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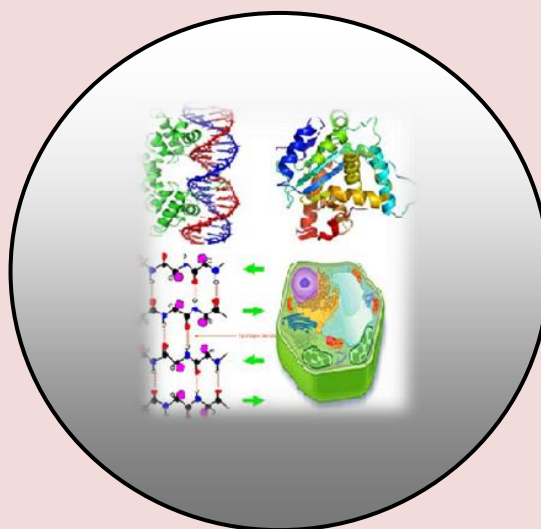
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## **Differential Effects of Light Intensity Fluence on Growth, Pigments and Photosynthesis in the Cyanobacterium *Aphanothece stagnina* under Cadmium Toxicity**

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### **ABSTRACT**

The present investigation is an attempt to study the response of differential light intensity fluence on growth, pigments and photosynthesis in cyanobacterium *Aphanothece stagnina* under different level of cadmium toxicity. Results suggested that the specific growth and photosynthetic pigments content enhanced progressively with light intensity ( $20-120 \mu \text{mol photon m}^{-2} \text{s}^{-1}$ ), however, different light intensities together with cadmium decreased the pigment contents. Uptake of cadmium metal depends on concentration and extent of light intensity. High light inhibited photosynthetic oxygen evolution in intensity and duration dependent manner. Photoinhibitory light inhibited photosynthetic electron transport activity. In the presence of elevated level of ( $50-100 \mu \text{M}$ ) of cadmium, the inhibition became stronger showing enhanced effect on whole chain activity and photosystem II (PS II) with the least effect on photosystem I (PS I). The  $^{14}\text{C}$ -fixation was found to be more sensitive to high light and Cd alone and in combination than photosynthetic oxygen evolution. Results 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 pigment content and thus growth of cyanobacterium.

**Keywords:** *Aphanothece stagnina*, Light intensity, Specific growth, Photosynthetic pigments, Photosynthetic oxygen evolution, Radioactive carbon fixation and Cadmium (Cd).

### **INTRODUCTION**

Almost all life on earth depends upon photosynthesis as it is important for generation of food, fuel and oxygen.

The advancement in photosynthetic research in the past several decades has been spectacular because it is one of the most important and popular area in plant science research at cellular and whole plant level. Like other process, photosynthesis is very sensitive to environmental factors in which light is one of the most critical factor, which profoundly influence the growth and development of plants (Pope, 1975).

Excess light is one of the general environmental stress factors. At many places on the globe including the experimental place, the amount of sunlight falling on a clear summer day is about  $1000 \text{ Wm}^{-2}$ . The natural light climate is extremely heterogenous because amount of isolation differs on equator and poles and in between them, and at different depths of ponds making biodiversity richer and prosperous. An alteration in optimum light intensity (whether a deficit or excess) would essentially effect plant's metabolic processes. Plants exposed to light levels in excess show superfluous excitation of electrons that can lead to an impairment and eventual damage. The light induced photoinhibition of the photosynthetic apparatus results in decreased biomass production / growth / viability. It has been reported, the effects of environmental factors such as temperature, salinity and metal stresses on photosystem II (PS II) are often intensified by high light intensity (Schoefs and Berfrand, 2005). In recent years, investigation related photoinhibition 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 stress is very common and complex their combinations cause intensification or overlapping of stress effects (Singh et al., 2012a).

