

## Effect of UV-B and high visible radiation on photosynthesis in freshwater (*Nostoc spongiaeforme*) and marine (*Phormidium corium*) cyanobacteria

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Human activity is causing depletion of ozone in stratosphere, resulting in increased UV-B radiation and global warming. However, impact of these climatic changes on the aquatic organism (especially marine) is not fully understood. Here, we have studied the effect of excess UV-B and visible radiation on photosynthetic pigments, fatty acids content, lipid peroxidation, nitrogen content, nitrogen reductase activity and membrane proteins, induction of mycosporine-like amino acids (MAAs) and antioxidant enzymes superoxide dismutase (SOD) and ascorbate peroxidase (APX) in freshwater (*Nostoc spongiaeform*) and marine (*Phormidium corium*) cyanobacteria. UV-B treatment resulted in an increase in photosynthetic pigments in *Nostoc* and decrease in *Phormidium*, but high light treatment caused photobleaching of most of the pigments in both the species. Unsaturation level of fatty acids of both total and glycolipids remained unchanged in both the cyanobacteria, as a result of UV-B and high light treatments. Saturated fatty acids of total and glycolipids declined slightly in *Nostoc* by both the treatments, but remained unchanged in *Phormidium*. No changes in the unsaturated lipid content in our study probably suggested adaptation of the organism to the treatments. However, both treatments resulted in peroxidation of membrane lipids, indicating oxidative damage to lipids without any change in the level of unsaturation of fatty acid in the cell membrane. Qualitative and quantitative changes were observed in membrane protein profile due to the treatments. Cyanobacteria were able to synthesize MAAs in response to the UV-B treatment. Both treatments also increased the activities of SOD and APX. In conclusion, the study demonstrated induction of antioxidants such as SOD and APX under visible light treatment and screening pigment (MAAs) under UV-B treatment, which might protect the cyanobacteria from oxidative damage caused by high light and UV-B radiation.

**Keywords:** Antioxidant enzymes, Cyanobacteria, Fatty acids, High light, Mycosporine-like amino acids, Photosynthetic active radiation, Photosynthetic pigments, Ultraviolet-B radiation.

Continuing depletion of the stratospheric ozone layer is resulting in an increasing incidence of solar ultraviolet-B (UV-B) radiations (280-320 nm) at the earth's surface<sup>1</sup>. Although UV-B is a small component of the solar radiation reaching the earth's atmosphere, the energy level of its photon is high, making it very photochemically active form of radiation. More importantly, it is absorbed by the biologically important molecules such as DNA, protein, chromophores, pigments, plastoquinone and plastoquinol. This leads to wide ranging effects, including alteration in the structure of proteins, DNA and depression of key physiological processes<sup>2</sup>. In algae, a number of physiological and biochemical

processes such as growth, survival<sup>3</sup>, pigmentation, photosynthesis, motility and orientation<sup>4</sup>, the enzymes of nitrogen and carbon metabolism<sup>5</sup>, phycobiliprotein composition and <sup>14</sup>CO<sub>2</sub> uptake<sup>6</sup> have been reported to be susceptible to UV-B.

In aquatic ecosystem, solar radiation affects physical, chemical and biological processes such as thermal stratification and the vertical distribution of nutrients, photoautotrophs, herbivore and consumers. Cyanobacteria, in general, have adapted for growth in relatively low light conditions<sup>4</sup> and their exposure to high light can lead to inhibition of photosynthesis, a process known as photoinhibition<sup>7</sup>. The photoinhibition results in generation of reactive oxygen species (ROS), which can oxidize membrane proteins, lipids and pigments resulting in membrane instability, as well as photobleaching of the photosynthetic pigments, affecting growth and survivability of organism.

Cyanobacteria provide a powerful model system to study responses and acclimation to stresses of various kinds<sup>8</sup>. In this study, we have investigated the effect of UV-B and visible radiation, to which the

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**Abbreviations:** APX, ascorbate peroxidase; MAAs, mycosporine-like amino acids; MDA, malonaldehyde; PAR, photosynthetic active radiation; ROS, reactive oxygen species; SOD, superoxide dismutase; UV-B, ultraviolet-B.

cyanobacteria are exposed routinely in nature, on the photosynthesis and related processes such as photosynthetic pigments, nitrogen content and proteins in a freshwater *Nostoc spongiaeforme* Agardh and marine *Phormidium corium* Agardh (Gomont). In addition, the effect of both treatments on defense mechanism such as induction of UV-B absorbing compounds like mycosporine-like amino acids (MAAs) and antioxidant enzymes has also been studied.

## Materials and Methods

### Culture conditions

The cultures were routinely grown in autoclaved liquid culture medium, BG 11 (for *Nostoc spongiaeforme* Agardh, a freshwater alga present in rice fields) and ASN III (for *Phormidium corium* Agardh (Gomont), a marine alga, isolated from coral reefs of Lakshadweep Island, India) as described previously<sup>9</sup>. Culture was maintained in 100 ml conical flasks filled to 40% of their volume and kept on a shaker set to a temperature of 30°C ± 2°C under cool white fluorescent tubes providing 80 µmol m<sup>-2</sup> s<sup>-1</sup> PAR (photosynthetic active radiation) at the culture level with a 12 h of photoperiod. *Nostoc* and *Phormidium* were allowed to grow for 14 and 30 days, respectively to obtain their logarithmic phase of growth (balance growth phase<sup>10</sup>).

### Exposure to UV-B radiation and high light

The algal culture was transferred to a petri-plate. The UV-B treatment of 0.8 ± 0.1 mW cm<sup>-2</sup> in the range of 280-320 nm with cut-off filters for UV-A and C (Vilbour-Lourmat, France T-6M source with a λ-max at 312 nm) was given in a BOD chamber at 30°C for 0-6 h, while keeping the algal culture continuously stirred. The UV-B radiation (312 nm) was measured using a UV-B radiometer specific for measuring radiation of 312 nm from the same manufacturer.

