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UV-B radiation and cadmium induced changes in growth, photosynthesis, and antioxidant enzymes of cyanobacterium *Plectonema boryanum*

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Abstract

UV-B (0.4 W m^{-2}) irradiation and cadmium (2 and 8 μM) treatments separately inhibited the survival, growth, pigment content, and photosynthetic electron transport in *Plectonema boryanum*. Phycocyanin was the main target to UV-B and Cd and it was followed by chlorophyll *a* and carotenoids. UV-B and Cd caused strong inhibition on activities of photosystem 2 (PS2) and the whole electron transport chain, whereas photosystem 1 (PS1) was the least affected. UV-B and Cd treatment accelerated respiration, lipid peroxidation, and the activities of superoxide dismutase and catalase. However, enhancement in catalase activity was considerably less (5 - 50 %) as compared to SOD activity. As compared to individual treatment, the effect of their combination (UV-B + Cd) was more detrimental to the above parameters. A synergistic interaction of UV-B and Cd is probably due to increased cadmium uptake as a result of increased membrane permeability caused by lipid peroxidation in *P. boryanum* after UV-B exposure.

Additional key words: carotenoids, catalase, chlorophyll, lipid peroxidation, oxidative stress, photosynthetic electron transport activity, photosystem 1 and 2, superoxide dismutase.

Introduction

The anthropogenic depletion of the stratospheric ozone layer (Frederick and Lubin 1988) has resulted in an increased level of UV-B radiation on earth surface. Enhancement in UV-B region (280 - 315 nm) is of particular interest in view of its adverse effect on biological systems (Coohill 1991). The impact of increasing UV-B irradiation on growth, motility, and pigmentation has been investigated in algae and cyanobacteria (Häder and Häder 1989). UV-B irradiation affects also DNA, proteins, lipids, and photosynthetic pigments and alters the key physiological processes (Döhler 1985, Karentz *et al.* 1991, Tyagi *et al.* 1992, Vincent and Roy 1993, Rai *et al.* 1995, Adhikary 2003/4) which regulate algae proliferation and development in aquatic ecosystems. Besides UV-B, aquatic ecosystem may also suffer from exposure to increased metal concentrations. Cadmium is one of the most toxic aquatic contaminants and is found in very high concentrations, 5 to 120 g m^{-3} as reported by Mathur *et al.* (1987) from river Ganges in Varanasi, India. High concentrations of metals disrupt algal metabolism either by inactivating the photosynthetic machinery or enzymatic pathways (Mallick and Rai 1994). Therefore, in an ecosystem cyanobacterial growth could be affected by the interaction of these stresses, not by a single stress. Recently, the response of cyanobacterium *Anabaena doliolum* to a combination of Pb/Cu along with elevated UV-B radiation has been examined (Rai *et al.* 1995, 1998). The individual effects of UV-B and heavy metals on various physiological activities of algae and cyanobacteria have been investigated up to greater extent but very little attention have been paid to photosynthesis, lipid peroxidation, and antioxidant enzymes in these microorganisms following simultaneous exposure to both UV-B and heavy metals, a situation likely to exist in aquatic environments.

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Abbreviations: ASC - ascorbate; Car - carotenoids; Chl - chlorophyll; DCMU - 3-(3'-4'-dichlorophenyl)-1,1-dimethyl urea; DCPIP - 2,6-dichlorophenol indophenol; MDA - malondialdehyde; MV - methyl viologen; *p*BQ - *p*-benzoquinone; PC - phycocyanin; PS - photosystem; UV-B - ultraviolet radiation (280-315 nm).

