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UV-B RADIATION INDUCED DIFFERENTIAL PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES IN *NOSTOC COMMUNE* AND *ANABAENA VARIABILIS*

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Keywords:

Nostoc, *Anabaena*, UV-B radiation, glutamine synthetase, nitrate reductase, Lipid peroxidation

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ABSTRACT: Batch cultures of two cyanobacterial species *i.e.*, *Nostoc commune* and *Anabaena variabilis* were used to evaluate the impact of UV-B radiation on growth, photosynthetic pigments, lipid peroxidation, proline content, protein content as well as on the enzymes nitrate reductase and glutamine synthetase. The essential targets of UV-B radiation are the photosynthetic apparatus, photosynthetic pigments, and the excitation energy transfer. The UV-B radiation adversely inhibited the growth and photosynthetic pigments in both cyanobacterial strains. The content of Malondialdehyde increased by two-fold in both cyanobacterial species upon prolonged UV-B exposure. Protein content showed varied responses in both cyanobacterial species on UV-B exposure. *Anabaena variabilis* showed a decrease in protein content, whereas in *Nostoc commune* protein content elevates with an increase in UV-B exposure duration. With the increase in the duration of UV-B exposure up to 72 hrs the proline content in both species of cyanobacteria increased. Following the different duration of UV-B exposure in the following species, the *in-vivo* Nitrate reductase activity increased while *in-vivo* Glutamine synthetase activity decreased, although the complete decline in glutamine synthetase activity was not observed even after 72 hrs of exposure. The results showed that damages at different levels are caused due to UV-B exposure in both *Anabaena variabilis* and *Nostoc commune*.

INTRODUCTION: Cyanobacteria, also known as blue-green algae are among the most diverse group of gram-negative photosynthetic autotrophs which are widely distributed globally. They are morphologically diverse and produce many secondary metabolites with different biological functions. Cyanobacteria, like all photoautotrophs, rely on sun radiation as their principal energy source.

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Due to the discharge of atmospheric pollutants such as chlorofluorocarbons (CFCs), chlorocarbons (CCs), and organobromide (OBs), the stratospheric ozone layer continues to deplete, increasing ultraviolet radiation (UVR 280–315 nm) reaching the earth's surface. UVR is a potential abiotic stress that deleteriously impacts crop productivity and living organisms.

UV-B and UV-A (315–400 nm) light can penetrate deep into the water, causing harm to aquatic ecosystems [1]. Both UV-B and UV-A radiation have been shown to affect a variety of vital functions in cyanobacteria, including survival, growth, photosynthetic pigmentation, cell differentiation, photosynthetic oxygen production, motility, nitrogen uptake, phycobiliprotein composition,

protein profiling, and CO_2 uptake [2]. UV-A is the least energetic of the three types of UV radiation (UV-A, UV-B, and UV-C), and it acts indirectly by creating reactive oxygen species (ROS). As a result, the current research is focused on UVB radiation. Increased solar UV radiation on the earth's surface is thought to be a major stressor for all photosynthetic organisms and the terrestrial ecosystem [3]. Solar photo energy (400–700 nm) is necessary for cell physiology and biochemistry to be maintained. With sun photo energy, cyanobacteria get a deadly dosage of UV radiation (280–400 nm), which damages cellular macromolecules such as DNA, proteins, lipids, and pigments directly and indirectly by creating free radicals or reactive oxygen species (ROS). UV-B light is primarily directed at the photosynthetic machinery of cyanobacteria. According to research, PSI is more resistant to UV-B radiation than PSII because the D1 and D2 reaction center protein subunits of PSII are destroyed following UV-B radiation [4].

Every photosynthetic organism, including cyanobacteria that are simultaneously exposed to PAR (400–700 nm) and UVR (UVR; 400–700 nm), have evolved methods to combat the harmful effects of UV-B radiation. Aside from photoactivation and excision repair for UV-induced DNA damage [5], carotenoids and detoxifying enzymes, as well as radical quenchers and antioxidants, protect oxygen species [6, 7]. UV-B light has been shown to stimulate the generation of reactive oxygen species (ROS) at numerous sites of photosynthetic electron transport and during many metabolic events in the cellular system [8]. These ROS are extremely harmful to cell structure and function [8, 9].

