

Responses of Cyanobacterium *Nostoc muscorum* to Photoinhibitory Light under Varying Levels of Cadmium

By

M.P.V. Vikram Singh

ISSN 2319-3077 Online/Electronic

ISSN 0970-4973 Print

Journal Impact Factor: 4.275

Global Impact factor of Journal: 0.876

Scientific Journals Impact Factor: 3.285

InfoBase Impact Factor: 2.93

Index Copernicus International Value

IC Value of Journal 47.86 Poland, Europe

J. Biol. Chem. Research

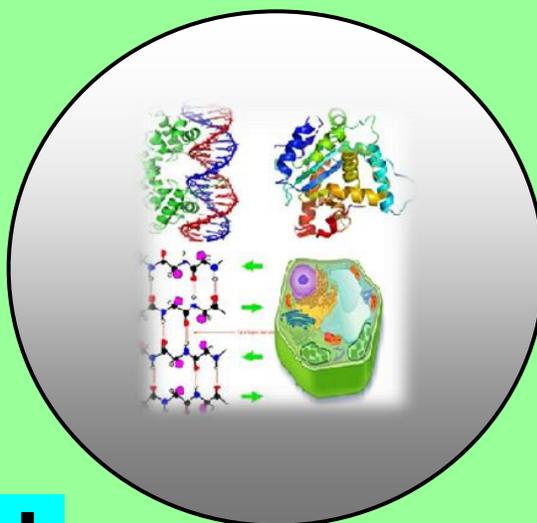
Volume 33 (1) 2016 Pages No. 175-187

Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and
National Scientific Databases

Published by Society for Advancement of Sciences®



J. Biol. Chem. Research. Vol. 33, No. 1: 175-187, 2016

(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)

Ms 32/2/112/2015

All rights reserved

ISSN 0970-4973 (Print)**ISSN 2319-3077 (Online/Electronic)**[http:// www.sasjournals.com](http://www.sasjournals.com)[http:// www.jbcr.in](http://www.jbcr.in)jbiorchemres@gmail.cominfo@jbcr.in

RESEARCH PAPER

Received: 10/06/2015

Revised: 31/10/2015

Accepted: 20/01/2016

Responses of Cyanobacterium *Nostoc muscorum* to Photoinhibitory Light under Varying Levels of Cadmium

M.P.V. Vikram Singh

Department of Botany, J. N. P. G. College, Lucknow, India 226001

ABSTRACT

The present study is investigated light induced inhibition of photosynthetic electron transport activity in heterocystous cyanobacterium Nostoc muscorum under cadmium stress along with growth, pigments content, cadmium uptake, photosynthetic oxygen evolution, energy transfer, and ¹⁴C fixation. Increasing light intensity (20-120 μmol photons m⁻²s⁻¹) decreased chlorophyll a and phycocyanin contents but increased the biosynthesis of carotenoids. However, different light intensities together with cadmium (Cd, 4 μM) decreased the content of all the photosynthetic pigments. Uptake of Cd depends on concentration and extent of light intensity. High light inhibited photosynthetic oxygen evolution in intensity and duration dependent manner. Electron transport activity in spheroplasts under photoinhibitory light (1000 μmol photon m⁻²s⁻¹, 30 min) and elevated level (50-150 μM) of Cd inhibited photosystem II (PS II) activity strongly, while photosystem I (PS I) activity appeared to be less sensitive and its partial restoration by artificial electron donors (DPC, NH₂OH, MnCl₂) suggests that these stresses (high light and Cd) blocked the electron flow at water oxidation side. However, less restoration in combination of these stresses indicated that the PS II reaction centre was also affected. The ¹⁴C -fixation was found to be more sensitive to high light and Cd alone and in combination than photosynthetic oxygen evolution. Result of the present study indicate that high light alone and together with Cd adversely affected photosynthetic activity by strongly arresting electron flow through PS II leading to the significant decrease in photosynthetic pigments content and thus growth of N. muscorum.

Keywords: *Nostoc muscorum, Cadmium, , Light intensity, Photosynthetic pigments, Photosynthetic electron transport activity and ¹⁴CO₂ fixation.*

INTRODUCTION

Photosynthesis is the only process on the earth by which solar energy is trapped by autotrophic organisms and converted into food for the rest of the organisms. About more than 170 million tones of dry matter is produced by this process annually, and 90% of it in the oceans by the phytoplanktons (Al-Qasmi et al. 2012; Sforza et al. 2012). This process (photosynthesis) is sensitive to various environmental factors, in which light is one of the critical factors, which profoundly influences the growth and development of plants (Pope 1975). An increase in light intensity accelerates the rate of photosynthesis. The relationship between light intensity and rate of photosynthesis follows logarithmic curve when all other factors are constant and not rate limiting (Clout 1994).

The exposure of photosynthesis organism to high light intensities gives raise superfluous excitations that can lead to an impairment and eventual damage of the photosynthetic apparatus. When light energy in excess of that utilized to drive photosynthetic electron transport is absorbed by plants, it causes photoinhibition that decreases the intrinsic efficiency and substantial reduction of the photosynthetic efficiency is termed as photoinhibition of photosynthesis (Pope 1975; Clout 1994). In recent years, investigation related to photo inhibition of photosynthesis has received increased interest due to strengthened advance in our knowledge, development of new techniques and complication of process as a result of overlapping of stresses. In nature, the interaction of multiple environment stresses is very common and complex. Their combinations cause intensification or overlapping of stress effects (Singh et al. 2012a).

