International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **4(4),** 52-59, April **(2015)** *Int. Res. J. Biological Sci.*

Evaluation of Ultraviolet -B radiation induced changes in biochemical response of *Arthrospira platensis* (Gomont)

Ganapathy Kavitha, Chidambaram Kurinjimalar, Rangaraja Thevanathan and Ramasamy Rengasamy
Centre for Advanced Studies in Botany, University of Madras, Chennai, INDIA

Available online at: www.isca.in, www.isca.me
Received 6th February 2015, revised 1st March 2015, accepted 8th April 2015

Abstract

In the current study, the influence of Ultraviolet - B radiation with intensity of 3 W/m^2 on growth, biochemical changes and antioxidant enzymes in Arthrospira platensis were examined. The additional UV-B radiation vitally reduced the development of the cell, total protein, pigments (i.e. Total chlorophyll, carotenoids and c - phycocyanin). The study revealed that rise in exposure period of UV-B radiation caused poorer growth of the organism. The outcomes demonstrated that photosynthetic complex was the vital focus of UV-B radiation causing deprivation of photosynthetic pigments. It was observed with an increase in lipid peroxidation, in contrast with untreated control of A. platensis. The antioxidant enzymes showed improved activities of peroxidase, superoxide dismutase and except catalase in UV-B illuminated cells. The test organism neutralizes the effect of free radicals produced under UV-B stress through increased activities of these antioxidant enzymes. The outcome of the result recommends that A. platensis was impervious to UV-B radiation and the conceivable negative impact on the growth of A. platensis have been adequately adjusted with increment in these antioxidant enzymes.

Keywords: UV-B radiation, *Arthrospira platensis*, carotenoids, chlorophyll and lipid peroxidation.

Introduction

The stratospheric ozone depletion and elevated solar UV-B radiation have a negative effect on living life forms. UV radiation is one of the unsafe components that cause hindrance to both flora and fauna on the earth[1]. Since the Cyanobacteria are widely dispersed group of light harvesting prokaryotes, where they absorb solar radiation to drive photosynthesis and nitrogen fixation, prone to damage during elevated levels of UV - B radiation. Many cyanobacteria have developed techniques to prevent the harmful effects caused by UV – B radiation[2]. UV-B radiation restrains cell metabolism, biosynthesis of chlorophyll and electron transport; however less consideration was given to lipid peroxidation and antioxidant enzymes in the species of photosynthetic cyanobacterium. It can adjust to different stress conditions including high saltiness, extreme dryness, increasing temperatures and high UV intensity, etc.[3]. It was already reported that these cyanobacteria have established a few tactics to survive under UV – B stress condition including DNA repair system, movement against UV radiation and induction of Ultraviolet absorbing pigments (scytonemins, MAAs)[4]. *Arthrospira platensis*, an important cyanobacterium, has been industrially delivered for a considerable span of time. Though, the increasing UV – B radiation decrease the photosynthetic rate and damage DNA, low levels can improve carbon sequestration in this phytoplankton[5-7]. In this work, the effects of UV – B radiation on the biochemical changes of *A. platensis* was explored, where it could give essential information for the developing conditions for the species to adapt with increasing UV-B radiation.

Material and Methods

Growth conditions: The cyanobacterium *Arthrospira platensis* (Gomont) (= *Spirulina platensis*), obtained from the CAS in Botany, University of Madras, Chennai was kept in Zarrouk's medium under 30µE m^{-2} s^{-1} light intensity, 12:12 light dark cycle and at 24 ± 2°C. UV-B irradiation was given to the culture having absorbance (> 0.15) at 750 nm.

Exposure to UV-B radiation: Exposure was given to the cynaobacterium, *Arthrospira platensis* taken in open petridishes (75mm) having a depth of 0.5 cm. The algal culture were kept at a height of 30 cm for 10, 15, 30, 45 and 60 mins exposed to an intensity of 3 Wm^{-2} of UV-B radiation (UV-B lights, Philips TL 20 W/12, Philips Gleolampenfabriken, USA.) The emission spectrum of UV-B source with a peak at 312 nm was used. The culture was shaken gradually to encourage uniform mixture. The culture grown at 24±2°C under flourescence light was used as control.

Growth measurement: The growth of the cyanobacterial cells drawn at predetermined intervals of 10, 15, 30, 45 and 60 mins was determined by reading the optical density of the culture at 750 nm using UV-Visible spectrophotometer (Ultrospec 4000).