For exposure to high light, the algal tissue was exposed to irradiance of 500 µmol m<sup>-2</sup> s<sup>-1</sup> PAR (Li-cor, Model Li-189) at the culture level at 30°C up to 6 h in a double-walled cuvette circulated with temperature controlled water. The light treatment was given using two slide projectors from opposite sides. Culture was kept constantly stirred during the treatment at a slow speed, to avoid mechanical damage.

### Analysis of photosynthetic pigments

Effect of light on photosynthetic pigments was studied as excess of light causes production of ROS, which leads to photobleaching of the pigments, thus

affecting the photosynthesis. Algal tissue was collected after centrifugation of culture at 8000 g for 15 min. The supernatant was discarded and pellet of algal cells was dried in lyophilizer. The 0.1 g of lyophilized algal cells was extracted in 1 ml of 80% (v/v) methanol in a homogenizer at 4°C under dim light, followed by centrifugation at 6000 g for 10 min at 4°C. The samples were filtered through 0.2 µm filter, prior to use in HPLC. The pigments were separated by HPLC as described<sup>11</sup> using reverse-phase column (Waters Spherisorb ODS 25 µm × 4.6 mm × 250 mm) and a PDA detector (Waters 2996).

### Extraction of total lipids, esterification of fatty acids and lipid peroxidation

Total lipids were extracted as described previously<sup>12</sup>, with slight modification for cyanobacteria<sup>13</sup>. The fatty acid methyl esters were prepared for GC analysis as described<sup>14</sup>. Lipid peroxidation of cell membrane lipids was determined by the production of thiobarbituric acid-malonaldehyde (TBA-MDA) adduct formation<sup>11</sup>.

### Estimation of nitrogen content and nitrate reductase activity

Nitrogen content was determined<sup>15</sup> using algal pellet equivalent to 0.5 g. The nitrate reductase enzyme assay was done according to the method described in the manual of 'National Facility for Marine Cyanobacteria', Tiruchirapally. Briefly, cultures were centrifuged at 4°C for 5 min at 5000 g and the pellet was suspended in 0.65 ml of carbonate buffer (pH 7.5). The cells were permeated with 0.1 ml of mixed alkyl trimethyl ammonium bromide (MTA) and incubated for 10 min at 4°C. Then added 0.1 ml of potassium nitrate, 0.1 ml of sodium dithionite and 0.05 ml of methyl viologen and incubated for 5 min at 30°C. The reaction was terminated by the rapid addition of 1 ml of sulphanilamide, followed by 1 ml of 0.02% (w/v) N-1-naphthyl)ethylene diamine hydrochloride (NED) and allowed the colour to develop for 30 min. The absorbance was recorded at 540 nm and the enzyme activity was expressed as mg of nitrite formed.

### SDS-PAGE of cell membrane proteins

Electrophoresis of cell membrane proteins was performed using a vertical gel system (Hoeffers, gel system) according to the method described by Laemmli<sup>16</sup>. Analysis of the gel was done using Alpha digiDoc gel documentation system.

### Extraction of mycosporine-like amino acids (MAAs)

Extraction and purification of mycosporine-like amino acid was carried out according to the previously described method<sup>13</sup>.

**Assay of superoxide dismutase (EC 1.15.1.1) and ascorbate peroxidase (1.11.1.7)**

Activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) was assayed as described<sup>11</sup> and expressed as  $\mu\text{mol mg}^{-1} \text{min}^{-1}$ .

**Results**

**Effect on photosynthetic pigments**

The effect of UV-B and high light on qualitative as well as quantitative changes in photosynthetic pigments is shown in Fig. 1A & B. The UV-B radiation for 6 h resulted in an increase in chlorophyll a, phycobilins,  $\beta$ -carotene and carotenoids in *N. spongiaeforme*, whereas in *P. corium* showed a decrease in the photosynthetic pigments. High light treatment for 6 h resulted in an over all decrease in photosynthetic pigments in both the species (Table 1).

**Effect on fatty acid**

The fatty acid present in *N. spongiaeforme* and *P. corium* were lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids. Unsaturation level of fatty acid composition of both total lipids as well as glycolipids remained unchanged in both the cyanobacteria on to exposure to UV-B and as high light treatment. However, in *N. spongiaeforme*, UV-B treatment resulted in larger decrease in the saturated fatty acids as compared to high light treatment. In *P. corium*, both saturated and unsaturated fatty acids showed no appreciable changes due to UV-B or high light treatment (Table 2).

**Effect on lipid peroxidation**

UV-B treatment to *P. corium* showed 73% increase in malonaldehyde (MDA) formation after 6 h of exposure, as compared to control, while the same