Heavy metals are natural elements that are found at various background levels and their persistence cannot be deleted from environment. So, the consequent effects of heavy metals in the biosphere have become a subject of concern, in which cadmium (Cd) is one of the more common and most toxic metals presents in soil and water bodies (Prasad and Zeeshan). The environmental problem resulting from Cd contamination may be more serious than some other heavy metals because modern industrial activities and agricultural practices increased levels of Cd in the soil and water bodies, which is readily incorporated in phytoplankton and in other plants, where they alter the normal physiological and biochemical process by inactivating the photosynthetic and enzymatic pathways (Singh et al. 2012a, b). Interaction of light with heavy metals is of common occurrence in water bodies where cyanobacteria, which are one of major phyllogenetically coherent groups of gram negative prokaryotes possessing oxygenic photosynthesis like eukaryotic algae and higher plants (Singh et al. 2012a, b). They are capable of both carbon assimilation and nitrogen fixation and have a great adoptability in response to high fluctuations of environmental factors (Singh et al. 2012a; Montgomery 2014).

Physiological studies under influence of metals are difficult because the site of toxic action, the active concentration of a metal or its salt is unknown as a result of chelating and the effects of pH on solubility. Cadmium toxicity to algae and cyanobacteria has been extensively studied by some worker on selected parameters (Prasad and Zeeshan 2005; Qian et al. 2009). None of the studies conducted earlier to give a comprehensive understanding of Cd toxicity together with high light condition, and nothing is known about its impact on the photosynthetic electron transport system and site of its action.

Therefore, considering the above facts, the present work was conducted with the aim to study the impact of light alone and together with Cd on growth, pigments content, Cd uptake, photosynthetic oxygen evolutions, photosynthetic electron transport system, energy transfer, and ^{14}C fixation in nitrogen fixing cyanobacterium *Nostoc muscorum*.

MATERIAL AND METHODS

Organism and culture conditions

Nostoc muscorum, a filamentous and heterocystous cyanobacterium was grown and maintained in Chu-10 medium under $75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, (PAR) at the center of culture flask at $27 \pm 1^\circ\text{C}$. The photoperiod of 14:10 h (light and dark cycle) was maintained by electronic automatic timer (MDS Switchgear Ltd., India).

Light treatment

High irradiance was provided by metal halogen lamp (Osram HQI-TS-500 W) mounted in a reflector. The desired photon flux densities (PFD) were achieved by varying the distance between the source and the glass tubes (2.5 cm diameter) containing cyanobacterium samples and immersed in water bath at $27 \pm 1^\circ\text{C}$, and the temperature of water bath was maintained by continuous water flow of desired temperature. The cyanobacterial samples in tubes were stirred gently. The PFD was measured with the help of Power Meter (Spectra Physics, USA model 407, A-2). Except pigment analysis, cell suspensions were incubated under $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (PAR) for 30 min in presence of different concentrations (10-150 μM) of Cd and thereafter, cultures were divided into two sets. Further one set of them was exposed to $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ light intensity for 30 min while other set was still irradiated with $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (PAR) for next 30 min. For pigment analysis, the cultures were incubated in growth medium containing 1-4 μM of Cd for 10 days under varying light intensity (20, 60 and $120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$).

Metal treatment

Stock solution of CdCl_2 was prepared in sterilized distilled water, and freshly prepared solution was filtered to sterilize (0.45 μm millipore membrane filter, USA) before the preparation of required working concentrations in culture medium. For the estimation of pigments, *N. muscorum* culture was incubated with 1, 2 and 4 μM of Cd whereas for other experiments 10-150 μM of Cd was used.

Cadmium uptake

Cadmium uptake was estimated by the method of Gabbrelli et al. (1991). Exponentially grown cyanobacterium was incubated in varying Cd concentrations (10, 50, 100 and 150 μM) containing medium for required time under normal ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and photo inhibitory light ($1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Cells were harvested by centrifugation at 4000 g for 10 min. Pellets were washed with sterilized distilled water, and 1 mM EDTA solution was used to remove absorbed Cd onto cell surface. Cells were dried at 80°C for 24 h and dried samples were digested at 300°C in acid solution containing nitric acid and perchloric acid (5:2, v/v). The process was repeated till the solution becomes colorless. The resulting colorless solution was analyzed by using atomic absorption spectrophotometer (Perkin-Elmer, 2380).

Measurement of growth and photosynthetic pigments

Growth was measured by estimating the protein content. Protein content was determined by the method of Lowry et al. (1951). Chlorophyll *a* (Chl *a*) was extracted in 80% acetone and the amount was determined by the method of Arnon (1949). Carotenoids (Car) content was assayed by measuring absorbance at 450 nm by the method of Jenson (1978). Phycocyanin (PC) was extracted in 2.5 mM phosphate buffer (pH 7.0) after repeated freezing and thawing, and the amount was determined by the method of Bennett and Bogorad (1973).

Measurement of photosynthetic oxygen evolution

Photosynthetic oxygen evolution was measured in a water jacketed electrode cell of 5 ml capacity by using a Clark-type O₂ electrode (Digital Oxygen System Model 10, Rank Brothers U.K.) in temperature controlled air tight reaction vessel at 27±1°C. For oxygen evolution measurement, 5 ml of treated or untreated cell sample was withdrawn at desired time interval (30, 60 and 90 min) and suspended in electrode cell. Actinic light was provided by a slide projector fitted with a 250 W halogen lamp which was fed by a constant current power supply. The light beam was passed through water jacket and the light intensity (360 μmol photons m⁻² S⁻¹) was set as desired at the center of the electrode cell.

