evolution. The rate of O_2 evolution was expressed in terms of nmol(O_2 evolved) mg^{-1}(protein) s^{-1}.

Hill activity was measured in terms of reduction of 2,6-dichlorophenol-1-indophenol (DCPIP) as described by Holt and French (1948). The suspension of permeaplasts (Wards and Myers 1972) having optical density of 0.7 at 665 nm in the HEPES-NaOH buffer (10 mM, pH = 7.0) containing $NaHCO_3$, $MgCl_2$, $CaCl_2$ (10 mM each), and NH_4Cl (1 mM) was kept in light for 90 min. The reaction was started with addition of 50 μM DCPIP. The rate of DCPIP reduction was measured in terms of change in absorbance at 600 nm against an appropriate blank in a spectrophotometer (*UV-Visible–1601*, *Shimadzu,* Japan). Hill activity was expressed in terms of nmol(DCPIP reduced) mg^{-1}(protein) s^{-1}. The effect of electron donors on Hill activity was also measured. *N. muscorum* cells pretreated with 60 μg mL^{-1} concentration of both the individual metals was used and supplemented with electron donors before the start of Hill activity. Either 1,5-diphenyl carbazide (DPC, 100 μM) or hydroxyl amine (50 μM) were used as the exogenous source of electron donors.

Fluorescence emission from Chl *a* and PBs: Fluorescence emission spectra (600–750 nm) of Chl *a* and PBs were measured in a spectrofluorometer (*UV-Vis SL-174, Elico*, India) by using excitation beam of 435 and 570 nm, respectively, as described by Singh *et al.* (1989). The cell suspension (optical density of 0.7 at 665 nm) was light incubated in the presence of different concentrations of Pb^{2+} and Cd^{2+} (25 and 100 μg mL^{-1}) for 90 min before recording the room temperature fluorescence emission. The emission spectra were obtained by using a band width of 5 nm and light path of 1 cm. At the time of measurement of fluorescence emission spectrum, the cell suspension in each sample was equalized in terms of Chl concentration and was continuously stirred in order to avoid the settling problem.

Statistical analysis: Experimental data obtained in triplicate were summarized as mean ± SE and were shown graphically. Data between the two metals were compared by one-way analysis of variance (*ANOVA*). They were considered statistically significant at $p<0.05$. All analyses were performed by *STATISTICA Windows version 6.0* (*StatSoft Inc*., USA).

Results

Effect of lead and cadmium on growth: Effect of different concentrations (0–60 μg mL^{-1}) of both Pb^{2+} and Cd^{2+} on the growth of *N. muscorum* was recorded separately at different concentrations (Fig. 1). The results showed that lower concentrations of metals (up to 5 μg mL^{-1}) did not inhibit the growth, while higher concentrations (10–60 μg ml^{-1}) caused a concentration dependent decline in the growth compared with the control (without metal). The I_{50} concentrations of both Pb^{2+} and Cd^{2+} were found to be 55 and 21 μg mL^{-1}, respectively. The results indicated that cells were more susceptible to Cd^{2+} in comparison to Pb^{2+} and there was statistically significant difference (p<0.001) between Pd^{2+} and Cd^{2+} toxicity throughout the concentration range.

Effect of lead and cadmium on pigments

Chl *a*: The *N. muscorum* cells growing in the presence of different doses of Pb^{2+} and Cd^{2+} (0–60 μg mL^{-1}) were taken for methanol extraction of Chl *a*. They showed concentration-dependent decrease in the Chl *a* content in the presence of both the metals (Fig. 2*A*). It was observed that Pb^{2+}-induced decline in Chl *a* content was sluggish up to 20 μg mL^{-1} concentration of Pb^{2+}, while Cd^{2+} induced a steep decline in the Chl *a* concentration at the lower dose (5 μg mL^{-1}). At 10 μg mL^{-1} concentration of both metals, inhibitory effect of Cd^{2+} on Chl *a* was found to be about two-fold higher than that of Pb^{2+}. Chl *a* showed higher sensitivity towards Cd^{2+}, which was significantly higher than Pb^{2+}.

