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Differential Responses of Three Cyanobacteria to UV-B and Cd

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Abstract Interactive effects of UV-B and Cd on growth, pigment, photosynthesis, and lipid peroxidation have been studied in *Anabaena*, *Microcystis*, and *Nostoc*; all the tested cyanobacteria showed differential sensitivity to different dosage of UV-B and Cd alone as well as in combination. Phycocyanin was severely affected by UV-B and Cd by all the strains; the degree of pigment bleaching was most pronounced in *Anabaena* followed by *Microcystis* and *Nostoc*. UV-B_2+Cd_2 produced nearly 83, 78, and 65% inhibition of phycocyanin in *Anabaena*, *Microcystis*, and *Nostoc*, respectively. The above treatment also significantly decreased the contents of Chl *a* and carotenoid. Presence of capsule in *Microcystis* protected the phycocyanin bleaching as compared to decapsulated cells. Laboratory-grown *Microcystis* revealed about 75 and 80% inhibition, following UV-B_2+Cd_2 treatment, respectively, in capsulated and decapsulated cells. Damage caused by Cd was more pronounced than UV-B. Inhibition of photosynthesis did occur in all the test strains, being maximum in *Anabaena*. PS II was the most sensitive component of the electron transport chain, showing 84, 80, and 70% inhibition in *Anabaena*, *Microcystis*, and *Nostoc*, respectively. As compared to control, significant lipid peroxidation (6.5-fold higher) was observed in *Anabaena* with UV-B_2+Cd_2. ^{14}C-uptake was more susceptible to Cd and UV-B than oxygen-evolution. Approximately 84, 80, and 76% inhibition of ^{14}C-uptake was observed in *Anabaena*, *Microcystis*, and *Nostoc*, respectively. Similarly, UV-B_2+Cd_2 inhibited ATP content of *Anabaena* by 87%. This study suggests that inhibition of carbon fixation was due to decreased ATP content of the test cyanobacteria by UV-B+Cd, where *Anabaena* was the most sensitive and *Nostoc* the most tolerant.

Key words: *Anabaena doliolum*, *Microcystis*, *Nostoc muscorum*, LC_{50}, UV-B irradiation, cadmium

The biological effectiveness of UV radiation increases logarithmically with decreasing wavelength. This increase is primarily due to increased overlap between the absorption spectra of nucleic acids and proteins. Earlier studies demonstrated that increased flux of UV-B (280–315 nm) not only hampers phytoplankton productivity [36], but also affects growth, motility, pigmentation, nitrogen metabolism, and carbon fixation of flagellated algae and cyanobacteria [12, 34]. In non-photosynthetic microorganisms, DNA is the main target for UV-B: It absorbs ~50% of the incident UV-B radiation. In contrast, photosynthetic cyanobacteria accumulate UV-A/UV-B screening pigments to attenuate UVR damage. The photoprotection is not complete, and the absorbance in the UV range is between 30 to 70% of the incoming radiation [1]. Light-harvesting protein complexes and chlorophyll are thought to absorb more than 99% of the incoming UV-B in photosynthetic organisms [16]. Research conducted to understand the mechanism of inhibition of photosynthesis has focused mainly on isolated chloroplast of higher plants and demonstrated that UV-B not only affects the dark steps of enzyme catalyzed CO_2 fixation, but also alters the light-driven primary photochemical reactions, mainly PS II, by affecting either the O_2-evolving site, as known for spinach and barley [33], or the PS II reaction center of soyabean and *Amaranthus* [15, 38].

Due to increased industrial activities, the level of heavy metals including Cd has increased in the aquatic ecosystem to such an extent as to produce measurable impacts on the indigenous biota [4]. Phytoplanktons are among the primary accumulators of metals in these habitats, because of the chemical reactivity of their transport ligands and comparatively high biomass. Toxic concentrations of trace metals are known to cause major changes in the metabolic variables of phytoplanktons apart from the production of metal binding compounds. Cd toxicity to algae and cyanobacteria has been extensively studied with respect to growth [28] and carbon fixation [39].

