UV-B INDUCED DIFFERENTIAL EFFECT ON GROWTH AND NITROGEN METABOLISM IN TWO CYANOBACTERIA UNDER COPPER TOXICITY

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Abstract

In the present study, impact of low (UV-B<sub>s</sub>: 0.1 µmol m<sup>-2</sup> s<sup>-1</sup>) and high (UV-B<sub>s</sub>: 1.0 µmol m<sup>-2</sup> s<sup>-1</sup>) fluence rates of ultraviolet-B on growth and nitrogen metabolism in two cyanobacteria: Phormidium foveolarum and Nostoc muscorum under copper toxicity (2 and 5 µM) was investigated after 24 and 72 h of experiments. Copper and UV-B<sub>s</sub> treatment suppressed growth but more in N. muscorum which was accompanied by significant accumulation of Cu. Nitrate and nitrite uptake rates and activities of nitrogen assimilating enzymes i.e. nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (GOGAT) except glutamate dehydrogenase activity (GDH; aminating) were decreased following treatments of Cu and UV-B<sub>s</sub>, and under combined treatments the effect was greater. On contrary, UV-B<sub>s</sub> declined Cu toxicity significantly. The study concludes that Cu and UV-B<sub>s</sub> suppressed the activity of NR, NiR, GS and GOGAT (except GDH) hence decreased growth. However, UV-B<sub>s</sub> showed cross tolerance in test organisms against Cu toxicity up to certain extent. Phormidium foveolarum is comparatively less sensitive against UV-B<sub>s</sub> and excess Cu, a situation likely exists in nature, hence it may be used as a biofertilizer for sustainable agriculture.

Key words: Copper toxicity, Cyanobacteria, Growth, High UV-B fluence rate, Low UV-B fluence rate, Nitrogen metabolism.

INTRODUCTION

Metal contamination of water bodies and soils is one of the main critical issues related to environment and public health (20). Aquatic ecosystems are particularly susceptible as they often act as a final receptor of these contaminants (20). Copper (Cu) is one of the most commonly used metals and its progressive increase in the aquatic ecosystems arises from various anthropogenic sources including industries and domestic products, copper-based pesticides, copper mine drainage and antifouling agents (20,24). Though Cu is an essential micronutrient for algae and plants being a component of several proteins and enzymes involved in number of metabolic pathways, however, at elevated levels, it can interfere with numerous physiological processes and cause toxicity to the cells (20,24,35). Copper is a redox-sensitive metal and its toxicity is mainly due to the existence of two readily interconvertible oxidation states makes it highly reactive (20). Copper is known to induce formation of highly toxic oxygen species (ROS) and thus causes damage to DNA, proteins, membranes and lipids, and does not involve specific receptors (11). In contrast, it has been reported that low UV-B fluence rate is capable of promoting metabolic and developmental changes such as biosynthesis of phenolic secondary metabolites and protects organisms against stress (3,11). Moreover, it has been shown that low UV-B fluence rate responses involve specific receptors and are not mediated by DNA damage signaling (3,11). Thus, it is clear that at one extreme, UV-B can cause damage, whereas on the other it can function as informational signal that initiates protective responses against other stress types.

Cyanobacteria are a phylogenetically primitive group of gram-negative prokaryotes having a cosmopolitan distribution (38). They are major biomass producers in aquatic ecosystems and represent more than 50% of the biomass in many ecosystems (13,38). In addition, their inherent capacity to fix atmospheric nitrogen makes them ecologically important for rice-growing countries where they add to fertility of rice fields as natural biofertilizer (38). Cyanobacteria are found on the surface of water and their presence in transparent water may vary up to 7 meter of depth (22). Studies have demonstrated that the penetration power of UV-B in water declines and only 10% of UV-B radiation can penetrate in transparent water up to 10 meter of depth (18,30,41). Therefore, cyanobacteria may be exposed to varied level of UV-B radiation and respond accordingly. Further, recent studies have shown that UV-B-induced responses in plants may depend on UV-B layer results in increased level of incident solar UV-B radiation at the Earth’s surface (13,14). It is well established that enhanced UV-B radiation damages nucleic acids, proteins and lipids that are essential for genetic, biochemical and physiological functions such as growth, pigmentation, photosynthesis and nitrogen metabolism within cells (13,14,21,35). Studies have demonstrated that high UV-B fluence rate responded by producing excess reactive oxygen species (ROS) and thus causes damage to DNA, proteins, membranes and lipids, and does not involve specific receptors (11). However, UV-B<sub>s</sub> showed cross tolerance in test organisms against Cu toxicity up to certain extent.
fluctuation rates (3,11). Therefore, the present study was
designed to investigate the impact of low (UV-B: 0.1 µmol • m⁻² • s⁻¹) and high (UV-B: 1.0 µmol • m⁻² • s⁻¹) fluctuation rates of
UV-B radiation on growth and enzymes involved in nitrogen
metabolism in two cyanobacteria under Cu (2 and 5 µM) toxicity. Besides, it is known that organisms differ in their degree of tolerance to stresses. Thus, for better exploitation of cyanobacteria as biofertilizer, an attempt was also made to investigate their degree of tolerance to Cu toxicity when exposed to low and high fluctuation rates of
UV-B radiation.