Cells create reactive oxygen species (ROS), such as superoxide radicals, which are then converted to hydrogen peroxide and hydroxyl radicals as a final product [10, 11]. Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) work together to inactivate O_2^{-2} and H_2O_2 molecules, preventing the generation of hydroxyl radicals, which are the most reactive of the ROS and cause cellular damage [12]. The goal of this study was to investigate how UV-B radiation affected growth, photosynthetic pigments, cellular damage determined by lipid peroxidation, proline and protein content, as well as its impact on the two important enzymes involved in nitrogen metabolism, *i.e.,* nitrate reductase and glutamine synthetase in two cyanobacteria, *Anabaena variabilis* and *Nostoc commune.*

MATERIALS AND METHODS: Cyanobacterial culture of *Anabaena variabilis* and *Nostoc commune,* a filamentous heterocystous cyano-bacteria, were procured from IARI, New Delhi. These were grown axenically in BG-11 medium [13] devoid of nitrogen source at 28°C±2°C supplied by Philips cool white fluorescent light with 8 hours light and 16 hours dark cycle in culture room. For performing different experiments, cells in the exponential growth phase were taken.

UV Treatment: Homogenized cultures of the above-mentioned cyanobacteria were taken in 100 mm open petridishes and exposed directly to UV-B radiation (280-350 nm) for various durations at influence rate of $0.4Wm^{-2}$ by using UV-B tubes (Philips, TL-40 W/12, RS UV-B Medical, Holland) at 315nm main output. *Anabaena variabilis* and *Nostoc commune* cells were exposed for 12-72 hours in triplicates. To avoid all incidents UV-C (<280 nm) radiation petri plates were covered with 0.14 mm cellulose acetate filter (Johnston Industrial Plastics, Canada).

Growth Measurement: To observe the effect of UV-B radiation on the growth, homogenous cultures of the cyanobacteria mentioned above were exposed to different duration of UV-B radiation ranging from 12 h to 72 h. An equal volume of cultures was withdrawn from each petri plate at regular interval of UV exposure. Growth was measured by recording the absorbance at 750 nm using a UV-Vis spectrophotometer.

Pigments Estimation: For the photosynthetic pigment estimation, cyanobacterial culture suspension was centrifuged at 15000 g for 7 min. and the pellet was suspended in precooled methanol and incubated at 4^oC for 30 min to extract the pigments from the cells. The suspension was centrifuged again at 10,000g for 5 min. The supernatant was collected, and its absorbance was measured at 665 and 480 nm for chlorophyll and carotenoid, respectively. Chl a content was calculated according to [14] and carotenoid content

was estimated, taking the specific absorption coefficient given by [15]. Phycocyanin was estimated by suspending the pellet in 0.5 M phosphate buffer (pH 7) followed by repeated freezing and thawing. After that the absorbance of the blue-coloured supernatant was recorded at 660 nm and then at 620 nm. The amount of phycocyanin was calculated according to [16].

$$C= [A_{660} – 0.474 (A_{620})] / 5.34$$

Lipid Peroxidation: Heath and Packer's approach was used to measure lipid peroxidation (1968). Cyanobacterial cells were homogenised in 1% trichloroacetic acid and centrifuged for 16 minutes at room temperature at 10,000 RPM. A new test tube was filled with an equal volume of supernatant and 0.5% Thiobarbituric acid (TBA) freshly prepared in 20 % Trichloro acetic acid solution [17].

The solution was heated at 95°C for 30 minutes in a water bath. The heated supernatant was again centrifuged for 5 minutes at 10000 RPM, and the absorbance was measured at 532 nm and 600 nm. As a blank, 5% TBA in 20% TCA was used. The extinction coefficient of 155 mm^{-1} cm^{-1} was used to determine MDA content.

Proline Estimation: Proline content was measured using treated, untreated cyanobacterial cells suspended in 3% sulfosalicylic acid and spun at 10,000 RPM for 10 minutes to remove cell debris. The reaction mixture contained 2 ml of supernatant, 2 ml of ninhydrin, 2 ml glacial acetic acid and was kept at boiling temperature for 1 hour before being extracted with toluene. From the toluene phase, proline was detected spectrophotometrically at 520 nm [18]

Protein Estimation: The protein content was measured at 700 nm using method of Lowry *et al.* (1951) [19].

Nitrate Reductase Test: The method of Camm and Stein (1974) was used to estimate *in-vivo* nitrate reductase activity. 2ml of the cyanobacterial culture exposed to different duration of UV-B radiation was withdrawn at regular intervals of time and mixed well with 4 ml of sulphanilamide. Thereafter 0.2% NEDD was added and left for 15 min. The absorbance of the pink colour developed was recorded at 540 nm in a spectrophotometer [20].