Estimation of c-Phycocyanin: Five milliliter of the culture from both treated and untreated cells of *A. platensis* was taken and centrifuged at 5000 rpm for 15 mins. The biomass (pellet) was washed with phosphate buffer (7.2 pH; 1.0 mM) and ruptured the cells by sonication for 2 min and incubated at 4°C

for 12 h. Again, the cells were spun at 5,000 rpm for 15 min. The amount of c-Phycocyanin (c-PC) was evaluated from the supernatant at 615 nm [8].

Estimation of Photosynthetic pigment: The photosynthetic pigments were determined using the algal pellet of both control and UV – B treated cells of *A. platensis* where it was mixed with 5mL of 80% acetone and homogenized in a sonicator. Then it was kept in dark for 12h at 4°C. Again the sample was centrifuged at 5000 rpm for 10 minutes where the supernatant was collected and the optical density were recorded at 644.8 nm, 661.6 nm and 470 nm [9].

Estimation of Total protein: Total protein was extracted using algal pellet obtained from centrifugation at 5000 rpm for 10 minutes. The pellet was homogenized with 5mL of 0.1 M phosphate buffer at pH 7.0 utilizing sonicator and separated by centrifugation at 5000 rpm for 15 minutes. The supernatant was taken for the estimation of total protein. To 0.2 mL of supernatant, 5mL of Comassie Brilliant Blue reagent (100 mg of CBB G-250 dissolved in 50ml of 93% ethanol mixed with 100 mL of 85% Phosphoric acid and made upto 1000 ml with distilled water) was added and mixed thoroughly. The optical density was read at 595 nm against a reagent blank The amount of total protein was calculated by using a standard graph with Bovine Serum albumin ranging from 10 to 100 µg /mL [10].

Estimation of total carbohydrate: The biomass for estimation of carbohydrate was extracted by centrifugation and the pellet was homogenized with 5mL of 0.1M phosphate buffer at pH 6.8 in a sonicator and followed by centrifugation. To the 1mL of supernatant, 1mL of 5% Phenol and 5mL concentrated Sulphuric acid was added and mixed thoroughly. The solution was kept at room temperature for 30 minutes and the Optical Density was recorded at 490 nm. Standard graph was prepared with different concentrations of D-glucose ranging from 10 to 100µgml^{-1} [11].

Estimation of proline: The cyanobacterial cells (treated and untreated) were for estimated for Proline content, where the cells were suspended in 5 mL of 3% Sulphosalicylic acid. The reaction mixture contained 2 mL of the extract and 1 mL of ninhydrin, followed by addition of 2 mL glacial acetic acid and kept at 100^{0}C for an hour and finally treated with toluene. The content of Proline was measured at 520 nm[12].

Lipid peroxidation: The harvested cyanobacterial cells were homogenized in 1% Trichloroacetic acid and centrifuged at 10,000 rpm for 15 min at 37^{0}C. 0.1 mL of the sample were treated with 0.5% Thiobarbituric acid and 20% Trichloroacetic acid, incubated at 95^{0}C for 30 minutes at 100^{0}C, followed by centrifugation at 10,000 rpm for 10 minutes. The absorbance of the resulting solution was recorded at 532 nm and 600 nm[13].

Preparation of crude extract: The extract for the enzymatic assays was prepared by homogenization of 0.2 g biomass of the *A.platensis* in 50 millimolar phosphate buffer (pH 7). The homogenates were centrifuged at 5000 rpm for 15 min and the solution was utilized as crude concentrate for enzymatic measure.

Catalase activity: The activity of Catalase was measured by recording the disintegration of hydrogen peroxide (H_2O_2) which was indicated by the reduction at 240 nm. The reaction mixture contained 0.1 M phosphate buffer at pH 7.2 millimolar hydrogen peroxide and 0.1 mL of crude extract. The reduction in hydrogen peroxide was recorded by decrease in the optical density at 240 nm and expressed in Units of micromoles of the substrate changed over mg chlorophyll[14].

Activity of Peroxidase enzyme: The reaction mixture contained 0.1 M phosphate buffer at pH 6.0, 7mM guaiacol, 10 mM H_2O_2 and 0.1 mL of crude extract made up to 3 mL solution. The change in the absorbance was measured at 470 nm after the addition of H_2O_2. The activity was expressed in units of micromoles of the substrate changed over per min per mg chlorophyll[14].

Activity of Superoxide dismutase: The photochemical decrease of nitro blue tetrazolium determine the activity of Superoxide dismutase (SOD) in UV –B illuminated cells. The reaction mixture contained 50 mM phosphate buffer at pH 7, 150 µM EDTA, 10 mM methionine, 10 µM riboflavin, 70 µM nitro blue tetrazolium, and 200 µl extract followed by the addition of Riboflavin. The tubes were shaken well and kept under illumination was used as a blank. The reaction was inititated under light condition for 10 min and ceased under dark. Absorbance of the reaction mixture was measured at 560 nm. The Superoxide dismutase activity was characterized by the measure of 50% inhibition without the enzyme substrate[15].