MATERIALS AND METHODS

Organisms and growth conditions
Phormidium foveolarum, a filamentous and non-hetero-
cystous cyanobacterium and Nostoc muscorum, a filament-
ous and heterocystous cyanobacterium were maintained in our laboratory. The axenic and homogenous cultures of both the cyanobacteria were grown in BG–11 medium in temperature controlled culture room at 25±2 ºC under 75
µmol photons m⁻² s⁻¹ photosynthetically active radiation (PAR, 400-700 nm) provided by white fluorescent tubes (Osram L 40W/25–1) with a 14:10 h of light:dark cycle. In case of P. foveolarum, BG–11 medium was externally added with NaNO₃ (1.5 g/l) as nitrogen source. All experi-
ments were conducted with exponentially growing cells.

Copper and UV-B radiation treatments
The exponentially grown cells of both cyanobacteria were harvested by centrifugation at 4,000 g for 10 min and gently washed twice with distilled water, and then cells were resuspended in nutrient medium containing 2 and 5
µM Cu (as copper chloride) together with Cu present in the medium as micronutrient (0.32 µM). Cu concentrations used in the present study are environmentally relevant and found in Cu-polluted water and are far above the maximum recommended limit (0.05 mg/l) for irrigation purposes (39). Just after metal treatment, Cu treated (2 and 5 µM) and untreated cells were placed in Petri dishes. Petri dishes were kept on a shaker to reduce the cell aggregation, sedi-
mentation and shedding effect and to ensure uniform expo-
sures then cells were irrigated with low and high UV-B fluence rates. In the present study, low (UV-B: 0.1 µmol
m⁻² s⁻¹) and high (UV-B: 1.0 µmol m⁻² s⁻¹) UV-B radiation were given for 4 h each day together with 75 µmol photons m⁻² s⁻¹ of PAR. The desired UV-B fluence rates were obtained by adjusting distance between cells’ sur-
face and UV-B lamp. Ultraviolet-B was provided by UV-B lamp (Q–Panel Co., USA) with its main output at 313
nnm while PAR provided by using white fluorescent tubes (Osram L 40W/25–1) with their main outputs at 400–700
nm. The irradiance was measured with the help of power
meter (Spectra physics, USA Model 407, A–2). To avoid any possible incidence of UV-C radiation (<290 nm), the UV-B lamp was wrapped with 0.127 mm cellulose acetate film (Johnston Industrial Plastics, Toronto, Canada). The UV-B fluorescent tubes (Q-Panel Co., USA) emit very low level of UV-A and blue light which has been found ineff-
icient to induce expression of chalcone synthase, a main
enzyme of flavonoids biosynthetic pathway (8). In the pre-
sent study, following combinations of Cu and UV-B were
made: control (without UV-B and Cu), 2 µM Cu, 5 µM Cu,
UV-B, UV-B+Cu, 2 µM Cu+UV-B, 2 µM Cu+UV-B+Cu, 5 µM
Cu+UV-B and 5 µM Cu+UV-B+Cu. After 24 and 72 h of
experiments, control (untreated) and treated cells of both the cyanobacteria were harvested by centrifugation and all parameters were analyzed.

Estimation of growth and Cu accumulation
Growth in control and treated cells of both the cyanobac-
teria was estimated in terms of relative growth rate (RGR) using equation:

\[
\text{RGR (day}^{-1}) = \ln W_2 - \ln W_i / (T_2-T_1)
\]

Where, \(W_2\) = dry weight at time \(T_2\), \(W_i\) = dry weight at time \(T_1\), \(T_1\) = initial time, \(T_2\) = final time, \(\ln\) = natural log.

For determination of Cu concentrations, treated and untreated culture [equivalent to 50±0.72 µg dry mass (ml culture)⁻¹ and 46±0.68 µg dry mass (ml culture)⁻¹, respectively, in unstressed P. foveolarum and N. muscorum cells at 0 h] was harvested by centrifugation at 4,000 g for 10 min. The cells were washed thrice with 1 mM of ethylene diaminetetra–acetic acid to remove surface bound metals. Oven dried cells of each sample were digested in tri-acid mixture (HNO₃, H₂SO₄, and HClO₄ in 5:1:1 ratio) at 80
ºC until a transparent solution obtained. The samples were maintained up to 5 ml with double distilled water. Concentrations of Cu in digested samples were estimated by using an atomic absorption spectrophotometer (Model 4141, ECIL, India). The instrument was calibrated by using stan-
dard stock solution of Cu.