Glutamine Synthetase Test: *In-vivo* glutamine synthetase activity was estimated by the Sampaio et al. (1979) method. During UV-B exposure, 2 ml of the cyanobacterial cultures were withdrawn at regular duration of exposure and mixed with 1 ml of toluene and incubated at 4°C for 15 min. The suspension was then centrifuged to remove the toluene layer. The pellet was resuspended in 0.3 ml of washing buffer containing 50 mM imidazole; 10 mM L-glutamic acid and 5 mM $MgCl_2$ having pH 7.0. After that 0.4 ml of assay mixture was added and incubated at 37°C for 30 min. Thereafter 3 ml of a solution containing 10% $FeCl_3$, 24% TCA and 6 N HCl was added to terminate the reaction. The absorbance of the pale-yellow color developed was recorded at 540 nm in a spectrophotometer [21].

Statistical Analysis: All the experiments were done in triplicates, and the results were expressed as mean± standard error (SD). The statistical software Graph Pad Prism version 5.0 (Graph Pad Software, USA) was used for analysis. P values were determined using the Student t-test; a p-value ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION:

Growth: The two selected cyanobacteria *Anabaena variabilis* and *Nostoc commune* showed inhibitory growth response to UV-B radiation exposureas shown in **Fig. 1**. It was observed that after 72 h of UV-B exposure growth declined by 60.41% and 49.78% in *Nostoc commune and Anabaena variabilis* respectively in comparison to control. These significant differences in the growth responses of these cyanobacteria to UV-B stress was attributed to direct or indirect damage caused by UV-B radiation to structural components, genetic material, proteins and physiological factors and photosynthetic apparatus [22, 23, 24].

In several cyanobacterial strains, the presence of UV-protecting pigments and the UV sunscreen role of mycosporine (MAA)-like compounds has been demonstrated [25, 26, 27] and different levels of these compounds in the cells could be one of the reasons for cyanobacterial sensitivity differences. Furthermore, morphological traits such as sheath, antioxidants, and the DNA repairing machinery could be used to explain the varied responses of these cyanobacteria to UV-B [28].

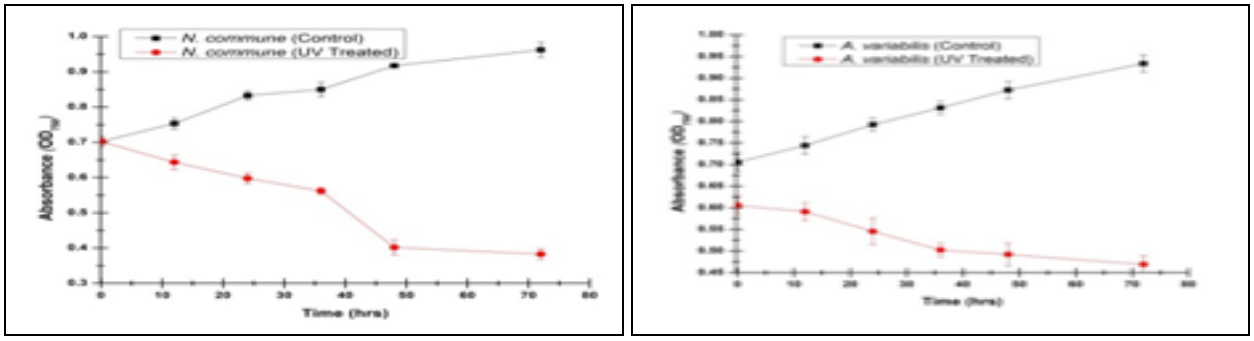

FIG. 1: EFFECT OF DIFFERENT DURATION OF UV-B RADIATION ON GROWTH OF *NOSTOC COMMUNE* AND *ANABAENA VARIABILIS*

Photosynthetic Pigments: In the above-mentioned cyanobacterial species, the Chlorophyll a and phycocyanin content decreased with increasing time of UV-B exposure. As the exposure duration increased from 12 h to 72 h, the chl a content decreased by 40.62% in *Anabaena variabilis* and 45.78% in *Nostoc commune* **Fig. 2A**.