Statistical analysis: The data are presented as mean with standard deviation (SD) using triplicate value. Results were analyzed using Microsoft excel 2013.

Results and Discussion

In the present investigation, Ultraviolet light - B induced changes in growth, pigments and Total protein content of cyanobacterium of *Arthrospira platensis* were studied. The test organism *A. platensis* was given exposure to Ultraviolet radiation (UV-B) for different time intervals of 10, 15, 30, 45 and 60 minutes. The changes in growth, pigment protein, and carbohydrate content were analyzed after treatment with Ultraviolet-B. The growth characteristic of *A. platensis* showed decrement with increase in duration of UV-B radiation as related to untreated cultures. But, in contrary, growth remained static up to 30 minutes UV-B exposure time followed by decline in subsequent 45 and 60 minutes time of UV-B exposure up to (28 – 40%) of the control causing severe damage to cellular system.

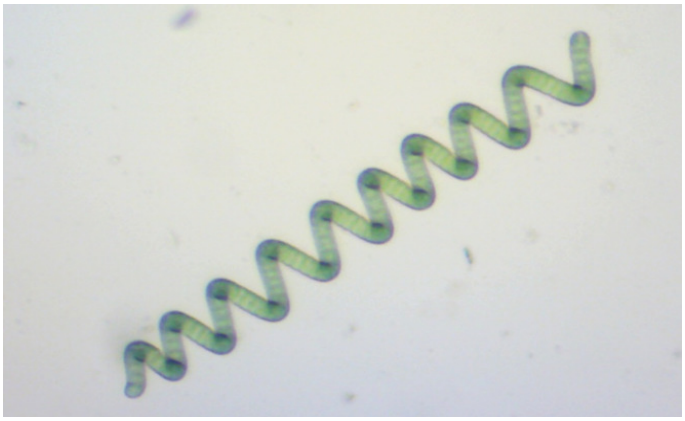

Figure-1
Microscopic observation of *Arthrospira platensis*

It was observed that growth pattern and total protein content of test organism *Arthrospira platensis* showed steady decrement with increasing duration as compared to control. These differences are due to diverse level of damage caused by UV-B radiation on total protein, nucleic acid and pigments as reported earlier[16].

The photosynthetic pigments, total Chlorophyll, total carotenoids and c - phycocyanin contents reduced with longer exposure time of UV-B radiation. *Arthrospira platensis* indicated decline in total chlorophyll at 60 mins of UV - B radiation up to 45%. The consequences of UV-B radiation directly influenced the light responses of light harvesting complex where it hindered the electron transport activity, causing disorientation of the thylakoid membrane. The Photosystem II and water oxidizing framework were the fundamental focuses of UV-B radiation inside these thylakoids in *A.platensis* [17].