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UV-B and heavy metals generate reactive oxygen species at various sites of respiratory and photosynthetic electron transport as well as during various biochemical reactions in cellular systems. These reactive oxygen species are highly deleterious for cell structures and functions (Halliwell and Gutteridge 1984, Hideg and Vass 1996, Foyer *et al*. 1997). In order to prevent the harmful effects caused by these stresses, organisms develop radical quenchers and antioxidants that provide protection by scavenging harmful radical or oxygen species. (Mittler and Tel-Or 1991, Middleton and Teramura 1993). Most of the available data on antioxidant response to increased UV-B and metals have been obtained separately for higher plants, and little is known for cyanobacteria. Therefore, this study has been undertaken to find out the individual and combined effects of UV-B and Cd on photosynthesis, antioxidant systems and lipid peroxidation in *P. boryanum.*

Materials and methods

Organism and growth conditions: *Plectonema boryanum* Gomont, a filamentous, non heterocystous cyanobacterium was isolated from rice field near Allahabad, India. Axenic culture of *P. boryanum* was maintained in the culture room at 27 ± 2 °C. For regular experiments, cultures were grown in N-containing BG-11 medium (pH 7.5) under photosynthetic photon flux density (PPFD) of 75 μmol m^{-2} s^{-1} and 14-h photoperiod.

UV-B and metal treatment: UV-B irradiation was provided by a single *Philips* (*TL 40W/12*, Eindhoven, The Netherlands) ultraviolet-B tube with main output at 312 nm. Culture suspension (dry mass 0.1 kg m^{-3}) taken in sterile 75 mm Petri dishes occupying the depth of 0.5 cm was irradiated under artificial irradiation by UV-B (280 - 315 nm). Samples were exposed for required time period to a PPFD of 20 μmol m^{-2} s^{-1} and 0.4 W m^{-2} UV-B. Culture suspension preincubated with tested dose of metal for 2 h was exposed to UV-B to observe combined effect. A second dose of UV-B was given on third day and the experiments were discharged on fourth day for studying various parameters. During UV-B irradiation, suspension was gently agitated by a magnetic stirrer to ensure uniform exposure. The desired dose of UV-B was obtained by adjusting the distance between UV-B source and cyanobacteria suspension. The radiation was filtered through 0.127 mm cellulose diacetate (*Johnston Industrial Plastics*, Toronto, Canada) to remove all incident UV-C (< 280 nm). Irradiance was measured with the help of power meter (*Spectra Physics*, USA, model *407*, *A-2*). Stock solution of $CdCl_2$ was prepared in glass sterilized distilled water and the solution was further sterilized by passing through *Millipore* membrane filter (0.22 μm). From the stock solution various required concentrations of Cd were prepared in BG-11 medium.

Survival and growth: To determine the survival, homogenised cyanobacterial cells were exposed to UV-B for 30, 60, 90, 120, and 180 min. After the predetermined time intervals, 0.05 cm^3 aliquot was withdrawn from each test sample and transferred to sterile solid agar plates supplemented with growth medium. For measuring survival against cadmium, cells were inoculated in agar plates containing 2, 4, 8, or 16 μM Cd. After 15 d of incubation in controlled conditions, the survival was recorded by counting the colonies of cyanobacterium with respect to untreated control. Growth experiments were performed in liquid medium and dry mass was determined after required time.

Photosynthetic pigment determination: Chlorophyll (Chl) *a* was extracted in 90 % methanol and its concentration was determined from absorbance at 663 nm using the method of Mackinney (1941). Total content of carotenoids (Car) was assayed by measuring absorbance of diethyl ether extract at 450 nm (Jensen 1978). Phycocyanin (PC) was extracted in 2.5 mM phosphate buffer (pH 7.0) after freezing and thawing and the amount was determined by the method of Bennett and Bogorad (1973).