It was observed that UV-B radiation has a more profound effect on phycocyanin content than chl a. After 72 h of UV-B exposure in *Anabaena variabilis*, the reduction in phycocyanin was 80.73% and in *Nostoc commune* it was recorded to be 60.91% as compared to control **Fig. 2B**.

The amount of carotenoid showed varied responses to the length of UV-B exposure. In *Anabaena variabilis*, it increased by 28.36 % up to 48 h and then declined by 51.4%. In contrast, in *Nostoc commune* it increased by 34.07 % up to 48 h and then declined by 47.3% after 72 h of UV-B exposure as compared to the control **Fig. 2C**.

It is evident that UV-B radiations have deleterious effect on photosynthetic pigments because of photobleaching or the formation of reactive oxygen species that mediates in peroxidation. (Nultsch and Agel 1986). The protective action of carotenoids in scavenging singlet oxygen may explain the rise in carotenoid concentration in response to UV-B in these cyanobacteria [29]. Singlet oxygen is usually created during normal photosynthesis, and its production may rise further under stress, such as UV-B radiation [30].

Out of three photosynthetic pigments, the severe effect on phycocyanin in these cyanobacteria was consistent with previous observations [31]. Direct interaction of UV-B with phycocyanin, located on the thylakoid membrane's outer surface, may have caused the significant loss of phycocyanin.

Because of its proteinaceous nature, phycocyanin can absorb UV-B light (280 nm and above) and be destroyed. Moreover, protein peroxidation caused by reactive oxygen species *i.e.*, ROS may also have a deleterious effect on phycocyanin.

UV-B radiation has been found to have a harmful effect on photosynthetic pigments in both brown and blue-green strains of *Nostoc spongiforme*, with the blue-green strain showing the most damage [32]. UV-B exposure causes a large amount of ROS to be produced by photosynthetic pigments and redox components.

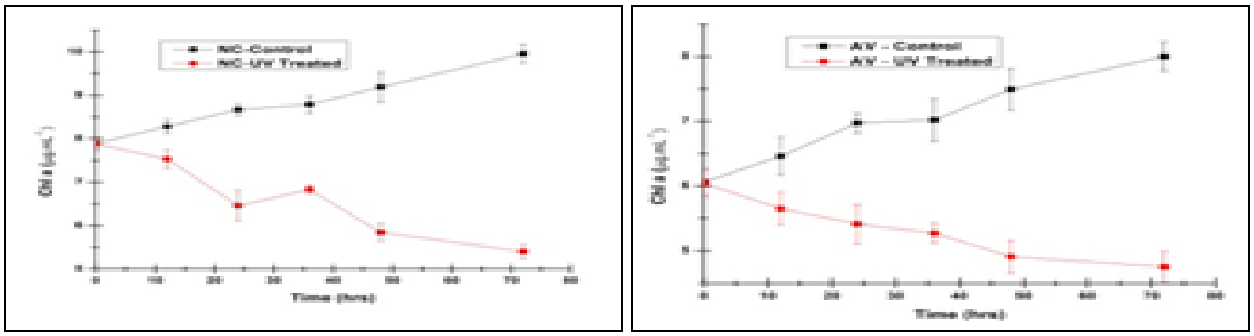

FIG. 2A: EFFECT OF DIFFERENT DURATION OF UV-B RADIATION ON CHL A CONTENT OF *NOSTOC COMMUNE* AND *ANABAENA VARIABILIS*

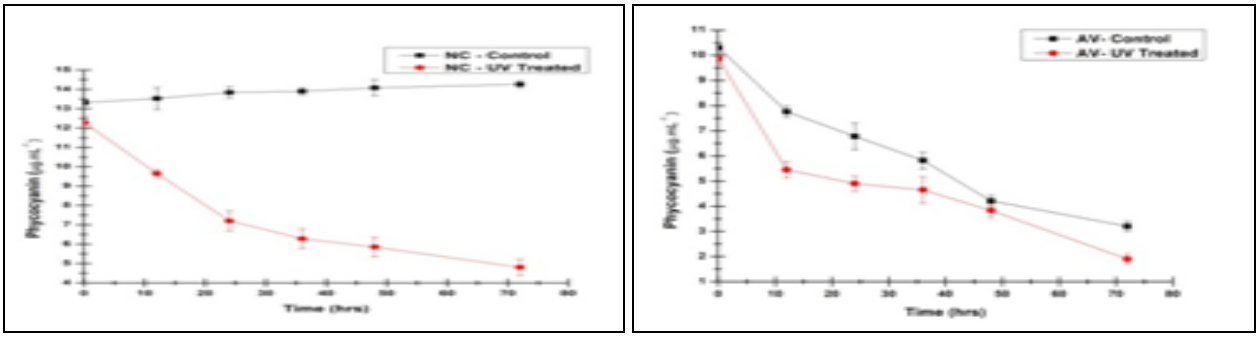

FIG. 2B: EFFECT OF DIFFERENT DURATION OF UV-B RADIATION ON PHYCOCYANIN CONTENT OF *NOSTOC COMMUNE* AND *ANABAENA VARIABILIS*