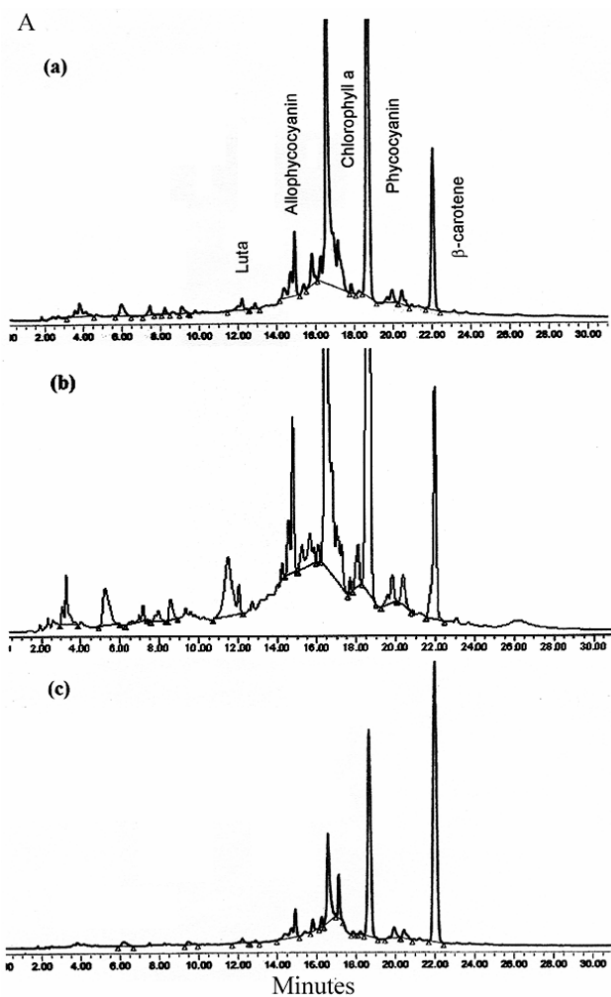


Fig. 1A—HPLC profile of photosynthetic pigments after 6 h of exposure to UV-B and high light in *Nostoc spongiaeforme* [a, Control; b, UV-B; and c, high light]

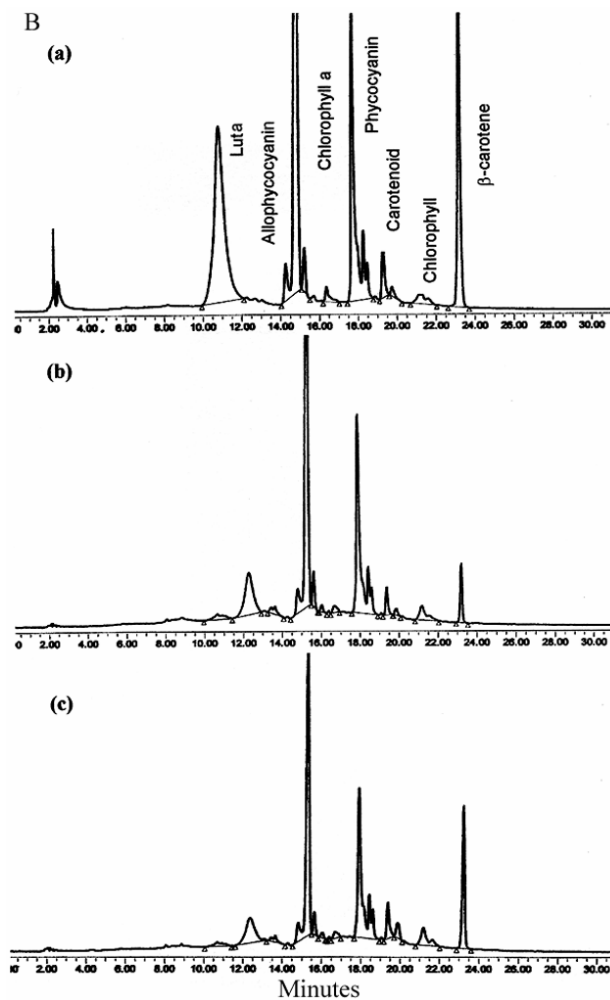


Fig. 1 B—HPLC profile of photosynthetic pigments after 6 h of exposure to UV-B and high light in *Phormidium corium* [a, Control; b, UV-B; and c, high light]

Table 1—Effect of UV-B radiation ( $0.8 \pm 0.1 \text{ mW cm}^{-2}$ ) and high light ( $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  PAR) for 6 h on photosynthetic pigments in *Nostoc spongiaeforme* and *Phormidium corium*[Values represent mean  $\pm$  S.D., n = 4]

Photosynthetic pigments ( $\mu\text{g}$ )	$\lambda_{\text{max}}$	<i>Nostoc spongiaeforme</i>			<i>Phormidium corium</i>		
		Control	UV-B	High light	Control	UV-B	High light
Lutein	475 nm, 505 nm	0.0178	0.2070	0.0051	0.7190	0.0220	0.0143
Allophycocyanin	480 nm	0.0921	0.1845	0.0404	0.0600	0.0140	0.0114
Chlorophyll a	430 nm, 660 nm	0.5792	1.4247	0.0993	0.4780	0.3150	0.2351
Phycocyanin	458 nm	0.4501	1.2242	0.2352	0.8980	0.5369	0.1090
Carotenoid	453 nm, 478 nm	0.0238	0.0460	0.0156	0.0122	0.0073	0.0260
Chlorophyll	430 nm, 660 nm	0.0191	0.0476	0.0120	0.0389	0.0383	0.0015
$\beta$ -Carotene	447 nm	0.1801	0.2993	0.3733	0.3514	0.0474	0.1299
Carotenoids/chlorophylls	-	0.3702	0.3751	3.539	2.094	0.2170	0.7193

Table 2—Effect of UV-B radiation ( $0.8 \pm 0.1 \text{ mW cm}^{-2}$ ) and high light ( $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  PAR) for 6 h on fatty acid composition of total and glycolipids in *Nostoc spongiaeforme* and *Phormidium corium* (mole percent)[Values represent the mean  $\pm$  S.D., n = 3]