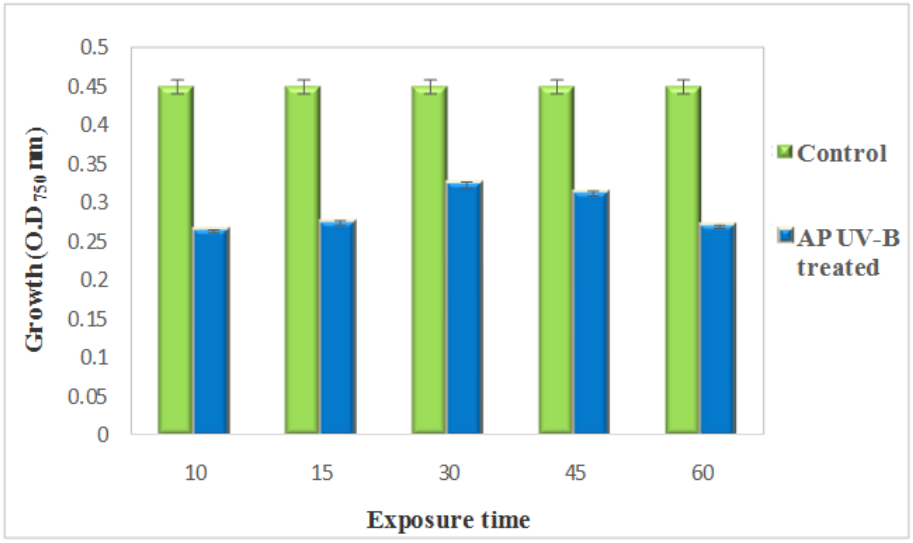

Figure-2
Effect of UV-B radiation on growth of *Arthrospira platensis*

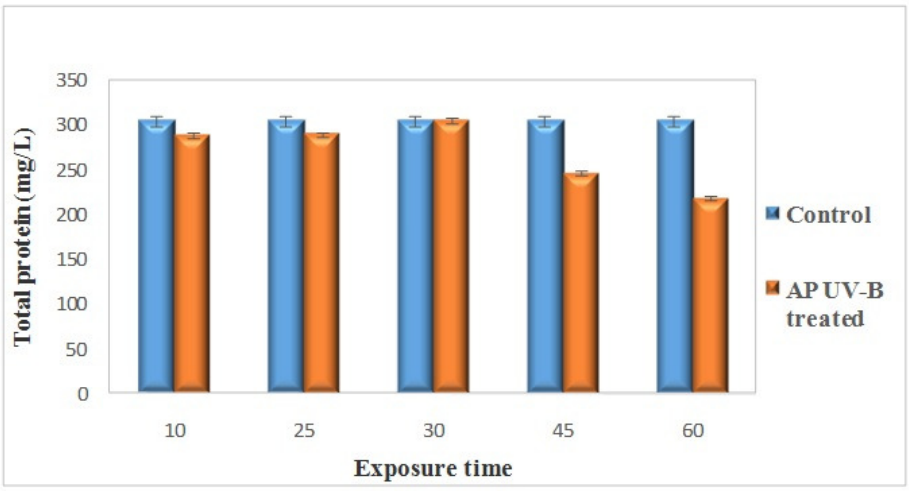

Figure-3
Effect of UV-B radiation on Protein content of *Arthrospira platensis*

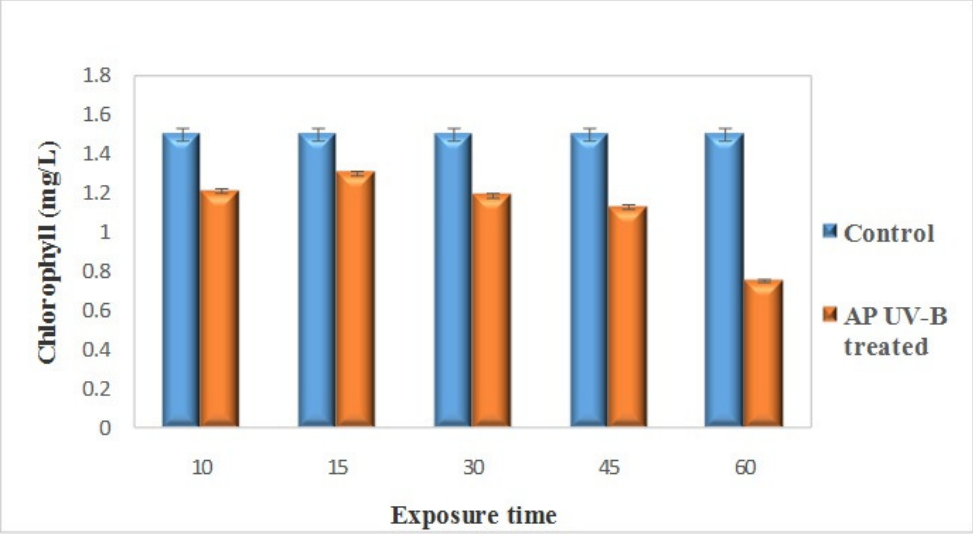

Figure-4
Effect of UV-B radiation on chlorophyll content of *Arthrospira platensis*

