Modulation of photosynthesis in *Synechocystis* and *Synechococcus* grown with chromium (VI)

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Synechocystis sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 exhibit dissimilar tolerance to Cr(VI) with a tenfold difference in their EC_{50} value for Cr(VI). This contrasting tolerance was attributed to the difference in the ability to transport Cr(VI) and to detoxify ROS. The present study used biochemical assays and chlorophyll fluorescence to investigate the effect of growth with Cr(VI) on photosynthesis in the two cyanobacteria. In absence of Cr(VI), all the measured parameters *viz.,* rates of CO_2 fixation, PSII and PSI activities were higher in *Synechocystis* in comparison to *Synechococcus,* suggesting intrinsic differences in their photosynthesis. Growth in the presence of Cr(VI) reduced the pigment content and photosystems' activities in both cyanobacteria. It was further observed that photosynthetic functions were more adversely affected in *Synechocystis* in comparison to *Synechococcus,* in spite of exposure to tenfold lower Cr(VI) concentration. The effective quantum yield of PSII and PSI obtained by chlorophyll fluorescence measurements increased in the presence of Cr(VI) in *Synechococcus* whereas it decreased in *Synechocystis.* However, the overall CO_2 fixation remained unchanged. These results indicated that, in addition to the intrinsic difference in photosynthetic rates, the two cyanobacteria exhibit differential modulation of photosynthetic machinery upon Cr(VI) exposure and *Synechococcus* could adapt better it's photosystems to counter the oxidative stress.

Keywords. Chromium (VI); chlorophyll fluorescence; cyanobacteria; Dual PAM; photosynthesis; PSII-PSI; *Synechocystis*; *Synechococcus*

Abbreviations: CEF, Cyclic electron flow; ETRI, Electron transport rate for PSI; ETRII, Electron transport rate for PSII; PAM, Pulse amplitude modulation; PAR, Photosynthetically active radiation; PSI, Photosystem I; PSII, Photosystem II; Y(I), Photochemical quantum yield of PSI; Y(II), Effective quantum yield of PSII; Y(NO), Quantum yield of non-regulated energy dissipation.

List of symbols: F_m, Maximum fluorescence; F_o, Minimum fluorescence after dark adaptation; F_v, Variable fluorescence; P_m, Maximal change in $P700^+$ signal.

1. Introduction

Cyanobacteria are the oldest photoautotrophs that carry out 'plant-like' oxygenic photosynthesis (Brocks *et al.* 1999). The success of cyanobacteria to survive in harsh environmental conditions on the earth for billions of years lies in the adaptability of their metabolic pathways to overcome various stresses in their growth milieu such as pH changes, nutritional imbalances,

variations in temperature and light intensity, exposure to UV, etc. (Marsac and Houmard 1993). Alterations to the environmental condition of these photoautotrophs are brought about by natural changes in the ecosystem, and augmented further by the pollutants generated from anthropogenic activities.

Chromium (Cr) is one of the major pollutants of water, air and soil due to its extensive use in leather, paper and metal alloy industries (Cervantes 1991). Being a transition metal, Cr exhibits various oxidation states from +2 to +6, of which +3 and +6 are predominant. The hexavalent chromium, Cr (VI), is highly mobile and bioavailable owing to its higher solubility, resulting in biological toxicity mainly due to oxidative damage to biomolecules (Cervantes *et al.* 2001). The microorganisms in the contaminated sites have evolved various mechanisms and metabolic adjustments for chromate tolerance. Among photoautotrophs, cyanobacteria and algae are able to tolerate chromate to a variable extent (Garnham and Green 1995; Khattar *et al.* 2004; Yewalkar *et al.* 2007; Anjana *et al.* 2007; Kiran *et al.* 2007, 2008; Ozturk *et al.* 2009).

Cell membranes and photosynthetic apparatus are known to be the targets of Cr (VI) (Appenroth *et al.* 2001). The acute stress of Cr (VI) is known to affect PSII function in various photoautotrophs. The inhibitory effect was mainly observed on D1 protein and oxygen-evolving complex of PSII in *Chlamydomonas reinhardtii* (Ali *et al.* 2006, Perreault *et al.* 2009). Effect of Cr (VI) on photosynthesis in fronds of *Spirodela polyrhiza* and on *Microcystis* sp. using chlorophyll fluorescence analysis was examined by Appenroth *et al.* (2001) and Wang *et al.* (2013) respectively. Cr (VI) affected several targets in PSII, especially the total number of active reaction centers, the efficiency of electron transport, yield of primary photochemistry and oxygen-evolving complex. The electron transport rate in PSI was affected to a lesser extent than PSII and cyclic electron flow was stimulated by Cr (VI). These studies were carried out by subjecting the samples to the acute stress of chromate. However, in nature, aquatic microflora rarely comes across acute metal stress; rather it is chronically exposed to toxic metals, highlighting the need for studying the long-term effects of those exposures.

Our earlier work has shown that two standard strains of unicellular cyanobacteria, *viz. Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 (hereafter referred to as *Synechocystis* and *Synechococcus* respectively) exhibit differential tolerance to Cr (VI) with EC_{50} values of 12±2 μM and 150±15 μM respectively. Cr (VI) is known to impart oxidative stress. Our investigations revealed the intrinsic level of antioxidants and the affinity of sulfate transporter for Cr to be the contributing factors (Gupta *et al.* 2013, 2015). While ROS generation/detoxification and ion transport are instantaneous processes, adaptation or modulation in response to changes in the environment are processes spanning longer time scales. Such adaptive changes brought about in photosynthesis by chronic exposure to Cr (VI) have not been studied. However, in our earlier study, we noticed morphological changes in thylakoid membranes in *Synechocystis* and *Synechococcus* after growth with potassium dichromate for over 9 days. The present study compared the long term effect of Cr (VI) exposure on photosynthetic parameters in these two cyanobacteria and concluded that the photosystem of *Synechococcus* showed better adaptation to Cr (VI) stress.