PC: Exponentially growing cells treated with different concentrations of Pb^{2+} and Cd^{2+} (5–60 μg mL^{-1}) were used for the measurement of the whole cell absorption spectra (400–700 nm). The content of PC was calculated from the absorption peak at 625 nm. A slight increase in the PC concentration (Fig. 2*B*) was observed at Pb^{2+} concentration up to 5 μg mL^{-1}, but higher concentrations (>10 μg mL^{-1}) showed a slight inhibitory effect on PC content. On the contrary, the PC content declined in the presence of lower concentrations of Cd^{2+} (5–10 μg mL^{-1}); it was followed by an increase between 10–20 μg mL^{-1} of Cd^{2+}. However, PC content of Cd^{2+}-treated cells remained always lower in comparison to Pb^{2+}-treated cells throughout the whole concentration range of both the metals (5–60 μg mL^{-1}). These results indicated that PC content was relatively more susceptible to Cd^{2+} than Pb^{2+}.

Car: We measured also changes in the Car content during the treatment of the *N. muscorum* cells with different concentrations of Pb^{2+} and Cd^{2+} (5–60 μg mL^{-1}). The results (Fig. 2*C*) showed a marginal increase in Car after Pb^{2+} treatment up to 10 μg mL^{-1}, which was followed by the sluggish decline throughout the rest of the Pb^{2+}

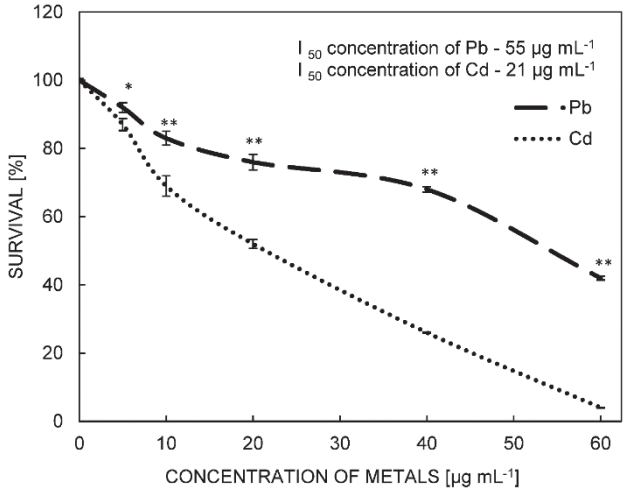

Fig. 1. Effect of lead and cadmium [0–60 μg mL^{-1}] on the growth of *Nostoc muscorum*. All the values are means ± SE of three replicates. * – p<0.05, ** – p<0.001. I_{50} – 50% inhibitory concentration.

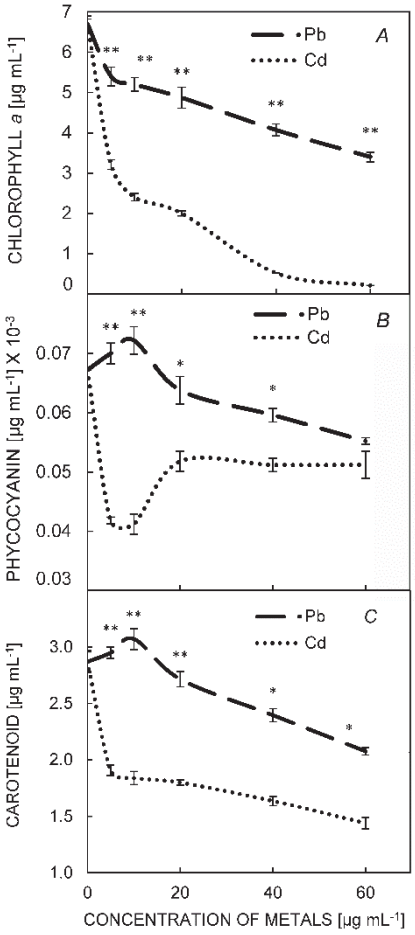

Fig. 2. Effect of lead and cadmium [0–60 μg ml^{-1}] on the chlorophyll *a* (*A*), phycocyanin (*B*), and carotenoid (*C*) content of *Nostoc muscorum*. All the values are means ± SE of three replicates (* – p<0.05, ** – p<0.001)

concentration range (10–60 μg mL^{-1}). On the other hand, the Car content gradually declined in response to Cd^{2+} concentration throughout the whole concentration range. In conclusion, the Car content exhibited similar pattern of susceptibility to metal toxicity as other measured pigments; the effect of Cd^{2+} was more pronounced than that of Pb^{2+}.