Measurement of photosynthetic activity: Photosynthetic oxygen evolution in treated and untreated cyanobacterial cells was monitored in temperature controlled airtight reaction vessel at 28 °C for 5 min by using a Clark type oxygen electrode (*Rank Brothers*, Cambridge, UK). The cyanobacterial cells were irradiated by projector lamp with 360 μmol m^{-2} s^{-1} PPFD at the surface of vessel. Spheroplasts were prepared by the method of Spiller (1980) and suspended in a medium containing 0.5 M sucrose, 10 mM HEPES-NaOH, 5 mM K_2HPO_4, 10 mM $MgCl_2$, 2 % m/v bovine serum albumin pH 6.9. The electron transport activity in treated and untreated (control) spheroplast suspension was measured polarographically at 28 °C by providing a PPFD of 360 μmol m^{-2} s^{-1}. Photosystem (PS2) activity was measured as O_2 evolution by using 1 mM pBQ as an electron acceptor. The rate of whole chain (H_2O → methyl viologen, MV) and PS1 (ASC/DCPIP → MV) mediated electron transport in spheroplasts was measured as O_2 uptake. For the whole chain electron transport, the reaction mixture of 3 cm^3 contained 0.05 mM NaN_3 and 0.1 mM MV. For PS1 measurement, the reaction mixture contained 0.05 mM DCPIP, 1 mM Na-ascorbate, 10 μM DCMU, 0.05 mM NaN_3, and 0.1 mM MV. In each assay, spheroplasts equivalent to 3 g Chl *a* m^{-3} were used.

Assay of catalase and superoxide dismutase (SOD) activity: Catalase activity was determined by following O_2 release from dissociation of H_2O_2 for 1 min after the addition of 5 cm^3 of 50 mM phosphate buffer (pH 7.0) containing 50 mM H_2O_2 to 1 cm^3 of cell suspension in darkness (Egashira *et al.* 1989). Oxygen release due to enzymatic dissociation of H_2O_2 was measured by a Clark type O_2 electrode (*Rank Brothers*). Temperature around the vessel was maintained at 28 °C. To determine the activity of SOD, test samples were harvested by centrifugation and homogenised at 4 °C in 100 mM EDTA phosphate buffer (pH 7.8). Supernatant obtained after centrifugation of the homogenate at 20 000 *g* for 30 min was used as a crude extract for SOD spectrophotometric assay of Giannopolitis and Ries (1977) using 3 cm^3 of reaction mixture containing 1.3 mM riboflavin, 13 mM L-methionine, 0.05 M Na_2CO_3 (pH 10.2), 63 μM p-nitroblue tetrazolium chloride, and 0.1 cm^3 of crude extract.

Measurement of malondialdehyde (MDA): Thiobarbituric acid reactive substances production in test samples was measured by the method of Heath and Packer (1968).

Respiration rate of treated and untreated cyanobacterial cells was determined by measuring the O_2 consumption in darkness with the Clark type oxygen electrode at 28 °C for 5 min.

Results

Survival was based on colony counts of *P. boryanum* following UV-B irradiation for different exposure time (Fig. 1*A*). After 30 min of UV-B irradiation there was about 90 % (LD_{10}) survival, after 60 min 85 % (LD_{15}), and after 90 min 75 % (LD_{25}). Complete killing was observed after 180 min of UV-B irradiation. Likewise, the percent survival of *P. boryanum* treated with different concentrations of Cd was 10 % (LD_{10}), 16 % (LD_{16}), 25 % (LD_{25}) and 50 % (LD_{50}) at 2, 4, 8, and 16 μM Cd, respectively (Fig. 1*B*). All the doses of UV-B and cadmium were inhibitory but only LD_{10} (UV-B_{30} and Cd_2) and LD_{25} (UV-B_{90} and Cd_8) were selected for further study.

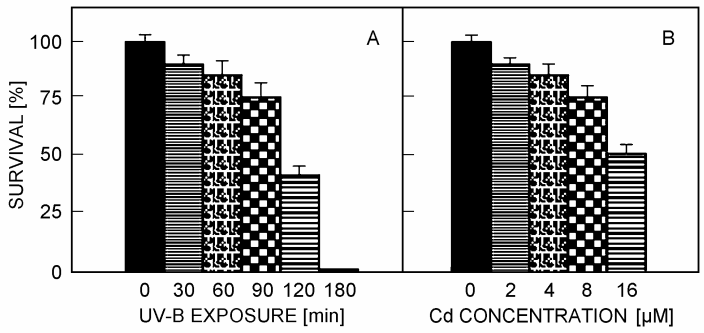

Fig. 1. Effects of UV-B (*A*) and Cd (*B*) on survival of *P. boryanum.* Means ± SE. All differences against the respective control are significant at $P < 0.01$.