Nitrate and nitrite uptake rate assay
The uptake of nitrate (NO₃⁻) and nitrite (NO₂⁻) was
assayed by measuring their depletion from the external medium. Treated and untreated cells were harvested by centrifugation at 4,000 g for 10 min, washed with 25 mM of Tricine–NaOH buffer (pH 8.3) and resuspended in the same buffer. Assays were carried out in open flasks under aerobic conditions with continuous shaking at 25 ºC and a
PAR of 75 µmol photons m⁻² s⁻¹. After 10 min of stabiliza-
tion, the experiments were started by the addition of 100
µM of KNO₃ or KNO₂ to the cell suspension at zero time
for NO₃⁻ and NO₂⁻ uptake, respectively. Samples were with-
drawn after 4 h of incubation, subjected to centrifugation at 4,000 g for 10 min and the cell–free supernatants ana-
lyzed for residual NO₃⁻ or NO₂⁻. NO₃⁻ and NO₂⁻ concentra-
tions were measured by the method of Cawse (5) and Snell and Snell (42), respectively.

Measurements of nitrogen assimilating enzymes
An in situ assay of nitrate reductase (NR; EC 1.6.6.1) and
nitrite reductase (NiR; EC 1.7.7.1) was performed with dithionite-reduced methyl viologen as reductant in
cells made permeable by including mixed alkyltrimethy-
lanmonium bromide (MTA) in the reaction mixture accord-
ing to the method of Herrero et al. (15,16) and Herrero and Guerrero (17), respectively . For NR assay, cell sus-
pension was added to a reaction mixture which contained
the following in a final volume of 1 ml: 75 µg of MTA, 100 µM of NaHCO₃–Na₂CO₃ buffer (pH 10.5), 20 µM of
KNO₃, 4 µM of methyl viologen, and 10 µM of Na₂S₂O₄
in 0.1 ml of 0.3 M NaHCO₃. The reaction mixture was
incubated for 5 min at 30 ºC, and NO₂⁻ was estimated in
corresponding cell free media. For NiR assay, cell sus-
pension was added to a reaction mixture which contained the
following in a final volume of 1 ml: 75 µg of MTA, 25 µM of MOPS/NaOH buffer (pH 7.2), 0.5 µM of KNO$_3$, 5 µM of methyl viologen and 20 µM of Na$_2$S$_2$O$_4$ in 0.1 ml of 0.3 M NaHCO$_3$. The reaction mixture was incubated for 10 min at 30 °C, and remaining NO$_2^-$ in cell free media was determined. For measurements of NR and NiR activities in heterocystous cyanobacterium *N. muscorum*, it was grown in BG–11 medium containing 20 mM KNO$_3$ or 0.2 mM NaNO$_2$ at beginning of experiment to induce NR and NiR enzymes, respectively. NO$_2^-$ ions in cell free media were measured according to the method of Snell and Snell (42). One unit (U) of NR activity is defined as 1 nmol NO$_2^-$ for min$^{-1}$. One unit (U) of NiR activity is defined as 1 nmol NO$_2^-$ consumed min$^{-1}$.

Glutamine synthetase (GS; EC 6.3.1.2) activity was determined by the formation of γ–glutamylhydroxamate (transferase assay) following the method of Mérida et al. (29). Treated and untreated cells were harvested by centrifugation at 4,000 g for 10 min, washed in nitrogen–free medium, and resuspended in HEPES–NaOH buffer (pH 7.0). Cells were disrupted by sonication (Sonics Vibra Cell, Model VCX–130PB, USA). The homogenate was centrifuged at 15,000 g for 20 min at 4 °C (Model CPR–30, Remi, India), and the resulting supernatant constituted the cell extract. Reaction mixture consisted of 60 µM of HEPES–NaOH buffer (pH 7.0), 40 µM of L–glutamine, 4 µM of MnCl$_2$, 60 µM of hydroxylamine, 1 µM of ADP, 20 µM of sodium arsenate, and 50 µl of cell extract. The reaction was started by adding the sodium arsenate, and the amount of γ–glutamylhydroxamate formed after 10 min of incubation at 28 °C was determined at 500 nm. One unit (U) of GS activity is defined as 1 nmol γ–glutamylhydroxamate formed min$^{-1}$.