2.   Materials and methods

2.1   *Culture and growth conditions*

The two strains of unicellular cyanobacteria, *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 were inoculated in 50 ml of BG-11 medium (Rippka *et al.* 1979) and grown under continuous illumination of $\sim$ 15μmol.m^{-2}·s^{-1} at 26±2°C for 9 days. *Synechocystis* was inoculated in a medium containing 0 or 12 μM potassium dichromate whereas *Synechococcus* was inoculated in 0 or 150 μM potassium dichromate.

2.2   *CO$_2$ fixation activity*

CO_2 fixation activity was determined by using NaH^{14}CO$_3$. Cell suspensions containing known chlorophyll content in the range of 0.5–1 μg chlorophyll in 200 μl of BG-11 medium containing the respective concentration of Cr (VI) was taken in transparent scintillation vials. NaH^{14}CO$_3$ (20 mM, specific activity 0.5 mCi.mmol^{-1}) was added to the culture and the reaction mixture was incubated for 10min in light (21 W.m^{-2}) at room temperature. The reaction was stopped by the addition of 400 μl of 6.0 N acetic acid. Contents of the vials were allowed to dry. Acid stable ^{14}C labeled product was re-suspended in 100 μl of distilled water and 3 ml of ethanol was added followed by 5 ml of scintillation cocktail (0.4% BBOT in toluene) and ^{14}C was estimated in a liquid scintillation counter. The rate of CO_2 fixation was expressed as n moles $^{14}CO_2$ fixed (mg chl) $^{-1}$·h^{-1}.

2.3   Estimation of cell density and pigments

Absorption spectra of the cultures were recorded from 400 to 800 nm using Shimadzu UV-1800 spectrophotometer. Data on pigments and pigment protein complexes like chlorophyll, carotenoid and phycobiliproteins on a per cell basis were calculated using absorbance at 680, 490 and 630 nm respectively.

2.4   Room temperature fluorescence

Room temperature fluorescence emission spectra of the cultures were recorded using Hitachi F-4010 spectrofluorimeter. The cultures were incubated in dark for 10 min before recording the spectra. PSII fluorescence emission was obtained by exciting the sample at 440 nm and recording emission between 600–800 nm. Energy transfer from phycobilisome to PSII was studied by exciting phycobilisomes at 580 nm and recording the emission between 600–800 nm.

2.5   Preparation of cell-free extract

Synechococystis and *Synechococcus* cells grown for 9 days with 0 or 12 μM and 0 or 150 μM potassium dichromate respectively were harvested from 50 ml culture by centrifugation at 6000×g for 5 min at room temperature. The pellet was washed with buffer 1 (30 mM sodium phosphate, pH 7.0) followed by washing with buffer 2 (600 mM sucrose, 30mM sodium phosphate, pH 6.8). The pellet was suspended in 100 μl of buffer 3 (15 mM TES/NaOH pH 7.0, 600 mM sucrose, 5 mM $MgCl_2$, 10 mM NaCl). Glass beads (100 μm acid washed) were added to the suspension and vortexed 3 to 5 times for 1 min with intermittent incubation on ice to break the cells. The cell-free extract was obtained by centrifugation of the suspension at $8000 \times g$ for 15 min at 4°C.

2.6   PSII activity assay

PSII activity was assayed spectrophotometrically by following the reduction of DCPIP at OD_{600} according to Xiao *et al.* (1997) with some modifications. 20 μl cell-free extract containing 8–10 μg chlorophyll was suspended in 1480 μl Tricine-KOH buffer (50 mM Tricine-KOH pH 7.6 containing 50 mM $MgCl_2$, 50 mM KCl). DCPIP was added in the end to the final concentration of 50 μM. This mixture was incubated

under saturating white light or dark with continuous stirring and OD_{600} was measured for 5 min immediately after the addition of DCPIP. The reaction mixture without DCPIP was taken as a reference. One unit of PSII activity was defined as the amount of the sample required to photo-reduce 1 μmol of DCPIP per minute under the reaction conditions.

2.7   PSI activity assay

PSI activity was assayed in cell-free extracts polarographically by following oxygen consumption in light and dark using Clarke type oxygen electrode from Hansatech Instruments, UK. The reaction mixture consisted of cell-free extract containing 8–10 μg chlorophyll suspended in Tricine-KOH buffer (50 mM Tricine-KOH pH 7.6 containing 50 mM $MgCl_2$, 50 mM KCl), 50 μM DCPIP, 2 mM sodium ascorbate, 2 mM sodium azide, 5 mM ammonium chloride, 5 μM DCMU and 50 μM methyl viologen in a 2 ml reaction volume. Buffer and buffer containing cell-free extract were taken as blank.

2.8   Dual-wavelength pulse amplitude modulated fluorescence to study PSII and PSI

The effect of growth with Cr (VI) on photosynthesis in the cyanobacteria was also studied by analysis of chlorophyll fluorescence using a dual-wavelength pulse amplitude modulated fluorescence monitoring system (Dual-PAM-100, Heinz Walz GmbH, Germany). Responses of PSII and PSI activities were simultaneously measured; quantification of PSII and PSI activities was carried out by using chlorophyll fluorescence and $P700^+$ absorbance changes respectively.

2.9   Measurement of PSII and PSI quantum efficiencies

Measurement of PSII and PSI efficiencies was carried out by placing around 2 ml of cell suspension (containing ~ 10 μg.ml^{-1} chlorophyll) in a quartz cuvette between the emitter head and detector head of the system. The sample was dark-adapted for 5 mins and the saturation pulse method was used for detection of maximal PSII fluorescence and maximal change in $P700^+$ signal (Klughammer and Schreiber 1994, 2008). Briefly, after dark adaptation minimum fluorescence (F_0) was determined by measuring light of intensity 24

$\mu mol.m^{-2}.s^{-1}$ followed by determination of maximum fluorescence (F_m) by applying a saturating pulse of irradiance of 10,000 $\mu mol.m^{-2}.s^{-1}$ for 300 ms. The corresponding maximal change in the $P700^+$ signal (P_m) was determined by the application of the satura-tion pulse after far-red pre-illumination for 10 s. For P700 analysis, the redox signal obtained by dual-wavelength (830/875) unit was used.