Contamination of agricultural land and aquatic bodies with heavy metals in one of the serious basic problem because metals are natural elements and found at various high background levels, they are persistent and cannot be deleted from the environment. Heavy metal contamination has become of the major environmental problems worldwide. Heavy metals have considerable effects on the flora and fauna of our ecosystem. Some heavy metals at low does are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition (Marschner, 1999). Potentially, toxic heavy metal with no known biological function has attracted more and more considerable attention for its widespread distribution and potential risk to the environment and human health, and cadmium (Cd) is one of them is common and persistent in soil.

The environmental problem resulting from cadmium contamination may be more serious than some other heavy metals because modern industrial activities and agricultural practices increase levels of cadmium in the soil and water bodies, which is readily incorporated in phytoplankton's and in other plants, where they alter the normal physiological and biochemical processes by inactivating the photosynthetic machinery or enzymatic pathway (Malik & Rai, 1994). 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., 2012 a, Montgomery, 2014).

Since cyanobacteria and algae are the primary producer in aquatic ecosystems, their susceptibility to heavy metal has attracted worldwide attention (Murthy and Mohanty, 1993). Despite their high degree of stress tolerance, cyanobacteria have been shown to be highly sensitive to heavy metal pollution (Reed and Gadd, 1990, De Filippis and Pollaghy, 1992). They can therefore serve as important biological indicators of the level of metal pollution in aquatic ecosystems.

The interaction between the high light intensity and cadmium metal study demonstrated that some important biochemical and physiological changes occurs in *Nostoc muscorum* (Singh et al., 2013). Results of the work encouraged us to widen the range of high light intensity and different concentration of cadmium on the photoinhibition of photosynthesis in cyanobacteria. Physiological studies under influence of metals or its salt is unknown as a result of chelating and the effect of pH on solubility. 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 on *Aphanothece stagnina* a unicellular, cylindrical and colonial cyanobactrium was chosen to evaluate the role of high light and cadmium alone and together on photoinhibition of photosynthesis. In order to elucidate the mechanism of photoinhibition of photosynthesis in cynaobacterium *A. Stagnina* under different intensities of light and different concentration of cadmium, various parameters like specific growth, photosynthetic pigment content, Cd uptake, photosynthetic oxygen evolutions, photosynthetic electron transport system, energy transfer and radioactive carbon fixation has been investigated.

## MATERIAL AND METHODS

### Organism and culture conditions

*Aphanothece stagnina*, a unicellular and non-heterocystous cynaobacterium was grown and maintained in modified nitrogen BG-11 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  $\mu \text{M}$ ) 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  $\mu\text{M}$  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  $\text{CdCl}_2$  was prepared in sterilized distilled water, and freshly prepared solution was filtered to sterilize (0.45  $\mu\text{m}$  millipore membrane filter, USA) before the preparation of required working concentrations in culture medium. For the estimation of pigments, *A. stagnina* culture was incubated with 1, 2 and 4  $\mu\text{M}$  of Cd whereas for other experiments 10-150  $\mu\text{M}$  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  $\mu\text{M}$ ) 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  $^{\circ}\text{C}$  for 24 h and dried samples were digested at 300 $^{\circ}\text{C}$  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).

#### **Specific Growth and Photosynthetic Pigments Estimation**

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).

#### **Photosynthetic Oxygen Evolution Measurement**

Photosynthetic oxygen evolution was measured in a water jacketed electrode cell of 5 ml capacity by using a Clark-type  $\text{O}_2$  electrode (Digital Oxygen System Model 10, Rank Brothers U.K.) in temperature controlled air tight reaction vessel at  $27 \pm 1$   $^{\circ}\text{C}$ . 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  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) was set as desired at the center of the electrode cell.