Glutamate synthase also known as glutamine 2–oxoglutarate aminotransferase (NADH–GOGAT; EC 1.4.1.14) activity was measured by the method of Meers et al. (28) with some modification. Treated and untreated cells were harvested by centrifugation at 4,000 g for 10 min. Cells were resuspended in 50 mM Tris buffer (pH 7.6) containing 10 mM mercaptoethanol and then thoroughly disrupted by sonication (Sonics Vibra Cell Model VCX–130PB, USA) and centrifuged at 15,000 g for 20 min at 4 °C, to remove cell debris. The resulting supernatant was used as enzyme. Reaction mixture consisted of 50 mM of Tris buffer (pH 7.6), 5 mM of L–glutamine, 5 mM of 2–oxoglutarate, 0.25 mM of NADH and enzyme. Reaction was carried out at 28 °C and enzyme activity determined by measuring oxidation of NADH at 340 nm. One unit (U) of GOGAT activity is defined as 1 nmol NADH oxidized min$^{-1}$.

Glutamate dehydrogenase (NADH-GDH; EC 1.4.1.2) aminating activity was determined by the method of Chávez and Candau (6). Treated and untreated cells were harvested by centrifugation at 4,000 g for 10 min. The cells were crushed in HEPES–NaOH buffer (pH 7.0) containing 10 mM CaCl$_2$, followed by centrifugation at 15,000 g for 20 min at 4 °C to remove cell debris. Supernatant obtained was used as enzyme. Reaction mixture consisted of 85 mM of Tris–HCl buffer (pH 8.5), 5 mM of 2–oxogluta-
rate, 50 mM of NH₄Cl, 0.2 mM of NADH and appropriate amount of enzyme. The reaction was started by the addition of NH₄Cl and oxidation of NADH followed spectrophotometrically at 340 nm. One unit (U) of GDH activity is defined as 1 nmol NADH oxidized min⁻¹. Protein in each sample was determined by the method of Lowry et al. (23) using bovine serum albumin as standard.

Statistical analysis
All the experiments were conducted in triplicate and repeated at least thrice to confirm the reproducibility of the results. A one-way ANOVA test was applied to confirm the significance of data. Comparison between control and treatment’s means was carried out by Tukey’s HSD (Honestly Significant Difference) test at p< 0.05 significance level using SPSS 10 software.

RESULTS

Growth
Impact of low (UV-Bₐ) and high (UV-Bₐ) fluence rates of UV-B radiation on growth of both the organisms under Cu toxicity was measured in terms of relative growth rate (RGR). Results showed that RGR in both the organisms decreased (P<0.05) following single and combined exposure of Cu and UV-Bₐ, but the effect was more prominent in N. muscorum (Fig. 1). In P. foveolarum, after 24 h of experiment, 2 and 5 µM of Cu decreased RGR by 39 and 96%, respectively, however, after 72 h the decrease in RGR was only 21 and 41%, respectively (Fig. 1). The effect was stronger in N. muscorum as RGR declined by 83% under 2 µM of Cu treatment after 24 h of experiment, and with 5 µM of Cu the RGR was even negative. Furthermore, after 72 h of experiment, the decrease in RGR was only 25 and 60% at 2 and 5 µM of Cu, respectively (Fig. 1). Similarly, UV-Bₐ alone decreased RGR up to 32% in P. foveolarum and 67% in N. muscorum, respectively (Fig. 1) after 24 h of commencement of experiment and after 72 h it showed only 13 and 25% decrease, respectively (Fig. 1). The RGR of both the cyanobacteria further declined under combined treatments of Cu and UV-Bₐ (P<0.05; Fig. 1) after 24 h of experiment. Maximum decrease in RGR was registered in N. muscorum under 5 µM Cu+UV-Bₐ treatment after 24 h of experiment, however, after 72 h a positive RGR was recorded (Fig. 1). Under UV-B treatment the RGR was unaffected (P<0.05; Fig. 1). There was significant (P<0.05) improvement in RGR of both the cyanobacteria when examined under the combined treatments of UV-Bₐ and Cu compared to Cu treatments alone (Fig. 1).