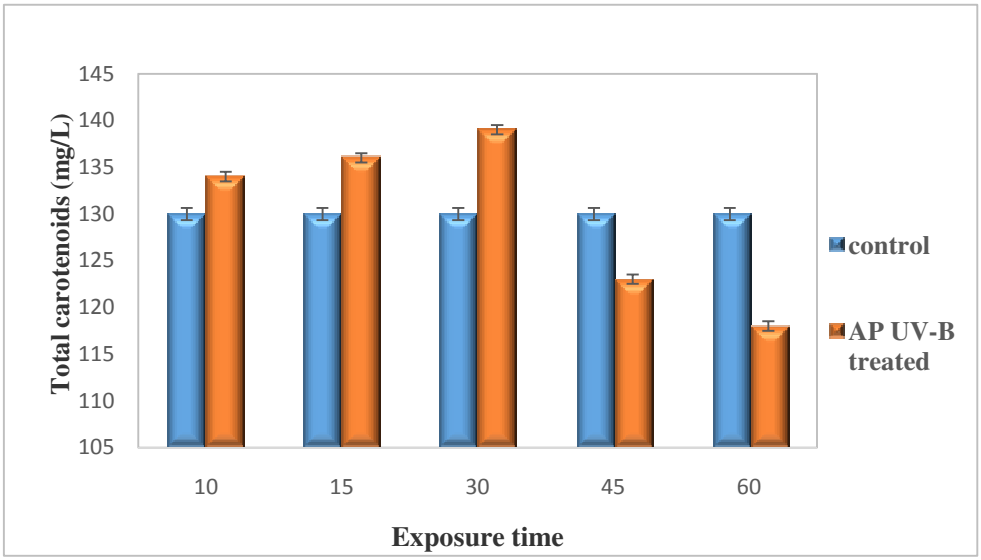

Figure-5
Effect of UV-B radiation on Total carotenoids content of *Arthrospira platensis*

Total Carotenoids revealed diverse response to UV-B radiation, where exposure upto 30 mins of UV-B radiation created negligible increase upto 6 % in *A. platensis*. While, similar treatment exhibited decrease up to 9% after 60 mins. Our outcomes showed that the influences of UV-B radiation on total chlorophyll was similar, however, contrasting observations were made on total carotenoids, which found to be decreasing at higher levels [18].

In this investigation, the phycobiliprotein, c - Phycocyanin was damaged upto 12 - 32% compared to total chlorophyll and carotenoids in *A. platensis* indicated lessening of phycocyanin in contrast with control. The damage of phycocyanin was due to direct absorption of UV-B radiation, which is protein in nature and limited on the external surface of thylakoid layer. Similar results were reported that there was a decline in phycobiliprotein and disassociation of the protein aggregate after UV-B treatment in various cyanobacteria. The fluorescence spectra of phycobiliproteins after UV-B illumination recommend that the electron transfer between the phycobiliprotein complex and photosynthetic reaction center. Similarly the absorption spectrum showed that the phycobiliproteins decrement during increasing exposure of UV-B radiation in *Nostoc*. Protein profile of phycocyanin and associated polypeptides of *Anabaena* also uncovered a loss of α and β subunits of c - phycocyanin, and other proteins in light harvesting complex after UV-B illumination[19].

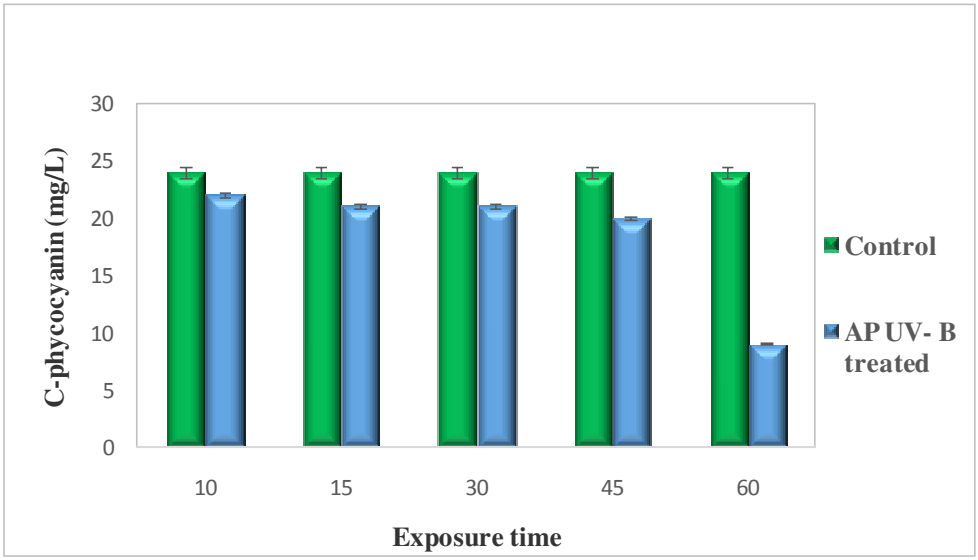

Figure-6
Effect of UV-B radiation on c-phycocyanin content of *Arthrospira platensis*