2.10 Recording of slow induction curve under constant actinic light

The induction curve based on the saturation pulse method was recorded for 240 s using the slow kinetics sequence of the Dual PAM software. Saturating pulses were applied after each 20s interval after the onset of actinic light of 53 $\mu mol.m^{-2}.s^{-1}$ for determination of maximum fluorescence (F_m') and maximum $P700^+$ (P_m') signal under the actinic light. The data derived after the 4th saturating pulse was used for the deter-mination of parameters, PSII and PSI efficiencies. A steady-state of the photosynthetic apparatus was reached after the 3rd saturating pulse. The quantum yields of PSII and PSI along with cyclic electron flow (CEF) were calculated as described in the Dual PAM manual and as described by Wang *et al.* (2013).

2.11 Recording of light curves of ETR(I) and ETR(II)

Information on the photosynthetic performance of the cyanobacteria with respect to light intensity was obtained by light response curves. Light response curves of ETR(I) and ETR(II) from PAR intensity 0 to 363 μmol photons.$m^{-2}.s^{-1}$ were recorded using the routine of the Dual PAM software after recording of induction curve. A saturating pulse of 10,000 μmol photons.$m^{-2}s^{-1}$ for 300 ms was given after 1.0 min exposure to each PAR and ETR(I) and ETR(II) were determined. The instrument measured the Y (I) and Y(II) after each PAR exposure. These were used to calculate the ETRs.

3. Results and discussion

### 3.1 Cr (VI) reduced overall photosynthetic activity in Synechocystis

The response of photosynthetic functions in *Syne-chocystis* and *Synechococcus* to Cr (VI) was analyzed by inoculating the same number of cells in the medium containing respective EC_{50} Cr (VI) along with control cultures (same cells in medium without chromate) for 9 days. All the experiments were conducted on these 9-day-old cultures and the results are presented below.

Overall photosynthetic activity was measured as light-dependent CO_2 fixation (figure 1). In absence of Cr (VI), control *Synechocystis* showed a higher CO_2 fixation rate as compared to *Synechococcus*. Both the cyanobacteria grown at their respective EC_{50} concentrations showed a decline in the rate of CO_2 fixation and the decline was 24% in *Syne-chocystis* and 12% in *Synechococcus*. The larger decline in the rate of CO_2 fixation indicated func-tional disturbance to a greater extent in *Synechocystis* when grown with Cr (VI). A 24 h exposure to Cr (VI) also brought a decline in CO_2 fixation activity in the two cyanobacteria. However, the decline was steep in *Synechocystis* and at low Cr (VI), whereas *Syne-chococcus* showed a slight increase followed by a slow decline in CO_2 fixation, indicating an adaptive response in the latter (Gupta *et al.* 2015).

3.2 Cr (VI) caused depletion of photosynthetic pigments

Fluorescence and absorption peaks are indicative of proper orientation of photosystems in the thylakoid membrane and of antenna supercomplexes over the thylakoid membrane. Therefore, in order to evaluate the modifications in photosynthetic apparatus after
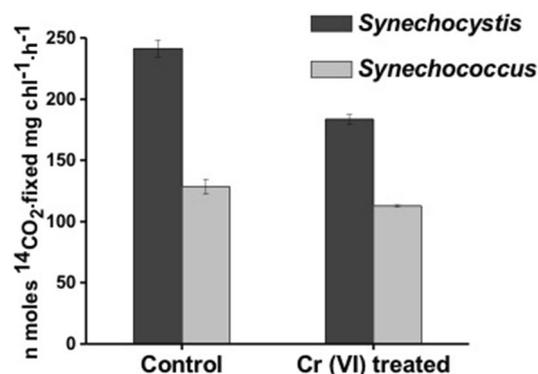
Figure 1. CO_2 fixation activity in *Synechocystis* and *Synechococcus*. CO_2 fixation activity was determined in *Synechocystis* grown with 0 or 12 μM and in *Synechococcus* grown with 0 or 150 μM potassium dichromate for 9 days. Activity is expressed as nmoles of $^{14}CO_2$ fixed (mg chlorophyll)$^{-1}.h^{-1}$.

chronic exposure to Cr (VI), absorption and fluorescence characteristic along with partial reactions of photosynthesis were investigated in *Synechocystis* and *Synechococcus* grown with Cr (VI).

Absorption spectra of *Synechocystis* and *Synechococcus* in response to growth with Cr (VI) are shown in figure 2. The spectra show characteristic absorption peaks of various pigments and pigment-protein complexes present in the cyanobacteria. The broad absorption band with a maximum at 440 nm followed by the shoulder at 490 nm was assigned to the Soret band of chlorophyll *a* and carotenoids respectively; phycobilisomes containing phycocyanin and allophycocyanin showed absorption at 630 nm. In addition, chlorophyll *a* absorbs at 680 nm.

Chlorophyll a, phycocyanin and carotenoids contents were determined on per cell basis from the absorption spectra. Both the cyanobacteria showed $\sim 25\%$ reduction in chlorophyll and phycocyanin content when grown with respective EC_{50} concentrations of Cr (VI). In *Synechocystis,* there was no decrease in carotenoids content on a per-cell basis when grown with Cr (VI), whereas *Synechococcus* showed about 25% reduction in carotenoid content (table 1). Growth with Cr (VI) caused a reduction in pigments but the shape and pattern of the spectra in both the cyanobacteria remained unchanged. This data suggested that growth with Cr (VI) caused a reduction in the proper functioning content of antenna pigments. Information on the relative content of phycocyanin and chlorophyll was obtained by fluorescence studies.