#### **Photosynthetic Electron Transport Measurement**

Photosynthetic electron transport activity in Cd and photoinhibitory light treated spheroplasts prepared from cyanobacterium was monitored in temperature controlled airtight reaction vessel at 27 $^{\circ}\text{C}$  for 5 min using Clark type oxygen electrode (Rank Brothers, U.K.). Photosynthetic active radiation (PAR) of 360  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  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  $K_2HPO_4$ , 10 mM  $MgCl_2$  and 2% (w/v) bovine serum albumin (pH 6.9). Photosystem II (PS II) activity was measured as  $O_2$  evolution by using 1 mM p-BQ as an electron acceptor. The whole chain ( $H_2O \rightarrow MV$ , where MV is methyl viologen) and PS I (DCPIP/ASC  $\rightarrow MV$ , where DCPIP/ASC is 2, 6-dichlorophenol indophenols/ascorbate) mediated electron transport in spheroplasts was measured as  $O_2$  uptake. For the whole chain electron transport activity, reaction mixture contained 0.05 mM  $NaN_3$  and 0.1 mM MV, while for PS I assay it contained 0.05 mM DCPIP, 1 mM ascorbate, 10  $\mu M$  DCMU, 0.05 mM  $NaN_3$  and 0.1 mM MV. In each assay, spheroplasts equivalent to 3  $\mu g$  Chl *a*  $ml^{-1}$  were used.

#### **$^{14}CO_2$ Fixation Measurement**

Carbon fixation in cyanobacterial cells was measured by recording the incorporation of  $^{14}CO_2$  from radioactive sodium bicarbonate ( $NaH^{14}CO_3$ , specific activity  $9.25 \times 10^4$  Bq  $ml^{-1}$ ) obtained from Bhabha Atomic Research Center, Mumbai, India) for 5 min into acid stable products. For this, 75  $\mu l$  of each  $NaH^{14}CO_3$  and nonradioactive carbon as bicarbonate ( $NaH^{12}CO_3$ ) was kept in centrally placed small container, and after placing the same amount of treated and untreated cyanobacterial cells, around this container, the apparatus ( $5 \times 5 \times 2$   $cm^3$ ) was made air tight. With the help of syringe, 0.5 ml 2N HCl was added in container so that  $^{14}CO_2$  was made available to the cells. The cyanobacterial cells were exposed to 400  $\mu mol$  photons  $m^{-2}s^{-1}$  (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  $^{14}CO_2$ . The radioactivity in acid stable photosynthates was counted by liquid scintillation counter (L.K.B Wallace 1209, Rockbeta, USA). The rate of  $^{14}CO_2$ -fixation is expressed as  $\mu mol$   $^{14}CO_2$ -fixed (mg Chl *a*) $^{-1}h^{-1}$ .

#### **Statistics**

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 specific growth and photosynthetic pigments under Cd stress**

Specific growth behavior and photosynthetic pigments content in *A. stagnina* cells was recorded at regular intervals for 10 days under various PFD (20, 60 and 120  $\mu mol$  photon  $m^{-2}s^{-1}$ ) alone and together with different concentrations (1, 2 and 4  $\mu M$ ) of Cd. Growth behavior was assessed by estimating total protein content (Table 1). *A. stagnina* responded maximally at 120  $\mu mol$  photons  $m^{-2}s^{-1}$  whereas, 60  $\mu mol$  photon  $m^{-2}s^{-1}$  light intensity support 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  $\mu M$  of Cd, growth in *A. stagnina* decreased by 33% compared to respective control under 120  $\mu mol$  photon  $m^{-2}s^{-1}$  light intensity.

Results indicated that Cd treatment induced a similar decline in chlorophyll *a* and phycocyanin contents in *A. stagnina* cells (Fig. 1 a and b).

As compared to control, Cd (1  $\mu\text{M}$ ) treated cells caused a significant decline of 5% in Chl *a* content in normal light exposed *A. stagnina*, while, it was 3% and 9% in low and high light exposed cells, respectively. The decline percentage of Chl *a* increased to 10, 14 and 22% in response to the high dose of Cd (4  $\mu\text{M}$ ) 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 as light intensity (Fig. 1 c). Cadmium untreated normal light exposed cells showed enhanced phycocyanin content over low and high light intensity exposed *A. stagnina* 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 *A. stagnina* cells was estimated after normal and photoinhibitory light exposure. It is clear from results (Table 2) that Cd accumulation in *A. stagnina* increased with the rise of Cd concentration (10, 50, 100 and 150  $\mu\text{M}$ ) in external medium under normal light exposure and the accumulation further increased under photoinhibitory light treatment.