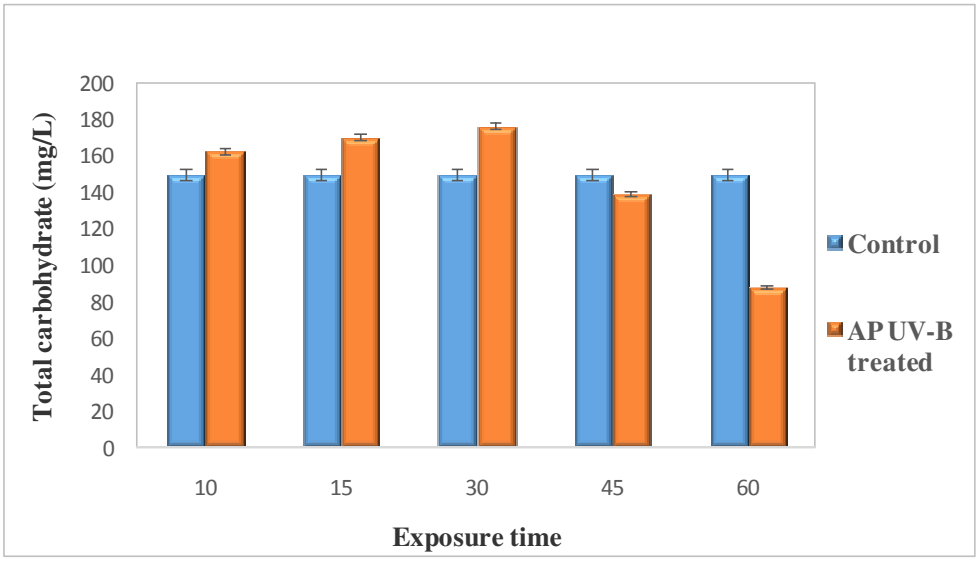

Figure-7
Effect of UV-B radiation on total carbohydrate content of *Arthrospira platensis*

Effect of 3 W m^{-2} UV-B radiation on the total carbohydrate content of *A. platensis* revealed noteworthy impact while at 45 mins, there was decrement resulting in the degradation of the total carbohydrate due to high illumination. After 60 min of UV-B illumination cells exposed to 3 W m^{-2} of UV-B radiation demonstrated a reduction in carbohydrate of 41.0% contrasted with control. Moreover, results showed that, little dosages of UV-B radiation stimulated the sugars in *A. platensis*. However, higher dosages repressed the starch generation. The results were in agreement with the earlier report where they studied the impact of UV-B radiation on polysaccharides in *Chlorella stigmatophora* and *Nostoc commune*[20, 21].

There was direct relationship between the generation of free radicals and enzymes such as ascorbate peroxidase, peroxidase, catalase including MDA accumulation during UV – B radiation. Proline is a stress indicator which accumulated intracellularlly in cyanobacteria and plants during unfavourable condition. It detoxify the destructive ROS directly than antioxidant enzymes. This is additionally in related with report on *Anabaena* sp.[22].

During oxidation stress, there was an increase in the level of MDA content due to lipid peroxidation cyanobacteria.

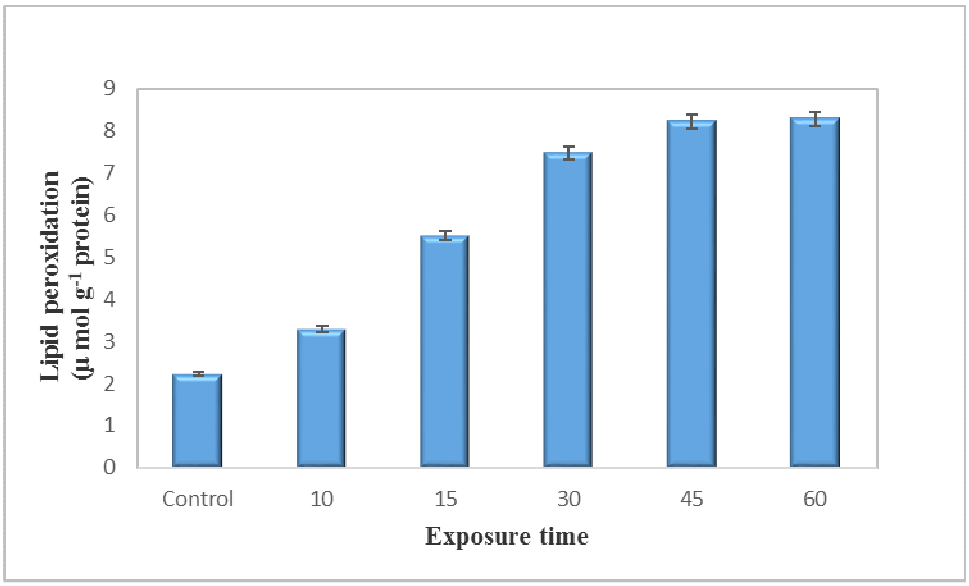

Figure-8
Effect of UV-B radiation on Lipid peroxidation of *Arthrospira platensis*