3.3 *Cr (VI) caused a shift in excitation energy transfer to photosystems in Synechococcus*

The fluorescence emission spectra show functional attributes of PSII and PSI complexes as well as energy transfer from accessory pigments. Dark incubated cultures were excited at 440 or 580 nm and emission spectra were recorded. At room temperature, the fluorescence arising from PSII can be observed after excitation with 440 nm. Reduction in fluorescence intensity from PSII (F690) was observed in both the cyanobacteria on growth with Cr (VI) (figure 3A and B). This may be due to a general reduction in chlorophyll content. On excitation of phycobilisomes at 580 nm (figure 3C), a decrease in F690 (energy transfer to PSII) and F653 (fluorescence of phycocyanin) was observed in *Synechocystis* grown with 12 μM potassium dichromate. *Synechococcus* grown with 150 μM dichromate, on excitation at 580 nm showed an increase in the allophycocyanin fluorescence with respect to PSII fluorescence (figure 3D). However, the fluorescence of PSII remained comparable to control culture. Such a change in fluorescence intensity suggests dissociation of phycobilisomes from PSII, an indication of modulation of photosynthetic functions.

3.4 *Photosystems activity was more adversely affected in Synechocystis in response to Cr (VI)*

Biochemical assays for PSII and PSI activities were also carried out further to analyze the functional integrity of the photosystems. PSII activity was
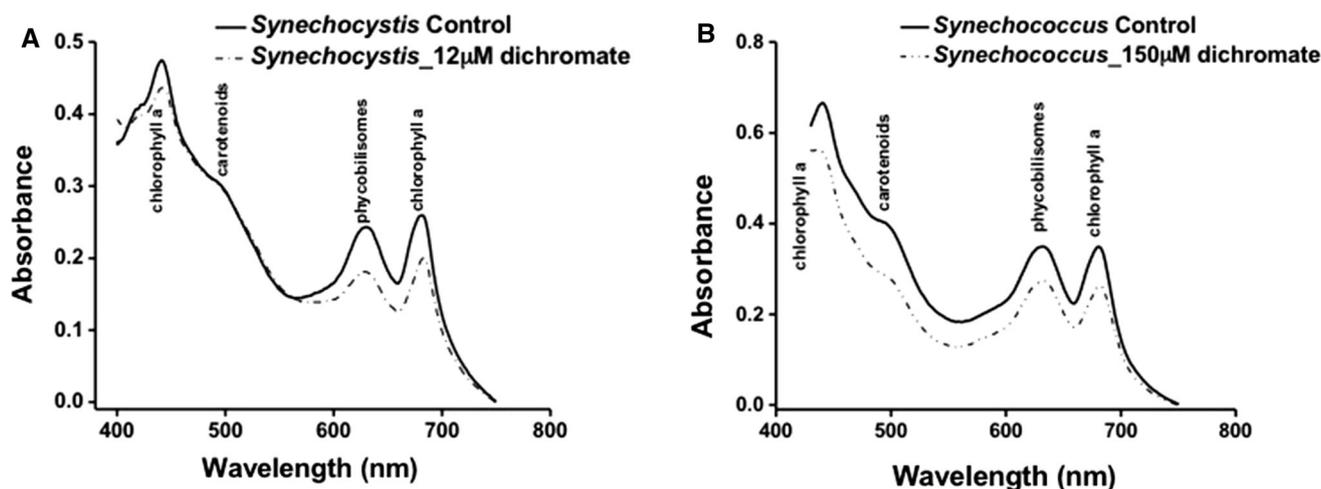
Figure 2. Absorption spectra of *Synechocystis* (**A**) and *Synechococcus* (**B**) grown with or without respective EC_{50} potassium dichromate for 9 days. $Peak_{440}$: Chlorophyll *a*; $Peak_{490}$: carotenoid; $Peak_{630}$: Phycobilisome; $Peak_{680}$: Chlorophyll *a*. All the spectra were normalized to OD_{730}.

Table 1. Effect of Cr (VI) on pigment composition

Pigments (% of control)	*Synechocystis* grown with 12 μM potassium dichromate	*Synechococcus* grown with 150 μM potassium dichromate
Chlorophyll *a*	76.9	75.3
Carotenoids	99.6	73.1
Phycocyanin	74.4	78.2

The percentage pigment contents shown in the cyanobacteria are relative to the respective control cultures. The pigment contents were derived from characteristic peak maxima, that is, 680 nm for chlorophyll *a*; 490 nm for carotenoid and 630 nm for phycocyanin from the absorption spectra recorded after 9 days of growth with or without potassium dichromate.

measured as DCPIP reduction (figure 4A). *Synechocystis* grown with 12μM dichromate showed a $\sim$70% decline in PSII activity as compared to control whereas *Synechococcus* showed a decline of up to 30%

in cells grown with 150 μM Cr (VI). Figure 4B shows that PSI activity was reduced by 20% in *Synechocystis* grown in presence of 12 μM potassium dichromate, whereas it was unaltered in *Synechococcus* grown in presence of 150 μM potassium dichromate. PSII and PSI activities were higher in *Synechocystis* as compared to *Synechococcus* on an equal chlorophyll basis (figure 4).