Rai *et al.* [30] provided first-hand information on the interactive effects of UV-B and Cu on photosynthetic activity of *Anabaena doliolum*. However, nothing is known about the interactive effect of UV-B and Cd on different cyanobacteria. The aim of the present investigation was to

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make a comparative assessment of the effect of UV-B and Cd on growth, survival, pigmentation, carbon fixation, O_2-evolution, electron transport system, ATP content, and lipid peroxidation of three cyanobacteria. It was further aimed to find out if *Nostoc* and *Anabaena* would show any difference in their metabolic behaviors following exposure to the two stresses, and how much these would be different from *Microcystis* (a naturally capsulated non-nitrogen fixing cyanobacterium).

MATERIALS AND METHODS

Organisms and Growth Condition

Anabaena doliolum and *Nostoc muscorum* were grown in modified Chu-10 medium [9] and *Microcystis* in DP medium [24] at 24±2°C under 72 μmol photon m^{-2} s^{-1} PAR light intensity with a photoperiod of 14:10 h. The cultures were shaken by hand 2–3 times daily. Stock solution of $CdCl_2 \cdot 2H_2O$ (100 μg ml^{-1}) was prepared in glass distilled water and sterilized by passing through a Millipore membrane filter (0.22 μm). All experiments were conducted in triplicate and repeated at least twice to confirm the reproducibility of the results.

Mode and Source of UV-B Irradiation

Culture suspensions (10 ml) were transferred to sterile 75-mm glass petridish and exposed to artificial UV-B radiation (from UV-B lamp, CAT No 34408, fotodyne, Inc. USA giving its maximum output at 310 nm). The desired dose (12.9 mWm^{-2} nm^{-1}) was obtained by adjusting the distance between the UV-B light source and the cyanobacterial suspension kept in an open petridish. The above dose was selected, based on the latitude of our workstation (25°N), and the mean percentage depletion of ozone layer as calculated by Crutzen [5] and Smith *et al.* [36]. The algal suspension was stirred continuously with a magnetic stirrer to allow uniform exposure.

Measurement of Survival and Growth

The cyanobacterial cells exposed to UV-B for 0–3 h duration were withdrawn at regular intervals and poured onto agar plates. For measuring their survival against Cd, cells were subjected to different concentrations (0–1 μg ml^{-1}) of Cd. The LC_{50} doses of UV-B and Cd were determined by plate colony count method [29]. Approximately 50% (LC_{50}) survival of the test cyanobacteria was observed after 26, 35, and 42 min for UV-B and at 0.02, 0.03, and 0.05 μg ml^{-1} Cd for *Anabaena*, *Microcystis*, and *Nostoc*, respectively. The doses of UV-B and Cd selected for further study were LC_{50} (denoted as UV-B_2 and Cd_2). Growth was determined by estimating protein content at regular intervals for 15 days. Protein content was quantified by the method of Lowry *et al.* [20] with lysozyme as standard. Unless otherwise stated, all experiments were performed with exponentially growing cultures having an initial dry weight of approximately 0.15 mg ml^{-1}.

Estimation of Pigments

For extraction of photosynthetic pigment, a known volume of algal culture was centrifuged and the pellet was suspended in a desired volume of acetone (80%). After overnight incubation at 4°C the suspension was centrifuged and the supernatant was used for the measurement of chlorophyll *a* and carotenoid contents by determining O.D. at 665 and 480 nm, respectively, with a Baush and Lomb Spectronic-20 colorimeter. Pellet was used for the extraction of phycocyanin by freezing and thawing, and the blue suspension formed in Milli 'Q' water was recorded at 610 nm. Chlorophyll *a* and carotenoid were calculated according to Mackinney [21] and phycocyanin according to Brody and Brody [3]. The total amount of carotenoid was calculated using the specific absorption coefficient, described by Myers and Kratz [23].

Measurement of O_2-Evolution and Carbon Fixation

The O_2-evolution was measured at 25°C under an illumination of 300 μmol m^{-2} s^{-1} PAR using a clark-type O_2 electrode (Digital O_2 system, Model-10, Rank Brothers, U.K.). For oxygen-evolution, a 5 ml culture was taken into the vessel and the oxygen evolved was measured for 5 min under light. Carbon fixation was measured according to the method described by Rai and Raizada [29]. A 0.2 ml sodium bicarbonate ($NaH^{14}CO_3$) solution was added to a 1 ml algal culture and kept in light for 2 h. The reaction was stopped by adding 0.2 ml of 50% acetic acid and bubbling with air for 5 min. This was then followed by the addition of 5 ml of scintillation cocktail. The counting was done in a Beckman model-LS6500 liquid scintillation counter and rate of ^{14}C uptake was expressed in cpm.

