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ISSN: 2637-7721

Journal of Plant Biology and Crop Research

Open Access | Research Article

Studies on the Photosynthetic Mechanism of *Spirulina Platensis* Under Metal Ions Toxicity

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Received: Aug 01, 2021

Accepted: Sep 15, 2021

Published Online: Sep 17, 2021

Journal: Journal of Plant Biology and Crop Research

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Energy transfer; Heavy metals; Photosynthesis; Spectral properties; Spirulina platensis.

Abstract

Spirulina platensis is a prokaryotic cyanobacterium and also a potent species indicator for the bioremediation process. In cyanobacterium are having major light harvesting protein pigments such as phycobilisomes. Which contains molecular structures of phycobiliproteins and important for the light energy transfer from Phycocyanin (PC) to chlorophyll *a* during photosynthetic process in *Spirulina platensis (S.platensis)*. The purpose of this study is to investigate the effect of two metals ions [Sodium arsenate (SA) and Nickel (II) nitrate (NN)] on the photosynthetic electron transport chain and photosynthetic energy transfer mechanism (*in vivo* and *in vitro*) of this blue-green algae. For *in vivo* studies; the cells were grown without (as control) and incubated with metal ions ((SA (10, 20, 30 µM) & NN (15, 30, 45 µM)) at 48 h. These PBPs are isolated from the control and treated samples and use these PBPs for the *in vitro* analysis incubate with two metal ions for 20 min. So based on spectral changes of PBPs at *in vivo* and *in vitro* studies, we concluded that SA is more toxic than NN at low concentrations. Which are able to change spectral alterations in the PC of the *S.platensis*. Therefore the changes in the spectral properties of the PC can be used as an indicator to assess the toxicity of heavy metal ions in *Spirulina* cells. Hence, an alteration Occured in *Spirulina* cells the changes of photosynthetic energy transfer mechanism from PC to Chl *a* under metal toxicity.

Introduction

Heavy Metals (HMs) are hazardous chemical elements for biological life in water reservoirs and man using resources [1-2]. Which are the main group of inorganic contaminants in the earth's crust, aquatic environment and soil contaminated by the use of sludge or municipal compost, pesticides, fertilizers and emissions from municipal wastes incinerates, exudates, residues from metalliferous mines and smelting industries [3-4]. All metals are toxic at higher concentrations; indiscriminate human activities have drastically altered metal geochemical cycles and biochemical balance. In addition, excessive levels of many metals can result in soil quality degradation, crop yield reduction, and poor quality of agricultural products, posing significant hazards to human, animal and ecosystem health [5]. Toxic HMs are entering the food chain due to uptake & accumulation by crops, posing a potential threat to human health [3,6-7]. Arsenic (As) metal is a ubiquitous in the environment and it is present mostly in its trivalent (As III) and pentavalent (As V) [8]. Depending on the conditions of the media, tri and pentavalent forms can be converted into one another. Some researchers are reported, metals are reduces the activities of photosystem I, II (PSII, PSI) and decreases the whole chain electron transport activities [9-10]. Nickel (Ni) is the 24th most abundant element in the Earth's

Cite this article: Middepogu A. Studies on the Photosynthetic Mechanism of *Spirulina Platensis* Under Metal ions Toxicity. Plant Biol Crop Res. 2021; 4(2): 1040.