#### **Effect of photoinhibitory light on photosynthetic $\text{O}_2$ evolution at varying time interval under Cd stress**

Results pertaining to photosynthetic oxygen evolution in *A. stagnina* 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  $\text{O}_2$  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, *A. stagnina* required 55 min of 1000  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  light intensity. This becomes more intense (Fig. 3) under similar condition in the presence of Cd in concentration dependent manner and required only 26 min of photoinhibitory light treatment (1000  $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ ) for 50% photoinhibition in the presence of 150  $\mu\text{M}$  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  $\text{O}_2$  evolution. It is clear from the results (Fig. 3) that even 20 min exposure of photoinhibitory light caused 28% 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 *A. stagnina* 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 *A. stagnina* 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 *A. stagnina* exposed to Cd and photoinhibitory light treatment. Reduction in  $^{14}\text{CO}_2$  fixation reached maximum (-89%) under 150  $\mu\text{M}$  Cd and photoinhibitory light treatment. Under normal light (control) the rate of  $^{14}\text{C}$ -fixation was  $6718 \pm 10$  [CPM (mg Chl *a*) $^{-1}$  h $^{-1}$ ] and rate of PS I, PS II and whole chain electron transport activity was  $440 \pm 4$ ,  $256 \pm 3$  and  $172 \pm 2$  [ $\mu\text{molO}_2$  evolution/consumption (mg Chl *a*) $^{-1}$  h $^{-1}$ ] respectively.

## DISCUSSION

Cyanobacterium *A. stagnina* 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 *A. stagnina* 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 *A. stagnina* is supported by similar decline recorded in photosynthetic pigments in dose dependent manner of Cd as well as light intensity.

*A. stagnina* shows maximum Chl *a* and phycocyanin and carotenoids content 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 except carotenoids. 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 (Choudhary 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).



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

Cadmium concentration ( $\mu\text{M}$ )	Light intensity ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ )		
	20	60	120
0	0.079 $\pm$ 0.003	0.095 $\pm$ 0.002	0.121 $\pm$ 0.0003
1	0.072 $\pm$ 0.004 (9)	0.082 $\pm$ 0.002 (14)	0.107 $\pm$ 0.004 (12)
2	0.070 $\pm$ 0.003 (17)	0.076 $\pm$ 0.003 (25)	0.101 $\pm$ 0.004 (17)
4	0.065 $\pm$ 0.004 (18)	0.070 $\pm$ 0.003 (31)	0.086 $\pm$ 0.003 (33)

All the values are means  $\pm$  SE. Data in parenthesis denote percent inhibition over control. All treatments are significantly different ( $P<0.05$ ) from control (student "t" test).

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.

**Table 2. Influence of normal and high light intensities on cadmium uptake in *A. Stagnina*.**

Cadmium concentration ( $\mu\text{M}$ )	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	0.42 $\pm$ 0.02	1.24 $\pm$ 0.02 (195)
50	5.72 $\pm$ 0.01	26.50 $\pm$ 0.05 (363)
100	15.44 $\pm$ 0.03	33.00 $\pm$ 0.08 (114)
150	17.66 $\pm$ 0.05	36.00 $\pm$ 1.00 (104)

All the values are means  $\pm$  SE. Data in parenthesis denote percent 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.

The extent of photoinhibition depends upon the light intensity and its duration. Under photoinhibitory light, *A. stagnina* 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 (Cloot, 1994).

*A. stagnina* requires 55 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 photo inhibitory 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 though photosynthesis (Singh, 2018).

Photoinhibitory light intensities inhibited whole cell  $\text{O}_2$  evolution in *A. stagnina* which become inhibitory with different concentrations of Cd. Photosynthetic whole cell  $\text{O}_2$  evolution depends on the functioning of  $\text{O}_2$  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,  $\text{O}_2$  evolving capacity denotes the overall status of photosynthesis under stress condition. Decline in whole cell  $\text{O}_2$  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.