Accumulation of Cu
Cells of P. foveolarum and N. muscorum accumulated appreciable amount (17.8–19.5 µg Cu g⁻¹ dry weight) of Cu from the nutrient medium (without additional Cu) and exhibited luxuriant growth (Fig. 1). Addition of 2 and 5 µM of Cu into the medium showed 47.9 and 111.4 µg Cu g⁻¹ dry weight in N. muscorum and 35.5 and 77 µg Cu g⁻¹ dry weight in P. foveolarum, respectively after 24 h of

Figure 2. Impact of low and high UV-B fluence rates on nitrate (NO₃⁻) and nitrite (NO₂⁻) uptake rate in P. foveolarum and N. muscorum under Cu toxicity. Data are means ± standard error of three independent experiments. Bars followed by different letter(s) are significantly different according to the Tukey HSD test at P<0.05 significance level.
Nitrate and nitrite uptake
Copper and UV-B_H exposure resulted in significant (P<0.05) suppression of NO_3^- and NO_2^- uptake in both the cyanobacteria; however, decline in uptake was more in _N. muscorum_ (Fig. 2). After 24 h of experiment, 2 and 5 µM Cu exposure decreased the uptake of NO_3^- by 25 and 37% and NO_2^- by 20 and 33% in _P. foveolarum_ (Fig. 2) and in _N. muscorum_ the reduction in NO_3^- uptake was up to 32 and 42% and NO_2^- uptake up to 26 and 36%, respectively (Fig. 2). After 72 h, there was considerable decrease in uptake; however, the negative impact of Cu was less than those obtained after 24 h. Ultraviolet-B at high fluence rate also significantly (P<0.05) inhibited NO_3^- and NO_2^- uptake by 19 and 15% in _P. foveolarum_ and by 22 and 16% in _N. muscorum_, respectively after 24 h of exposure, and after 72 h there was partial recovery in NO_3^- and NO_2^- uptake but still less than control. The combined treatment (Cu and UV-B_H) further exacerbated inhibitory (P<0.05) effect on NO_3^- and NO_2^- uptake in both the cyanobacteria. The UV-B exposure did not show significant (P<0.05) effect on NO_3^- and NO_2^- uptake in both the cyanobacteria but UV-B_H and Cu combinations exhibited appreciable decrease in uptake rate and it was less (P<0.05) than those recorded in presence of Cu alone (Fig. 2).

Nitrate and nitrite reductase activities
Activity of NR and NiR declined (P<0.05) up to 18-29% and 22-34% in _P. foveolarum_, and 25–36% and 27-39% in _N. muscorum_, respectively when cells were exposed to...
2-5 µM Cu for 24 h (Fig. 3). After 72 h of experiment the pattern was similar, however, inhibitory effect of Cu on NR and NiR was appreciably lower than those recorded after 24 h. Under high fluence rate of UV-B exposure the enzyme activity declined significantly (P<0.05) in both the cyanobacteria, however, toxic effect on both the enzymes was less after 72 h than that recorded after 24 h of experiment. Under combined exposure of Cu and UV-B the inhibitory effect was further raised (P<0.05). There was significant (P<0.05) alleviation in Cu-induced inhibitory effect on the activity of these enzymes when cultures were exposed with Cu (2 and 5 µM) and UV-B_L together (Fig. 3).

**Activities of ammonia assimilating enzymes**

The results pertaining to the activity of GS, NADH–GO

GAT and NADH–GDH under UV-B and Cu treatments are presented in figures 4 and 5. The GS and GOGAT activity was inhibited significantly (P<0.05) under Cu and UV-

B_L exposure while GDH activity showed reverse trend. After 24 h of experiment, the inhibition of 26 and 37% in *P. foveolarum* and 33 and 41% in *N. muscorum* in GS activity following 2 and 5 µM Cu treatments, respectively was noticed (Fig. 4). Under similar treatments the activity of GOGAT was inhibited by 21 and 29% in *P. foveolarum* (Fig. 4) and 26 and 34% in *N. muscorum*, respectively (Fig. 4). After 72 h, there was considerable improvement in GS and GOGAT activity in both the cyanobacteria but the activity in presence of Cu (2 and 5 µM) was still less than their respective controls. High fluence rate of UV-B also significantly (P<0.05) inhibited GS and GOGAT activity as it was declined by 28 and 24% in *P. foveolarum* and by 35 and 29% in *N. muscorum*, respectively after 24 h of experiment. The inhibitory effect on GS and GOGAT activity in both the cyanobacteria under combined doses of Cu and UV-B was further enhanced (P<0.05). The GS and GOGAT activity appeared to be unaffected under low UV-B fluence rate (P<0.05). Copper-induced inhibitory effect on these enzymes was alleviated appreciably when cultures were exposed with Cu and UV-B_L together (Fig. 4).