To get a deeper insight into the effect of Cr (VI) on the modulation of photosynthetic functions of thylakoid membranes, pulse amplitude modulated (dual PAM) fluorimetry was used. Maximal quantum yield of PSII (F_v/F_m) and maximal P700 change (P_m) were higher in *Synechocystis* as compared to *Synechococcus* (table 2). Cr (VI) caused $\sim$ 25–30% decline in F_v/F_m in comparison to the respective controls. P_m of the two cyanobacteria showed different behavior. In *Synechocystis*, P_m showed 50% reduction in response to Cr (VI), whereas in *Synechococcus*, it did not cause any significant change in P_m. These results with PAM fluorometry were in agreement with the *in vitro* measurements of PSII and PSI activities.
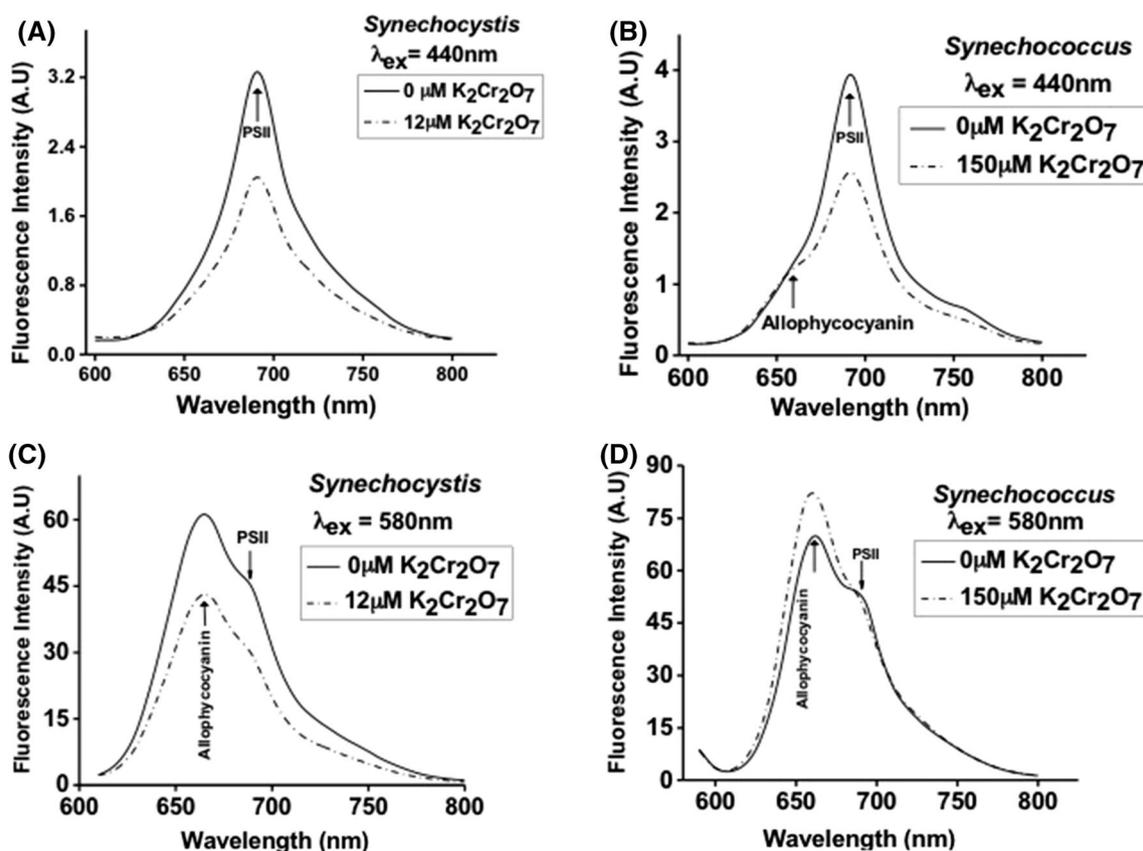
Figure 3. Fluorescence emission spectra of *Synechocystis* and *Synechococcus* grown with or without potassium dichromate. *Synechocystis* and *Synechococcus* were grown with 0 or 12 μM and 0 or 150 μM potassium dichromate respectively in the media for 9 days. (**A, B**) excitation at 440 nm and (**C, D**) excitation at 580 nm. Fluorescence intensity is expressed in arbitrary units (A.U.).
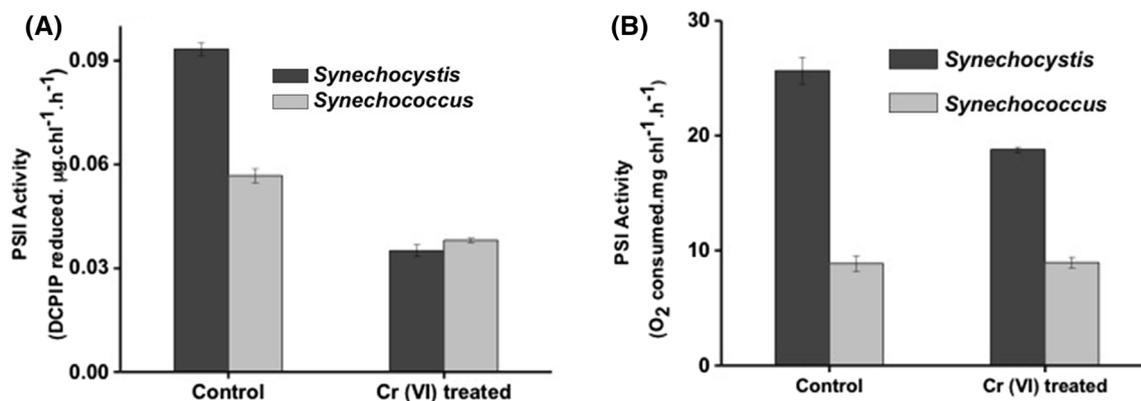
Figure 4. PSI and PSII activity in *Synechocystis* and *Synechococcus*. (**A**) PSII activity in *Synechocystis* and *Synechococcus* grown with or without dichromate. (**B**) PSI activity in *Synechocystis* and *Synechococcus* grown with or without dichromate.PSII activity was measured spectrophotometrically as rate of DCPIP reduction and PSI activity was measured polarographically as oxygen consumption.

Chlorophyll fluorescence measurements using Pulse Amplitude Modulated fluorometers is a non-invasive and fast method of measuring photosynthetic electron transport. However, in cyanobacteria, the interpretation of chlorophyll fluorescence data as a direct indicator of photosynthetic electron transport is not straight forward. This is due to the contribution of phycobilisomes and Isi A (iron starvation-induced protein) toward F_0 signal, which affects the calculation of parameters like F_v/F_m and Y (II), etc. (Campbell *et al.* 1998; Chen *et al.* 2018). Phycobilisomes act as a major antenna complex and have absorption overlap with chlorophyll a. IsiA is a chlorophyll-binding protein and is known to function as an antenna to PSI under iron starvation conditions. In addition to iron starvation, Isi A expression is also induced by oxidative stress (Chen *et al.* 2018). In our experimental setup, IsiA may not express in both the cyanobacteria as growth medium BG -11 provides iron and PAM measurements were carried out after 9 days of growth with Cr (VI) when the cyanobacteria have acclimated to the oxidative stress caused by Cr (VI). The contribution of phycobiliproteins to F_0 signal is responsible for the low characteristic F_v/F_m ratio in cyanobacteria in comparison to plants; the higher the phycobiliproteins, the lower is the F_v/F_m. Indeed, a phycocyanin mutant of *Synechococcus* 7942 showed F_v/F_m close to that of plants (Campbell *et al.* 1996). We observed that growth with Cr (VI) caused a decline in phycocyanin content in *Synechocystis* and *Synechococcus* (table 1). It also caused a decline in F_v/F_m in both the cyanobacteria. Therefore, the reduction in F_v/F_m could be attributed to processes other than a mere reduction in phycocyanin content.