Table 1. Effects of UV-B and Cd, singly and in combination, on growth and photosynthetic pigments of *P. boryanum.* Means ± SE, values in parentheses are [%]. All treatment are significantly different ($P < 0.01$) and (*$P < 0.05$) from control (Student's *t*-test). χ^2-test revealed that the interactive effects of UV-B with cadmium are significantly ($P < 0.01$ and **$P < 0.05$) higher than their additive values. ns - not significant.

Treatment	Growth [g(d.m.) m^{-3}]	Chl *a* [g m^{-3}]	Car [g m^{-3}]	PC [g m^{-3}]
Control	200 ± 5	3.47 ± 0.05	0.93 ± 0.01	22.50 ± 0.90
UV-B_{30}	170 ± 5 (15)*	2.91 ± 0.02 (16)	0.78 ± 0.01 (16)	18.00 ± 0.40 (20)
UV-B_{90}	140 ± 3 (30)	2.60 ± 0.05 (25)	0.72 ± 0.02 (22)	15.97 ± 0.60 (29)
Cd_2	180 ± 5(10)*	3.22 ± 0.02 (7)*	0.91 ± 0.01 (2)ns	19.80 ± 0.20 (12)*
Cd_8	140 ± 4 (30)	2.77 ± 0.04 (20)	0.87 ± 0.01 (6)*	18.00 ± 0.30 (20)
UV-B_{30} + Cd_2	110 ± 3 (45)	2.15 ± 0.03 (38)	0.60 ± 0.02 (35)	12.37 ± 0.60 (45)**
UV-B_{30} + Cd_8	80 ± 3 (60)**	1.73 ± 0.03 (50)**	0.51 ± 0.01 (45)**	1.01 ± 0.10 (55)**
UV-B_{90} + Cd_2	80 ± 3 (60)	1.67 ± 0.01 (52)	0.51 ± 0.01 (45)	7.87 ± 0.20 (65)
UV-B_{90} + Cd_8	40 ± 2 (80)	1.11 ± 0.01 (68)	0.41 ± 0.02 (56)	6.75 ± 0.30 (70)

Growth of *P. boryanum* was studied at various exposure times of UV-B and doses of Cd (data not shown). Only two selected doses of UV-B (UV-B_{30} and UV-B_{90}; 30 and 90 min exposure, respectively) and Cd (Cd_2 and Cd_8; 2 and 8 μM, respectively) were applied to demonstrate the effect of these stresses singly as well as in combination on growth (Table 1). UV-B and Cd reduced the growth of cyanobacterium significantly with increasing UV-B exposure time and cadmium concentration. The inhibitory effect became more severe when UV-B was combined with cadmium, as growth was decreased by 45 - 80 % under UV-B and Cd combinations. The χ^2 test revealed that the values for interactive effects of UV-B and Cd were statistically significant ($P < 0.01$ and $P < 0.05$).

Photosynthetic pigments: Contents of Chl *a*, Car and PC were reduced by UV-B_{30} and Cd, either alone or in combination (Table 1). Further decrease in contents of photosynthetic pigments was noticed with increasing exposure time of UV-B. The inhibitory effect of UV-B on the pigments became more pronounced when both applied doses of UV-B were combined separately with Cd_2 and Cd_8. The combinations generated synergistic reduction in pigment content. Phycocyanin was severely affected which was followed by Chl *a* and Car.

Photosynthetic activity: Photosynthetic oxygen evolution was decreased by 19 and 31 % following UV-B_{30} and UV-B_{90} exposure, respectively, whereas Cd_2 and Cd_8 doses reduced the photosynthetic O_2 evolution by 9 and 24 %, respectively. When both the stresses were combined together, more severe effect on oxygen evolution rate was observed, as it was declined by 44 - 73 % with the tested UV-B and Cd combinations (Fig. 2*A*, Table 2). On exposing spheroplasts to UV-B_{30} and UV-B_{60} as well as Cd_2 and Cd_8 singly, the activities of PS 2 and whole electron transport chain were drastically affected. Further deterioration in PS 2 and whole chain activities was recorded when the spheroplasts were treated with UV-B and Cd simultaneously. In contrast to this, marginal decrease in the PS1 activity was observed following the exposure of spheroplasts with these stresses, both singly and in combination.