Measurement of photosynthetic electron transport activities

Photosynthetic electron transport activity in Cd and photoinhibitory light treated spheroplasts prepared from cyanobacterium was monitored in temperature controlled airtight reaction vessel at 27°C for 5 min using Clark type oxygen electrode (Rank Brothers, U.K.). Photosynthetic active radiation (PAR) of 360 μmol photons m⁻² s⁻¹ was received at the surface of vessel. Spheroplasts for the measurement of photosynthetic electron transport activity were prepared by the method of Spiller (1980) and spheroplasts were resuspended in medium containing 0.5 M sucrose, 10 mM HEPES-NaOH, 5 mM K₂HPO₄, 10 mM MgCl₂ and 2% (w/v) bovine serum albumin (pH 6.9). Photosystem II (PS II) activity was measured as O₂ evolution by using 1 mM p-BQ as an electron acceptor. The whole chain (H₂O→MV, where MV is methyl viologen) and PS I (DCPIP/ASC→MV, where DCPIP/ASC is 2, 6-dichlorophenol indophenols/ascorbate) mediated electron transport in spheroplasts was measured as O₂ uptake. For the whole chain electron transport activity, reaction mixture contained 0.05 mM NaN₃ and 0.1 mM MV, while for PS I assay it contained 0.05 mM DCPIP, 1 mM ascorbate, 10 μM DCMU, 0.05 mM NaN₃ and 0.1 mM MV. In each assay, spheroplasts equivalent to 3 μg Chl *a* ml⁻¹ were used.

Measurement of ¹⁴CO₂ fixation

Carbon fixation in cyanobacterial cells was measured by recording the incorporation of ¹⁴CO₂ from radioactive sodium bicarbonate (NaH¹⁴CO₃, specific activity (9.25 x 10⁴ Bq ml⁻¹) obtained from Bhabha Atomic Research Center, Mumbai, India) for 5 min into acid stable products. For this, 75 μl of each NaH¹⁴CO₃ and nonradioactive carbon as bicarbonate (NaH¹²CO₃) was kept in centrally placed small container, and after placing the same amount of treated and untreated cyanobacterial cells, around this container, the apparatus (5x5x2 cm³) was made air tight. With the help of syringe, 0.5 ml 2N HCl was added in container so that ¹⁴CO₂ was made available to the cells. The cyanobacterial cells were exposed to 400 μmol photons m⁻²s⁻¹ (PAR) light for 20 min at 25°C.

In the end, the cells were withdrawn quickly and crushed in test tubes containing 2 ml acidified (0.5 ml 2 N HCl) 80% ethanol solution. The liquid samples were flushed with air for 30 min to remove the dissolved $^{14}\text{CO}_2$. The radioactivity in acid stable photosynthates was counted by liquid scintillation counter (L.K.B Wallace 1209, Rockbeta, USA). The rate of $^{14}\text{CO}_2$ -fixation is expressed as $\mu\text{mol } ^{14}\text{CO}_2\text{-fixed (mg Chl } a)^{-1}\text{h}^{-1}$.

Statistical analysis

Data obtained from analysis have been evaluated statistically at factorial level by means of analysis of variance (ANOVA) followed by Student's t-test and their significance levels were determined at $P < 0.05$.

RESULTS

Effect of different light intensities on growth and photosynthetic pigments under Cd stress

Growth behavior and photosynthetic pigments content in *N. muscorum* cells was recorded at regular intervals for 10 days under various PFD (20, 60 and 120 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$) alone and together with different concentrations (1, 2 and 4 μM) of Cd. Growth behavior was assessed by estimating total protein content (Table 1). *N. muscorum* responded maximally at 60 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ whereas, 120 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ light intensity inhibited the growth considerably. However, Cd treatment with these various PFD caused a decline in growth of cyanobacterium in a metal concentration dependent manner. After 10 days of treatment with 4 μM of Cd, growth in *N. muscorum* decreased by 58% compared to respective control under 120 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ light intensity.

Results indicated that Cd treatment induced a similar decline in chlorophyll *a* and phycocyanin contents in *N. muscorum* cells (Fig. 1 a and b). As compared to control, Cd (1 μM) treated cells caused a significant decline of 12% in Chl *a* content in normal light exposed *N. muscorum*, while, it was 7% and 19% in low and high light exposed cells, respectively. The decline percentage of Chl *a* increased to 16, 32 and 38% in response to the high dose of Cd (4 μM) under low, normal and high light intensities, respectively. Carotenoids on the other hand showed a continuously increasing trend in response to both, Cd concentrations as well light intensity (Fig. 1 c). Cadmium untreated normal light exposed cells showed enhanced phycocyanin content over low and high light intensity exposed *N. muscorum* cells. Comparing the effect of the Cd with different light intensities on photosynthetic pigments content, phycocyanin content was the most severely affected, suggesting that phycocyanin was more sensitive.

Effect of normal and photoinhibitory light on Cd uptake

Cadmium uptake in *N. muscorum* cells was estimated after normal and photoinhibitory light exposure. It is clear from results (Table 2) that Cd accumulation in *N. muscorum* increased with the rise of Cd concentration (10, 50, 100 and 150 μM) in external medium under normal light exposure and the accumulation further increased under photoinhibitory light treatment.