Measurement of Photosynthetic Electron Transport Chain

Electron transport activities were measured according to the methods described by Rai and Raizada [29] and Lien [19]. The activity was determined by three basic assays. A cell-free thylakoid membrane was prepared by sonication following the method of Lien [19]. Activity of photosystem I (PS I) was determined in the presence of DCMU (10^{-5} μM) by recording O_2 consumption after the addition of ascorbate (5 μM), 2,6-dichlorphenol indophenol (DCPIP, 50 μM), and methyl viologen (MV, 1.4 mM). Likewise, photosystem II (PSII) activity was measured in terms of O_2-evolution in the presence of p-bezoquinone (PBQ, 2 mM). The whole chain electron transport (PSI and PSII dependent) was measured in the presence of potassium ferricyanide [K_3 Fe $(CN)_6$; 80 μM] as electron acceptor and H_2O as electron donor.

The overall reaction can be expressed as:

1. Ascorbate → DCPIP → MV (PSI)
2. H_2O → PBQ (PSII)
3. H_2O → Fericyanide (whole chain)

Depletion of *Microcystis* Capsule

The *Microcystis* capsule (slime layer or mucilaginous covering) was successfully removed by suspending the cells in deionized water for 1-2 h, until they rise to the surface. The cells were skimmed off and put on a 100% polyester fabric (Gloria Shirting, Bombay Dyeing Corporation, India) that was presoaked several times in Milli 'Q' water. Deionized distilled water (1-2 liters) was passed through the cell biomass and the presence of capsule was examined microscopically after staining with India ink. This step was repeated several times, until the capsule was fully removed from the cells.

Measurement of ATP Content

The size of the ATP pool of the test cyanobacteria was determined immediately after treatment with UV-B and Cd according to the method of Larson and Olsson [17]. ATP was extracted by 4% TCA supplemented with 2 mM EDTA, and the total content was measured by a Luciferin Luciferase assay using a LKB 1250 Luminometer.

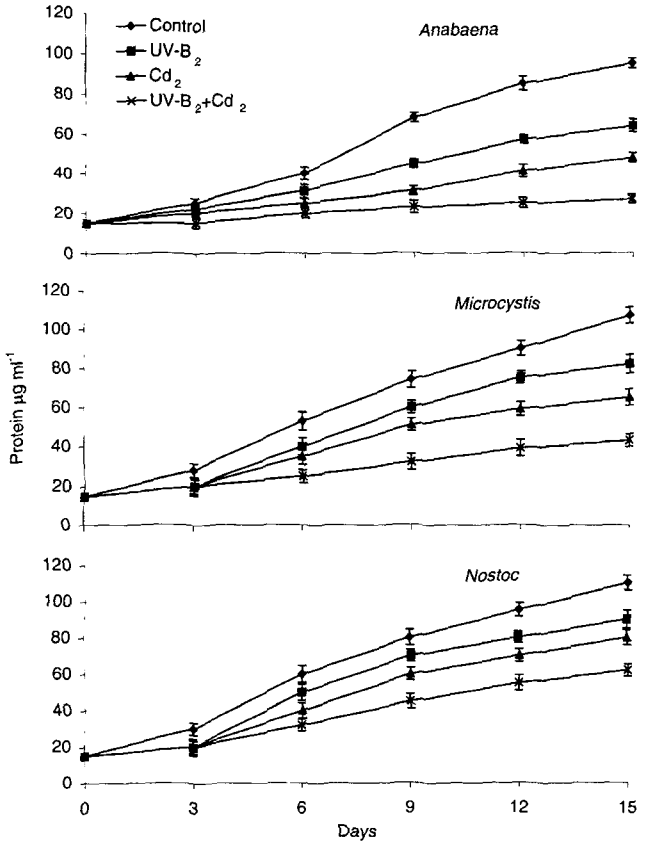

Fig. 1. Growth behavior of *Anabaena, Microcystis*, and *Nostoc* under UV-B and Cd.