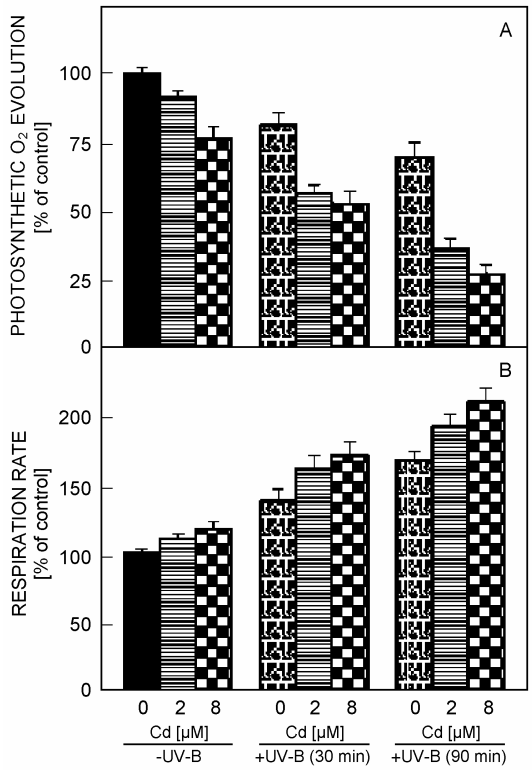

Fig. 2. Effects of UV-B and Cd, singly and in combination, on photosynthetic O_2 evolution (*A*) and respiration (*B*). Photosynthetic O_2 evolution and respiration rates in untreated control were 3.19 ± 0.1 μmol(O_2 evolved) g^{-1}(protein) s^{-1} and 1.23 ± 0.06 μmol(O_2 consumed) g^{-1}(protein) s^{-1}, respectively. Mean ± SE. All the values are significant at $P < 0.01$.

Table 2. Effects of UV-B and Cd, singly and in combination, on photosynthetic electron transport rate [μmol(O_2 evolved or consumed) g^{-1}(Chl *a*) s^{-1}] in spheroplasts of *P. boryanum.* Means ± SE, values in paranthesis are [%]. All treatment are significantly different ($P < 0.01$) and (*$P < 0.05$) from control (Student's *t*-test). χ^2-test revealed that the interactive effects of UV-B with cadmium are significantly (**$P < 0.01$ and ***$P < 0.05$) higher than their additive values. ns - not significant.

Treatment	PS1 (DCPIP/Asc → MV)	PS2 (H_2O → *p*BQ)	Whole chain (H_2O → MV)
Control	164 ± 1.0	78 ± 0.5	57 ± 0.3
UV-B_{30}	162 ± 0.5 (1)ns	60 ± 0.3 (23)	41 ± 0.2 (28)
UV-B_{90}	160 ± 0.5 (2)*	51 ± 0.2 (35)	33 ± 0.2 (42)
Cd_2	159 ± 1.0 (3)*	69 ± 0.3 (12)	47 ± 0.2 (17)
Cd_8	156 ± 0.5 (5)	62 ± 0.3 (20)	42 ± 0.1 (26)
UV-B_{30} + Cd_2	157 ± 0.8 (4)	39 ± 0.2 (50)***	23 ± 0.2 (60)***
UV-B_{30} + Cd_8	152 ± 0.5 (7)	31 ± 0.2 (60)**	18 ± 0.1 (68)***
UV-B_{90} + Cd_2	151 ± 0.5 (8)	26 ± 0.3 (67)**	14 ± 0.1 (75)***
UV-B_{90} + Cd_8	148 ± 0.3 (10)	19 ± 0.2 (76)**	7 ± 0.1 (88)***

Rate of respiration: In contrast to the photosynthetic activity, the rate of respiration in the cyanobacterial cells was increased significantly and was further enhanced in the cells treated with combined doses of UV-B and Cd (Fig. 2*B*).