Effect of photoinhibitory light on photosynthetic O_2 evolution at varying time interval under Cd stress

Results pertaining to photosynthetic oxygen evolution in *N. muscorum* treated with photo inhibitory light and varying concentration of Cd and exposing them to constant light intensity of 360 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ for 5 min in Fig. 2.

The different PFD (250, 500 and 1000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) for different time show different sensitivity and susceptibility when cyanobacterium was exposed to different levels of Cd. The photoinhibition commenced within a few minute of transfer, and initially rapid characteristic decline in photosynthetic activity. The initial rapid phase of O_2 evolution inhibition was much more pronounced at 1000 than 500 and least at 250 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ light intensity. It is clear from the results that the cyanobacterium showed different degree of photoinhibition in time and light intensity dependent manner. For 50% inhibition of photosynthetic oxygen evolution, *N. muscorum* required 30 min of 1000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ light intensity. This becomes more intense (Fig. 2) under similar condition in the presence of Cd in concentration dependent manner and required only 12 min of photo inhibitory light treatment (1000 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$) for 50% photo inhibition in the presence of Cd. This supports the view that the photoinhibitory condition becomes more severe when cyanobacterium was exposed to high light in combination with other stress such as Cd. Photoinhibition of photosynthesis after photoinhibitory light treatment in the presence of different concentration of Cd was determined by whole cell O_2 evolution. It is clear from the results (Fig. 3) that even 20 min exposure of photoinhibitory light caused 47% inhibition in photosynthetic oxygen evolution which becomes more intense with various concentrations of Cd.

Effect of photoinhibitory light on photosynthetic electron transport activity under Cd stress

For the characterization of site of photoinhibition of photosynthesis, the photosynthetic electron transport activities were studied in the spheroplasts of *N. muscorum* subjected to photoinhibitory light (1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 30 min) with and without Cd. The PS II, PS I and whole chain electron transport activities declined significantly under photoinhibitory light treatment and damaging effects were more prominent on whole chain than PS II followed by PS I. The damaging effects of photoinhibitory input were further increased with Cd in concentration dependent manner.

Photoinhibitory light induced decrease in $^{14}\text{CO}_2$ fixation in *N. muscorum* cells in the presence and absence of Cd was estimated and the results are presented in Table 3. Results reveal that there was a significant decrease in $^{14}\text{CO}_2$ fixation in cells of *N. muscorum* exposed to Cd and photo inhibitory light treatment. Reduction in $^{14}\text{CO}_2$ fixation reached maximum (-99%) under 150 μM Cd and photoinhibitory light treatment. Under normal light (control) the rate of ^{14}C -fixation was $23313 \pm 5 [\text{CPM} (\text{mg Chl } a)^{-1} \text{ h}^{-1}]$ and rate of PS I, PS II and whole chain electron transport activity was 543 ± 4 , $238 \pm$ and $180 \pm 3 [\mu\text{molO}_2 \text{ evolution/consumption} (\text{mg Chl } a)^{-1} \text{ h}^{-1}]$ respectively.

DISCUSSION

Cyanobacterium *N. muscorum* grown under different light intensities with and without Cd differs significantly in growth response (Table 1). The results suggest a possible role of growth irradiance and toxic effects of Cd. Photoautotrophic growth of *N. muscorum* was severely inhibited by Cd treatment under varying light intensities after 10 days with concurrent loss of photosynthetic pigments. Maximum reduction in growth was observed under high light intensity with highest concentration of Cd. This decline in growth of *N. muscorum* is supported by similar decline recorded in photosynthetic pigments in dose dependent manner of Cd as well as light intensity.

N. muscorum shows maximum Chl *a* and phycocyanin contents at normal ($60 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) light intensity whereas the content of carotenoids increase at high light intensity ($120 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) (Fig. 1 a, b and c). However, the maximum reduction of all these photosynthetic pigments was observed when the maximum tested dose of Cd ($4 \mu\text{M}$) was coupled with high light intensity. Lu and Zhang (2000) found that *Spirulina platensis* under salt stress had substantial decrease in phycocyanin and chlorophyll contents, which become more pronounced with excess light. This resulted due to the photooxidative damage to photosynthetic apparatus. A loss in light harvesting pigments as a consequence of heavy metal treatment has been reported in a cyanobacterium (Prasad and Zeeshan, 2005) as well as in a green alga (Vavillin et al. 1995). Heavy metals inhibit several metabolic processes by causing oxidative stress and inhibiting the action of enzymes, and these are important causes of growth inhibition (Choudhari et al. 2007). For instance, Cd inhibits chlorophyll biosynthesis through inhibition of *delta*-aminolevulinic acid dehydrate protochlorophyllide (Pchlde) and NADPH/Pchlde oxidoreductase (Schoefs and Bertrand, 2000) by its interference with the sulfhydryl groups. Photosynthetic pigments such as Chl *a* and carotenoids are integrated in the membranes, while phycocyanin is found attached to the outer surface of thylakoid membrane that is why phycocyanin was more affected than Chl *a* and carotenoids. In the present study, carotenoids amount increased at high light intensity suggests its photoprotective role and thus help in sustaining photosynthetic efficiency (Prashad and Zeeshan 2005).