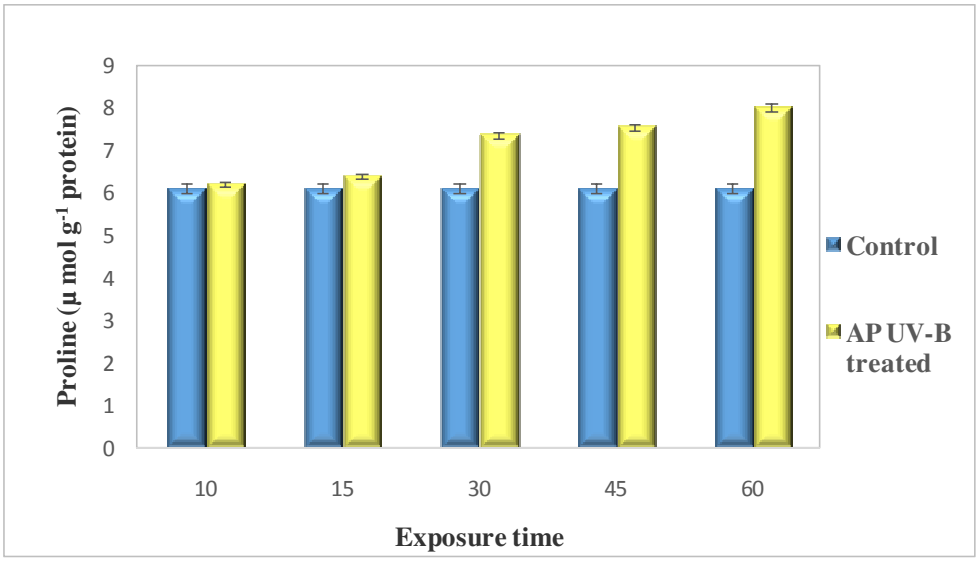

Figure-9
Effect of UV-B radiation on proline content of *Arthrospira platensis*

Table-1
Effect of different times of exposure to UV-B on Superoxide dismutase activity, Peroxidase content and catalsase *in Arthrospira platensis*

Exoposure Time (mins)	SOD (U)	Peroxidase (U)	Catalase (U)
10	2.82±0.08	4.94±0.14	0.92±0.03
15	5.54±0.16	5.49±0.16	0.82±0.02
30	7.48±0.35	7.34±0.22	0.79±0.02
45	9.75±0.29	9.56±0.28	0.64±0.02
60	10.67±0.35	10.08±0.31	0.66±0.12

It was demonstrated in this investigation where H_2O_2 and O_2 were produced where it triggered the activity of several antioxidant enzymes such as Superoxide dismutase, Catalase and Peroxidase (table-1.). High accumulation of these enzymes in *A.platensis* could be connected with stress tolerance efficiency. Similar results were obtained with prominent increment in the activity of Superoxide dismutase and Peroxidase in *P. boryanum* and *N. muscorum* and also in *Aphanothece* sp. reported in previous studies[23,24].

Conclusion

It is often neglected that any change in environment or climate

will be first noticed at the micro level and hence, there is a need to focus research precisely at this level to find solutions in the mitigation of the negative effects of the climatic changes at global level. It is asserted that UV-B radiation created huge impact in biochemical organization of the cyanobacterium, *Arthrospia platensis* which was found to withstand longer duration. It is suggested that microalgae has also evolved ways of protecting themselves against UV-B damage by producing screening compounds. Results indicate that there is cumulative increase in the carbohydrate content of *A. platensis*. Resistance induced to UV-B radiations in microalgae of commercial significance also have the potential to be used for carbon sequestration. Carbon allocation in this algae remains to be explored.

Acknowledgements

This work was supported by Department of Science and Technology (DST), Government of India. The authors were grateful for the financial aid facilitated for this study.

References

1. Wang G., Hu C., Li D., Zhang D., Chen K. and Liu Y., The response of antioxidant systems in *Nostoc sphaeroides* against UV-B radiation and the protective effects of exogenous antioxidants, **39**, 1034-1042 **(2007)**

2. Vaishampayan A., Sinha R.P., Hader D.P., Dey T., Gupta A.K., Bhan U. and Rao L., Cyanobacterial biofertilizers in rice agriculture, *Bot Rev,* **67,** 453-516 **(2001)**

3. Gorton H.L. and Vogelmann T.C., Ultraviolet radiation and the snow alga *Chlamydomonas nivalis* (Bauer) Wille, *Photochem. Photobiol*., **77,** 608-615 **(2003)**