crust, comprising about 3% of the composition of the earth. The Ni and it compounds have many industrial and commercial uses and the progress of industrialization has led to increased emission of pollutants into ecosystems. 0.1 to 1.0 mgl^{-1} nickel caused a significant increase in chlorophyll *a* content of *Chlorella pyrenoidosa*. Some of these HMs are micronutrients for plants (Zn, Cu, Mn, Ni & Co), while others have not involved in any biological function, which are toxic (Cd, Pb and Hg) [11]. The cyanobacteria comprise a major proportion of the total phytoplankton biomass. *S.platensis* (*Arthrospira platensis*) is a photolithotropic, trichomatous cyanobacterium, family of *Ossillotoriace* and potent indicator in bioremediation process, which is an economically produced as a source of human food, animal feed and cosmetic colorants. Considerable efforts have been made to optimize the growth conditions of this cyanobacterium for massive production [12]. It also is a single cell protein (SCP), edible source of vitamins (64%), minerals, proteins, polyunsaturated fatty acids (gamma-linolenic acid), therapeutic properties, antioxidant activity and voluble food supplement for human beings and animals too. In *S. platensis* two photosystems work in series to convert the energy of solar photons into storable chemical energy**.** PBSs serve as the primary light-harvesting antennae for photosystem II in cyanobacteria and red algae these macromolecular structures composed with PBPs [13-13a]. In terms of their spectral properties as well as pigment compositions, PBPs were divided into four groups, Allophycocyanin (APC-Blue), Phycocyanin (PC-Blue), Phycoerythrin (PE-Red) and Phycoerythrocyanin (PEC). In general hemidiscoidal structure of PBSs consists of core (constituted of APC) surrounded by PE and PC organized in a periferric structure known as rod [14]. PC and APC were composed of two polypeptides, such as α and β polypeptides (in some PEs, there is a special type of subunit, the γ subunit) in rod side of PBSs, are a brilliantly colored group of disc-shaped proteins bearing covalently attached open-chain tetra pyrroles known as Phycobilins. Whereas PC and APC are always present in cyanophyceae and Rhodophyceae, PE may be absent in the former. The PBS absorbs light in the 500-650 nm wavelength range. Primary dynamics in PSII and energy transfer processes in photosynthetic apparatus of cyanobacteria have been extensively studied for decades [13]. PC is an antioxidant, fluorescent protein and major constitute of the PBSs while, APC holds the bridging pigments between PBS and photosynthetic lamellae. The photosynthetic energy transfer in the PBPs from PC to Chl *a* (PS II) can be influenced by different type of HM ions, Hg [15] and Cr [16]. In cyanobacteria, metals are exerting toxic action mostly by damaging chloroplast and disturbing photosynthesis [8]. Photosynthetic pigments are easily measurable and frequently used to determine stress for regulatory purposes copper inhibits the photosynthesis, ostensibly on the oxidizing side of PS II. Photosynthetic pigments were found to be reduced the activity under the excessive concentrations of various HMs [8]. There are few reports that showed the enhancement of pigments after the exposure to metals [3]. The inhibition of *Chl* biosynthesis by metals has been well described by De Filippis *et al.* [17] and Cheng et al. [3]. Direct interaction of HM either with the donor or with the acceptor side of PS II leads to the interruption of electron transport. In the photosynthetic electron transport chain, PS is the site most sensitive to metal ions. In this paper was an attempt has been made to study the target pigment protein through PS II catalyzed electron transport and energy transfer (PC→Chl *a*) under HM toxicity of *S.platensis* biomass, by the using the electron transport (Whole chain, PSII) and absorption, fluorescence emission studies.

Materials and methods

Organisms and culture conditions

Spirulina platensis was obtained from National Centre for Conservation and Utilization of Blue Green Algae Laboratory (CCUBGA) at the Indian Agricultural Research Institute (IARI), New Delhi, India. Procured strain was maintained on Zarrouk's agar mediaslantsat $4^{O}C$. *Spirulina* culture were inoculated in 1 L of conical flask containing 500 ml of Zarrouk's Medium [18],, trichomes were grown at $26\pm2^{O}C$ under continuous illumination (15 Wm^{-2}) sterile condition.

Preparation of stock solutions and treatment

The inoculum was taken at Log growth phase of *Spirulina* culture and the stock solutions were prepared, SA (Na_3AsO_4) (for *in vivo* studies 20 μML^{-1} and *in vitro* studies 12 μML^{-1}) and NN ($Ni(NO_3)_2$) (for *in vivo* studies 30 μML^{-1} and *in vitro* studies 20 μML^{-1}) added a appropriate double distilled water. Log phase *S.platensis* cells were taken and treated with different concentrations of HMs (SA (Na_3AsO_4) (10, 20, 30 µM) and NN ($Ni(NO_3)_2$) 15,30,45 µM) incubated at an intensity of light 32 µM photons/m^2/S for 48h., whole chain and PS II catalyzed electron transport activities analyzed after 48 h using by clark type oxygen electrode & calculated by the method of Shim *et al.* [19]. For photosynthetic energy transfer studies, biomass was treated with NN (30 µM), SA (20 µM) for 48 h. Maintain algal samples to illuminated with light intensity (100 to 400 µM photons/m^2/S), then measured using oxygen (O_2) electrode (Hansatechoxygraph).

Assay of electron transport activities

The oxygen monitor (YSI oxygen electrode) was used to measure the rate of O_2 uptake and evolution in intact cells and thylakoid fragments. Assays of electron transport activities were carried out using the thermostated (26±1°C) glass cuvette, fitted within a clark type oxygen electrode (Hanstech Instrument Co., India). Three ml of reaction mixture consisted of 25 mM Hepes-NaOH (pH 7.5) 20 mM NaCl and intact cells equivalent to 12 to 15 µg of Chl *a*. The assay mixture was continuously stirred during measurement. Whole chain electron transport assay (H_2O→MV) was studied in terms of O_2 consumption due to photoreduction of methylviologen (MV) and its subsequent auto oxidation. Para benzoquinone (pBQ) was used to measure the PS II catalyzed electron transport (H_2O→pBQ) in the intact cells. Being a lipophilic compound pBQ enters into the intact cells and accepts electrons at plastoquinone (PQ) position [20-21].