Cadmium uptake was estimated after normal ($50 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) and photoinhibitory light ($1000 \mu\text{mol photons m}^{-2}\text{s}^{-1}$, 30 min) treatment conditions (Table 2). The amount of Cd absorbed by cyanobacterial cells increased with increasing external Cd concentration and light intensity. Qian et al. (2009) observed an increase in Cd bioaccumulation by *Chlorella vulgaris* over the entire range of increasing concentration. External factors such as light not only interfere with the metabolism of cyanobacteria but also enhance metal uptake due to increased cell membrane permeability.

The extent of photoinhibition depends upon the light intensity and its duration. Under photoinhibitory light, *N. muscorum* showed decrease in photosynthetic efficiency than those experienced during their normal requirement. Reduction in photosynthetic oxygen evolution in response to photoinhibitory light suggests the changes in the photosynthetic activity due to varying degree of susceptibility of organism. This supported the hypothesis that the photoinhibition occurs when the rate of oxidation exceeds the dissipation capacity of the electron transport chain in excess light condition. The antenna pigments of photosynthetic apparatus absorb excess light (Pope 1975; Sforza et al. 2012) and saturate the rate of photosynthesis (Clout 1994). *N. muscorum* requires 30 min of photoinhibitory light treatment ($1000 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) for 50% photoinhibition. Cadmium mediated high light induced photoinhibitory result also proved the hypothesis that even moderate photoinhibitory light along with Cd causes severe photoinhibitory effect on growth of the tested cyanobacterium because under these conditions a given light level which was previously not inhibitory becomes inhibitory. This Cd mediated inhibition of photosynthesis might have resulted due to decreased utilization of photonic energy through photosynthesis.

Photoinhibitory light intensities inhibited whole cell O₂ evolution in *N. muscorum* which become inhibitory with different concentrations of Cd. Photosynthetic whole cell O₂ evolution depends on the functioning of O₂ evolution complex, photosynthetic electron transport as well as the utilization of generated assimilatory power (ATP and NADPH) in the sink reactions of carbon fixation. Thus, O₂ evolving capacity denotes the overall status of photosynthesis under stress condition. Decline in whole cell O₂ evolution may be correlated to the substitution of the central atom (Mg) of chlorophyll molecules by Cd preventing photosynthetic light harvesting in the affected molecules. Lu and Zhang (1999) reported that increase in metal concentration led to a decrease in quantum yield of PS II. Under high light, *N. muscorum* not only utilizes vast majority of the absorbed light for photosynthesis but it gives rise superfluous excitations that can lead to an impairment and eventual damage to the photosynthetic apparatus and substantially increased by Cd. This could be explained on the basis of enhanced binding of Cd with thylakoid membranes. It is known that excess light is harmful to photosynthetic apparatus in view of its action in generating reactive oxygen species (ROS) (Pope 1975).

Table 1. Specific growth rate ($\mu\text{g protein ml}^{-1}$) in cyanobacterium *N. muscorum* cells grown under low, normal and high light intensities and cadmium for 10 days.

Cadmium concentration (μM)	Light intensity ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)		
	20	60	120
0	0.080 \pm 0.002	0.161 \pm 0.004	0.149 \pm 0.004
1	0.075 \pm 0.001 (6)	0.145 \pm 0.002 (10)	0.115 \pm 0.004 (23)
2	0.070 \pm 0.131 (13)	0.131 \pm 0.002 (19)	0.104 \pm 0.003 (30)
4	0.066 \pm 0.001 (18)	0.090 \pm 0.002 (44)	0.074 \pm 0.003 (50)

All the values are means \pm SE. Data in parenthesis denote per cent inhibition over control. All treatments are significantly different ($P < 0.05$) from control (student's "t" test)

Table 2. Influence of normal and high light intensities on cadmium uptake in *N. muscorum*.

Cadmium concentration (μM)	Cadmium uptake [$\mu\text{M Cd (mg dry wt)}^{-1}$]	
	Normal light (50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	High light (1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$)
10	0.36 \pm 0.005	0.048 \pm 0.003 (+33)
50	2.56 \pm 0.02	10.23 \pm 0.02 (+300)
100	11.46 \pm 0.05	18.89 \pm 0.05 (+65)
150	13.08 \pm 0.06	19.31 \pm 0.07 (+48)

All the values are means \pm SE. Data in parenthesis denote per cent increase over the respective values of low light treatment. All treatments at high light intensity are significantly different ($P < 0.05$) from their respective treatments at low light intensity.