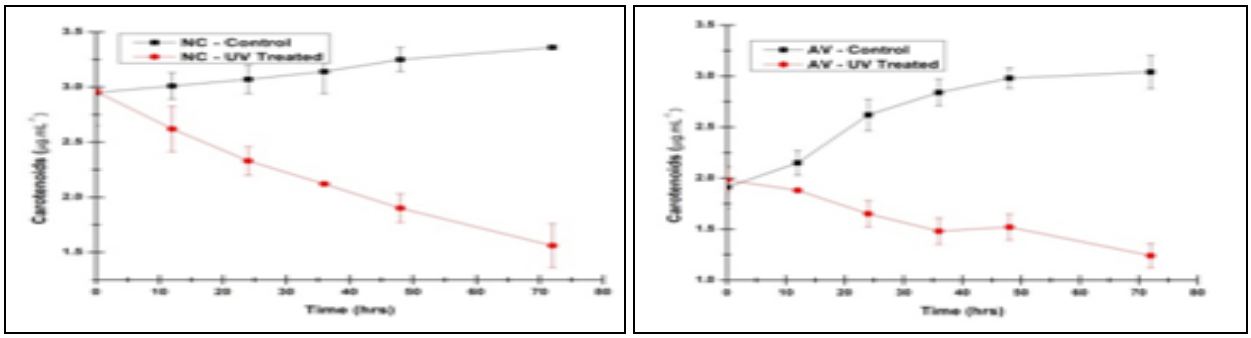

FIG. 2C: EFFECT OF DIFFERENT DURATION OF UV-B RADIATION ON CAROTENOID CONTENT OF NOSTOC COMMUNE AND ANABAENA VARIABILIS

Lipid Peroxidation: In UV-B exposed cyanobacteria, the level of lipid membrane damage evaluated as malondialdehyde (MDA) content revealed an increase in lipid peroxidation. MDA levels in both cyanobacterial species increased twofold. After 72 hours of UV-B radiation, MDA content in *Anabaena variabilis* increased by 131 percent, and MDA concentration in *Nostoc commune* increased by 103 percent **Table 1**. The oxidative breakdown of polyunsaturated fatty acids in the cyanobacterial membrane caused the increased MDA level in UV-B-treated cyanobacteria [33]. UV-B radiation increased the concentration of MDA in both *Anabaena variabilis* and *Nostoc commune*, indicating oxidative stress in both species.

TABLE 1: EFFECT OF DIFFERENT DURATION OF UV-B EXPOSURE ON LIPID PEROXIDATION IN TWO CYANOBACTERIA

Organism	UV-B duration (h)	Lipid peroxidation (nmol MDA/mg protein)
Nostoc commune	Control	2.05
	12	2.10
	24	2.14
	36	3.18
	48	3.25
	72	4.16
Anabaena variabilis	Control	1.08
	12	1.12
	24	1.75
	36	2.02
	48	2.25
	72	2.50

Proline Content Estimation: Proline is a -amino acid with a well-known function in stressful situations. Proline is a good osmolyte and functions as a metal chelator, an antioxidant defence molecule and a signalling molecule when under stress [34, 35]. Proline is utilised in numerous pharmacological and biotechnological applications because it is an important osmoprotectant in the fight against arteriosclerosis, which lowers the pressure created by blockages and, as a result, lowers the risk of heart disease (Wickham 2011). Proline content in these cyanobacteria was also

examined because of its significance to humans. Proline is a vital amino acid for primary metabolism. It builds up in adverse circumstances and abiotic stressors, such as UV-B radiation. Proline content increased substantially with increasing doses of UV-B radiation in both *Anabaena variabilis* and *Nostoc commune* **Fig. 3**. Upto 72 hours of UV-B exposure, proline content in *Anabaena variabilis* increased by 101.8% compared to control. In contrast, it increased by 118.9 percent in *Nostoc commune*. Many cyanobacteria and higher plants have accumulated proline due to increased synthesis and decreased breakdown under stress conditions, particularly UV-B radiation. Under stressful conditions, proline accumulates due to a decrease in the electron transport system activity [36].