Isolation of phycobilisomes

PBSs were isolated according to the method of Rajagopal *et al*. [22] with slight modifications. One gram of *Spirulina* cells were suspended in 10 ml of 1 M-K phosphate buffer at P^H 7.0 containing 1 mM PMSF, 1mM sodium azide, 1 mM EDTA and 2% 2-Mercaptoethanol. After cells were disrupted by sonication for 5 min. Sonicated cells were incubated with 2% Triton X-100 for 40 min. Cell debris was separated by centrifugation at 35,000 xg for 35 min. The supernatant was then layered on a buffered sucrose density gradient (2.0, 1.0, 0.5 and 0.25 M). The PBSs were concentrated in the 1.0 M region after spinning the gradients at 1,40,000 xg for 6 h at $25^{O}C$. The PBPs were pooled after removal from the 1M region and dialyzed overnight in 0.75 M phosphate buffer PC and APC were separated by using hydroxy apatite column.

Statistical analysis

All experiments were run in triplicate and the results were presented as a mean ± standard deviation. The differences between the control and the treatments were analysed by using Excel, Origin 8.5 version. Statistical analysis of the experimental data utilized Independent-Samples T Test. Each of the toxicity data was compared to its corresponding control. Statistical significance (*) was accepted when the probability of the result assuming the null hypothesis (p) was less than 0.05.

Results

S.platensis PBSs harvest light and cause energy migration usually toward PS II reaction centers. PS II receives more than 90% of the excitation energy emitted from PBSs [23]. Hemidiscoidal structures of PBSs are major light harvesting pigment protein complexes attached in PS II of the cyanobacterium, *S.platensis* (Figure 1). Any alterations in the spectral properties lead to the inhibition of PS II catalyzed electron transport (H_2O→pBQ). Therefore, after creating the HM ion stress, a comparative study has been made among electron transport activities by using various donors, acceptors (P-benzoquinone, methylviologen) and inhibitors (diuron, hydroxylamine). The artificial electron acceptor, MV which accepts at the reducing side of PS II has free access to the thylakoid membrane, even in the case of intact cells of *S platensis* [24]. Many sites in the photosynthetic membrane, especially the PS II, are highly sensitive to SA toxicity. SA can bind strongly to the thiol groups of proteins in both the donor and the acceptor sides of PS II and consequently disturbs their functions.

Electron transport measurements to identify the target site

The effect of selected HM ions (SA and NN) on the whole chain electron transport activity (H_2O→MV) in control and treated with heavy metal ions was studied (Figure 2). Control cells showed a high rate of O_2 consumption involving whole chain electron transport activity [253 µM of O_2 consumed (mg Chl)$^{-1}$ h^{-1}].

The treatment of NN showed a concentration dependent inhibition in whole chain electron transport activity. Various concentrations of NN: 15, 30, 45 µM caused 17%, 49%, 62% inhibition in whole chain electron transport activity respectively. The 51% inhibition was noticed with 20 µM of SA and further rise in the concentration caused 66% inhibition with 30 µM of Arsenic metal. From above figures it is clear that the reason for the inhibition of whole chain electron transport activity could be due to two possibilities a) Either the alteration at the level of PS II catalyzed reaction center or at the level of LHC b) both. In verifying the above proposition the partial electron transport reaction of PS II has been measured by using pBQ as hill acceptor. pBQ is an artificial electron acceptor which accepts electrons from PQ pool [20]. Control cells exhibited a high rate of PS II dependent oxygen evolving catalyzed electron transport activity (369 µM of O_2 evolved mg Chl^{-1}h^{-1}). The treatment of NN as expected showed concentration dependent inhibition in PS II catalyzed electron transport activity. 57% inhibition in PS II activity was noticed with 30 µM of Ni toxicity (Figure 3). Further rise in the concentration to 45 µM induced 74% loss in the PS II activity.

In cyanobacteria PBPs constitute the major light harvesting pigment proteins, which are attached to the outer surface of thylakoid membrane [25]. The light energy absorbed by the PBSs is transferred to the reaction center of PS II in the following sequence PC→ APC→APC B (L_{CM})→Chl *a* (PS II) [26]. A variety of environmental factors are known to influence the energy transfer from PC to Chl *a* i.e., high temperature [27] low temperature [28], HMs [16].