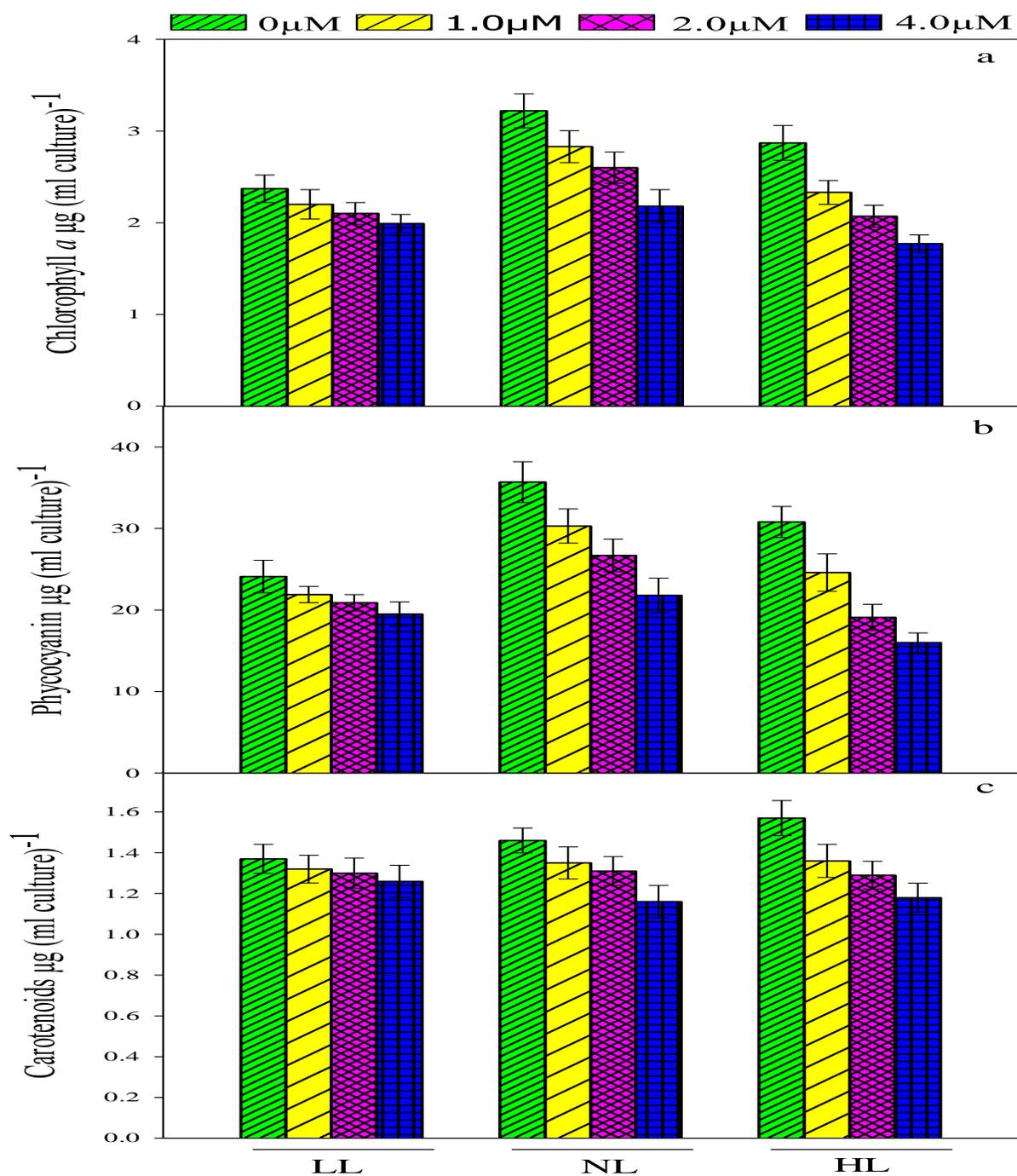


Figure 1. Effect of different concentrations of Cd on chlorophyll *a* (a), phycocyanin (b) and carotenoids (c) content in cyanobacterium *N. muscorum* under low, normal and high light intensities after 10 days. Data are means \pm standard error of three independent experiments. All treatments are significantly different ($P < 0.05$) from control (student's "t" test).

Table 3. Effect of photoinhibitory light ($1000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) on photosynthetic electron transport activity and $^{14}\text{CO}_2$ fixation of *N.muscorum* treated with cadmium.

Treatment	$^{14}\text{CO}_2$ fixation [CPM (mg Chl <i>a</i>) $^{-1}$ h $^{-1}$]	Photosynthetic electron transport activity O $_2$ evolution /consumption		
		ASC/DCPIP \rightarrow MV (PSI)	H $_2$ O \rightarrow p-BQ (PSII)	H $_2$ O \rightarrow MV (Whole chain)
Photoinhibitory light	66254 \pm 11	499 \pm 4	123 \pm 3	59 \pm 2
+50 μM Cd	8995 \pm 10 (-61)	483 \pm 4 (11)	97 \pm 2 (70)	50 \pm 2 (72)
+100 μM Cd	698 \pm 5 (-97)	461 \pm 4 (15)	72 \pm 3 (78)	30 \pm 1 (83)
+150 μM Cd	305 \pm 4 (-99)	456 \pm 4 (16)	36 \pm 3 (89)	68 \pm 1 (96)

All the values are means \pm SE. Data in parenthesis denotes per cent increase over the respective values of low light treatment. All treatments at high light intensity are significantly different ($P < 0.05$) from their respective treatments at low light intensity.

Slower utilization of ATP and NADHP during Calvin cycle might initiate the formation of ROS at several sites of photosynthetic electron transport systems under high PFD leading to per oxidation of several proteins and lipids involved in photosynthesis. However, photoproduction of ROS is unavoidable even under favorable conditions. Moreover, when metabolism is inhibited by toxicants, light energy is not fully used in the photosynthetic reactions. This results into the formation of ROS which ends in photodestruction of chlorophyll and cell structures (Pope 1975).