Treatment	Fatty acid composition (mole percent)						
	Lauric acid (12:0)	Myristic acid (14:0)	Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)
<i>Nostoc spongiaeforme</i>							
	<b>Total lipids</b>						
Control	11.46 $\pm$ 0.6	18.21 $\pm$ 0.4	15.42 $\pm$ 0.6	9.51 $\pm$ 0.4	3.42 $\pm$ 0.6	20.79 $\pm$ 0.6	3.52 $\pm$ 0.3
UV-B	11.26 $\pm$ 0.4	17.73 $\pm$ 0.5	14.94 $\pm$ 0.7	9.21 $\pm$ 0.6	3.41 $\pm$ 0.5	20.74 $\pm$ 0.4	3.51 $\pm$ 0.5
High light	10.37 $\pm$ 0.5	17.61 $\pm$ 0.3	14.83 $\pm$ 0.3	9.14 $\pm$ 0.7	3.41 $\pm$ 0.4	20.78 $\pm$ 0.3	3.52 $\pm$ 0.4
	<b>Glycolipids</b>						
Control	17.90 $\pm$ 0.4	20.64 $\pm$ 0.5	15.14 $\pm$ 0.3	25.62 $\pm$ 0.5	8.83 $\pm$ 0.4	37.73 $\pm$ 0.6	9.19 $\pm$ 0.7
UV-B	12.33 $\pm$ 0.6	14.20 $\pm$ 0.4	10.42 $\pm$ 0.4	17.90 $\pm$ 0.4	8.82 $\pm$ 0.3	37.72 $\pm$ 0.4	9.18 $\pm$ 0.4
High light	16.75 $\pm$ 0.3	19.40 $\pm$ 0.6	14.10 $\pm$ 0.4	25.38 $\pm$ 0.3	8.80 $\pm$ 0.6	37.71 $\pm$ 0.5	9.19 $\pm$ 0.4
<i>Phormidium corium</i>							
	<b>Total lipids</b>						
Control	11.38 $\pm$ 0.6	17.63 $\pm$ 0.4	14.83 $\pm$ 0.6	9.41 $\pm$ 0.4	3.28 $\pm$ 0.6	19.98 $\pm$ 0.6	3.38 $\pm$ 0.3
UV-B	11.36 $\pm$ 0.4	17.61 $\pm$ 0.5	14.81 $\pm$ 0.7	9.13 $\pm$ 0.6	3.28 $\pm$ 0.5	19.97 $\pm$ 0.4	3.38 $\pm$ 0.5
High light	11.29 $\pm$ 0.5	17.49 $\pm$ 0.3	14.71 $\pm$ 0.3	9.07 $\pm$ 0.7	3.27 $\pm$ 0.4	19.95 $\pm$ 0.3	3.37 $\pm$ 0.4
	<b>Glycolipids</b>						
Control	11.62 $\pm$ 0.4	13.40 $\pm$ 0.5	9.82 $\pm$ 0.3	16.63 $\pm$ 0.5	5.76 $\pm$ 0.4	35.06 $\pm$ 0.6	5.92 $\pm$ 0.7
UV-B	11.48 $\pm$ 0.6	13.40 $\pm$ 0.4	9.82 $\pm$ 0.4	16.63 $\pm$ 0.4	5.76 $\pm$ 0.3	35.06 $\pm$ 0.4	5.93 $\pm$ 0.4
High light	11.60 $\pm$ 0.3	13.37 $\pm$ 0.6	9.80 $\pm$ 0.4	16.60 $\pm$ 0.3	5.75 $\pm$ 0.6	35.03 $\pm$ 0.5	5.92 $\pm$ 0.4

treatment to *Nostoc* resulted in an increase of 270% in lipid peroxidation (indicated as MDA formation; Fig. 2). High light treatment for 6 h showed only 40% increase in lipid peroxidation in both *Nostoc* and *Phormidium*, compared to their respective controls. Important observation was that basal level of peroxidation in *Phormidium* was higher than in *Nostoc*.

#### Effect on nitrogen and nitrate reductase activity

Both UV-B radiation and high light treatments caused decrease in the nitrogen content, as compared to control and extent of decrease in the nitrogen content in both the species was more or less same (Fig. 3a). The activity of nitrate reductase increased

by 25% after 1 h of the treatment, followed by a decrease of 45% after 6 h of UV-B treatment in *N. spongiaeforme* (Fig. 3b), whereas high light treatment resulted in a linear decrease of 61% in the activity of nitrate reductase. In *P. corium*, UV-B treatment for 6 h resulted in 70% decrease in nitrate reductase activity, while high light treatment showed an initial increase, followed by a 16% decrease (Fig. 3c).

#### Effect on cell membrane proteins

UV-B radiation and high light treatment showed qualitative as well as quantitative changes in the SDS-PAGE profile of cell membrane proteins in both the organisms (Fig. 4). UV-B treatment to *Nostoc* did not

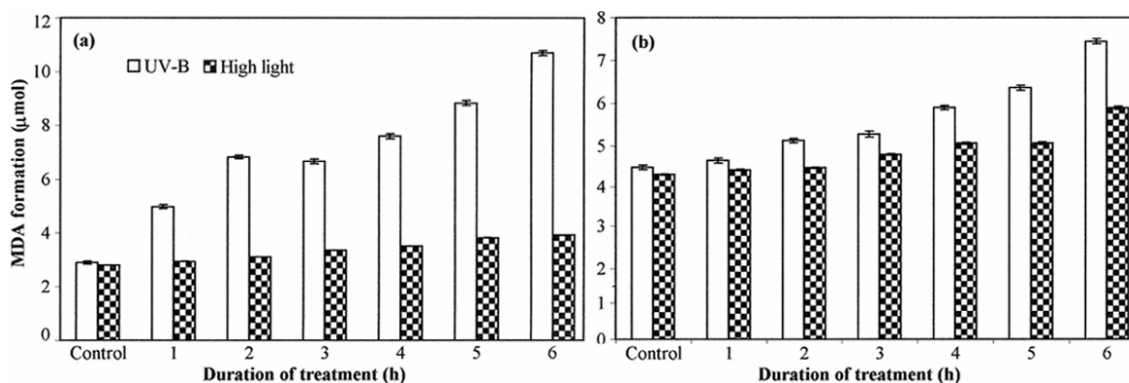


Fig. 2—Effect of UV-B and high light treatment up to 6 h on lipid peroxidation in *Nostoc spongiaeforme* (a) and *Phormidium corium* (b) [Each bar represents mean ± S.D., n = 6]