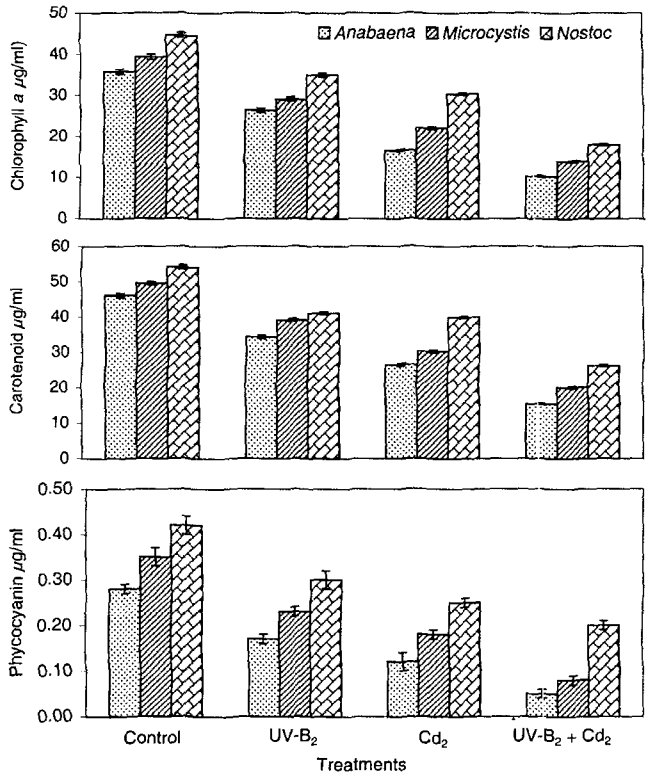

Fig. 2. Pigment contents of *Anabaena, Microcystis*, and *Nostoc* under UV-B and Cd.
Symbols: $UV\text{-}B_2$=LC_{50} of UV-B, Cd_2=LC_{50} of Cd.

Lipid Peroxidation

Peroxidation of lipids by UV-B and Cd individually and in combination was measured according to the TBA-rm (thiobarbituric acid-reactive material) method of De Vos *et al.* [6] and the values were presented as the differences between the peroxidized lipids in the control and in the treated cells.

Results

Survival and Growth Behaviour

Approximately 50% survival of *Anabaena doliolum*, *Microcystis*, and *Nostoc* was noticed after 26, 33, and 42 min of UV-B exposure, respectively. However, in the case of Cd, the LC_{50} concentrations were 0.02, 0.03, and 0.05 µg ml^{-1} for *Anabaena*, *Microcystis*, and *Nostoc*, respectively. Figure 1 shows inhibition of growth of all the cyanobacteria under UV-B and Cd; inhibition induced by UV-B and Cd together reached upto 72, 63, and 59% for *Anabaena*, *Microcystis*, and *Nostoc*, respectively.

Figure 2 shows inhibition of photosynthetic pigments by all the test cyanobacteria under UV-B and Cd stress. The pattern of inhibition of all the pigments was similar, except for phycocyanin which was maximally affected. This was

Table 1. Phycocyanin content in capsulated and decapsulated *Microcystis* from lab and field samples.

Treatments	*Microcystis* (Lab)	
	Capsulated (Phycocyanin μg/ml)	Decapsulated (Phycocyanin μg/ml)
Control	0.24±0.01	0.20±0.01
UV-B_2	0.14±0.01 (42)	0.11±0.02 (45)
Cd_2	0.10±0.02 (59)	0.09±0.02 (55)
UV-B_2+Cd_2	0.06±0.01 (75)	0.05±0.01 (80)
	Microcystis (Field)	
	Capsulated (Phycocyanin μg/ml)	Decapsulated (Phycocyanin μg/ml)
Control	0.28±0.01	0.34±0.03
UV-B_2	0.20±0.01 (29)	0.19±0.01 (45)
Cd_2	0.16±0.01 (43)	0.15±0.01 (56)
UV-B_2+Cd_2	0.09±0.03 (68)	0.10±0.01 (72)

Data in parentheses denote percent inhibition. All the treatments are significantly different ($p<0.05$) from their respective controls. All the values are mean±SD. UV-B_2 and Cd_2 are LC_{50} dose of UV-B radiation and cadmium, respectively.

followed in turn by chlorophyll *a* and carotenoids in all the three strains. Pigment inhibition was most pronounced in *Anabaena* followed by *Microcystis* and *Nostoc*. Following treatment with UV-B_2+Cd_2 in combination, there was approximately 83, 78, and 65% inhibition of phycocyanin and 67, 60, and 52% of carotenoid, respectively, in *Anabaena*, *Microcystis*, and *Nostoc*. Thus, the combination of LC_{50} of UV-B and Cd produced an additive inhibition (x^2 significant, $p<0.025$) of the pigments.