4. Sinha R.P., Singh N., Kumar A., Kumar H.D. and Hader D.P., Impacts of ultraviolet-B irradiation on nitrogen-fixing cyanobacteria of rice paddy fields, *J. Plant Physiol.,* **150,** 188-193 **(1997)**

5. Villafane E.V., Sundback K., Figueroa F. and Helbling W., Photosynthesis in the aquatic environment as affected by UVR. In: Helbling, W., Zagarese, H. (Eds.), UV Effects in Aquatic Organisms and Ecosystems, The Royal Society of Chemistry, Cambridge, UK, 357-397 **(2003)**

6. Hader D.P. and Sinha R.P., Solar ultraviolet radiation-induced DNA damage in aquatic organisms: potential environmental impact, *Mut. Res. Fund. Mol.Mech. Mutag*., **571**, 221-233 **(2005)**

7. Gao K., Li G., Helbling E.W. and Villafan V.E., Variability of UVR Effects on Photosynthesis of Summer Phytoplankton Assemblages from a Tropical Coastal Area of the South China Sea, **103,** 802-809 **(2007)**

8. Bennett A. and Bogorad L., Complimentary Chromatic Adaptation in a Filamentous Blue, Green Alga, The *Jour of Cell Biol*., **58**, 419 **(1973)**

9. Lichtenthaler H.K., Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, In: Packer, L. and Douce, R. eds., *Methods in Enzymology*, Washington, Academic Press, **148,** 350-382 **(1987)**

10. Bradford M.M., A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding, *Anal Biochem*, **72**, 248-254 **(1976)**

11. Dubois M., Giles K.A., Hamilton J.K., Robeus R.A. and Smith E., Calorimetric methods for determination of sugars and related substance, *Anal Biochem.,* **28**, 305-365 **(1956)**

12. Bates L.S., Wadern R.P. and Teare I.D., Rapid estimation of free proline for water stress determination, *Plant Soil*., **39**, 205-207 **(1973)**

13. Heath R.L. and Packer L., Photoperoxidation on in isolated Chloroplasts, I. Stoichiometry of fatty acid peroxidation, *Arch Biochem. Biophys*., **125,** 189-98 **(1968)**

14. Kato M. and Shimizu S., Chlorophyll metabolism in higher plants, VII. Chlorophyll degradation in senesc- ing Tobacco leaves, phenolic-dependent peroxidative degradation, *Can. J. Bot*., **65**, 729-735 **(1987)**

15. Beauchamp C. and Fridovich I, Superoxide dismuttases: improved assays and an assay applicable to acrylamide gels, *Anal. Biochem*., **44**, 276-287 **(1971)**

16. Callaghan T.V., Bjorn L.O., Chemov Y., Chapin T., Christensen T.R, Huntley B., Ims R.A., Johansson M., Jolly D., Jonasson S., Matveyeva N., Panikov N, Oechel W., Shaver G., Elster J., Jonsdottir I.S., Laine K. Taulavuori K. and Zockler C., Responses to projected changes in climate and UV-B at the species level, *Ambio*., **33,** 418-435 **(2004)**

17. Bornman J.F., Target sites of UV-B radiation in photosynthesis of higher plants, *J. Photochem. Photobiol.* **4**, 145-148 **(1989)**

18. Dohler G., Effect of UV-B radiation on the marine diatoms *Lauderia annulata* and *Thalassiosira rotula* grown in different salinities, *Mar. Biol*., **83**, 247-253 **(1984)**

19. Sinha R.P., Lebert M., Kumar A., Kumar H.D. and Hader D.P., Disintegration of phycobilisomes in a rice field cyanobacterium *Nostoc* sp. following UV irradiation, *Biochem. Mol. Biol. Int.,* **37,** 697-706 **(1995)**

20. Ehling-Schulz M., Bilger W. and Scherer S., UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*, *J. Bacteriol*., **3**, 1940-1945 **(1997)**

21. Kaplan D., Christiaen D. and Arad S., Chelating properties of extracellular polysaccharides from *Chlorella* sp. *Appl. Environ. Microbiol*., **12,** 2953-2956 **(1987)**

22. Tripathi B.N. and Gaur J.P., Relationship between Copper and Zinc: Induced Oxidative Stress and Proline Accumulation in *Scenedesmus* sp., *Planta*, **219**, 397-404 **(2004)**

23. Girotti A.W., Photodynamic lipid peroxidation in biological systems, *Photochem. Photobiol*., **51,** 497-509 **(1990)**

24. Chris A., Zeeshan M., Abraham G. and Prasad S.M., Proline accumulation in *Cylindrospermum* sp., *Environ Exp Bot*. **57**, 154-159 **(2006)**