**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 *A. stagnina* treated with cadmium.**

Treatment	$^{14}\text{CO}_2$ fixation [CPM (mg chl a) $^{-1} \text{n}^{-1}$ ]	Photosynthetic electron transport activity $\text{O}_2$ evolution/consumption		
		ASC /DCPIP $\rightarrow$ MV (PS I)	$\text{H}_2\text{O} \rightarrow$ p-BQ (PS II)	$\text{H}_2\text{O} \rightarrow$ MV (Whole chain)
Photoinhibitory light	16475 $\pm$ 9	414 $\pm$ 4	125 $\pm$ 3	77 $\pm$ 2
+50 $\mu\text{M}$ Cd	253 $\pm$ 6 (-62)	400 $\pm$ 4 (9)	94 $\pm$ 2 (63)	55 $\pm$ 2(68)
+100 $\mu\text{M}$ Cd	978 $\pm$ 5 (-85)	382 $\pm$ 4 (13)	79 $\pm$ 2 (69)	46 $\pm$ 2 (73)
+150 $\mu\text{M}$ Cd	720 $\pm$ 4 (-89)	378 $\pm$ 4 (14)	64 $\pm$ 2 (75)	29 $\pm$ 2 (83)

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

Lu and Zhang (1999) reported<sup>1</sup> that increase in metal concentration led to a decrease in quantum yield of PS II. Under high light, *A. stagnina* 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). 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.