Unlike the response of NR, NiR, GS and GOGAT to Cu and UV-B_L doses, GDH activity (aminating) exhibited appreciable enhancement (Fig. 5). Copper at 2 and 5 µM concentrations and UV-B at high fluence rate singly significantly (P<0.05) enhanced the GDH activity by 20, 42 and 32% in *P. foveolarum* and by 45, 174 and 52% in *N. muscorum*, respectively after 24 h of experiment, and upon combination of these stresses the activity of enzyme was further accelerated (P<0.05; except 5 µM Cu+UV-B_H), however, it was non-additive. Furthermore, under similar condition, the stimulation in enzyme activity was noticed...
DISCUSSION

Growth of both cyanobacteria *P. foveolarum* and *N. muscorum* declined significantly (*P*<0.05) with increasing concentration (2.5 µM) of Cu, but the level of toxicity was found to be decreased appreciably with the increasing exposure time in batch culture (Fig. 1). In earlier study, Cu induced growth reduction was also noticed in *Anabaena dolitoliu* (35). It has been suggested the Cu exhibits toxic effect on plant tissues when cellular accumulation of Cu exceeds 25 µg Cu g⁻¹ dry weight (25). The cellular Cu accumulation in *N. muscorum*, growing in the medium (without supplemented Cu), varied from 17.8–18.4 and in *P. foveolarum* it ranged from 18.6–19.5 µg Cu g⁻¹ dry weight which appeared to be quite low and thus, both the organisms showed luxuriant growth (Fig. 1). Copper treatment increased (*P*<0.05) cellular Cu several fold showing 47.9–121.8 µg Cu g⁻¹ dry weight in *N. muscorum* and 35.5–94.6 µg Cu g⁻¹ dry weight in *P. foveolarum* and thus significant (*P*<0.05) reduction in the growth of both the organisms was recorded (Fig. 1). The greater toxicity in *N. muscorum* than *P. foveolarum* could be correlated with the more accumulation of cellular Cu (Fig. 1). It has been suggested that Cu toxicity in cells under excess Cu is regulated by membrane bound Cu transporter proteins i.e. CtaA–ATPase in cell membrane and PacS–ATPase in thylakoid membrane in cyanobacteria (4,34,44). Higher accumulation of Cu in *N. muscorum* may be explained on the basis of Cu transporter proteins located in cell membrane that were probably present in greater amount in *N. muscorum* and allowed more Cu to enter into the cells. Other stress UV-B (UV-Bₜ) at high fluence rate diminished the growth and the effect was also more prominent in *N. muscorum* than *P. foveolarum*. Earlier studies have also demonstrated UV-B induced damaging effect on growth which was explained on the basis of UV-B induced direct effect on photosynthesis, light harvesting pigments and alteration in energy transfer mechanism in the antenna system (14,35). Besides this, DNA and RNA may also be the target of UV-B radiation hence cellular activities declined appreciably (14,21). Further, the stress may also enhance the generation of ROS which damage biologically important components such as lipids, proteins, enzymes and nucleic acids (14,35,45). The less impact of high UV-B fluence rate on growth in *P. foveolarum* could be due to its better ability to withstand against stress (37). The results are in consistent with earlier findings where UV-B induced differential sensitivity on growth was recorded in three cyanobacteria (45). Copper and UV-Bₜ stress when applied simultaneously growth of both the cyanobacteria further declined (*P*<0.05; Fig. 1). Under combined treatment greater toxicity in *N. muscorum* and *P. foveolarum* was obvious as both the stresses are acting simultaneously and are capable to inhibit the metabolic processes (14,24,35,45). Furthermore, greater toxicity may also be correlated with higher rate of Cu accumulation under combined treatment (Fig. 1). The result also suggests that UV-Bₜ dose might have caused a conformational change in cell membrane bound protein CtaA–ATPase thereby the transport of Cu into the cell enhanced up to 44.9–111.8 in *P. foveolarum* and 66.7–157.5 µg Cu g⁻¹ dry weight in *N. muscorum* after 24 and 72 h of experiments, respectively.
It has been shown that UV-B can modify the soluble and membrane bound proteins (19). Interestingly, at the low fluence rate of UV-B, Cu induced damaging effects on the cyanobacteria alleviated significantly (P<0.05) in both the cyanobacteria (Fig. 1). The results point out that the low fluence rate of UV-B modified the cell membrane bound Cu transporter protein to regulate Cu transport across the cell membrane under Cu stress which results in significant (P<0.05) decline in Cu accumulation and hence the alleviation in growth was observed. Recently, low fluence rate of UV-B has been reported to stimulate the protective responses that increase plant resistance to other stresses (3,11). Furthermore, stimulation in the non-protein thiols observed under Cu+UV-B combinations (data not shown) may also regulate the level of free Cu in cells and thus protected cells against Cu stress.

