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UVB-Induced Reduction in Biomass and Overall Productivity of Cyanobacteria

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Effect of middle wave ultraviolet radiation (UVB) was studied in three different species of cyanobacteria (Nostoc, Anabaena and Scytonema) by estimating their growth pattern, biomass yield, chlorophyll content, total starch and protein content. The results show that exposure of the cyanobacteria with UVB dose corresponding to an increase or decrease of 20% in its environmental flux will have drastic effects on biomass production, photosynthetic rate and nitrogen fixation. Cyanobacteria are primary sources of marine food web and an important biofertilizer; therefore, their protection from increasing threat of stratospheric ozone depletion will be necessary to maintain the ecological balance.

Algae, including cyanobacteria are a group of prokaryotic, autotrophic organisms that are abundant in terrestrial, fresh water and marine environment (1). They contribute significantly towards the welfare of human being by providing organic food to the entire marine food web and perform over 90% of the total photosynthetic activity (2). Apart from that, selected algal species are endowed with nitrogenase enzyme complex to allow the fixation of atmospheric nitrogen to generate ammonia (3). The unicellular green algae also possess high quality of food value. Besides being a N_2 fixer, it is being used for the reclamation of barren and alkaline soil, as a biofertilizer and as organic manure. Cyanobacteria require relatively low photon flux densities to utilize sunlight for photosynthesis (4). In nature, these species are vulnerable to the exposure of intense sunlight including UVB, long wave ultraviolet radiation (320-400 nm), visible radiation (400-750 nm) and heat (5-9). The environmental level of UVB are increasing due to release of man-made ozone depleting substances (10). Although, attempts are being made to restrict the use of chlorofluorocarbons, but their current level in the stratosphere are likely to remain unchanged for the next 20-25 years (11, 12). In view of above, it was considered of interest to examine the role of UVB on selected algal species. We report the results of the effect of UVB on Nostoc, Anabaena and Scytonema species at an intensity corresponding to a three year average solar UVB output, determined near our laboratory (26°45′N latitude and 80°50′E longitude at 140 m above the mean sea level). Studies were also conducted to investigate the effect of UVB on algal species at a dose corresponding to a 20% increase or a 20% decrease in its intensity at the ground level.

MATERIALS AND METHODS

Chemicals. Sodium molybdate and bovine serum albumin were purchased from Sigma Chemical Company (St. Louis, MO, USA). Potassium phosphate, magnesium sulfate, calcium chloride, boric acid, manganese chloride, zinc sulfate, cupric sulfate, sodium potassium tartrate, phenol, acetone, trichloroacetic acid, EDTA, sodium hydroxide, perchloric acid, sulfuric acid and other chemicals and reagents used throughout this investigation were purchased from BDH, Analar and Merck (Merck India Private Ltd., Bombay).

Culture. Nostoc, Anabaena and Scytonema species were isolated from paddy fields, purified, identified and maintained in Fogg's medium (13). The culture was grown in a nitrogen free medium ($^{-}$N media) and maintained in a growth chamber at 28±2°C under fluorescent light (2500 lux) and dark for 16/8 hrs day under controlled humidity(60%). The inoculum consisting of 1 ml homogeneous suspension of a 6 days old culture (exponential phase) was inoculated into fresh media and allowed to grow for 3 days. The initial growth was measured turbidometrically in a UV/Vis Spectrophotometer at 663 nm.

UV irradiation and dosimetry. The test organism were transferred into a sterilized petridish (10 × 1.5 cm) and irradiated individually under three different intensity of UVB radiation (0.4 mW/cm^2, 0.5 mW/cm^2 and 0.6 mW/cm^2) for a dose totalling 2 Joule (J) and 4 J. The UVB irradiation system comprised an array of three 4-ft long lamps (T-40 M) manufactured by Vilbar Lourmat (Marne Lavalle, France). The spectral emission of UVB source ran from 280-320 nm with a peak at 312 nm. The intensity of UVB was measured with a RMX-3W Radiometer (Vilber Lourmat) equipped with a UVB-detecting probe.

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Abbreviations used: J, Joule; UVB, 290-320 nm ultraviolet radiation.