Energy transfer studies

Table 1 shows the effect of light illumination on NN metal induced PS II catalysed electron transport activity. From this study it is clear that the inhibition between light saturating and light limiting conditions in the case of NN 13 % loss was noticed. Similar results are also observed in the case of SA stressed cyanobacterial cells. In the case of SA the differences of inhibition between light saturating conditions and light limiting conditions was 12% (Table 2). The main reason for the inhibition of PS II activity at light limiting conditions in the two cases is due to alterations in the LHC of PSII. PBSs act as LHC in PS II. Therefore, any alterations in the PBSs can lead to the inhibition of its function i.e. PS II catalyzed electron transport activity.

***In vivo* experiments**

Spirulina is a fantastic source of antioxidants, which can protect against oxidative damage. Its main active component is called PC. This antioxidant substance also gives *Spirulina* its unique blue-green colour. PC can fight free radicals and inhibit production of inflammatory signalling molecules. As shown in Figure 4a, the absorption spectrum of PBSs exhibits a main peak at 623 nm. The treatment of NN 30 µM caused a small decrease in the absorption of PC and shifted the peak position from 623 to 612 nm. This effect is more pronounced in the case of PBSs isolated from SA (20 µM) exposed *Spirulina* cells (Figure 4b). Arsenic caused almost 38% decreases in the absorption capacity of PC and APC caused the shift towards the blue region by 21 nm. Since the absorption properties are related to the fluorescence emission of PC, PBSs samples which were isolated from NN (30 µM) exposed *S.platensis* cells were used for the measurement of PC fluorescence emission. NN (30 µM) tested biomass caused the slight decrease in fluorescence emission intensity and caused 4 nm blue shift in the peak position (Figure 4c). But in the case of SA (20 µM) the HM is able to cause a similar decrease in the fluorescence intensity as in the case of NN, but there is a huge peak shift from 672 nm to 664 nm. The decrease in the fluorescence intensity indicates the changes of energy transfer and blue shift gives information about structural changes in the PBPs (Figure 4d).

***In vitro* studies**

Since PBSs are made up of PC and APC to identify the exact target pigment protein of PBSs, we have gone for the study of the above HMs individually on PC and APC after their separation from PBSs. After dissociation of PBSs, they were loaded on the hydroxy apatite column and both PC and APC were separated by gradient elution using 5 mM Na-PO_4 buffer. To examine the purity of PC and APC we have used the spectral criteria. The PC was eluted from the column by passage of 10 mM K-PO_4 (P^H 7.0).

Figure 5a shows the spectral characteristics of PC, it is exhibited a single peak at 616 nm in the absorption spectrum without any shoulder at 650 nm indicating only the presence of PC in the collected fraction. When this fraction was excited at 610 nm the emission peak at 642 nm was observed (Figure 5b). This indicates that the observed light energy is emitted by PC at 654 nm instead of 678 nm. This is due to the absence

of other associated pigment proteins (APC) which accepts energy from PC. This indicates the emission at 662 nm arises from APC but not from PC. After taking the PC fraction it was given treatment with 20 µM of NN for 15 min in the dark and absorption spectra has been measured. NN caused the slight decrease in absorption capacity of PC and shifted the peak towards the lower wavelength of 18 nm (Figure 5a). According to Schirmer *et al*. [28], the spectrum of PC at shorter wavelength region is contributed by chromophore of 155th position in α subunit. The longer wavelength region was contributed by chromophores of both 84th position of β subunit and 155th position of subunits of PC. So my results strongly suggest that the above selected metal ions are causing the bleaching of β subunit-84, hence the absorption was observed only from α subunit of PC. The

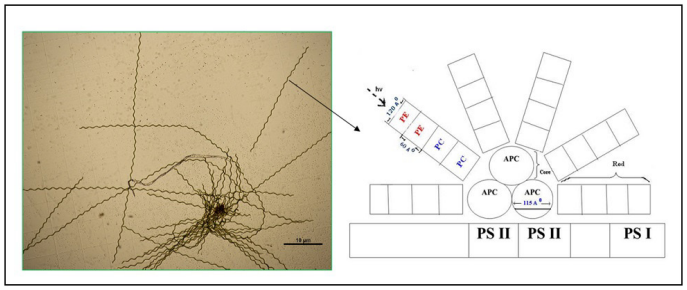

Figure 1: Fluorescence microscopic view of *S. Platensis* with hemidiscoidal structure PBSs.