Table 2. Effect of growth with potassium dichromate on maximal quantum yield of PSII and PSI in the cyanobacteria

Sample	Maximum Quantum Yield of PSII (F_v/F_m)	Maximal P700 change PSI (P_m)
Synechocystis Control	0.245 ± 0.005	0.453 ± 0.015
*Synechocystis*_12 μM $K_2Cr_2O_7$	0.172 ± 0.014	0.223 ± 0.001
Synechococcus Control	0.186 ± 0.011	0.360 ± 0.006
*Synechococcus*_150 μM $K_2Cr_2O_7$	0.144 ± 0.002	0.368 ± 0.021

F_v/F_m and P_m were determined in the dark adapted cultures for 5 minutes by applying saturation pulse method using the Dual PAM software. $F_v/F_m = (F_m-F_o)/F_m$, where F_o is fluorescence yield of dark adapted sample and F_m is fluorescence yield of dark adapted sample after application of saturation pulse. P_m represents maximal P700 change. The values (mean ± SD) are representative data of an experiment with minimum three replicates.

3.5 *Effective quantum yields suggest adaptation in Synechococcus in response to Cr (VI)*

Effective quantum yields [Y(II)] of energy conversion in PSI and PSII were determined at steady-state from the slow induction curves using Dual PAM. The Y(II) of PSII, in *Synechocystis* was three times higher as compared to that of *Synechococcus* (table 3). Y(II) decreased by 24% on growth with Cr (VI) in *Synechocystis* along with a minor increase in the Y(NO). In contrast, in *Synechococcus*, more than a two-fold increase was observed in Y(II) on growth with 150 μM potassium dichromate along with a corresponding

decrease in Y(NO). Y(NO) is an indicator of PSII damage (Huang *et al.* 2010; Perreault *et al.* 2009; Suzuki *et al.* 2011). Higher values of Y(NO) in *Synechocystis* in response to Cr (VI) suggested that the photochemical energy conversion and protective regulatory mechanisms were inefficient. Whereas a decrease in Y(NO) in *Synechococcus* grown with Cr (VI) indicate adaptive mechanisms working towards higher photochemical energy conversion. Y(II) determination was also carried out with increasing light intensity (figure 5). With an increase in irradiance, Y(II) decreased in the two cyanobacteria. In *Synechocystis*, growth with Cr (VI) caused a steady decline in Y (II) as irradiance was increased. However, in *Synechococcus*, growth with Cr (VI) caused an increase in Y(II) at low irradiance of up to 46 μmol.m^{-2}·s^{-1}, thereafter Y(II) exhibited a steady decline with higher irradiance. Decline in Y(II) with irradiance is thought to be due to relative increase in heat dissipation (Ralph and Gademann 2005). The data on *Synechococcus* acclimated to Cr (VI) therefore indicated a relatively lower heat dissipation in it at a lower light intensity.

In control conditions, the effective quantum yield of PSI, Y(I), was also higher in *Synechocystis* as compared to that of *Synechococcus*. In *Synechococcus*, similar to Y(II), growth with Cr (VI) caused an increase in the Y(I). These results suggested that although intrinsically the photosynthesis efficiency may be higher in *Synechocystis*, the adaptability of the photosystems is better in *Synechococcus* in response to Cr (VI).

During acute exposure, Cr (VI) is reported to have different sites of inhibition associated with PSII, PSI and electron transport sink beyond photosystems (Perreault *et al.* 2009). The present study, however, has been carried out on the cyanobacteria grown in the presence of Cr (VI) in the media using concentrations that allowed them to grow, albeit at a slower rate.

Therefore, photosynthetic parameters signaled adaptations in *Synechococcus* in response to Cr (VI) in addition to the intrinsic differences between the two cyanobacteria under study.

Electron transport rates (ETR) of PSII and PSI were calculated using the relation

Electron transport rate on PSII, ETR (II)

$$= Y(II) \times PAR \times 0.84 \times 0.5$$

Electron transport rate on PSI, ETR(I)

$$= Y(I) \times PAR \times 0.84 \times 0.5$$

The response of ETR (I) and ETR (II) with increasing photochemically active radiation (PAR) was recorded using the light curve function of the Dual PAM (figure 6). ETR of *Synechocystis* and *Synechococcus* showed contrasting responses when grown with Cr (VI). In *Synechocystis* cells, ETR (I) did not change with increase in PAR, whereas ETR (II) showed a decline with increase in PAR; the decrease was more pronounced at PAR >77 μmol photons.m^{-2}·s^{-1}; possibly due to photoinhibition. *Synechococcus* grown with Cr (VI) showed increase in both ETR (I) and ETR (II) beyond 77 μmol photons.m^{-2}·s^{-1}. With increase in PAR, ETR increases only if reaction centers are open. Higher ETR (I) and ETR (II) may reflect higher rates of photosynthesis. This was in agreement with higher effective quantum yields in response to Cr (VI) in the case of *Synechococcus*, again suggesting adaptive changes in the photosynthetic apparatus.