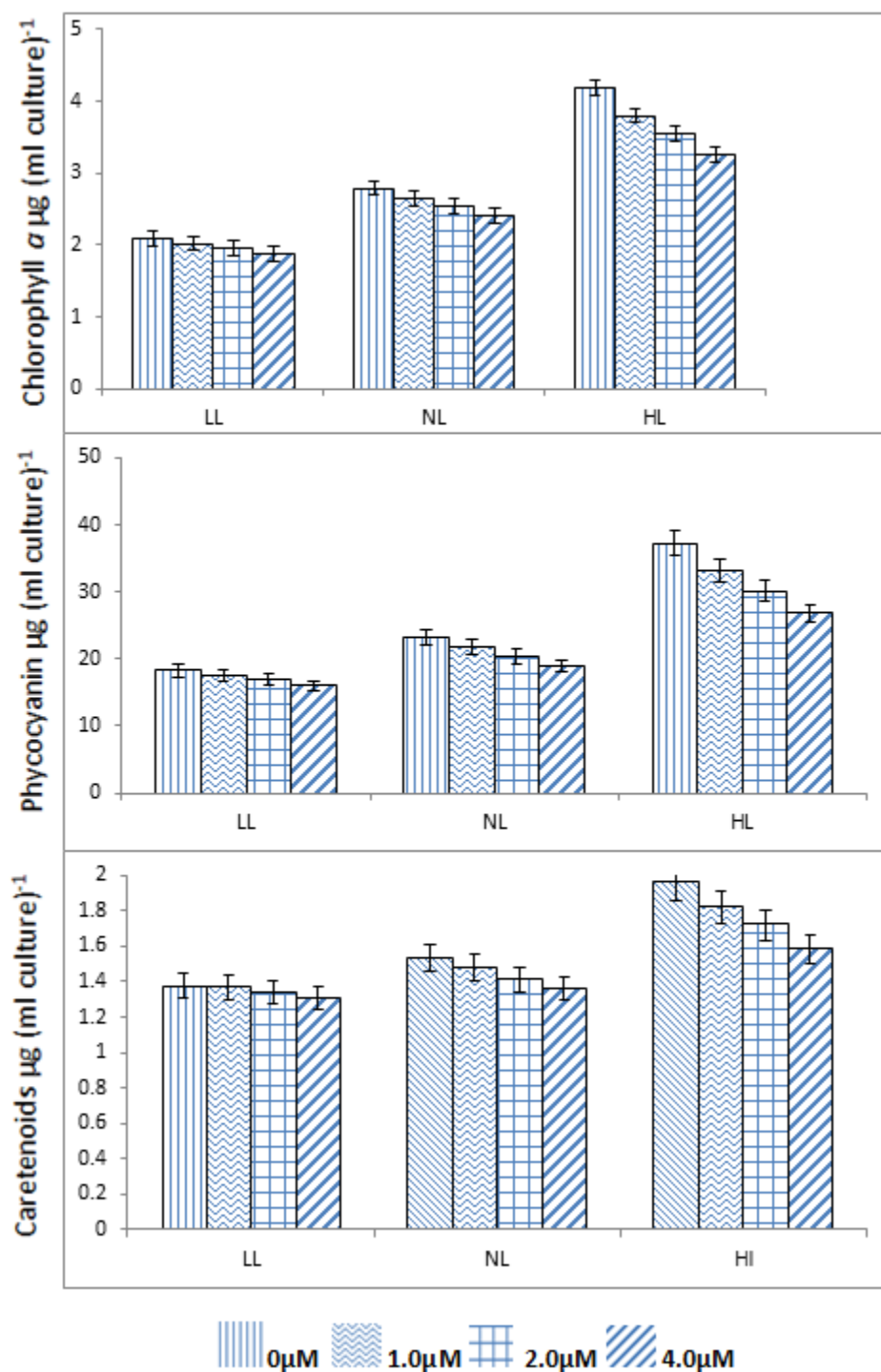


Figure 1. Effect of different concentrations of Cd on chlorophyll a (a), phycocyanin (b) and carotenoids (c) content in cyanobacterium *A. stagnina* under low, normal and high light intensities after 10 days. Data are means  $\pm$  standard error of three independent experiments. All treatments are significantly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

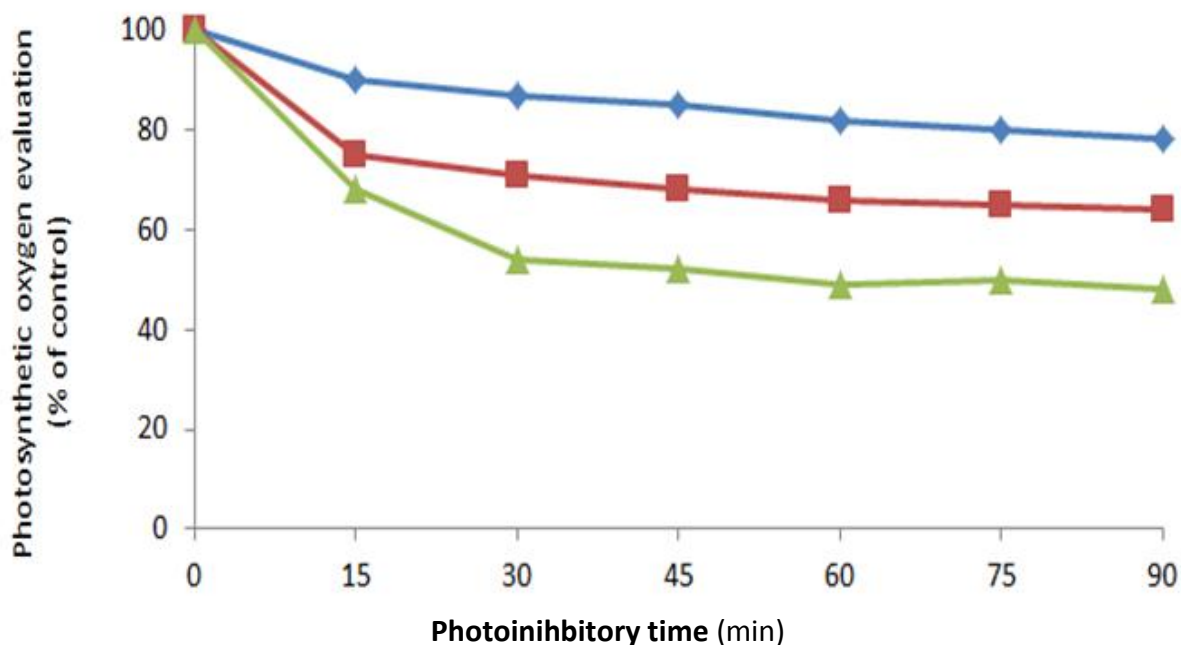


Figure 2. Photosynthetic  $\text{O}_2$  evolution *A. stagnina* versus time of photoinhibitory light. Control rate of  $\text{O}_2$  evolution was  $210 \pm 5 \mu\text{mol (mg chl a)}^{-1} \text{h}^{-1}$ . The PPFD of the photoinhibitory light were 250 (—◆—), 500 (—■—) and 1000 (—▲—)  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ .