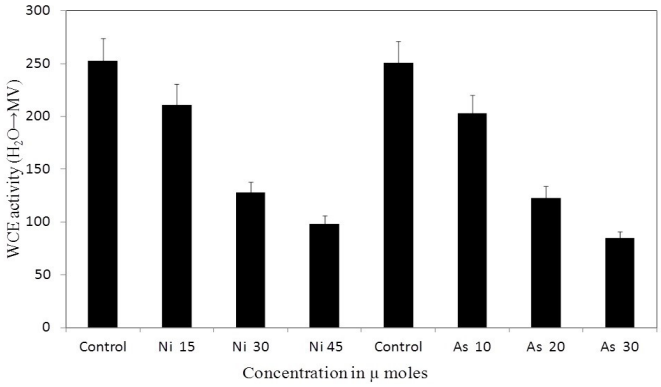

Figure 2: Effect of various concentrations of NN & SA metal stress treated on thylakoid membranes of whole chain electron transport activity (H_2O→MV) in *S.platensis*. The SD is not more than activity 10%. (Percentage: 0, 17, 49,62**,** 0,19,51,66).

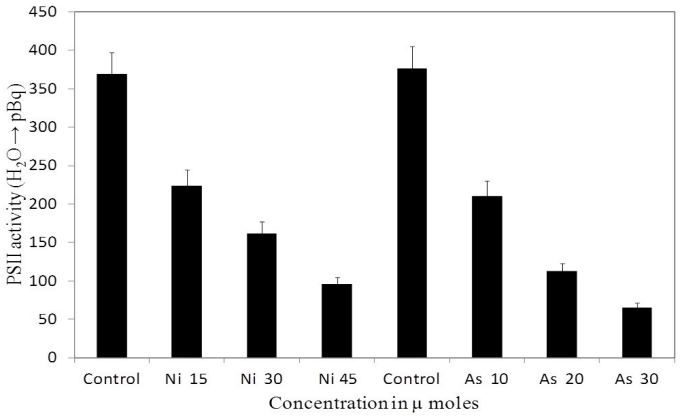

Figure 3: Effect of different concentrations of NN and SA metals treated on PS II catalysed electron transport activity (H_2O→pBQ) in the *S.platensis.* The SD is not more than activity 10%. (Percentage 0, 42,57,74,0,44,70,82).

treatment of NN caused a shift in the peak position by 21 nm towards the blue region of spectrum (Figure 5b). Unlike NN and SA (12 µM) induced drastic decrease in blue shift by 27 nm towards the blue region of absorption spectrum and caused 40 % decrease in the PC fluorescence emission (Figure 5c & 5d). In addition the blue shift of 3 nm noticed in the PC fluorescence emission from 646 to 644 nm. Thus, both the selected HM ions are able to cause the bleaching of the chromophore of β subunit and thereby affected the photochemistry of PC. Thus, both the selected heavy metal ions are causing alterations in the energy transfer by particularly acting at the level of β subunit in the cyanobacterium *S.platensis*.

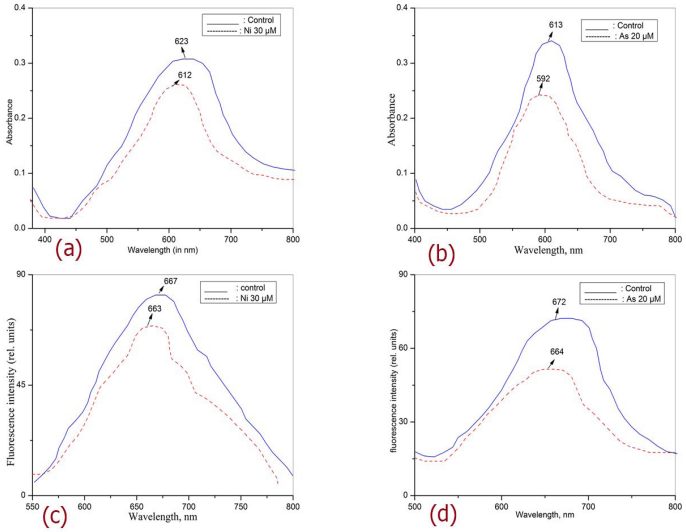

Figure 4: (a) The room temperature absorption spectra of PBSs isolated from control and NN ion (30 µM) exposed with *S.platensis* cells.; **(b)** The room temperature absorption spectra of PBSs isolated from control and SA metal (20 µM) exposed *S.platensis* cells; **(c)** Fluorescence emission spectra of PBSs isolated from control and NN (45 µM) treated cells; **(d)** Fluorescence emission spectra of PBSs isolated from control and SA (20 µM) treated cells.