Table 1 shows the phycocyanin content in capsulated and decapsulated *Microcystis* from laboratory and field. The field isolate contained a higher amount of phycocyanin than the lab for both decapsulated and capsulated cells. However, the inhibition of phycocyanin content was more in the lab than in the field sample. Combination of UV-B_2 and Cd_2 reduced the phycocyanin content by 75 and 80% and 68 and 72% in capsulated and decapsulated cells from lab and field, respectively.

Photosynthesis

Table 2 presents data on the oxygen-evolution, carbon-fixation, and ATP content of *Anabaena*, *Microcystis*, and *Nostoc* exposed to UV-B alone as well as in combination with Cd. The O_2-evolution of *Anabaena*, *Microcystis*, and *Nostoc* was reduced to the extent of 82, 77, and 70%, respectively, following exposure to UV-B_2+Cd_2. ANOVA indicated that the inhibition was significant ($p<0.01$); this being higher for Cd than UV-B (Table 2). $^{14}CO_2$-fixation was reduced by 42, 38, and 32% at LC_{50} of UV-B, and by 51, 48, and 42% with LC_{50} of Cd, respectively, for *Anabaena*, *Microcystis*, and *Nostoc*. Interactively, UV-B_2 and Cd_2 inhibited ($p<0.01$) the $^{14}CO_2$-fixation by 84, 80, and 76% for *Anabaena*, *Microcystis*, and *Nostoc*, respectively. Inhibition produced jointly by UV-B and Cd was additive in nature. Inhibition of the ATP content was also dose dependent. It was inhibited by 87, 83, and 79% with UV-

Table 2. Interactive effects of UV-B and Cd on oxygen evolution, CO_2-fixation, and ATP content of *Anabaena doliolum*, *Microcystis*, and *Nostoc muscorum*.

Treatments	O_2-evolution (μmol O_2 evolved μg^{-1} protein h^{-1})			CO_2-fixation (CPM×10^3)			ATP pool (μmol ATP μg^{-1} protein)		
	Anabaena	*Microcystis*	*Nostoc*	*Anabaena*	*Microcystis*	*Nostoc*	*Anabaena*	*Microcystis*	*Nostoc*
Control	50.6±0.5	60.2±0.4	85.2±0.6	15.0±0.6	25.6±0.5	42.0±0.4	2.62±0.02	3.49±0.02	4.62±0.04
UV-B_1	40.2±0.4 (21)	50.0±0.5 (17)	72.0±0.6 (16)	12.0±0.2 (20)	21.0±0.2 (18)	35.7±0.3 (15)	2.04±0.01 (22)	2.79±0.02 (20)	3.78±0.03 (18)
UV-B_2	30.4±0.3 (40)	39.7±0.3 (35)	59.9±0.3 (30)	8.7±0.2 (42)	15.8±0.2 (38)	28.5±0.3 (32)	1.29±0.01 (51)	2.02±0.01 (43)	2.96±0.02 (36)
Cd_1	35.2±0.3 (39)	45.2±0.4 (25)	67.2±0.2 (22)	10.9±0.2 (27)	19.9±0.2 (22)	33.6±0.3 (20)	1.93±0.01 (26)	2.68±0.02 (23)	3.64±0.02 (21)
Cd_2	27.4±0.3 (46)	35.2±0.3 (42)	52.0±0.4 (40)	7.3±0.4 (51)	13.3±0.4 (48)	24.3±0.4 (42)	1.20±0.01 (55)	1.71±0.01 (51)	2.12±0.02 (44)
UV-B_1+Cd_1	25.0±0.2 (51)	35.0±0.3 (43)	53.0±0.3 (38)	6.6±0.5 (54)	12.8±0.1 (50)	22.6±0.2 (46)	1.20±0.03 (54)	1.74±0.01 (50)	2.44±0.02 (47)
UV-B_1+Cd_2	17.4±0.2 (66)	25.2±0.2 (59)	40.2±0.3 (53)	5.1±0.5 (66)	10.7±0.3 (58)	21.4±0.2 (49)	1.10±0.01 (60)	1.60±0.01 (54)	2.31±0.02 (50)
UV-B_2+Cd_1	20.2±0.2 (60)	28.5±0.2 (53)	42.6±0.3 (50)	5.7±0.3 (62)	11.2±0.4 (56)	22.1±0.3 (52)	1.10±0.01 (58)	1.57±0.02 (55)	2.26±0.02 (51)
UV-B_2+Cd_2	9.2±0.3 (82)	14.3±0.1 (77)	25.6±0.3 (70)	2.4±0.2 (84)	5.1±0.3 (80)	10.0±0.1 (76)	0.36±0.01 (87)	0.62±0.01 (83)	0.97±0.01 (79)