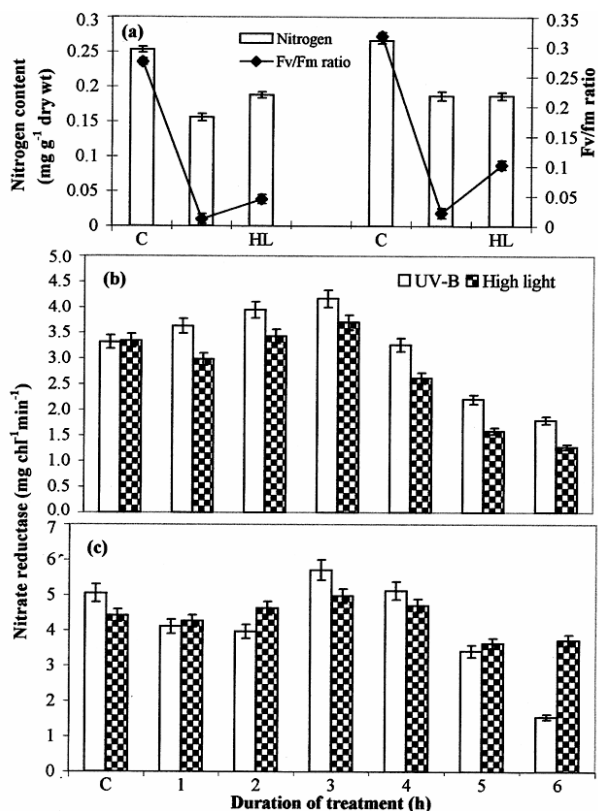


Fig. 3—Effect of UV-B and high light treatment up to 6 h on nitrogen content and Fv/Fm ratio (a) and activity of nitrate reductase in *Nostoc spongiaeforme* (b) and *Phormidium corium* (c) [Nitrogen content and Fv/Fm ratio are shown by bar graph and line graph respectively. Each bar represents mean ± S.D., n = 4]

cause any significant changes in the protein profile of the cell membrane as compared to control. However, UV-B treatment to *Phormidium* caused slight induction of 45 kDa and significant decrease in the 50 kDa protein, as compared to control (Fig. 4). High light treatment quantitatively increased 45 kDa protein in *Nostoc*, but in *Phormidium* 45 kDa protein

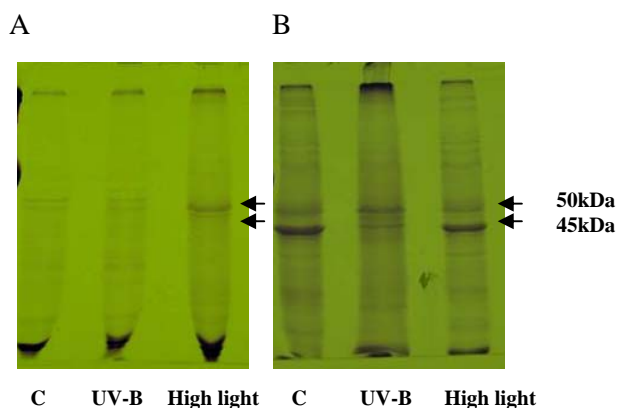


Fig. 4—Effect of UV-B and high light treatment up to 6 h on cell membrane proteins in *Nostoc spongiaeforme* (a) and *Phormidium corium* (b) [Arrows represent 45 kDa and 50 kDa protein bands]

decreased, while 50 kDa protein increased significantly.

**Effect on mycosporine-like amino acids (MAAs)**

Fig. 5 shows the HPLC profile of mycosporine-like amino acids (MAAs) from *N. spongiaeforme* and *P. corium*. Exposure to UV-B radiation for 6 h resulted in an increase in the amount of MAAs in both the species. Increase in the MAAs was considerably higher in *P. corium* (124%) than in *N. spongiaeforme* (42%), compared to their respective controls. High light treatment decreased the MAAs content in *Phormidium* by 60%, but in *Nostoc*, it remained more or less constant as observed in control.

**Effect on superoxide dismutase and ascorbate peroxidase**

UV-B treatment to *N. spongiaeforme* for 3 h resulted in 30% increase in the SOD activity, as compared to control. However, it increased only to 19% after 6 h of the treatment (Fig. 6a). In contrast, high light treatment resulted in continuous increase