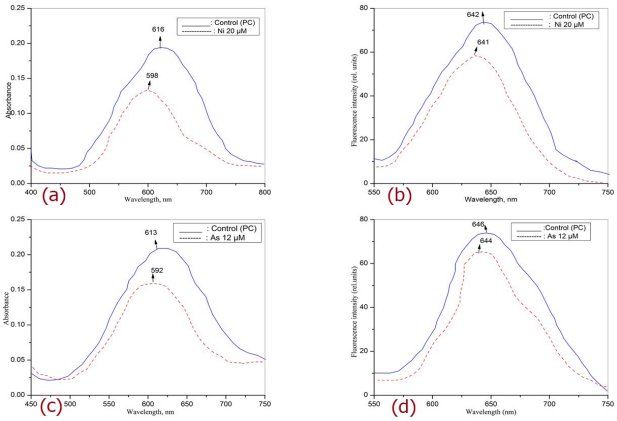

Figure 5: (a) Absorption spectra of control and NN treated PC fraction. PC was incubated in the presence and absence of NN metal (20 µM) for 20 min in the dark condition before measuring the spectrum; **(b)** Fluorescence emission spectra of control and NN (20 µM) treated PC fraction. PC was incubated in 10 mM of K-PO_4 buffer (P^H 7.0) in the presence and absence of NN metal (20) for 20 min in the dark condition before measuring the spectrum; **(c)** Effect of SA on absorption spectra of PC fraction. PC was incubated in the presence and absence of SA metal (12 µM) for 20 min in the dark condition before measuring the spectrum; **(d)** Effect of SA on fluorescence emission spectra of PC fraction. PC was incubated in 10 mM of K-PO_4 buffer (P^H 7.0) in the presence and absence of SA metal (12 µM) for 20 min in the dark condition before measuring the spectrum.

Table 1: Effect of illuminated light intensity on nickel treated PS II catalyzed electron transport activity (H_2O → pBQ) of *S.platensis*.

Light intensity (moles photons/m2/S)	PS II catalysed electron transport activity H2O->BBQ (µ moles O2 evolved mg $Ch1^{-1}h^{-1}$		Loss of percentage
	Control	Ni (30 M) treated	
100	43 ± 3	25 ± 2	41
200	106 ± 9	57 ± 5	46
300	185 ± 16	91 ± 9	51
400	370 ± 35	170 ± 17	54

Table 2: Effect of illuminated light intensity of arsenic induced inhibition of PS II catalyzed electron transport activity (H_2O → pBQ).

Light intensity (µ moles photons/m^2/S)	PS II catalyzed electron transport activity H2O → pBQ (µ moles O_2 evolved mg $Ch1^{-1}h^{-1}$		Loss of percentage
	Control	As treated (20 µ)	
100	43=4	24+2	41
200	102+9	56=5	45
300	190+18	95=9	50
400	365=34	172+17	53

Discussion

High concentrations of heavy metals in soil as well as in water are inhibitory for both plant growth and development [29-31]. Therefore, there is a need for the development of probes to detect the presence of heavy metals in the environment. To achieve this it is essential to know the specific targets and impairments induced by selecting HM ions on selected experimental system cyanobacteria, so that they can be used as indicators of heavy metal ion pollution. Our knowledge on these aspects is quite scanty and thus the study of heavy metal ion stress is being followed physiological, biochemical and genetic level by several researchers. Being prokaryotes and oxygenic photosynthetic organism in nature, cyanobacteria offer several advantages for studying mechanisms of photosynthesis. These cyanobacteria contain specialized photosynthetic pigments sensitive to environmental stress and exhibit the altered spectral properties. Among cyanobacteria, *S. platensis* is a easily cultivated in large quantities for biochemical investigations [30]. In addition *S.platensis* has been used for the studies on photosynthetic electron transport, photophosphorylation and chromatic adaptation [16]. Therefore, in the present study, NN and SA metal ions have been used to identify the alterations in the PBSs and analyzed their effect on the energy transfer process of PS II in the above cyanobacterium. Since NN is present in excess quantity in the environment due to its use power plants and trash incinerators into the air, it has been chosen as one of the stress factors. Similarly SA is also used in mining, smelting and coal-fired power, volcanoes; therefore SA is also identified as stress component. By choosing the above two heavy metal ions, attempts were made by incubating the cells and isolated pigment proteins for 48 h light conditions at different concentrations. I discuss here the specific effects of selected two HMs on energy transfer in photosynthetic pigment and photosynthetic electron transport activity.