Data in parentheses denote percent inhibition. ANOVA significant at $p<0.02$; Cd_1 and UV-B_1 are LC_{25} dose, while Cd_2 and UV-B_2 are LC_{50} doses of Cd and UV-B, respectively. All the values are mean±SD.

Table 3. Interactive effects of UV-B and Cd on electron transport chain of *Anabaena doliolum*, *Microcystis*, and *Nostoc muscorum*.

Treatments	*Anabaena*			*Microcystis*			*Nostoc*		
	PS-I activity (μmol O_2 consumed μg^{-1} protein h^{-1})	PS-II activity (μmol O_2 evolved μg^{-1} protein h^{-1})	Whole chain (μmol O_2 evolved μg^{-1} protein h^{-1})	PS-I activity (μmol O_2 consumed μg^{-1} protein h^{-1})	PS-II activity (μmol O_2 evolved μg^{-1} protein h^{-1})	Whole chain (μmol O_2 evolved μg^{-1} protein h^{-1})	PS-I activity (μmol O_2 consumed μg^{-1} protein h^{-1})	PS-II activity (μmol O_2 evolved μg^{-1} protein h^{-1})	Whole chain (μmol O_2 evolved μg^{-1} protein h^{-1})
Control	51.2±0.5	62.3±0.3	46.3±0.4	59.7±0.6	65.7±0.7	54.3±0.5	72.3±0.7	88.2±0.6	70.2±0.6
UV-B_1	38.9±0.3 (24)	45.5±0.5 (27)	35.1±0.3 (24)	47.1±0.4 (21)	49.2±0.4 (25)	42.4±0.4 (22)	59.2±0.5 (18)	68.8±0.6 (22)	56.9±0.6 (19)
UV-B_2	29.2±0.2 (44)	27.3±0.2 (57)	26.2±0.4 (46)	35.4±0.3 (41)	30.8±0.3 (53)	32.2±0.3 (41)	42.2±0.4 (40)	44.2±0.3 (50)	49.2±0.4 (30)
Cd_1	35.8±0.3 (30)	42.3±0.4 (32)	32.8±0.4 (29)	44.7±0.4 (25)	46.6±0.4 (29)	41.3±0.4 (24)	57.8±0.4 (20)	66.1±0.4 (25)	56.2±0.5 (20)
Cd_2	24.9±0.2 (52)	24.6±0.2 (61)	20.9±0.2 (55)	31.3±0.3 (48)	29.2±0.2 (56)	28.6±0.4 (48)	39.7±0.6 (45)	39.9±0.3 (55)	40.2±0.4 (43)
UV-B_1+Cd_1	22.5±0.2 (56)	25.5±0.3 (59)	18.6±0.2 (57)	28.6±0.2 (52)	31.5±0.3 (52)	26.6±0.2 (51)	38.3±0.3 (47)	46.7±0.4 (47)	37.9±0.3 (46)
UV-B_1+Cd_2	20.4±0.2 (60)	22.4±0.4 (64)	18.9±0.2 (59)	26.2±0.3 (56)	26.2±0.2 (60)	27.1±0.2 (50)	36.1±0.3 (50)	39.7±0.3 (55)	38.6±0.4 (45)
UV-B_2+Cd_1	21.0±0.2 (59)	24.6±0.3 (62)	19.4±0.4 (58)	26.8±0.4 (55)	26.9±0.2 (59)	27.7±0.2 (49)	36.8±0.3 (49)	42.3±0.5 (52)	40.7±0.4 (42)
UV-B_2+Cd_2	14.2±0.1 (73)	10.2±0.2 (84)	14.2±0.2 (70)	18.0±0.1 (70)	14.2±0.2 (80)	18.0±0.1 (67)	23.1±0.2 (68)	20.2±0.3 (78)	26.2±0.3 (62)

Data in parentheses denote percent inhibition. ANOVA significant at $p<0.05$. Symbols are the same as in Table 2. All the values are mean±SD.