The quantum yield of cyclic electron flow, Y(CEF) was calculated from Y(II) and Y(I) values obtained at steady-state from the slow induction curves. As observed for Y(II) and Y(I), Y(CEF) was also higher in *Synechocystis* as compared to *Synechococcus*. Y(CEF) increased in both the cyanobacteria on growth with Cr

Table 3. Complementary quantum yields of energy conversion in PSII and PSI of *Synechocystis* and *Synechococcus* grown with or without chromate

| Sample | Quantum yields in PSII | | Quantum yields in PSI |
	Y(II)	Y(NO)	Y(I)
Synechocystis Control	0.234± 0.002	0.792± 0.001	0.741 ± 0.019
*Synechocystis*_12µM K$_2$Cr$_2$O$_7$	0.173± 0.016	0.848± 0.001	0.740 ± 0.026
Synechococcus Control	0.076± 0.008	0.948 ± 0.001	0.373 ± 0.002
*Synechococcus*_150µM K$_2$Cr$_2$O$_7$	0.165± 0.001	0.866± 0.001	0.731 ± 0.041

Effective quantum yields of energy conversion in PSI and PSII were determined at steady state under actinic light during the recording of slow induction curve as described in methods. The values (mean ± SD) are representative data of an experiment with minimum three replicates.
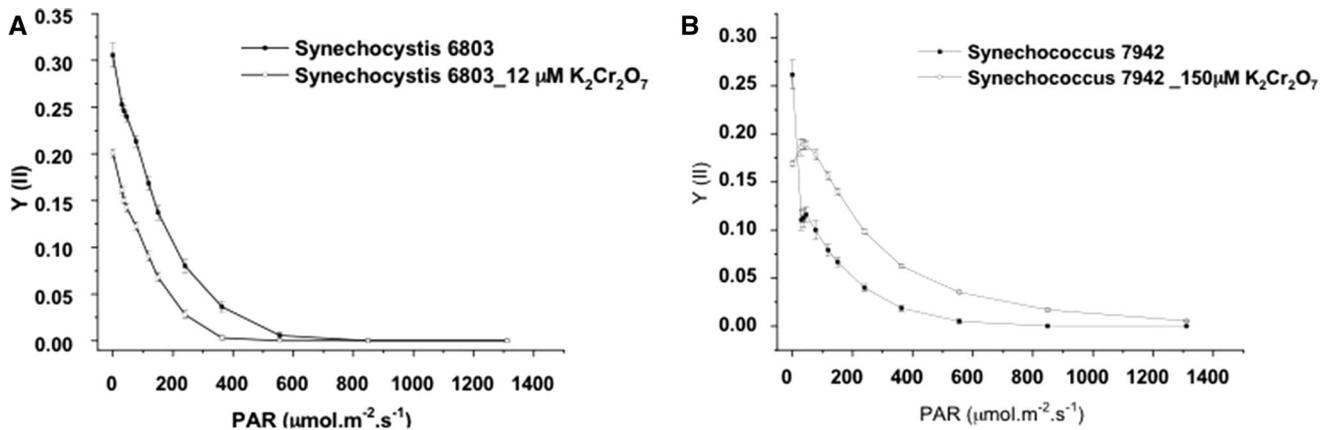
Figure 5. Effective quantum yield of PSII, Y (II) in the cyanobacteria with variation in light intensity. (**A**) Y (II) in *Synechocystis* control culture and after growth with 12 μM $K_2Cr_2O_7$. (**B**) Y (II) *Synechococcus* control culture and after growth with 150 μM $K_2Cr_2O_7$. The Y(II) values were measured during the acquisition of light curves (figure 6). The cultures were exposed to increasing light intensity from 0 to 363 μmol photons m^{-2} s^{-1} and Y(II) was measured after 1 min exposure to each light intensity.
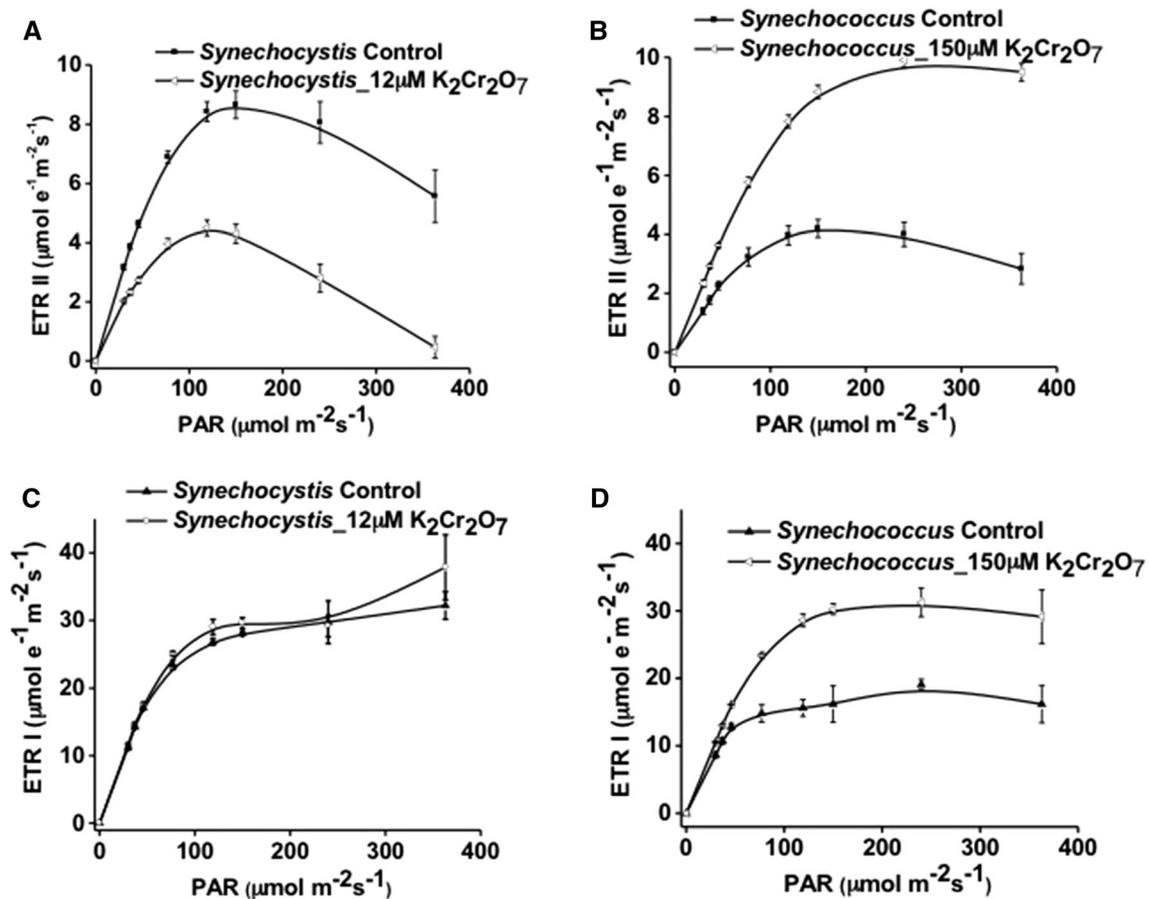
Figure 6. Light curves of electron transport rate of PSI and PSII [designated as ETR(I) and ETR(II) respectively] in the cyanobacteria in response to dichromate. (**A**) Light curve of ETRII in *Synechocystis*. (**B**) Light curve of ETRII in *Synechococcus*. (**C**) Light curve of ETRI in *Synechocystis*. (**B**) Light curve of ETRI in *Synechococcus*. Light curves of electron transport rate (ETR) of PSI and PSII in the cultures were recorded by exposing them to increasing photochemically active radiation (PAR) from 0–363 μmol photons m^{-2} s^{-1} using the Dual PAM software.