Oxygen evolution: A short-term effect of Pb^{2+} and Cd^{2+} on the functional properties of photosynthetic apparatus of *N. muscorum* cells was studied by treating the cells for 90 min in the presence of varying concentrations (10–100 μg mL^{-1}) of Pb^{2+} and Cd^{2+}, which was done to avoid any long-term effect of these metals on the size of pigment antenna and other electron transfer components. The metal-treated cells were used for measurement of photosynthetic O_2 evolution. Results showed a concentration-dependent sharp decline in the O_2-evolving capacity of the cells in the presence of both Pb^{2+} and Cd^{2+} (Fig. 3). However, Cd^{2+}-induced decline in the rate of O_2 evolution (46.8%) was more pronounced than that of Pb^{2+} (40.4%) at 10 μg mL^{-1} concentration of both the metals. In case of Cd^{2+}-treated cells, the rate of O_2 evolution reached its lowest level at and above 20 μg mL^{-1} concentrations, whereas the cells treated with Pb^{2+} showed a concentration-dependent gradual decline in the rate of O_2 evolution throughout the concentration range (10–100 μg mL^{-1}). I_{50} concentration (50% inhibitory concentration) of Pb^{2+} and Cd^{2+} for O_2 evolution was found to be 16 and 10 μg mL^{-1}, respectively, indicating that the photosynthesis in *N. muscorum* was more susceptible to Cd^{2+} than Pb^{2+} during short-term.

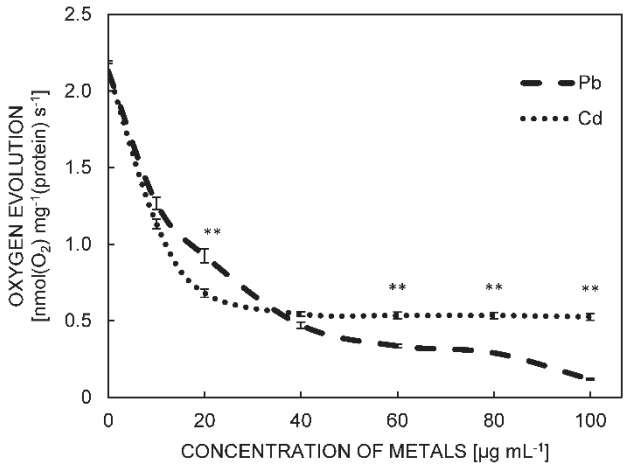

Fig. 3. Effect of different concentrations of lead and cadmium [0–100 μg mL^{-1}] on the rate of oxygen evolution [nmol(O_2) mg^{-1}(protein) s^{-1}] of *Nostoc muscorum*. All the values are means ± SE of three replicates (* – p<0.05, ** – p<0.001)

Hill activity: Similarly to oxygen evolution, *N. muscorum* cells pretreated with both Pb^{2+} and Cd^{2+} (10–100 μg mL^{-1}) for 90 min were used for measurement of Hill activity [nmol(DCPIP reduced) mg^{-1} (protein) s^{-1}]. Results (Fig. 4)

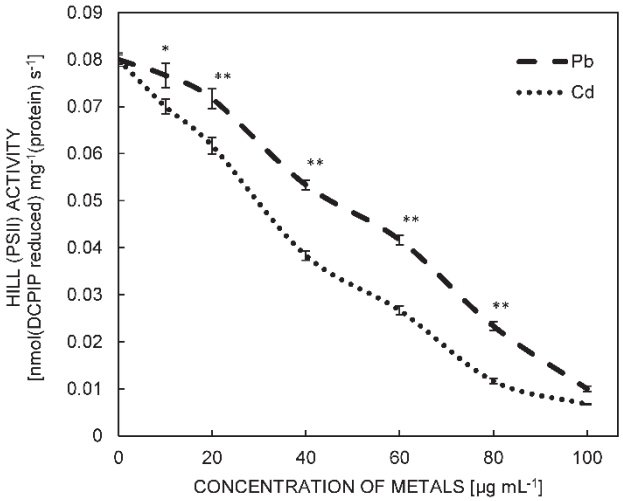

Fig. 4. Effect of different concentrations of lead and cadmium [0–100 μg mL^{-1}] on Hill activity [nmol(DCPIP reduced) mg^{-1}(protein) s^{-1}] in the *Nostoc muscorum*. All the values are means ± SE of three replicates (* – p<0.05, ** – p<0.001).