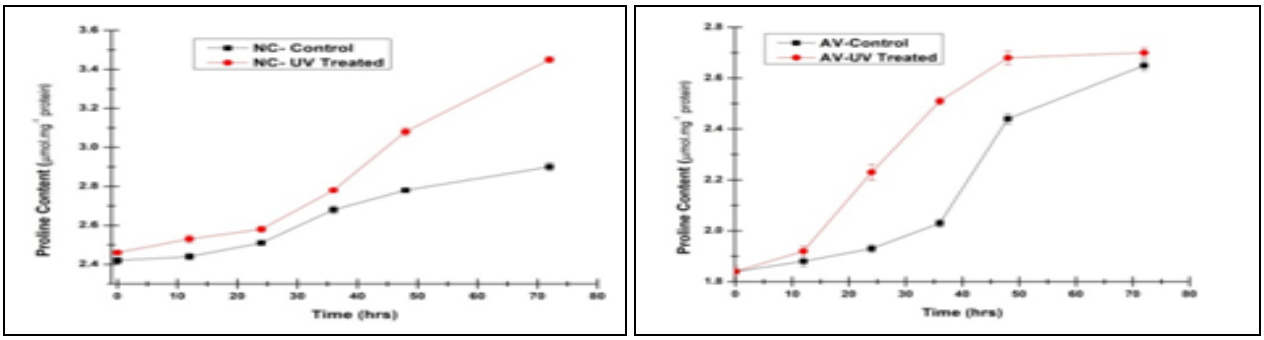

FIG. 3: EFFECT OF DIFFERENT DURATION OF UV-B RADIATION ON PROLINE CONTENT OF *NOSTOC COMMUNE* AND *ANABAENA VARIABILIS*

Protein Content Estimation: In both the cyanobacteria species *Anabaena variabilis* and *Nostoc commune*, UV-B radiation caused considerable changes in protein content when compared to non-exposed cyanobacteria. With increasing length of UV-B exposure up to 72 hours, protein content in *Anabaena variabilis* reduced up to 81.29% of control. *On the other hand, Nostoc commune* showed a 78.11 percent fall in concentration until 72 hours of UVB exposure **Fig. 4**. The protein composition of both species of cyanobacteria changed after exposure to UV-B radiation, according to the findings. This is because under stress conditions, such as brief and long-term UV-B exposure, numerous proteins are destroyed, and few new proteins are generated [37].