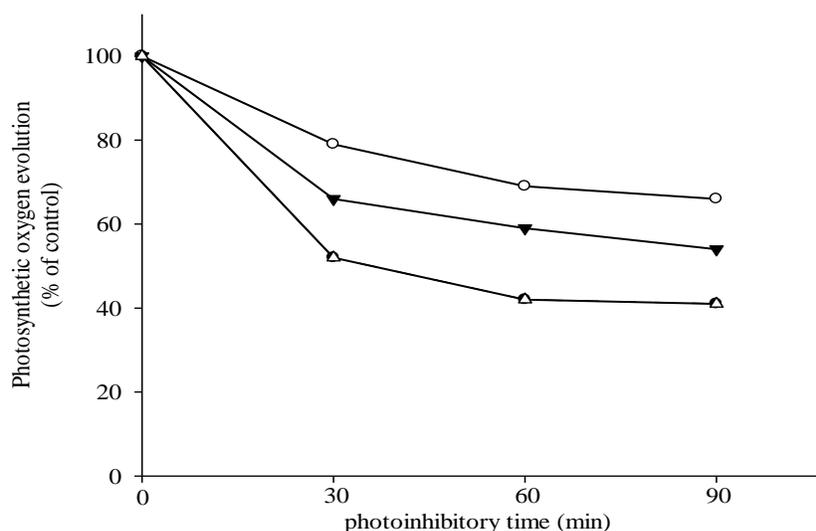


Figure 2. Photosynthetic O $_2$ evolution in *N. muscorum* versus time of photo inhibitory light. Control rate of O $_2$ evolution was $392 \pm 6 \mu\text{mol (mg Chl } a)^{-1} \text{ h}^{-1}$. The PPFD of the photo inhibitory light were 250 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, 500 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ and 1000 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$.

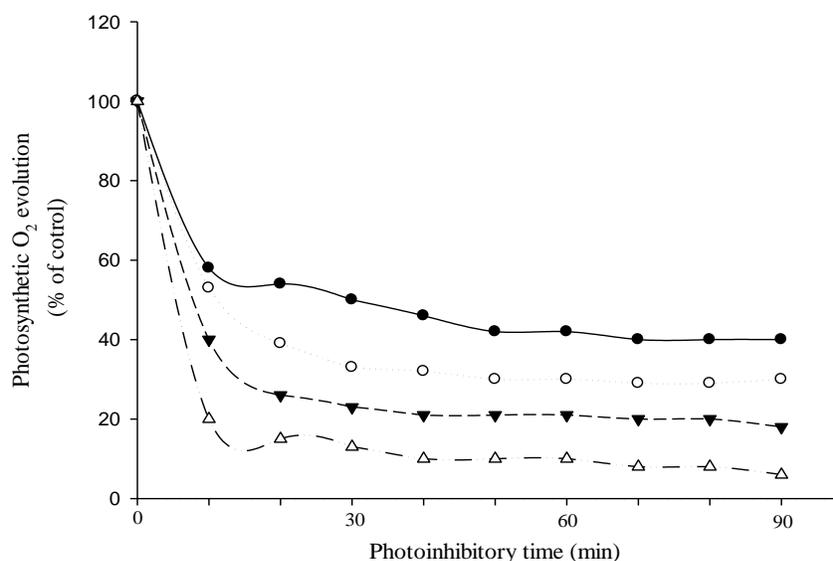


Figure 3. Effect of photo inhibitory light ($1000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) treatment on photosynthetic O_2 evolution rate in *N. muscorum* with varying concentrations of cadmium and exposure time. Rate of O_2 evolution in control sample was $390 \pm 7 \mu\text{mol (mg Chl a)}^{-1} \text{h}^{-1}$. Control —●—, 50 μM ○....., 100 μM ---▼---, 150 μM —△— of cadmium.

Photosynthetic electron transport activities (PS II, PS I and whole chain) in spheroplasts of the tested cyanobacterium were adversely affected by photoinhibitory light ($1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) alone and in combination with Cd (50, 100 and 150 μM) (Table 3). Whole chain electron transport ($\text{H}_2\text{O} \rightarrow \text{MV}$) and PS II ($\text{H}_2\text{O} \rightarrow \text{p-BQ}$) activity was severely inhibited under studied condition. The decrease in PS II activity under excessive irradiance may be due to induction of photoprotective mechanism of thermal energy dissipation (Pope 1975) or light mediated damage of PS II reaction centers (Montgomery 2014). Cyanobacterium exposed to high light intensity showed more photo inhibition in presence of Cd because it might have acted at different sites of electron transport chain and interrupted the electron flow. Thus, a given light intensity which was previously not photoinhibitory caused strong photoinhibitory effect. Cadmium induced alterations may also cause lesser utilization of light energy in ATP and NADPH generation. The presence of Cd increased the intensity of inhibition. In a similar observation, Singh et al. (2012a) reported that mercury toxicity on PS II photochemistry was greatly stimulated by light. The PS I (DCPIP/ASC \rightarrow MV) was found to be resistant to photo inhibitory light but a considerable inhibition was observed with high dose of Cd. However, PS I in comparison to the PS II appeared to be more resistant against the stress because of its greater stability and lesser abundance in thylakoid membrane. Similar pattern of results was obtained in $^{14}\text{CO}_2$ fixation rate suggested that photoinhibitory light alone and together with Cd not only affect the photochemical reaction of photosynthesis but also the Calvin cycle enzymes due to greater accumulation of ROS (Han et al. 2000).

Another possible explanation for Cd toxicity is that it triggers the formation of ROS and induces oxidative stress. The rate of photodamage and repair of PS II depend on light intensity. Rate of repair of PS II was weak under high light intensity and reduced significantly by environmental factors (Sforza et al. 2012; Montgomery 2014). Retardation of photofixation of carbon in response to Cd and high light intensity may result into, limitation of ATP and NADPH consumption and therefore, down regulation or even feedback inhibition of PS II photochemistry.