Table 1. Effect of electron donors on DCPIP reduction in the presence of Pb and Cd. The rate of activity measured for each electron donor is the average value ± SE of three independent experiments. The values given in parenthesis indicate the percent activity in relation to control. DCPIP – 2,6 dichlorophenol-1-indophenol; DPC – 1,5-diphenyl carbazide; NH_2OH – hydroxyl amine.

Treatment	DCPIP reduction [nmol(DCPIP reduced) mg^{-1}(protein) s^{-1}]	
	Pb^{2+}	Cd^{2+}
Control	0.080 ± 0.39 (100)	0.080 ± 0.39 (100)
Metal alone (60 μg mL^{-1})	0.048 ± 0.21 (60.00)	0.029 ± 0.07 (36.25)
Metal + DPC (100 μM)	0.069 ± 0.42 (86.25)	0.043 ± 0.16 (53.75)
Metal + NH_2OH (50 μM)	0.065 ± 0.25 (81.25)	0.064 ± 0.30 (80.00)

revealed a concentration-dependent gradual decline in the Hill activity of the cells in the presence of each metal. The Cd^{2+}-induced decline in the Hill activity was again more pronounced than Pb^{2+}. The I_{50} concentration (50% inhibitory concentration) for the Hill activity was observed at 39 and 61 μg mL^{-1} of Cd^{2+} and Pb^{2+}, respectively. There was a complete inhibition of the Hill activity at concentration of 100 μg mL^{-1} for both the metals. A comparison of the results for each metal suggested that photosynthetic electron transport in the cells was more susceptible to Cd^{2+} than to Pb^{2+}.

Effect of electron donors on lead- and cadmium-induced inhibition of Hill activity was studied in *N. muscorum* cells treated with or without metals. The cells pretreated with Pb^{2+} and Cd^{2+} (60 μg mL^{-1}) for 90 min were supplemented with electron donors, DPC and NH_2OH (hydroxyl amine) (100 and 50 μM, respectively),

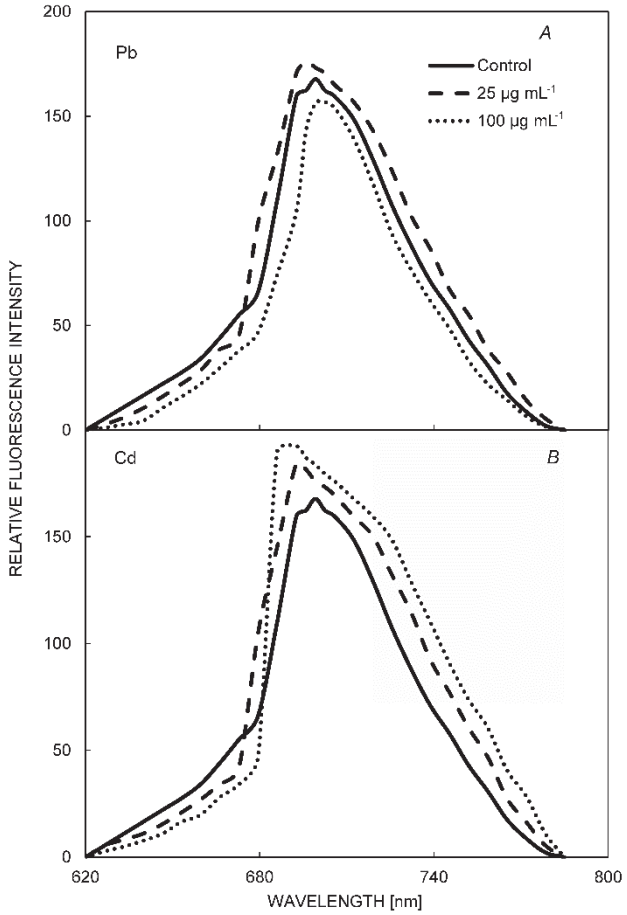

Fig. 5. Room temperature chlorophyll *a* fluorescence emission spectra of the *Nostoc muscorum* cells treated with different concentration of lead (*A*) and cadmium (*B*).