Results showed that exposure of both organisms to Cu and UV-B significantly (P<0.05) decreased NO\textsubscript{3}{\textsuperscript{--}} and NO\textsubscript{2}{\textsuperscript{--}} uptake compared to their respective controls (Fig. 2). Flores and Herrero (10) showed that entry of NO\textsubscript{3}{\textsuperscript{--}} and NO\textsubscript{2}{\textsuperscript{--}} in cells of cyanobacteria is facilitated by ABC-type transporter that likely uses ATP as a source of energy. Thus, Cu and UV\textsubscript{B}{\textsuperscript{H}} induced decrease in NO\textsubscript{3}{\textsuperscript{--}} and NO\textsubscript{2}{\textsuperscript{--}} uptake (Fig. 2) may be attributed to a decreased ATP pool as a consequence of damaged photosynthetic electron transport chain as suggested by Rai et al. (35) in Anabaena doliolum under Cu and UV-B stress. Copper and UV\textsubscript{B}{\textsuperscript{H}} induced severe damage to photosynthetic electron transport chain in both the organisms was also observed (data not shown). On the contrary, UV-B alone did not significantly (P>0.05) influence NO\textsubscript{3}{\textsuperscript{--}} and NO\textsubscript{2}{\textsuperscript{--}} uptake in both the organisms compared to their respective controls (Fig. 2). Furthermore, UV-B\textsubscript{H} together with both doses of Cu appreciably alleviated Cu–induced inhibition in NO\textsubscript{3}{\textsuperscript{--}} and NO\textsubscript{2}{\textsuperscript{--}} uptake (Fig. 2). Under Cu+UV-B\textsubscript{H} combinations, less damage to photosynthetic electron transport chain than Cu treatments alone (data not shown) could provide better ATP pool thus an improvement in NO\textsubscript{3}{\textsuperscript{--}} and NO\textsubscript{2}{\textsuperscript{--}} uptake in both the organisms was noticed.

NO\textsubscript{3}{\textsuperscript{--}} is the available form of nitrogen which is most widely used by cyanobacteria. Like other NO\textsubscript{3}{\textsuperscript{--}} utilizing organisms, in cyanobacteria reduction of NO\textsubscript{3}{\textsuperscript{--}} occurs in two successive steps. In first step, NO\textsubscript{3}{\textsuperscript{--}} is reduced to NO\textsubscript{2}{\textsuperscript{--}} in a two electron reaction catalyzed by NR; in second step, resulting NO\textsubscript{2}{\textsuperscript{--}} is reduced to ammonium (NH\textsubscript{3})\textsuperscript{--} in a six electron reaction catalyzed by NiR (12,17). Exposure of both the organisms to Cu and UV\textsubscript{B}{\textsuperscript{H}} decreased NR and NiR activities but the effect was more in N. muscorum (Fig. 3). It has been shown that sulphydryl (–SH) groups are required for catalytic activity of NR (43), thus it is possible that Cu with and without UV\textsubscript{B}{\textsuperscript{H}} affect the NR activity by binding to functional –SH groups present in the active sites of this enzyme (43). Besides this, decrease in NR and NiR activities might have occurred due to the effect of Cu and UV\textsubscript{B}{\textsuperscript{H}} on the enzyme synthesis and/or alteration in enzyme. Appenroth et al. (1) have shown that UV-B exposure decreases NR and NiR in Spirodelta polyrhiza L. Results showed that low UV-B fluence rate alone did not influence (P<0.05) NR and NiR activities (Fig. 3).

Further, Cu–induced inhibition on NR and NiR activities in both organisms were alleviated significantly (P<0.05) by UV-B\textsubscript{H} that could be correlated with less availability of free cellular Cu.