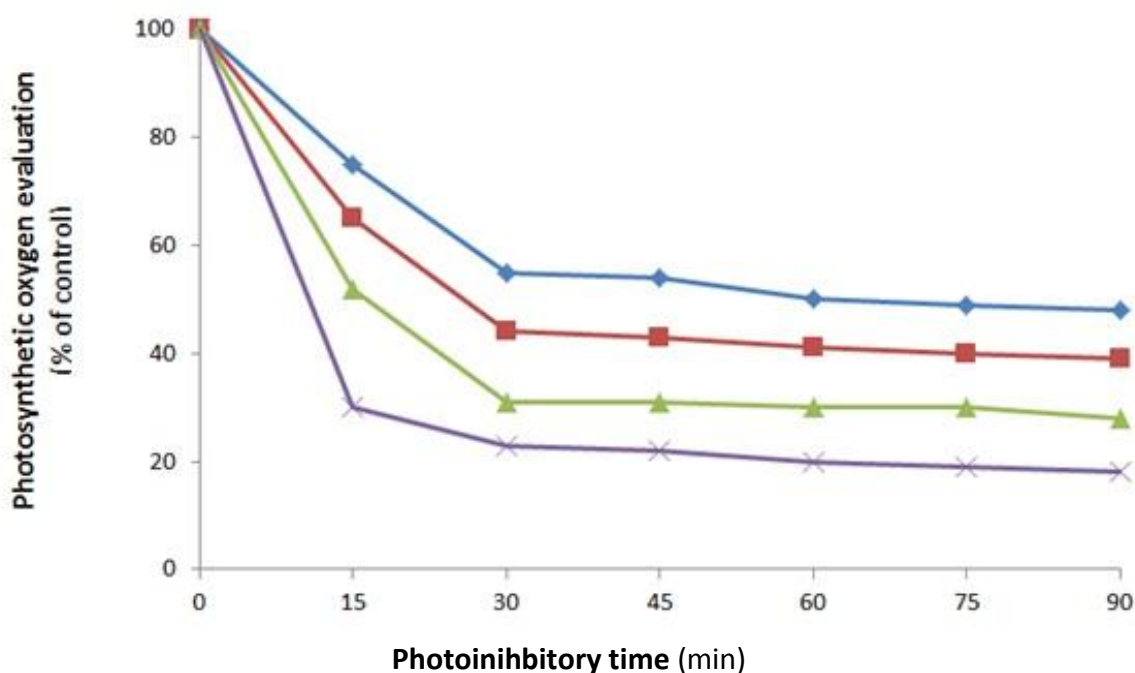


Figure 3. Effect of photoinhibitory light ( $1000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) treatment on photosynthetic  $\text{O}_2$  evolution rate in *A. stagnina* with varying concentrations of cadmium and exposure time. Rate of  $\text{O}_2$  evolution in control sample was  $200 \pm 6 \mu\text{mol (mg chl a)}^{-1} \text{h}^{-1}$ . Control (—◆—), 50  $\mu\text{M}$  (—■—), 100  $\mu\text{M}$  (—▲—), 150  $\mu\text{M}$  (—×—) of cadmium.

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).

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  $\mu\text{M}$ ) (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 photoinhibition 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 photoinhibitory 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 *A. stagnina*. 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. Result obtained in the present study may have further wide spread application if we use *A. stagnina* as a biological indicator to monitor the large scale input of hazard heavy metals (particularly Cd) in water bodies. This study also suggests clearly that the toxic effect of Cd is influenced by light intensity and even low doses prove to be more toxic at high light intensity.

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