B_2+Cd_2 for *Anabaena*, *Microcystis*, and *Nostoc*, respectively. Of all the photosynthetic variables, ATP content depicted the highest and highly significant ($p<0.01$) inhibition.

Hill Reaction Assay

All the three reaction centers of the electron transport system were inhibited severely by UV-B and Cd (Table 3); inhibition was less than the additive type. Inhibition of PS II and whole chain due to a combination of UV-B_2+Cd_2 was 84 and 70, 80 and 67, 78 and 62% for *Anabaena*, *Microcystis*, and *Nostoc*, respectively. The oxygen consumption in PS I was inhibited by 73, 70, and 65% in *Anabaena*,

Table 4. Interactive effects of UV-B and Cd on lipid peroxidation of *Anabaena*, *Microcystis* and *Nostoc*.

Treatments	Lipid peroxidation TBA-rm ($E_{532-600}$ mg^{-1} protein)		
	Anabaena	*Microcystis*	*Nostoc*
Control	0.04±0.01	0.05±0.01	0.04±0.01
UV-B_1	0.10±0.01	0.06±0.01	0.05±0.01
UV-B_2	0.17±0.02	0.11±0.02	0.08±0.02
Cd_1	0.15±0.01	0.10±0.02	0.07±0.01
Cd_2	0.20±0.02	0.15±0.01	0.10±0.02
UV-B_1+Cd_1	0.28±0.02	0.19±0.01	0.11±0.02
UV-B_1+Cd_2	0.32±0.01	0.23±0.01	0.14±0.02
UV-B_2+Cd_1	0.30±0.02	0.21±0.03	0.13±0.02
UV-B_2+Cd_2	0.39±0.03	0.25±0.02	0.15±0.03

All the treatments are significantly different ($p<0.01$) from their respective controls. All the values are mean±SD. Symbols are the same as in Table 2.

Microcystis, and *Nostoc*, respectively. The inhibition produced by the UV-B and Cd individually as well as in combination was significant ($p<0.01$); PS II was maximally inhibited in all the three test cyanobacteria.

Lipid Peroxidation

Table 4 shows significant ($p<0.01$) inhibitory effects of UV-B and Cd on lipid peroxidation. Approximately 3.3, 3.0, and 2.5 and 2.8, 2.2, and 2.0 fold increase in lipid peroxidation following Cd_2 and UV-B_2 treatment was observed, respectively, for *Anabaena*, *Microcystis*, and *Nostoc*. A combination of UV-B_2+Cd_2 increased the peroxidation by 6.5, 5.0, and 3.7 fold for *Anabaena*, *Microcystis*, and *Nostoc*, respectively. The interactive effect of UV-B and Cd was significant ($p<0.01$).

DISCUSSION

Difference in the growth behavior and survival of three cyanobacterial strains following UV-B and Cd treatment might be due to the presence or absence of (i) sheath and (ii) UV protective compounds such as scytonemin or mycosporine-like amino acids (MAAs). Reduction of Cd toxicity by mucilagenous sheath was reflected by a greater sensitivity of *Anabaena* than *Nostoc* and *Microcystis*, which contain sheath. Growth parameters investigated showed significant inhibition ($p<0.01$) by UV-B and Cd. This can be explained by the proposal put forward by several

workers that cellular constituents responsible for absorbing radiation between 280 and 320 nm are destroyed by UV-B, which further affect membrane permeability and protein, eventually resulting in the death of the cell [35].