Alka Gupta et al.
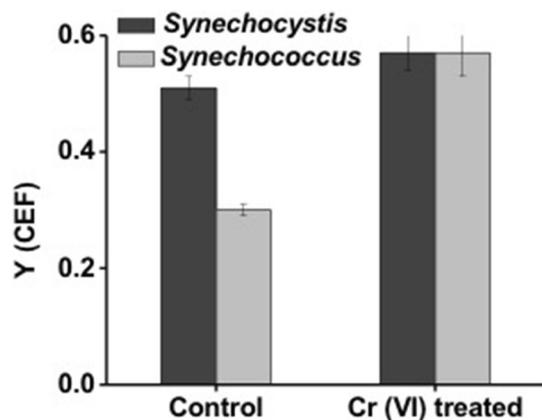
Figure 7. Quantum yield of cyclic electron flow in *Synechocystis* and *Synechococcus* in response to growth with dichromate. The quantum yield of cyclic electron flow (CEF) [Y(CEF)] of the cultures was calculated from the respective Y(I) and Y(II) values at steady state during recording of the slow induction curve as described in methods. Quantum yield of cyclic electron flow Y (CEF) = Y (I) − Y (II).

(VI) (figure 7); and the increase was more pronounced in *Synechococcus*.

Cr (VI) creates oxidative stress for cells and thylakoid membrane functions are the major targets of oxidative stress. Growth with Cr (VI) affected levels of pigment in both the cyanobacteria but not the characteristic absorption maxima. However, PAM measurements signaled modulation of photosynthetic parameters, all of which suggest adaptation in *Synechococcus* in response to growth with Cr (VI). Further insight on the sites of Cr (VI) affected site was provided by the parameters obtained from slow kinetics and light curves. The results suggested that PSII structure-function may be altered significantly in *Synechocystis* in presence of Cr (VI).

In the absence of Cr (VI), all the measured parameters were higher in *Synechocystis*, viz., rates of CO_2 fixation and PSII and PSI activities on chlorophyll basis, the maximal quantum yield of PSII (F_v/F_m) and maximal P700 change (P_m), effective quantum yields YII, YI and ETR II and I, in comparison to *Synechococcus* suggesting that the efficiency of photosynthetic machinery of the former is higher than the latter. However, photosynthesis in *Synechocystis* was more susceptible to Cr (VI) stress. The higher sensitivity of *Synechocystis* may be attributed to its more labile PSII, which is not able to sustain Cr (VI) stress even at 10 times lower concentration as compared to *Synechococcus*.

On growth with Cr (VI), CO_2 fixation rates and PSII, PSI activities were reduced as compared to controls in *Synechocystis,* so also the maximal quantum yield of PSII and P_m, Y(II), ETR(II). *Synechococcus*, on growth with Cr (VI), showed reduction in CO_2 fixation, PSII activity and maximal quantum yield of PSII. However, contrary to the expectation, the effective quantum yields PSII and PSI showed an increase.

It is likely that the increase in electron transport rates observed in response to Cr (VI) or variations in cyclic electron flow reflect other pathways of utilizing electrons such as ATP production. In *Synechococcus*, there may exist an energy-dependent process related to uptake and efflux of Cr (VI). The increase in electron transport rates as well as the effective quantum yield of PSII, PSI and CEF do not result in increase in rates of CO_2 fixation in *Synechococcus* in response to Cr (VI). This may be due to the diversion of electrons for other processes related to survival in Cr (VI)-containing environment.

4. Conclusions

The effect of long-term exposure to Cr (VI) on photosynthetic machinery was analyzed in *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 in the present study with the help of photochemical and biochemical assays. The results suggest that Cr (VI) affects the overall yield of photosynthesis measured in terms of carbon dioxide fixed in both the cyanobacteria. A careful dissection of photochemical functions revealed several adjustments at the level of photosystems, mainly suggesting better adaptive tendency to the presence of Cr (VI) in growth medium in the case of *Synechococcus*. This data is in agreement with our earlier observation of morphological disturbance of thylakoid membranes of *Synechocystis* in response to Cr (VI) (Gupta *et al.* 2013). In addition, our analysis of oxidative stress tolerance parameters has shown *Synechococcus* to have intrinsically higher levels of antioxidants (Gupta *et al.* 2015). Therefore, in summary, our observations suggest that between *Synechococcus* and *Synechocystis*, the former performs better due to its adaptability of the photosynthetic apparatus and stronger oxidative stress tolerance mechanisms. The photosynthetic electron transport which takes place in thylakoid membrane components does generate ROS and cyanobacteria have evolved various ways of detoxifying the ROS (Latifi *et al.* 2009). Whether the observed hardiness of photosynthesis in *Synechococcus* in response to Cr (VI) stress is

due to its better oxidative stress tolerance, can be a subject of future study.

Acknowledgements

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