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TABLE 1

Effect of UVB on Growth Pattern of Various Species of Cyanobacteria

Species	Control**	0.4 mW/cm2 2J	0.4 mW/cm2 4J	0.5 mW/cm2 2J	0.5 mW/cm2 4J	0.6 mW/cm2 2J	0.6 mW/cm2 4J
			Nostoc				
1st Day	.057 ± .001	.061 ± .001	.055 ± .033**	.059 ± .003	.047 ± .003	.050 ± .001	.049 ± .003
2nd Day	.067 ± .001	.064 ± .024**	.056 ± .002**	.064 ± .002	.055 ± .002	.049 ± .002	.053 ± .001
3rd Day	.115 ± .003	.086 ± .002	.056 ± .002	.090 ± .001	.052 ± .001	.054 ± .002	.039 ± .002
4th Day	.137 ± .001	.104 ± .002	.067 ± .003	.097 ± .002	.041 ± .002	.062 ± .003	.029 ± .003
			Anabaena				
1st Day	.091 ± .002	.084 ± .002	.049 ± .002	.065 ± .002	.046 ± .003	.046 ± .002	.037 ± .003
2nd Day	.125 ± .001	.100 ± .002	.055 ± .002	.083 ± .002	.047 ± .001	.052 ± .002	.044 ± .002
3rd Day	.138 ± .002	.102 ± .002	.072 ± .001	.095 ± .002	.042 ± .001	.060 ± .001	.039 ± .001
4th Day	.148 ± .001	.109 ± .001	.077 ± .001	.055 ± .001	.056 ± .001	.087 ± .002	.042 ± .001
			Scytonema				
1st Day	.069 ± .001	.067 ± .008**	.062 ± .003	.060 ± .008	.053 ± .005	.047 ± .001	.049 ± .002
2nd Day	.112 ± .002	.089 ± .001	.051 ± .008	.088 ± .001	.057 ± .001	.054 ± .005	.051 ± .001
3rd Day	.139 ± .001	.103 ± .002	.082 ± .001	.086 ± .005	.049 ± .005	.062 ± .001	.080 ± .001
4th Day	.151 ± .008	.111 ± .001	.083 ± .001	.098 ± .001	.060 ± .001	.090 ± .001	.039 ± .001

Note. Measured by determining optical density at 663 nm. Values are arithmetic mean ± SD of 3 determinations. $P > 0.001$ in comparison to control.

Growth pattern. The UVB irradiated and control samples were transferred into 15 ml test tubes and mixed thoroughly by vigorously shaking to get a homogeneous suspension. Growth pattern was measured turbidemetrically at 663 nm (14).

Chlorophyll content. 10 ml irradiated or control (unirradiated) sample of algae were centrifuged (5000 rpm) for 10 minutes. The pallet was washed twice with distilled water, resuspended in methanol (96%, 10 ml) and kept in a water bath for 30 min at 60°C. The material was cooled at room temperature and centrifuged again to remove cell debris. The supernatent was estimated for chlorophyll content by measuring optical density at 665 nm using methanol (96%) as a reference blank (15).

Protein estimation. Protein content of the irradiated and unirradiated samples was estimated by the method of Lowry et al (16). The interference by pigments was eliminated by washing the trichloroacetic acid precipitate twice with acetone (90%). Bovine serum albumin was used as a standard.

Estimation of total starch. Starch was estimated by the procedure as follows: Irradiated or control algal samples (10 ml) were centrifuged (2000 rpm, 10 min.) and the pellet was washed twice with ethanol (80%), followed by gelatinizatin by heating for 10 min in 1 ml H_2O. The gelatinized material was hydrolyzed with perchloric acid (52% in H_2O, 60°C, 1 min) and centrifuged (2000 rpm, 10 min). The supernatent (0.1 ml) was added in a mixture of phenol (80%, 0.1 ml), H_2O (1.9 ml), H_2SO_4 (5 ml) and allowed to stand in room temperature for 30 min. Starch was estimated by recording the OD of the resulting mixture at 490 nm (17).

RESULTS AND DISCUSSION

Effect of UVB on the growth pattern of cyanobacteria. The data for growth pattern of cyanobacteria species prior to UVB irradiation and after exposure to varying dose and intensity of UVB are described in Table 1. The average rate of growth in control Nostoc, Anabaena and Scytonema after 4 days was 296%, 324% and 338%, respectively. However, UVB irradiation of Nostoc at 0.4, 0.5, 0.6 mW/cm^2 for a total dose of 2J per day for 4 consecutive days resulted in the reduction of growth by 25%, 28% and 55%, respectively. Further increase in the UVB dose to 4J per day at 0.4 mW/cm^2 resulted in further reduction in growth to 50%. When the inten-

TABLE 2

Effect of UVB on Chlorophyll Content (μg/ml) of Cyanobacteria

Species	Control	0.4 mW/cm2 2J	0.4 mW/cm2 4J	0.5 mW/cm2 2J	0.5 mW/cm2 4J	0.6 mW/cm2 2J	0.6 mW/cm2 4J
Nostoc	.281 ± .001	.201 ± .001	.189 ± .002	.192 ± .002	.102 ± .001	.099 ± .001	.049 ± .001
Anabaena	.292 ± .002	.211 ± .002	.186 ± .001	.182 ± .002	.121 ± .003	.096 ± .002	.049 ± .003
Scytonema	.262 ± .003	.203 ± .003	.181 ± .001	.170 ± .001	.101 ± .002	.083 ± .002	.046 ± .001

Note. Values are arithemetic mean ± SD of 3 determinations. $P < 0.001$ in comparison with control.