30 min prior to the start of Hill activity. The results showed Pb^{2+}- and Cd^{2+}-induced inhibition of the Hill activity by approximately 40 and 65%, respectively, when compared with that of control (100%, without metal) (Table 1). Addition of 100 µM DPC resulted in the reversal of metal-induced inhibition of Hill activity (26 and 17% for Pb^{2+} and Cd^{2+}, respectively). The addition of 50 µM of NH_2OH caused also restoration of Pb^{2+}-and Cd^{2+}-induced inhibition of Hill activity by approx. 22 and 44%, respectively, as compared to the cells treated with metals only (without electron donor). Results clearly depicted that addition of DPC and NH_2OH could restore the metal-induced inhibition of Hill activity in both the Cd^{2+}-and Pb^{2+}-treated cells, albeit to different extent.

The results suggested that Pb^{2+} and Cd^{2+} inhibited the PSII in the *N. muscorum* cells at or before electron donation sites of DPC and NH_2OH as the inhibitory effect of both the metals could be reversed by both electron donors. However, reversal of Cd^{2+}-induced inhibition of Hill activity was relatively more pronounced in the presence of NH_2OH than by DPC as electron donor, while reversal of Pb^{2+}-induced inhibition of Hill activity was almost the same in presence of both the electron donors.

Chl *a* fluorescence emission: The cells were incubated in the presence of different concentrations (25 and 100 µg mL^{-1}) of Pb^{2+} and Cd^{2+} for 90 min at room temperature (25ºC) before recording the Chl *a* fluorescence emission spectra (620–800 nm). The Chl *a* fluorescence emission spectra upon 435 nm excitation revealed a characteristic peaks at 695 nm and a small hump at 657 nm (Fig. 5). Results showed that 25 µg mL^{-1} concentration of both metals caused stimulation in the fluorescence emission intensity at 695 nm. But at higher concentration of Pb^{2+} (100 µg mL^{-1}), a decrease in fluorescence emission was observed, while the same concentration of Cd^{2+} resulted in almost similar response as observed at its lower concentration (25 µg mL^{-1}). The cells treated with Cd^{2+} also showed a blue shift of about 4 nm. These results suggested a Cd^{2+}-induced inhibition of energy transfer from Chl *a* to photosynthetic reaction center. However, the Pb^{2+}-induced quenching of Chl *a* fluorescence emission at higher concentration (100 µg mL^{-1}) might be due to damage of Chl containing complexes.

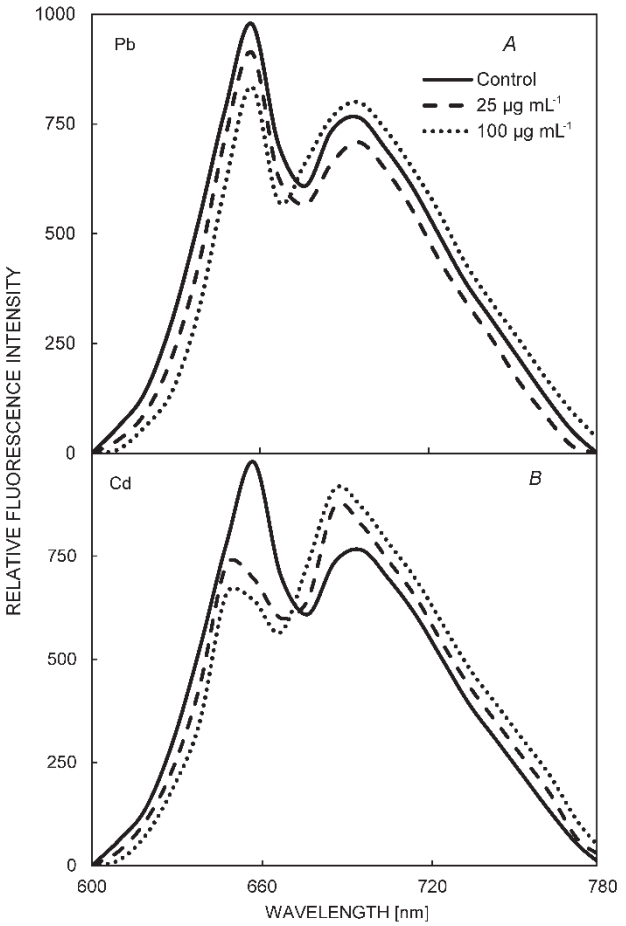

Fig. 6. Room temperature phycobilisome emission spectra of the *Nostoc muscorum* cells treated with different concentration of lead (*A*) and cadmium (*B*).