Ammonium ions produced from NO\textsubscript{3}{\textsuperscript{--}} by successive action of NR and NiR are toxic to cell so they must rapidly be assimilated into organic compounds. Like other NH\textsubscript{3}– assimilating organisms, in cyanobacteria NH\textsubscript{3}– ions are incorporated into carbon skeletons mainly through GS–GOGAT cycle (26). Glutamine synthetase catalyzes the ATP–dependent amination of glutamate, leading to formation of glutamine, while GOGAT performs reductive transfer of amino groups of glutamine to 2–oxoglutarate to produce two molecules of glutamate. Studies have demonstrated that Fd–GOGAT activity is dominant in the cyanobacteria (32). However, Okuhara et al. (33) have reported comparable activities of both GOGATs i.e. Fd and NADH–GOGAT in the cyanobacterium Plectonema boryanum. We have also observed comparable activity of NADH–GOGAT in untreated cells of both the cyanobacteria (Fig. 4) as observed by Okuhara et al. (33) in cells of P. boryanum. Copper and UV-B\textsubscript{H} treatments significantly (P<0.05) decreased GS and NADH–GOGAT activities; however, the activities were less affected in P. foveolarum (Fig. 4). It is well known that GS/GOGAT pathway plays key role in NH\textsubscript{3}– assimilation and is a primary route of NH\textsubscript{3}– assimilation in cyanobacteria. Thus, under Cu and UV-B\textsubscript{H} exposure, decrease in GS and GOGAT activities may disturb carbon/nitrogen balance and growth in both the organisms as these enzymes are known to generate glutamate for synthesis of several amino acids. The GS/GOGAT pathway in cyanobacteria depends on photosynthesis for ATP and reducing power (31), however, in the present experimental set up, decreases in GS and GOGAT activities due to the Cu and UV-B\textsubscript{H} treatments (Fig. 4) could be attributed to oxidative modifications. It has been reported that cadmium stress oxidatively modified GOGAT and GS activities and alters NH\textsubscript{3}– assimilation in soybean plants (2). Reverse to this, under Cu+UV-B treatments, Cu–induced inhibition on GS and GOGAT activities was alleviated appreciably compared to the Cu treatments alone (Fig. 4). Improved activity of GS and GOGAT leads better NH\textsubscript{3}– assimilation than those of Cu alone treated cells hence; improved RGR was recorded under Cu + UV-B\textsubscript{H} treatments.

In contrast to GS and GOGAT activities, aminating activity of GDH in both the organisms greatly increased (P<0.05) after 24 h of experiment under Cu and UV-B\textsubscript{H} exposures alone and in combination, and after 72 h of experiment it showed declining trend; however GDH activity was still higher than their respective controls (Fig. 5). Under Cu and UV-B\textsubscript{H} exposure, greater increase in GDH activity suggested that it might have partially compensated NH\textsubscript{3}– assimilation which declined considerably due to decreased GS and GOGAT activities. In the literature, contradictory results on the existence of GDH activity have been reported in cyanobacteria. Workers showed that the enzyme was either absent or present only at very low level (36). Reverse to this, other group claimed that GDH (amingating activity) was responsible for glutamate synthesis in Anacystis nidulans (27). Chávez et al. (7) suggested that presence of GDH activity is a selective advantage in Synechocystis sp. strain PCC 6803 under non–exponential growth condition and could be an alternative pathway to the GS/GOGAT cycle for NH\textsubscript{3}– assimilation. Recently, Skopelitis et al. (40) reported that under stress conditions when GS/GOGAT system is not fully operative, increase in GDH activity may result into glutamate formation that is responsible for synthesis of several protective compounds.
(eg. proline). Thus, under Cu and UV-B concentration, higher GDH activity may be a selective advantage of both organisms to sustain NH$_4^+$-assimilation under adverse conditions as evidenced by its continuous higher activity. On the contrary, exposure of both the organisms to UV-B alone did not significantly (P<0.05) influence GDH activity (Fig. 5). Moreover, exposure of cells to UV-B$_{340}$ together with Cu showed lower GDH activity in comparison to the activity of Cu alone treated cells (Fig. 5). The results suggest that cells grown under Cu+UV-B treatments have improved primary route of NH$_4^+$-assimilation i.e. GS/GOGAT pathway than in Cu alone treated cells.

Present study concludes that UV-B may play dual role based on its fluence rates. The results clearly demonstrated that low and high UV-B fluence rates differentially modulated Cu toxicity in P. foveolarum and N. muscorum. Cu and UV-B$_{340}$ alone affected growth, NO$_3^-$ and NO$_2^-$ uptake and nitrogen metabolism in both the organisms. Besides, we observed that when UV-B$_{340}$ was given together with Cu, it exacerbated damaging effects of Cu. However, recovery in studied parameters was noticed after 72 h of experiment showed adaptation in both the cyanobacteria against Cu and UV-B$_{340}$. In contrast, UV-B$_{340}$ alone did not influence growth, nutrient uptake and nitrogen metabolism in both the organisms. Besides, UV-B$_{340}$ exposure alleviated toxic effects of Cu on growth, nutrient uptake and nitrogen metabolism. The UV-B$_{340}$-mediated protective role against Cu toxicity is due to initiation of protective responses that regulate Cu accumulation in cells. This study further demonstrates that P. foveolarum has higher growth rate and is comparatively less affected by Cu in presence and absence of UV-B$_{340}$ which could be correlated with its potential for avoiding accumulation of Cu when compared to N. muscorum hence P. foveolarum may be preferred as a biofertilizer for sustainable agriculture.

ACKNOWLEDGEMENTS
Authors are thankful to The Head, Department of Botany, University of Allahabad, Allahabad for providing necessary laboratory facilities. Authors are also thankful to UGC, New Delhi for financial support to VPS and PKS in the form of SRF.

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