The drastic reduction in phycocyanin compared to any other pigment is in accordance with earlier work on cyanobacteria [8] and cryptophyceae [11]. It has been shown that strong UV radiations can photooxidize and bleach all types of photosynthetic pigments [13]. Phycocyanin is a protein having a rapid turnover of degradation and resynthesis. Its rate of synthesis is affected by protein synthesis. Carotenoid synthesis depends upon preexisting enzyme, and regulation of the synthesized carotenoid depends on its concentration. In the accessory pigments, the phycobillins were bleached first, indicating that the major accessory pigments of cyanobacteria were affected much more than other pigments in other algal groups. Our data on carotenoids, which serve the dual function of photoprotection and energy transduction to the chlorophyll reaction centers, are supported by the findings of Haberlein and Häder [11] on some members of cryptophyceae.

The composition of the capsule layer resembles the plant polysaccharide pectin, which is the methyl ether of pectic acid having a chain of at least 2,001–2,004 cross-linked D-galacturonic acid units. Pectin reacts strongly with metal cations and biosorbs them. The major constituents of *Microcystis* capsule are galacturonic acid and neutral carbohydrate. Pronounced inhibition of physiological variables in decapsulated as compared to capsulated cells suggests that a major portion of Cd is adsorbed onto the surface of cells, [25], hence reducing the toxicity of Cd. Present result can be explained by the findings of Rai *et al.* [32] on capsulated/decapsulated *Microcystis* treated with Cd and Ni. The capsule present on the cells also provides protection against UV-B and Cd. UV-B is thought to be unable to affect the linkage of acid and carbohydrate in capsulated cells.

It is evident from the results (Table 2) that $^{14}CO_2$-uptake in *Anabaena* was severely affected by UV-B and Cd exposure as compared to *Microcystis* and *Nostoc*. It has been postulated that UV-B exposure has a deleterious effect on the photosynthetic apparatus leading to a reduction in the supply of ATP and $NADPH_2$ [15]. UV-B-induced inhibition of $^{14}CO_2$-uptake in cyanobacteria may be due to reduced supply of ATP and $NADPH_2$. Our findings are supported by earlier work on cyanobacteria, [7] algae, and some plants [27]. The UV-B and Cd-induced reduction in O_2-evolution might be due to the inhibition of photosystem II as a result of thylakoid membrane damage of the reaction center [31]. This is supported by a significant correlation between oxygen-evolution and the PS II activity ($r=0.80$; $p<0.01$).

Decrease in ATP pool after UV-B and Cd treatment, as observed in all the three strains, may be due to the inhibition of electron transport system [31]. Maximum inhibition in *Anabaena* was followed by *Microcystis* and *Nostoc*; this trend was also noticed in the electron transport system. A significant positive correlation between ATP content and inhibition of whole chain ($r=0.58$; $p<0.001$) supports to our findings. It is known that UV-B causes an inactivation of the PS II reaction center, while PSI appears to be more resistant. Disruption of thylakoid integrity as a result of UV radiation may partly or wholly destroy the components required for photosythesis, which may in turn affect the rate of CO_2-fixation [15, 7].

Results pertaining to the inhibition of electron transport chain by UV-B irradiation resembled the findings of Kulandaivelu and Noorudeen [15] on *Amaranthus* and Bornman [2] on spinach. A significant inhibition of PS II activity not only supports the findings of Strid *et al.* [37] on UV-B and Prasad *et al.* [26] on Cd, but also confirms that PS II was more sensitive than PS I in all the above cases. Thus, UV-B and Cd-induced inhibition of the electron transport system seems to be the cause for reduction of ATP pool of the cells that conceivably resulted in inhibition of all the energy-requiring metabolic processes and ultimately the growth. The test cyanobacteria treated with UV-B and Cd together showed consumption, instead of evolution, of O_2.

UV-B radiation has been shown to increase the peroxidation of lipids in animal membrane system [14, 22] in algae and in some plants [27]. The UV-B-induced increase in lipid peroxidation suggests that fatty acids in thylakoid membranes are targets for UV-B. Degradation of fatty acids in photosynthetic organism or in reconstructed membrane systems following exposure to UV-B has been observed earlier [10]. All these can be achieved via the energetic change of covalent bonds [18] by UV-B-generated amino acid radicals that can oxidize fatty acids or through the activation of toxic O_2 molecules that can attack fatty acid chains. Whether lipid molecules were direct or indirect targets of UV-B/Cd damage in this study is not known.

We can conclude that continued ozone depletion resulting from increased solar UV-B radiation and increased metal concentration might adversely affect the cyanobacterial population, which in turn affects the productivity in the habitats jointly stressed by UV-B and Cd.

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