PB fluorescence emission: The PB fluorescence emission spectrum (600–780 nm) of *N. muscorum* in presence of different concentration of Pb^{2+} and Cd^{2+} (25 and 100 μg mL^{-1}) was recorded at room temperature (25ºC) using an excitation wavelength of 570 nm. The results showed two characteristic emission peaks at 645 nm, emanating from PC, and at 685 nm, attributable to terminal fluorescence emitters of PBs (special allophycocyanin) and PSII (Fig. 6). Treatment of cells with Pb^{2+} caused only negligible changes in the PB fluorescence emission spectra, while PB fluorescence emission peak in Cd^{2+}-treated cells at 685 nm was stimulated, which can indicate interruption in energy transfer from PB to PSII reaction center or inhibition of energy transfer from pigment Chl *a* to photosynthetic reaction center.

Discussion

Photosynthetic organisms are known to be highly sensitive to Cd^{2+} and Pb^{2+} ions (Rai and Chandra 1992, Poskuta *et al.* 1996, Gaur and Rai 2001, Pinto *et al.* 2003). However, it has been considered that the mechanisms of heavy-metal toxicity and resistance vary in various photoautotrophs depending upon the type of metal and nature of organisms (Neelam and Rai 2003).

The present study revealed that both Pb^{2+} and Cd^{2+} inhibited the photoautotrophic growth of *N. muscorum* cells. At the exponential phase, cells were found to be more susceptible to Cd^{2+} toxicity than to Pb^{2+} as it is evident from the 50% growth inhibitory concentration of Pb^{2+} and Cd^{2+} (I_{50} value of 55 and 21 μg mL^{-1}, respectively). A stress-induced inhibition of cyanobacterial growth occurs due to damage of the cellular constituents or inactivation of vital processes, such as nutrient uptake, enzyme activities, and photosynthesis (Pahlsson 1989, Takano *et al*. 1995, Thapar *et al.* 2008). It has been considered that metal-induced disorganization of thylakoids and decrease in the size of pigment antenna, leading to reduced photosynthetic activity, makes the photoautotrophs more susceptible to metal toxicity (Matoo *et al.* 1989, Murty *et al.* 1989, Küpper *et al.* 1996, Leborans and Novillo 1996).

Both Cd^{2+} and Pb^{2+} were found to reduce Chl, PC, and Car content in the present study. Similar results were observed by other researchers (Pahlsson 1989, Leborans and Novillo 1996, Lamai *et al.* 2005). It is well known that Cd^{2+} can cause disorganization of chloroplasts leading to a reduction in the content of photosynthetic pigments (Leborans and Novillo 1996). It has been suggested that decrease in Chl *a* content is perhaps due to Cd^{2+}-induced replacement of Mg^{2+} from Chl molecules (Küpper *et al.* 1996). The toxicity symptoms of Pb^{2+} and Cd^{2+} to *Cladophora fracta* were reported in terms of reduced number of chloroplasts, disintegrated cell wall, and death.