Lipid peroxidation: UV-B and Cd separately induced the formation of MDA indicating enhanced lipid peroxidation in treated cells (Fig. 3*C*). Content of MDA was increased by 20 and 40 % with $UV\text{-}B_{30}$ and $UV\text{-}B_{90}$ exposures whereas with Cd_2 and Cd_8 treatment, the increase was 12 and 29 %, respectively. It was more pronounced when UV-B was combined with Cd. All the four tested combinations of UV-B with Cd accelerated peroxidation of lipids by 43 - 90 % over control sample, respectively.

Antioxidation enzymes: Exposure of cyanobacterium to $UV\text{-}B_{30}$ and $UV\text{-}B_{90}$ enhanced the activity of SOD by 25 and 40 % and catalase by 18 and 25 %, respectively. Similar to this, Cd enhanced the activity in a concentration dependent manner. Further enhancement in SOD and catalase activity was observed when UV-B was combined with cadmium. All the four combinations of UV-B with Cd enhanced SOD activity by 55 - 90 % and catalase activity by 20 - 62 %. The enhancement in the activity of SOD was comparatively larger than that of catalase (Fig. 3*A*,*B*).

Discussion

The decreasing trends in survival (Fig. 1*A*) following UV-B irradiation might be due to damage of the cellular constituents or inactivation of various vital processes, eventually causing death of the cells (Newton *et al.* 1979, Caldwell 1981, Häder *et al.* 1986). Decrease in survival following Cd exposure (Fig. 1*B*) indicated that Cd could have arrested the physiological and biochemical processes in *P. boryanum*. Cadmium causes damage to various metabolic processes (Stratton and Corke 1979, Wong 1979), pigment synthesis (Dubey 1997), and ultrastructure of membrane (Rai *et al.* 1990).

Like survival, growth of the *P. boryanum* was also inhibited following enhanced UV-B irradiation (Table 1) which showed similar inhibitory trend as observed in many cyanobacteria (Quesada *et al.* 1995, Sinha and Häder 1998). The results reveal that UV-B induced inhibition on growth of cyanobacterium might be due to irreparable damage to DNA, proteins, and photosynthetic apparatus as observed by Caldwell (1979), Newton *et al.* (1979), Melis *et al.* (1992), Friso *et al.* (1994) and Adhikary (2003/4). Cadmium at 2 and 8 μM significantly reduced the growth of *P. boryanum* which is in agreement with the results of Husaini and Rai (1991). Reduction in dry mass of the cyanobacterium following Cd treatment could have resulted from the inhibition of Chl synthesis and photosynthetic activity (Bazzaz and Govindjee 1974, Husaini and Rai 1991, Dubey 1997). These processes were further deteriorated when UV-B and Cd were applied simultaneously causing synergistic effect. In contrast to this, the increased respiration rate (Fig. 2 *B*) in *P. boryanum* following UV-B and Cd treatment, separately and in combination, indicated increased energy demands for active exclusion or sequestration of the heavy metal as noticed by Lösch and Köhl (1999). Significant decrease in the photosynthetic electron transport activity (Table 2) may also reduce photophosphorylation under Cd stress as reported in *N. muscorum* by Prasad *et al*. (1991). Hence, additional demand of ATP in this cyanobacterium following UV-B and Cd treatment may be based on energy supply through respiratory electron transport system and hence these stresses enhanced the respiration rate in *P. boryanum.* Similar to this, increased respiration rate was also noticed in *A. doliolum* and in some terrestrial plants following UV-B and Cd treatment (Rai *et al.* 1995, Lösch and Köhl 1999).