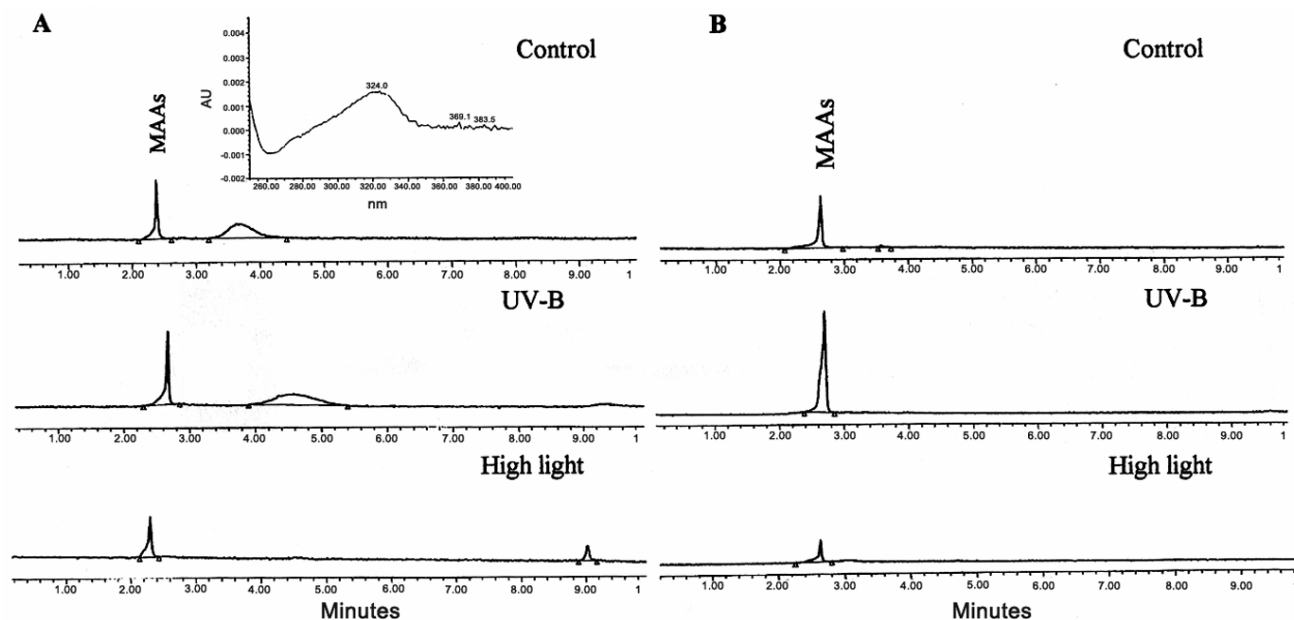


Fig. 5—HPLC profile of mycosporine-like amino acids (MAAs) after 6 h of exposure to UV-B and high light in *Nostoc spongiaeforme* (a) and *Phormidium corium* (b)

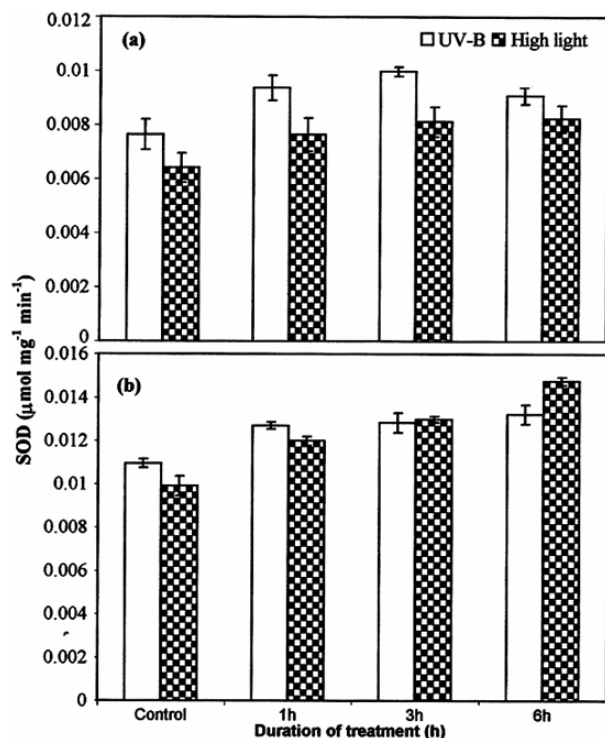


Fig. 6—Effect of UV-B and high light treatment up to 6 h on superoxide dismutase (SOD) activity in *Nostoc spongiaeforme* (a) and *Phormidium corium* (b) [Each bar represents mean  $\pm$  S.D.,  $n = 5$ ]

(28%) in the SOD activity in *Nostoc* over a period of 6 h. In *P. corium*, SOD activity increased linearly, as a result of the UV-B and high light treatments (Fig. 6b). Results showed that UV-B treatment showed

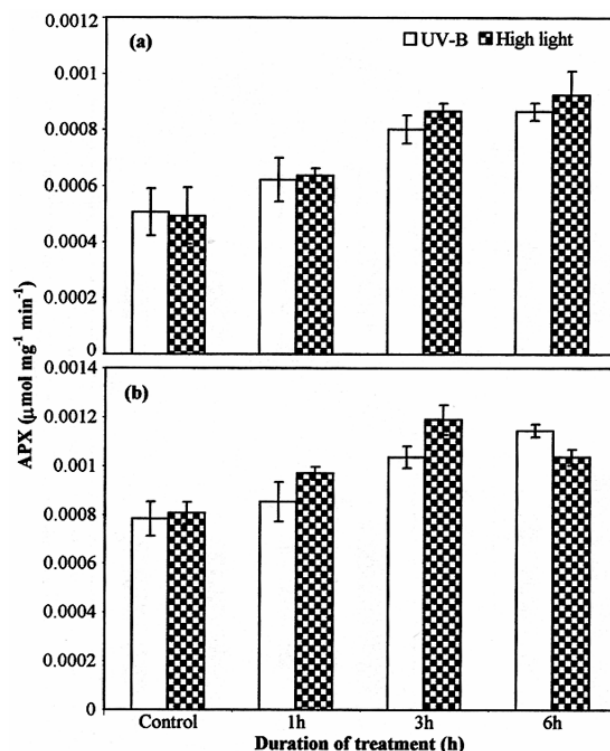


Fig. 7—Effect of UV-B and high light treatment up to 6 h on ascorbate peroxidase (APX) activity in *Nostoc spongiaeforme* (a) and *Phormidium corium* (b) [Each bar represents mean  $\pm$  S.D.,  $n = 5$ ]

more increase in SOD activity in *Nostoc* while high light treatment resulted in greater activity of SOD in *Phormidium*. In *Nostoc*, the UV-B treatment resulted in a linear increase in the APX activity (46% after 6 h of

h of treatment), while high light treatment showed an initial increase (47%) in the APX activity up to 3 h, declined on further exposure (Fig. 7a). In *Phormidium*, APX activity increased linearly as a result of UV-B and high light treatments (Fig. 7b).

### Discussion

An increase in photosynthetic pigments, mainly chlorophyll, carotenoids and to some extent phycobillins in *Nostoc* under UV-B treatment would imply that their synthesis is induced, directly or indirectly, by UV-B radiation (Fig. 1A & B). The increase in carotenoid may be to protect the organisms against the singlet oxygen species generated from oxidative damage by UV-B. Earlier studies<sup>5,17</sup> reported that UV radiation results in an increase in the various photosynthetic pigments, especially carotenoids and phycobilins in various algae and this is correlated with protection against UV damage. Pigments, such as phycoerythrin under UV-B stress can protect DNA from oxidative damage by forming a peripheral layer around it<sup>18</sup>.  $\beta$ -Carotene can act as antioxidant to scavenge the free radicals. An overall increase in carotenoids and phycobilins, in this study may suggest protection against the UV-B radiation by acting as antioxidant<sup>19</sup> and by exhibiting chromatic adaptation<sup>18</sup>, respectively.