In conclusion, present study showed that photoinhibitory light alone and in combination with Cd produced negative effects on growth, photosynthetic electron transport system and $^{14}\text{CO}_2$ fixation in *N. muscorum*. The extent of reduction in these parameters further gets more worsened with the increasing doses of Cd coupled with high light. These inhibitory effects on growth of the studied test organism may adversely affect the processes of organic matter accumulation and nitrogen fixation in paddy fields by *N. muscorum* in particular and cyanobacteria in general. Result obtained in the present study may have further wide spread application if we use *N. muscorum* as a biological indicator to monitor the large scale input of hazard heavy metals (particularly Cd) in water bodies.

ACKNOWLEDGEMENTS

The author is thankful to the Head, Department of Botany, University o of Allahabad, Allahabad. Author is also thankful to Professor L.C. Rai, CAS in Botany, Banaras Hindu University, Varanasi for providing facility for $^{14}\text{CO}_2$ estimation.

REFERENCES

- Al-Qasmi, M., Raut, N., Talebi, S., Al-Rajhi, S. and Al-Barwani, T. 2012.** A review of effect of light on microalgae growth. *Proc World Cong Eng*, Vol I WCE, 4-6.
- Arnon, D.J. 1949.** Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*, 24:1-15.
- Bennett, A. and Bogorad, L. 1973.** Complementary chromatic adaptation in filamentous blue-green algae. *J Cell Biol*, 58:419-435.
- Choudhary, M., Jetley, U.K., Abash Khan, M., Zutshi, S. and Fatma, T. 2007.** Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. *Ecotoxicol Environ Saf*, 66:204-209.
- Cloot, A. 1994.** Effect of light intensity variations on the rate of photosynthesis of algae: A dynamical approach. *Math Comp Model*, 19:23-33.
- Gabbrelli, R., Mattioni, C. and Verganano, O. 1991.** Accumulation mechanisms and heavy metal tolerance of nickel hyperaccumulator. *J Plant Nutr*, 14:1067-1080.
- Han, B.P., Virtanen, M. and Koponen Straskraba, J.M. 2000.** Effect of photoinhibition on algal photosynthesis: a dynamic model. *J Plankton Res* 22:865-885.
- Jensen, A. 1978.** Chlorophylls and Carotenoids. In: Hellbust JA, Craige JS (eds), Handbook of phycological methods, Cambridge University Press, Cambridge, pp 419-432.

- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951.** Protein measurement with the Folin phenol reagent. *J Biol Chem*, 193:265-275.
- Lu, C. and Zhang, J. 2000.** Role of light in the response of PS II photochemistry to salt stress in the cyanobacterium *Spirulina platensis*. *J Exp Bot*. 51:911-917.
- Lu, C.M. and Zhang, J.H. 1999.** Copper induced inhibition of PS II photochemistry in cyanobacteria *Spirulina platensis* is stimulated by light. *J Plant Physiol*, 154:173-178.
- Montgomery, B.L. 2014.** The regulation of light sensing and light-harvesting impacts the use of cyanobacteria as biotechnology platforms. *Front Bioeng Biotechnol*, 2:22.
- Pope, D.H. 1975.** Effects of light intensity, oxygen concentration, and carbon dioxide concentration on photosynthesis in algae. *Microb Ecol*, 2:1-16.
- Prasad, S.M. and Zeeshan, M. 2005.** UV-B radiation and cadmium induced change in growth, photosynthetic and antioxidant enzymes of cyanobacterium *Plectonema bonyanum*. *Biol Plant*, 49:229-236.
- Qian, H., Li, J., Sun, L., Chen, W., G., Sheng, D., Liu, W. and Fu, Z. 2009.** Combined effect of copper and cadmium on *Chlorella vulgaris* growth and photosynthesis-related gene transcription. *Aquat Toxicol*, 94:56-61.
- Schoefs, B. and Bertrand, M. 2000.** The formation of chlorophyll from chlorophyllide in leaves containing proplastids is a four-step process. *FEBS Lett* 486:243-246.
- Sforza, E., Simionato, D., Giacometti, G.M., Bertuccio, A. and Morosinotto, T. 2012.** Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in photobioreactors. *PLoS One*, 7(6): e38975.
- Singh, R., Dubey, G., Singh, V.P., Srivastava, P.K., Kumar, S. and Prasad, S.M. 2012a.** High light intensity augments the mercury toxicity in cyanobacterium *Nostoc muscorum*. *Biol Trace Elem Res*, 149:262-272.
- Singh, V.P., Srivastava, P.K. and Prasad, S.M. 2012b.** Differential effects of UV-B radiation fluence rates on growth, photosynthesis, and phosphate metabolism in two cyanobacteria under copper toxicity. *Toxicol Environ Chem* 94:1511-1535.
- Spiller, H. 1980.** Photophosphorylation capacity of stable spheroplast preparations of *Anabaena*. *Plant Physiol* 66:446-450.
- Vavillin, D.V., Polynov, V.A., Matorin, D.N. and Venediktov, P.S. 1995. Sublethal concentrations of copper stimulate photosystem II photoinhibition in *Chlorella pyrenoidosa*. *J Plant Physiol* 146:609-614.

Corresponding author: Dr. M.P.V. Vikram Singh, Department of Botany, J. N. P. G. College, Lucknow, India.

Email: vikramsingh693@yahoo.com