The treatment of NN showed a concentration dependent effect on whole chain electron transport activity and 49% inhibition was noticed with 30 µM of NN. Similarly SA is able to cause 51% inhibition with 20 µM concentrations (Figure 2). The possible reason for the inhibition of whole chain electron transport could be either alterations at the level of PS II catalyzed electron transport or due to alterations at PS I or both. To verify the susceptibility of photosystem (s), the partial electron transport reactions catalyzed by PS II have been made using thylakoid membranes. The treatment of NN and SA independently caused 74% & 82% inhibition of pBQ supported Hill reaction at 45µM of NN and 30 µM of SA respectively (Figure 3). The loss in the PS II activity under heavy metal ion toxicity could be due to three reasons a) alterations at the level of WOC b) changes at the level of PS II reaction center and c) modifications at the level of reducing side of PS II. From the above mentioned studies it is quite clear that PS II is the main target for HM action in this cyanobacterium. Since our objective is energy transfer from PC to Chl *a* in *S.platensis*, we have characterized the spectral alteration related to the Hill activity by exposing the cells to different illumination intensity of white light (100 to 400 µ mole photons/m^{-2}/S) by using neutral density filters. These results suggest that the reason for the inhibition of PS II activity at low light intensity (100 µM) could be alterations at the light harvesting complexes (Table 1 and 2). Therefore an attempt has been made to correlate the *in vivo* studies with *in vitro* studies regarding the spectral alterations induced with NN and SA. The spectral alterations were noticed only PC, but not in the case of APC. Low concentrations of NN and SA an induced shift in the absorption peak towards the shorter wavelength region (towards 595 nm) indicate the specific bleaching of β 84 chromophore of PC (Figure 4d). A similar observation of the selective bleaching of β-84 chromphore of a PC was observed due to the effect of reducing agent dithionine in the cyanobacterium, *Mastigo cladus laminosus* [28]. Long term incubation of *S.platensis* cells with low concentrations of SA induces not only spectral changes in the pigment proteins but also ultimately leads to the dissociation of proteins specifically the β polypeptide of PC. Pecci & Fujimori. [31-32] also indicated that organic mercurial (phynyl mercuric acetate)

Conclusion

Among the tested concentrations of heavy metals (NN and SA) minimum to maximize ability to induce inhibition in PS II catalyzed electron transport activities of *S.platensis*. Light intensity measurements with NN and SA treated samples indicate that the reason for the inhibition of PS II catalyzed electron transport is alterations at the LHC of PS II. Between two photosystems, PS II is more sensitive to heavy metal toxicity when compared to that of PSI catalyzed electron transport. Spectral measurements in terms of absorption and fluorescence indicate that the energy transfer from PC to Chl *a* is main target for heavy metal action under *in vivo* and *in vitro* conditions. NN and SA inhibit the energy transfer by affecting the pigment protein interaction of PC under *in vivo* (intact cell level) *in vitro* (isolated pigment protein level) conditions.

References

1. Lasat MM. Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. Journal of Hazardous Materials. 2000; 2: 1-25.
2. Azeh Engwa G, Udoka Ferdinand P, Nweke Nwalo F, Unachukwu NM. Mechanism and Health Effects of Heavy Metal Toxicity in Humans- New Tricks for an Old Dog? Ozgur Karcioglu and Banu Arslan. 2019.
3. Cheng W, Zhang G, Yao H, Wu W, Xu M. Genotypic and environmental variation in cadmium, chromium, arsenic, nickel, and lead concentrations in rice grains. Journal of Zhejiang University SCIENCE B. 2006; 7: 565-571.
4. Middepogu A. Impact of heavy metal poisoning on cyanobacterial photosynthesis and its detoxification-A Review. Innoriginal International Journal of Sciences. 2016: 4: 18-23.
5. McLaughlin MJ, Singh BR. Cadmium in soil and plants: a global perspective. In: 'Cadmium in soils and plants'. 1999: 13-21.
6. Jackson AP, Alloway BJ. The Transfer of Cadmium from Agricultural Soils to the Human Food Chain. In: Adriado DC, editor. Biogeochemistry of Trace Metals. Boca Raton. FL: Lewis Publishers. 1992: 109-158.
7. Sponza D, Karaoğlu N. Environmental geochemistry and pollution studies of Aliağa metal industry district. Environ International. 2002; 27: 541-553.
8. Ma Z, Lin L, Wu M, Yu H, Shang T, et al. Total and inorganic arsenic contents in seaweeds: absorption, accumulation, transformation and toxicity. Aquaculture. 2018; 497: 49-55.
9. Ye ZP, Yu Q, Kang HJ. Evaluation of photosynthetic electron flow using simultaneous measurements of gas exchange and chlorophyll fluorescence under photorespiratory conditions. Photosynthetica. 2012; 50: 472-476.
10. Alidadi H, Tavakoly Sany SB, Zarif Garaati Oftadeh B, Mohamad T, Shamszade H, et al. Health risk assessments of arsenic and toxic heavy metal exposure in drinking water in northeast Iran. Environmental Health and Preventive Medicine. 2019; 24.
11. Gaur A, Adholeya A. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Current Science. 2004; 86: 528-534.
12. Godlewska K, Michalak I, Pacyga P, Baśladyńska S, Chojnacka K. Potential applications of cyanobacteria: Spirulina platensis filtrates and homogenates in agriculture. World Journal of Microbiology and Biotechnology. 2019; 35.
13. Middepogu A, Hou J, Gao X, Lin DH. Effect and mechanism of TiO2 nanoparticles on the photosynthesis of Chlorella pyrenoidosa. Ecotoxicology Environmental Safety. 2018; 161: 497-506.