TABLE 3

Effect of UVB on Protein Content (μg/ml) of Cyanobacteria

Species	Control	0.4 mW/cm2 2J	0.4 mW/cm2 4J	0.5 mW/cm2 2J	0.5 mW/cm2 4J	0.6 mW/cm2 2J	0.6 mW/cm2 4J
Nostoc	9.25 ± .02	8.43 ± .02	6.55 ± .03	7.73 ± .03	5.59 ± .03	4.58 ± .02	3.75 ± .01
Anabaena	9.48 ± .02	8.73 ± .02	8.46 ± .03	7.61 ± .02	6.74 ± .02	5.76 ± .01	3.94 ± .01
Scytonema	8.89 ± .14	7.83 ± .01	6.12 ± .01	8.12 ± .01	5.90 ± .01	5.12 ± .02	3.10 ± .01

Note. Values are arithemetic mean ± SD of 3 determinations. $P < 0.001$ in comparison with control.

sity of UVB was increased to 0.5 mW/cm^2 or 0.6 mW/cm^2, total inhibition was observed in the growth of Nostoc. The effect of UVB on the growth pattern of Anabaena and Scytonema was more or less the same as was observed with Nostoc. The growth rate of UVB (2J) exposed Anabaena was reduced by 25%, 30% and 41% after irradiation at an intensity of 0.4 mW/cm^2, 0.5 mW/cm^2 and 0.6 mW/cm^2, respectively. Upon increasing the dose of UVB to 4J, the inhibition in the growth rate got nearly doubled . The inhibition in growth rate of Scytonema species at a dose of 2J per day, at 0.4, 0.5, 0.6 mW/cm^2 was 27%, 36% and 42%, respectively. Likewise, upon increasing the UVB dose to 4J per day, the growth was found to be inhibited 47%, 60%, 0% at 0.4, 0.5 and 0.6 mW/cm^2, respectively.

Effect of UVB on chlorophyll content of the cyanobacteria. The alteration in chlorophyll content of the cyanobacteria was recorded in the 4 day irradiated samples of algal sps (2J and 4J at 0.4, 0.5 and 0.6 mW/cm^2). The chlorophyll content of unirradiated Nostoc, Anabaena and Scytonema after 4 days was 0.281, 0.292 and 0.262 μg/ml, respectively. A dose dependent inhibition in the chlorophyll content was observed in the UVB irradiated cyanobacteria. The chlorophyll content was reduced by 25.5±3%, 34.5±3% and 67±1.5% when the cyanobacteria sps were exposed to UVB (2J) at 0.4, 0.5 and 0.6 mW/cm^2, respectively. An increase in the dose to 4J under similar condition led to a 33.5±3%, 60.5±2% and 82.5±5% decrease in chlorophyll content of the cyanobacteria (Table 2).

Effect of UVB on cellular protein content of the cyanobacteria. The protein content of all the cyanobacterial sps decreased progressively as the UVB exposure dose increased from 2J to 4J and the intensity of UVB increased from 0.4 mW/cm^2 to 0.5 mW/cm^2 or 0.6 mW/cm^2 (Table 3). The difference in the rate of inhibition of protein content between the cyanobacterial species after 4 day exposure was not a great deal, nevertheless, the pattern was of the following order: Scytonema> Nostoc> Anabaena.

UVB effect on total starch content of the cyanobacteria. In Table 4 we show the effect of UVB on various species of the cyanobacteria after 4 days. The level of total starch decrease progressively as the intensity of UVB increased. Anabaena was most sensitive towards UVB and even low dose exposure (2J at 0.4 mW/cm^2) resulted in 11% reduction in the total starch content. Under similar conditions, the loss of total starch content in Scytonema and Nostoc sps was upto 4% and 6%, respectively. The order of decrease in total starch content of cyanobacteria upon exposure to UVB was found to be of the following order: Anabaena>Scytonema>Nostoc.