The decrease in photosynthetic O_2 evolution (Fig. 2 *A*) and photosynthetic electron transport activity (Table 2) may be correlated with the decrease in contents of photosynthetic pigments (Table 1) as observed in the present study. A decrease in photosynthetic pigment contents, particularly Chl *a*, Car, and PC (Table 1), was reported in cyanobacteria following enhanced UV-B irradiation (Tyagi *et al.* 1992, Sinha and Häder 1998). Chlorophylls exist in a highly organised state by forming complexes with proteins and lipids; therefore, the UV-B induced degradation of protein and lipids complexes associated with pigments in the thylakoid membrane resulted in the decrease of Chl *a* (Friso *et al.* 1994, Nedunchezhian *et al.* 1995, Rai *et al.* 1998). Car were less affected than the Chl *a* and PC in response to enhanced UV-B radiation, indicating the role of Car in cyanobacteria as a general defence against photooxidation under stress (Quesada *et al.* 1995). Phycocyanin was more sensitive than Chl *a* and Car, which is in consonance with the reports of Tyagi *et al.* (1992), and Sinha and Häder (1998). Strong damaging effect of UV-B on PC might be due to its localisation on the outer surface of thylakoid membrane which make it more prone towards stresses. Heavy metals degrade Chl *a* and Car when plants and cyanobacteria are exposed to their elevated concentration (Moustakas *et al.* 1997). In addition to this, Cd inhibits Chl and Car synthesis (Dubey 1997). A significant decrease in Chl and Car contents in *P. boryanum* supported the above view. However, both the stresses together might have caused synergistic effect on degradation and synthesis of Chl *a*, PC and Car. Strong inhibitory effect on PC could be due to the

binding of Cd to its sulphohydryl group, as reported for the other metals (Rai *et al*. 1990). Carotenoids protect Chl and photosynthetic membrane from photooxidative damage. Therefore, decline in Car content could have serious consequence on Chl, PC as well as thylakoid membrane which may lead to the reduction in photosynthetic efficiency of *P. boryanum.*

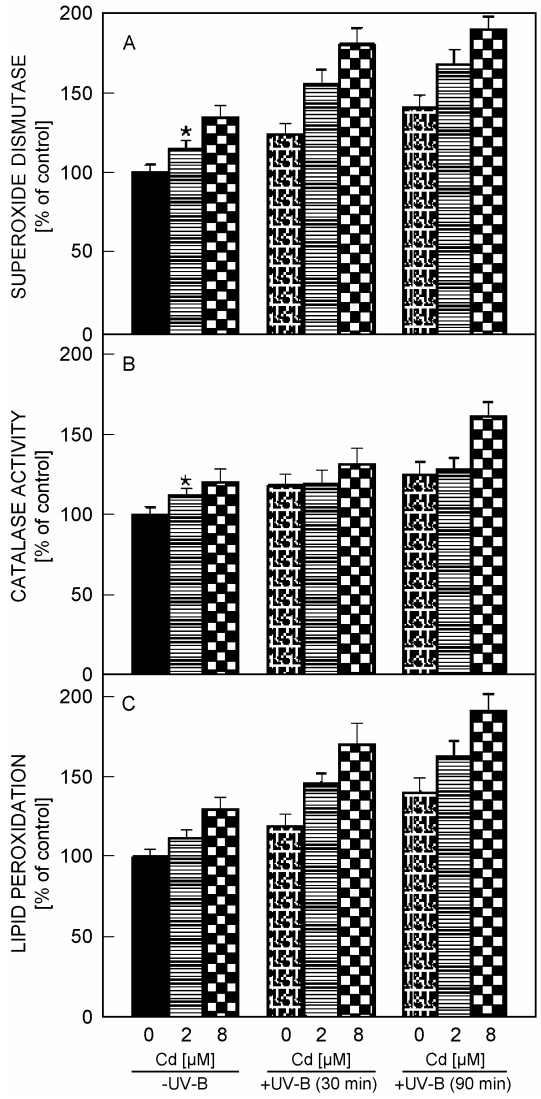

Fig. 3. Effects of UV-B and Cd, singly and in combination, on superoxide dismutase activity (*A*), catalase activity (*B*) and lipid peroxidation (*C*). Superoxide dismutase activity, catalase activity and lipid peroxidation in untreated control were, 56.0 ± 0.2 mmol(SOD) kg^{-1}(protein), 2000 ± 33 mmol(O_2 released) kg^{-1}(Chl *a*) s^{-1}, and 1.62 ± 0.04 mmol(MDA) kg^{-1}(protein), respectively. Mean ± SE. All the values are significant at $P < 0.01$ and $*P < 0.05$.