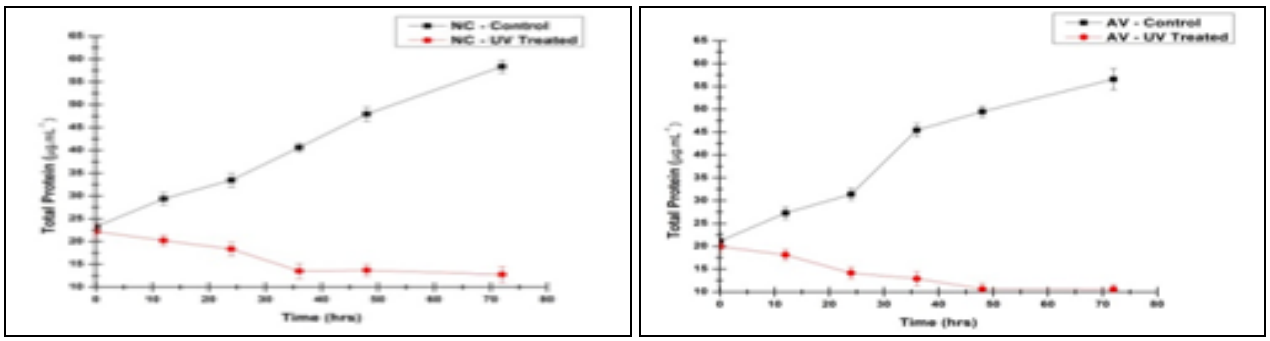

FIG. 4: EFFECT OF DIFFERENT DURATION OF UV-B RADIATION ON PROTEIN CONTENT OF *NOSTOC COMMUNE* AND *ANABAENA VARIABILIS*

Nitrate Reductase Test: It was observed that the nitrate reductase activity was increased after being exposed to UV-B radiations. It was indicated that nitrate reductase activity was stimulated upto 48 hours of UV-B exposure and then became steady. **Fig. 5** depicts the effect of various UV-B exposure durations on *in-vivo* nitrate reductase activity. In both the strains studied thus far, activity increased linearly with increasing UV-B exposure time. The activity of nitrate reductase in *Anabaena variabilis* was much higher than in *Nostoc commune*. In the current study, UV-B-mediated stimulation of nitrate reductase activity was observed. The observed enhancement of Nitrate reductase activity in response to UV-B exposure is still unknown. UV-B radiation of cyanobacterial cultures has been shown to have negative effects on nearly all metabolic processes examined thus far [38]. However,

Crotalaria juncea, an angiospermic plant, has been shown to stimulate Nitrate reductase activity after UV-B treatment [39]. In *Neurospora crassa*, photoactivation of Nitrate reductase has been seen [40]. The same enzyme has been shown to be inhibited by UV-B in marine diatoms [41]. Exposure of *Oscillataria princeps* to blue light has also been observed to stimulate Nitrate reductase activity [42]. Hence more research is needed to determine the specific mechanism of UV-B-mediated Nitrate reductase activity stimulation.

Glutamine Synthetase Activity: Glutamine synthetase is the principal ammonia assimilating enzyme and is involved in nitrogenase and NR activity regulation, it was important to see how UV-B affected this enzyme. In contrast to nitrate reductase activity, it was observed that the glutamine synthetase showed a decrease in its activity with a increase in the duration of UV-B exposure **Fig. 6**. Even after 72 hrs of UV-B exposure there was no complete inactivation of its activity. The suppression of GS activity by UV-B has been seen in a variety of marine diatoms [43]. Our observations with cyanobacterial strains support the previous conclusion. GS activity is also inhibited by other stressors, such as NaCl [44]. However, even after extended hours of UV-B irradiation, there was no full suppression. To date, it is unclear if UV-B's effects on nitrogen metabolism enzymes are due to a direct impact on enzyme activity or whether enzyme production is affected by RNA damage or other yet undiscovered factors.

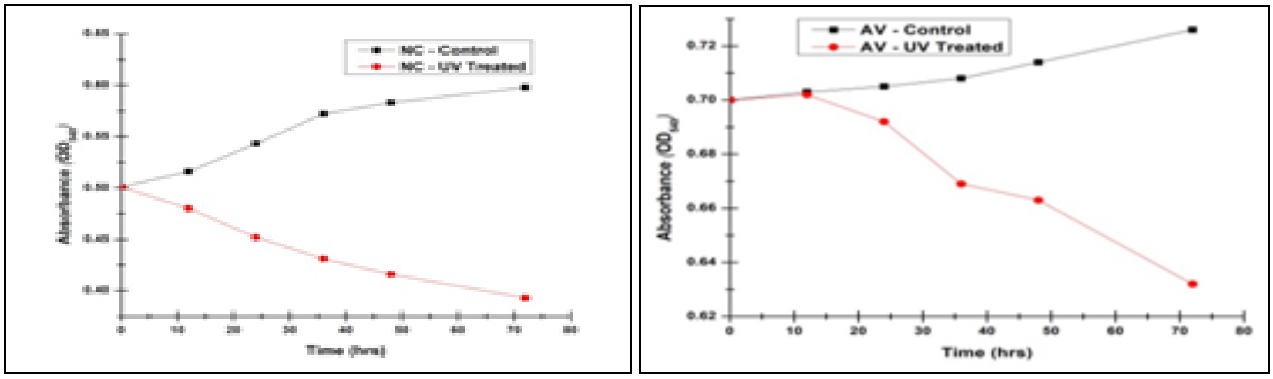

FIG. 4: EFFECT OF DIFFERENT DURATION OF UV-B RADIATION ON GLUTAMINE SYNTHETASE OF *NOSTOC COMMUNE* AND *ANABAENA VARIABILIS*

CONCLUSION: In the present study, we measured the effect of UV-B radiations on growth, photosynthetic pigment content, lipid peroxidation, proline content, and protein content, along with its effect on nitrate reductase and glutamine synthetase enzymes in two different cyanobacterial species. It is seen that the two cyanobacterial species showed different degrees of sensitivity to UV-B radiations.

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CONFLICTS OF INTEREST: Nil

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