However, decrease in almost all the photosynthetic pigments observed during high light treatment indicate greater production of ROS, leading to photooxidation/photobleaching of the pigments. Under high light conditions, plants tend to decrease the chlorophyll content in antenna size to prevent overexcitation in the reaction center. Therefore, decrease in pigment content may be an adaptation strategy against high light. These results were in agreement with the previous study<sup>20</sup>.

No damage to unsaturated fatty acid composition has been observed in both the species under the UV-B and high light treatments (Table 2), both of which result in production of ROS. This indicates a role of membrane integrity in protection against the stresses. Data indicate that adaptation against UV-B and high light probably does not cause any change in the phase transition of the membrane, which is acceptable as no temperature fluctuations are involved in the study. The UV-B affects the integrity of the membrane, thereby causing a decrease in the lipid content and membrane transport system<sup>21</sup>. A decrease in lipid content has been reported in diatom species *Odontella*

*weissflogii* due to degradation of complex lipid after exposure to UV-B treatment<sup>22</sup>. Although we have not studied the fatty acid composition of phospholipids, the phospho and glycolipids contents have shown decrease as a result of treatments in both the cyanobacteria (data not shown).

Slight decrease in the saturated lipid content and no decrease in the unsaturated lipid content (Table 2) on high light treatment indicate possibility of an adaptation against the membrane sol-gel phase transition rather than against the photooxidative damage, which would have resulted changes in unsaturated fatty acids, as they are more sensitive to oxidative damage. Changes in fatty acid composition during light acclimation of algae have been observed<sup>23</sup>. The light intensity influences the content of saturated and tetra unsaturated fatty acids in green alga *Ulva pertusa*<sup>24</sup>. The variation in fatty acid composition of total lipids, which is advantageous for adaptation against abiotic stresses may be due to cellular response to reduce the susceptibility of membranes to photo-oxidation<sup>25</sup>. However, changes in fatty acid composition in response to stress conditions still remain a less studied process and need to be further studied.

Oxidative nature of stresses imposed in this study is verified by an increase in the level of lipid peroxidation of cell membrane (Fig. 2). Lipid peroxidation occurs especially at sites where polyunsaturated fatty acids (PUFA) occur in high concentrations<sup>26</sup>. Earlier study<sup>27</sup> reported that UV-B promotes the formation of lipid oxidation products that can destroy the lipid soluble antioxidants as well. Decrease in nitrogen content (Fig 3a) under UV-B and high light treatments may be due to decrease in both nitrate reductase activity (Fig. 3b & 3c) as well as photosynthesis (Fv/Fm ratio, Fig 3a). UV-B is known to cause decrease in nitrogen-fixing enzymes<sup>28</sup>. Decrease in nitrogen content may also be due to oxidation of proteins<sup>29</sup>.

Changes in protein profile indicate that cellular proteins are also one of the targets of UV-B and high light exposure (Fig. 4). UV radiation damages enzymes and proteins, especially those rich in aromatic amino acids such as tryptophan, tyrosine, phenylalanine and histidine, which show strong absorption in the UV range from 270-290 nm<sup>30</sup>. The ROS, which are produced as a result of the treatments, may lead to oxidation of proteins, resulting in disruption of their structural entity (membrane proteins) and inactivation of proteins (enzymes)<sup>31</sup>.

The present study shows that both the species are able to synthesize MAAs in response to UV-B radiation (Fig. 5), suggesting that they adapt to daily fluctuations in solar UV-B radiation, which have an impact on their natural environment. The protection against UV-B stress in some cyanobacteria has been attributed to the synthesis of MAAs and scytonemin<sup>32,33</sup>. However, presence of scytonemin is not found in our study. The presence of high concentration of MAAs has been reported<sup>34,35</sup> in various algae exposed to high levels of solar radiation. MAAs acts as a UV-absorbing sunscreen in preventing 3 out of 10 photons from hitting cytoplasmic targets<sup>36</sup>. In marine organisms<sup>37</sup>, mycosporine glycine may also function as a biological antioxidant, suggesting that MAAs play more than one role in the cellular metabolism. However, the actual role of MAAs in algae needs to be studied further. In our study, white light (halogen lamp) has shown no induction of MAAs, as it does not contain UV fraction.

The increase in the SOD and APX activities in response to the UV-B and high light treatments (Fig. 6 & 7) indicates that these enzymes play an important role in an organism's defense mechanism by metabolizing the ROS<sup>38</sup>. The UV-B treatment has also shown increase in the SOD activity in green alga<sup>39</sup>. Activities of SOD and APX are also increased under high light conditions<sup>40</sup>.

### Conclusion

Results suggest that UV-B and high light cause oxidative damage as indicated by MDA formation in both freshwater and marine cyanobacteria. Oxidative stress to the organisms is also suggested by the induction of antioxidant enzymes such as SOD and APX. However, no change in the composition of unsaturated fatty acids, but slight decrease in saturated fatty acids under the treatment conditions indicate adaptation by the organisms against the treatment conditions rather than the oxidative damage. Increase in UV-B absorbing pigments such as MAAs due to UV-B treatment suggest specific role for MAAs as screening pigment against UV-B radiation. The contrasting results with respect to MAAs and antioxidant enzymes in *Nostoc* and *Phormidium* may be due to differences in their tolerance and sensitivity levels as a result of adaptation to their respective growth environments and thus responding differently with respect to oxidative damage and protective mechanism. The work contributes towards understanding of cyanobacterial response to changing

environmental conditions mainly UV-B, since they are responsible for large part of global photosynthetic productivity and carbon sink.