Photosynthetic oxygen evolution in intact cells (Fig. 2*A*) and photosynthetic electron transport activity in spheroplasts (Table 2) of *P. boryanum* were adversely affected by UV-B irradiation and Cd treatment. Similar effects were noticed in *A. doliolum* following UV-B and Cu treatment (Rai *et al.* 1995). In our study, the whole chain as well as the PS 2 activities were markedly affected when spheroplasts were treated with UV-B and Cd singly as well as in combination. The greater sensitivity of PS 2 towards Cd and UV-B could be due to interaction of these stresses with the oxygen evolving complex, carriers of oxidising as well as reducing side of PS 2 and reaction centre itself. In the earlier findings, Cd and UV-B singly caused deleterious effects on PS 2 activity in cyanobacteria (Husaini and Rai 1991, Rai *et al.* 1995) and in higher plants (Melis *et al.* 1992, Friso *et al.* 1994). However, the synergistic effect following combined treatment was probably either due to the action of these stresses on the same site or multiple sites of PS 2, as suggested by Prasad and Strzalka (1999). PS 1 is a highly conserved core complex (Almog *et al*. 1991), and inhibitory effects are either very small or even sometimes it is resistant against stresses. We found only a marginal inhibitory effect on PS 1 activity following UV-B and Cd, singly and in combination.

The treatment of UV-B and Cd might have resulted into the formation of active oxygen species (Halliwell and Gutteridge 1984, Dai *et al.* 1997). The generation of active oxygen species poses an intrinsic threat to plant tissue because many components within the cells are susceptible to oxidative damage (Foyer *et al.* 1997). Polyunsaturated fatty acids in membranes are prone to oxidative degradation (Girotti 1990) which results in the formation of various degradation products, particularly aldehydes like MDA. Its content increased after UV-B and Cu/Pb exposure (Rai *et al.* 1998, Mallick and Rai 1999). Similarly, in the present study Cd and UV-B, singly and in combination, enhanced the content of MDA (Fig. 3*C*). Increased content of MDA following lipid peroxidation indicates oxidative stress in *P. boryanum.* During oxidative stress protective systems get accelerated to keep the deleterious effect under control by scavenging free radicals. High SOD activity could be linked with stress tolerance efficiency. The increase in the activity of SOD of the *P. boryanum* observed in our study following Cd and UV-B treatment, singly and in combination (Fig. 3*A*), may be a consequence of production of O_2^- in the cells. A similar increased SOD activity has been demonstrated in the leaves of *Arabidopsis thaliana* and in marine microalgae *Tetraselmis gracilis* following Cd treatment (Okamoto *et al.* 1996, Skórzyńska-Polit *et al.* 2003) and in UV-B exposed cucumber seedlings (Kondo and Kawashima 2000). SOD is prominent bio-marker of defence against oxidative stress. It dismutates O_2^- radicals to H_2O_2. Catalase in turn destroys the toxic H_2O_2. A significant rise in catalase activity of *P. boryanum* following UV-B and Cd treatments (Fig. 3*B*) suggested that *P. boryanum* synthesised large amounts of these enzymes to scavenge the excess of O_2^- and H_2O_2. The increased activity of catalase in the cyanobacterium *A. doliolum* under Cu stress was reported by Mallick and

Rai (1999).

In conclusion, this study has demonstrated that UV-B and Cd caused significant reduction in survival, growth, contents of photosynthetic pigments, and activities of PS 2 and whole electron transport chain in *P. boryanum*. In contrast to this, respiration rate, lipid peroxidation, and SOD and catalase activities were enhanced. Both the stresses together exhibited synergistic effect. Thus, present findings suggest that UV-B and Cd, singly and together, alter the key physiological and biochemical processes of *P. boryanum*. The study also demonstrated, that other stresses should be taken into consideration while investigating the damaging impact of UV-B on living system.

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