Effect of UVB on biomass yield of cyanobacteria. Anabaena produced highest level of biomass in comparison to other cyanobacterial sps under controlled conditions (Table 5). Although, low exposure dose of UVB (2J at 0.4 mW/cm^2) resulted in only 11% reduction in the biomass yield of Anabaena, an increase in UVB intensity (0.5 mW/cm^2) resulted in a sharp decline (27%) in biomass production. Under similar conditions (2J at 0.5 mW/cm^2), Nostoc and Scytonema produced 12% and 10% loss of the biomass, respectively. Scytonema was found to be most resistant towards UVB exposure. At higher exposure dose (4J at 0.6 mW/cm^2), Nostoc's biomass production was depleted by 38%. The

TABLE 4

Effect of UVB on Starch Content (μg/ml) of Cyanobacteria

Species	Control	0.4 mW/cm2 2J	0.4 mW/cm2 4J	0.5 mW/cm2 2J	0.5 mW/cm2 4J	0.6 mW/cm2 2J	0.6 mW/cm2 4J
Nostoc	34.83 ± .02	32.80 ± .01	30.09 ± .02	28.12 ± .02	24.18 ± .01	23.29 ± .02	20.40 ± .02
Anabaena	35.78 ± .02	31.91 ± .01	27.70 ± .01	25.16 ± .02	21.41 ± .02	22.62 ± .02	18.26 ± .03
Scytonema	34.26 ± .01	32.98 ± .02	24.40 ± .02	26.60 ± .02	24.67 ± .02	23.07 ± .02	18.22 ± .03

Note. Values are arithemetic mean ± SD of 3 determinations. $P < 0.001$ in comparison with control.

TABLE 5

Effect of UVB on Biomass Yield (mg/100 ml) of Cyanobacteria

Species	Control	0.4 mW/cm2 2J	0.4 mW/cm2 4J	0.5 mW/cm2 2J	0.5 mW/cm2 4J	0.6 mW/cm2 2J	0.6 mW/cm2 4J
Nostoc	130 ± 2	111 ± 2.5	102 ± 2	115 ± 4	101 ± 2.5	92 ± 1.2	81 ± 2
Anabaena	166 ± 2	148 ± 3	138 ± 1.6	121 ± 3	118 ± 3	120 ± 3.1	113 ± 2.4
Scytonema	128 ± .8	123 ± 2	109 ± 2	117 ± 1.7	112 ± 1.4	108 ± 1.7	93 ± 2

Note. Values are arithemetic mean ± SD of 3 determinations. $P < 0.001$ in comparison with control.

order of reduction in the biomass yield of the phytoplankton sps upon UVB exposure was as follows: Nostoc>Anabaena>Scytonema.

Visible and ultraviolet solar radiation are essential for all forms of life on the earth and photochemical reactions are some of the most important processes taking place in our environment today. However, depletion of the ozone layer by man-made chemicals is contributing to increased penetration of UVB on the surface of Earth. Ambient solar UVB radiation is currently an important limiting ecological factor and even a small increment in its level could result in significant changes (18). In marine ecosystem, UVB penetrates approximately the upper 10% of the marine euphotic zone before it is reduced by 99% of its surface irradiance (19). This clearly indicates that in aquatic ecosystem both phytoplankton and zooplankton are equally vulnerable to UVB effects (20). A three year long study to determine the ground level intensity of UVB has demonstrated that the solar UVB intensity ranged between 0.4-0.6 mW/cm^2 (average 0.5 mW/cm^2) near our laboratory (V. Babu, R.B. Misra, R.S. Ray, P.C. Joshi; unpublished data). This observation formed the basis for studying the effect of UVB on cyanobacteria at 0.4, 0.5 and 0.6 mW/cm^2, representing a 20% loss, ambient UVB and 20% gain, respectively in the UVB level on the surface of earth. Nostoc, Anabaena and Scytonema species of cyanobacteria were selected due to their world wide abundance in marine and terrestrial ecosystem. A two way analysis of variance of data reported in Table 1-5 showed significant interaction effect ($P<0.001$) between the intensity of UVB and the exposure dose (intensity × time). Study also revealed significant ($P<0.001$) changes in the growth, chlorophyll, protein, starch contents and biomass yield in all the exposed groups. The inhibition of specific growth rate was in accordance with the inhibition of photosynthesis. We believe that a significant ($P<0.001$) positive correlation between growth rate and carbon fixation could be responsible for the retardation of growth rate. The low photosynthetic pigment content of the cells could also lead to decrease in the level of other parameters, a result of decreased carbon and N_2 assimilation or due to enhanced catabolism. Anabaena flos-Aqual nitrogenase activity has been found to decline specifically during irradiation under low level of UVB (8). Therefore, the concern that phytoplankton communities confined to near-surface waters of the marginal ice zone in Antarctic water will be harmed by increased UVB irradiance pertaining to ocean surface, thereby altering the dynamics of Antarctic marine ecosystems (21) is substantiated from our observation. Phytoplankton serve as the source of food for primary consumers which in turn are consumed by the secondary and tertiary consumers in the marine food web. Therefore, any change in the biomass content will have an adverse effect in the marine food supply. The loss of phytoplankton biomass will also affect soil fertility, fixing the N_2 from environment and cleaning up the pollution load through biodegradation.

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