13a. Middepogu A, Murthy SDS, Prasanna Reddy B. Structural organization and functions of phycobiliproteins in cyanobacteria. International. Journal Plant Animal Environmental Sciences. 2012; 2: 9-17.

14. McCall R. Cyanobacterial phycobilisomes. Journal of Structural Biology. 1998; 124: 311-334.
15. Murthy SDS. Studies on bioenergetic processes of cyanobacteria: Analysis of the effect of selected heavy metal ions on energy linked process.1991.
16. Middepogu A, Murthy SDS. Altered energy transfer in phycobilisomes of the cyanobacterium, Spirulina platensis under the influence of chromium (III). Journal of Pure and Applied Sciences. 2011; 19: 1-3.
17. De-Filippis LF, Hampp R, Ziegler H. The effects of sublethal concentrations of zinc, cadmium and mercury on Euglena growth and pigments. Journal of Plant Physiology. 1981; 1: 37-47.
18. ZarroukC. Contribution to the study of influence of cyanophyce diverse physical and chemical factors on growth and photosynthesis of Spirulina maxima (Setch and Gardner) Geitler, Ph.D. Thesis, University of Paris. 1966.
19. Shim H, Cao J, Govindjee E, Debrounner PG. Purification of highly active oxygen-evolving photosystem II from Chlamydomonas reinhardtii. Photosynthesis Research. 1990; 26: 223-228.
20. Trebst A. Energy conservation in photosynthetic electron transport of chloroplasts. Annual Review of Plant Physiology. 197; 25: 423-458.
21. Singh M, Shyam R, Sane PV. Inhibition of photosynthetic electron transport by diethyl pyrocarbonate in chloroplasts. Indian. Journal of Experimental Biology. 1990; 28: 162-165.
22. Rajagopal S, Jha IB, Murthy SDS, Mohanty P. Ultraviolet-B Effects on Spirulina platensis Cells: Modification of Chromophore–Protein Interaction and Energy Transfer Characteristics of Phycobilisomes. Biochemical and Biophysical Research Communications. 1998; 249: 172-177.
23. Glazer AM. Light harvesting in phycobilisomes. Annual Review of Biophysics and Biophysical Chemistry. 1985; 14: 47-77.
24. Robinson SJ, DeRoo CS, Yocum CF. Photosynthetic electron transfer in preparations of the cyanobacterium Spirulina platensis. Plant Physiology. 1980; 70: 154-161.
25. Gantt E. Phycobilisomes. The Annual Review of Plant Biology. 1981; 32: 327-347.
26. Fork DC, Mohanty P. Fluorescence and other characteristics of blue-green algae (Cyanobacteria), red algae and cryptomonads. In: Light Emission by Plants and Bacteria. 1986: 451-496.
27. Singhal GS, Mohanty P, Govindjee. Effect of preheating intact cells on pigments revealed by absorption and fluorescence spectra. Z pflanzen-Physiology. 1981; 103: 217-228.
28. Schirmer T, Bode W, Huber R, Silder W, Zuber H. X-ray crystallographic structure of the light-harvesting biliprotein C-phycocyanin from the thermophilic cyanobacterium Mastigocladus laminosus and its resemblance to globin structures. Journal of Molecular Biology.1985; 184: 257-277.
29. Suresh Kumar K, Dahms HU, Won E-J, Lee JS, Shin KH. Microalgae - A promising tool for heavy metal remediation. Ecotoxicology and Environmental Safety. 2015: 113: 329-352.
30. Venkataraman L. V.Spirulina in India. In: Proc. Natl. Seminar Cyanobacter Res. - The Indian Scene. Natl. Facility for Marine Cyanobacteria, Bharathidasan Univ, Tiruchirapalli, India. 1992: 92-116.
31. Pecci J, Fujimori E. Spectral changes of phycoerythrin from Hydrochocoleum species and its relationship to protein dissociation. Biochimica Biophysica Acta. 1968; 154: 332-341.