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### References

- 1 Madronich S, Mckenzie R L, Bjorn L O & Caldwell M M (1998) *J Photochem Photobiol B: Biol* 460, 5-19
- 2 Sinha R P & Häder D-P (2002) *Adv Space Res* 3, 1547-1556
- 3 Sinha R P & Häder D-P (2000) *Recent Res Devel Photochem Photobiol* 4, 239-246
- 4 Sundback S, Odmark K, Wul A, Nilsson C & Wangberg S A (1997) *Mar Biol* 128, 171-179
- 5 Bischof K, Krabs G, Wiencke C & Hanelt D (2002) *Planta* 215, 502-509
- 6 Häder D-P, Kumar H D, Smith R C & Worrest R C (1998) *J Photochem Photobiol B: Biol* 46, 53-68
- 7 Krause G H (1994) In: *Photoinhibition of Photosynthesis: from Molecular Mechanism to the Field* (Baker N R & Boyer J R, eds), pp 331-348, Oxford, BIOS Scientific Publishers
- 8 Glatz T, Windhovel U, Boger P & Sandmann G (1999) *Plant Physiol* 120: 599-602
- 9 Rippka R, Deruelles J, Waterbery J B, Herdman M & Stanier R Y (1979) *J Gen Microbiol* 111, 1-61
- 10 Vonshak A (1985) In: *Techniques in Bioproductivity and Photosynthesis* (Cooms J, Hall D O, Long S P & Scurlock J M O, eds), 2<sup>nd</sup> edn, pp 188-200, Pergamon Press
- 11 Bhandari R & Sharma P K (2006a) *Photochem Photobiol* 82, 702-710
- 12 Turnham E & Northcote D H (1984) *Phytochem* 23, 35-39
- 13 Bhandari R & Sharma P K (2006b) *Indian J Expt Biol* 44, 330-335
- 14 Christie W W (ed) (1982) *Lipid Analysis*, 2<sup>nd</sup> edn, pp 51-56, Pergamon Press, Oxford
- 15 Tandon H L S (ed) (1993) *Methods of Analysis of Soils, Plants, Waters and Fertilizers*, 2<sup>nd</sup> edn, Fertilizer Development and Consultation Organization, New Delhi
- 16 Laemmli U K (1970) *Nature* 227, 680
- 17 White A L & Jahnke L S (2000) *Plant Cell Physiol* 43, 877-884
- 18 Aráoz R & Häder D-P (1997) *FEMS Microbiol Ecol* 23, 301-313
- 19 Nonnengießer K, Schuster A & Koenig F (1996) *Bot Acta* 109, 115-124
- 20 Han T, Sinha R P & Häder D-P (2003) *Photochem Photobiol Sci* 2, 649-654
- 21 Murphy T M (1983) *Plant Physiol* 58, 381-388
- 22 Skerratt J H, Davidson A D, Nichols P D & Mcmeekin T A (1998) *Phytochem* 49, 999-1007
- 23 Klyachko-Gurvich G L, Tsoglin L N, Doucha J, Kopetskii J, Ryabkha I B S & Semenenko V E (1999) *Plant Physiol* 107, 240-249
- 24 Dawes C, Kovach C & Friedlander M (1993) *Bot Mar* 36, 289-296



- 25 Gombos Z, Kanervo E, Tsvetkova N, Sakamoto T, Aro E M & Murata N (1997) *Plant Physiol* 115, 551-556
- 26 Yamashoji S, Yoshida H & Kajimoto G (1979) *Agric Biol Chem* 43, 1249-1254
- 27 Salmon S, Maziere J C, Santus P, Morliere P & Bouchemal N (1990) *Photochem Photobiol* 52, 541-545
- 28 Tyagi R, Kumar A, Tyagi M B, Jha P N, Kumar H D, Sinha R P & Häder D-P (2003) *J Basic Microbiol* 43, 137-147
- 29 Solheim B, Johanson U, Callaghan T V, Lee J A, Jones D G & Bjorn L O (2002) *Oecologia* 133, 90-93
- 30 Vass I (1997) In: *Handbook of Photosynthesis* (Pessaraki M, ed.), pp. 931-949, Dekker, New York
- 31 Kulkarni R D & Golden S S (1994) *J Bacteriol* 176, 659-963
- 32 Sinha R P, Klisch /M, Helbling E W & Häder D-P (2001) *J Photochem Photobiol B: Biol* 60, 129-135
- 33 Klisch M, Sinha R P, Richter P R & Häder D-P (2001) *J Plant Physiol* 158, 1449-1454
- 34 Rajeshwar P, Sinha R P, Sinha A, Klisch M & Häder D-P (2003) *J Photochem Photobiol B: Biol* 71, 51-58
- 35 Rozema J, Björn L O, Bornman J F, Gaber A, Häder D-P, Tro T, Germ M, Klisch M, Gröniger A, Sinha R P, Lebert M, HeY Y, Buffoni-Hall R, de Bakker N V J, van de Staaij N V J & Meijkamp B B (2002) *J Photochem Photobiol B: Biol* 66, 2-12
- 36 Garcia-Pichel F, Wingard C E & Castenholz R W (1993) *Appl Environ Microbiol* 59, 70-176
- 37 Dunlap W C, Chalker B E, Bandaranayake W M & Wu Won J J (1998) *Int J Cosmetic Sci* 20, 41
- 38 Canini A, Leonardi D & Caiola M G (2001) *New Phytol* 152, 107-116
- 39 Bischof K, Janknegt P J, Buma A G J, Rijstenbil J W, Peralta G & Breeman A M (2003) *Sci Mar* 67, 353-359
- 40 Sankhaliker S & Sharma P K (2002) *Indian J Exp Biol* 40, 1260-1268