The present findings confirmed the metal-induced damage to the photosynthetic apparatus as the rate of O_2 evolution as well as Hill activity declined in response to short-term treatment of cells with both Pb^{2+} and Cd^{2+}. However, O_2 evolution was found to be relatively more susceptible to metal toxicity than the Hill activity as it is evident from I_{50} values obtained for all processes. The phenomenon might be explained in terms of short interference of cations with the charge separation property of the PSII reaction centre, unlike the quinone and plastoquinone component of the reducing site of PSII. It has been earlier suggested that metal cations interfere with the O_2-evolving complex by displacing the essential cations responsible for water oxidation (Singh *et al*. 1987, 2002; De Philippis *et al*. 2001). Further results demonstrated that effect of Cd^{2+} on photosynthetic activity in *N. muscorum* cells was more pronounced than that of Pb^{2+}. Earlier findings also suggested that Cd^{2+} ions directly inhibited the PSII reaction center (Peters and Chin 2003), perhaps by replacing essential divalent cations required by the O_2-evolving complex (Sersen and Kralova 2001). In order to delineate the action site of both Pb^{2+} and Cd^{2+}, the Hill activity in the cells was measured in the presence and absence of exogenous electron donors, such as DPC and NH_2OH (Verma and Singh 1995). The results exhibited reversal of Pb^{2+}-induced inhibition of photosynthetic electron transport in the presence of both DPC and NH_2OH, suggesting that action site of Pb^{2+} was located prior to electron donation sites of these two electron donors. On the other hand, reversal of Cd^{2+}-induced inhibition of Hill activity was relatively higher in the presence of NH_2OH than DPC, indicating the inhibition site of Cd^{2+} before the electron donation site of NH_2OH – the electron donor to PSII reaction center (Verma and Singh 1995). These results suggested that inhibition site of Cd^{2+} was located on the oxidizing side of PSII reaction center, but before the electron donation site of NH_2OH. Inhibition site of Pb^{2+} was apparently located before the water-oxidation complex as it is evident from the reversal of Pb^{2+}-induced inhibition of Hill activity in the presence of both DPC and NH_2OH.

Chl *a* fluorescence emission in *N. muscorum* showed a characteristic peak at 695 nm associated with the inner core of pigment antenna involving energy transfer to the PSII reaction center (Pakrasi and Sherman 1985, Singh *et al.* 1993). The stimulation of Chl *a* emission intensity at 695 nm in the lower concentration of both Cd^{2+} and Pb^{2+} (25 μg mL^{-1} each), along with about 4 nm blue shift in presence of Cd^{2+}, indicated inhibition of energy transfer from Chl *a* to PSII center as well as conformational changes in the core pigment antenna. Similar results were reported in the cyanobacterium *Anacystis nidulans* by Pakrasi and Sherman (1985) and in *Anabaena flos-aquae* by Singh *et al.* (1993). However, unlike Cd^{2+}, a higher

concentration of Pb^{2+} (100 μg mL^{-1}) resulted in quenching of emission peak at 695 nm, which might be either due to nonphotochemical quenching or due to thermal dissipation of energy caused by structural damage to the inner core of pigment antenna (Oxborough and Horton 1986).

The fluorescence emission spectra of PBs in *N. muscorum*, obtained in the presence of Cd^{2+}, exhibited decline in the emission intensity at 645 nm emanating mainly from the PC component of PBs. On the contrary, fluorescence emission peak at 685 nm associated with terminal emitter component of the PBs or PSII (Murty *et al.* 1989) was stimulated by Cd^{2+}, suggesting inhibition of energy transfer from allophycocyanin to other components of PBs. These results are partly in agreement with the observation of Singh *et al.* (1993) on the Hg^{+2}- and Cd^{+2}-induced damage to the PC component of PBs in *Anabaena flos-aquae,* resulting in reduced emission intensity. Our results (Fig. 2) also suggested metal-induced PC decrease in *N. muscorum* cells. Similar observations were made by Murty *et al.* (1989) in the cyanobacterium *Spirulina platensis*; they suggested a greater sensitivity of PC to metals than the allophycocyanin-containing components of the PBs. Taken together, the present results indicated that lower concentrations of these metals bring about cessation of energy transfer from Chl *a* to PSII center, whereas higher concentrations might cause damage in the photosynthetic complex.

Conclusion: The present investigation revealed that photoautotrophic growth of *N. muscorum* was susceptible to Pb^{2+} and Cd^{2+} toxicity. The growth, pigments, and photosynthetic activity in *N. muscorum* cells were more inhibited by Cd^{2+} than by Pb^{2+}. The O_2-evolving complex was relatively more sensitive to metal toxicity than the photosynthetic electron transport system. Fluorescence emission from Chl *a* and PBs demonstrated that energy transfer in the pigment antenna of PSII reaction center was more susceptible to Cd^{2+} than Pb^{2+}. Results on the reversal of Pb^{2+}- and Cd^{2+}-induced inhibition of Hill activity in the presence of exogenous electron donors indicated that action sites of both the metals are located on the oxidizing side of PSII.

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