*Cell. Mol. Biol.* 58 (1): 85-95
Published on December 22, 2012
DOI 10.1170/T925

Cellular & Molecular Biology
http://www.cellmolbiol.com


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

V. P. SINGH, P. K. SRIVASTAVA AND S. M. PRASAD✉

Ranjan Plant Physiology and Biochemistry Laboratory, Department of Botany, University of Allahabad, Allahabad-211002, India.

**Abstract**

In the present study, impact of low (UV-$B_L$: 0.1 µmol $m^{-2}$ $s^{-1}$) and high (UV-$B_H$: 1.0 µmol $m^{-2}$ $s^{-1}$) 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-$B_H$ 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-$B_H$, and under combined treatments the effect was greater. On contrary, UV-$B_L$ declined Cu toxicity significantly. The study concludes that Cu and UV-$B_H$ suppressed the activity of NR, NiR, GS and GOGAT (except GDH) hence decreased growth. However, UV-$B_L$ showed cross tolerance in test organisms against Cu toxicity up to certain extent. *Phormidium foveolarum* is comparatively less sensitive against UV-$B_H$ 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.

**Article information**

Received on May 5, 2012
Accepted on June 13, 2012

✉ **Corresponding author**
Tel: +919450609911
Fax: +91-532 -2461009
E-mail:profsmprasad@gmail.com

## 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 hydroxyl radicals ($^{\bullet}OH$) from superoxide radicals ($O_2^{\bullet-}$), or hydrogen peroxide ($H_2O_2$) via Haber-Weiss reaction (9). Hydroxyl radicals are highly reactive and can oxidize biological macromolecules leading to major cellular damages such as DNA alterations, oxidation of proteins and lipid peroxidation (9). It has been reported that Cu at concentrations beyond a certain threshold (25 µg $g^{-1}$ dry weight) (25), causes toxic symptoms in algae such as inhibition in growth, photosynthesis and fertility, disruption in development and nitrogen metabolism and damage to membranes (9,24). Therefore, it is essential that intracellular level of free Cu be kept at minimum.

Besides metal contamination, rapid industrialization in past few decades has resulted in an increase in anthropogenically released chlorofluorocarbons, chlorocarbons and organobromides causing depletion of the stratospheric ozone layer (14,38). Depletion of the stratospheric ozone 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). 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

fluence rates (3,11). Therefore, the present study was designed to investigate the impact of low (UV-$B_L$: 0.1 µmol $m^{-2}$ $s^{-1}$) and high (UV-$B_H$: 1.0 µmol $m^{-2}$ $s^{-1}$) fluence 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 fluence rates of UV-B radiation.

## MATERIALS AND METHODS

### *Organisms and growth conditions*

*Phormidium foveolarum*, a filamentous and non-heterocystous cyanobacterium and *Nostoc muscorum*, a filamentous 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 °C 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 $NaNO_3$ (1.5 g/l) as nitrogen source. All experiments 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, sedimentation and shading effect and to ensure uniform exposures then cells were irradiated with low and high UV-B fluence rates. In the present study, low (UV-$B_L$; 0.1 µmol $m^{-2}$ $s^{-1}$) and high (UV-$B_H$; 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' surface and UV-B lamp. Ultraviolet-B was provided by UV-B lamp (Q–Panel Co., USA) with its main output at 313 nm 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 inefficient to induce expression of chalcone synthase, a main enzyme of flavonoids biosynthetic pathway (8). In the present study, following combinations of Cu and UV-B were made: control (without UV-B and Cu), 2 µM Cu, 5 µM Cu, UV-$B_L$, UV-$B_H$, 2 µM Cu+UV-$B_L$, 2 µM Cu+UV-$B_H$, 5 µM Cu+UV-$B_L$ and 5 µM Cu+UV-$B_H$. 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 cyanobacteria was estimated in terms of relative growth rate (RGR) using equation:

$$RGR\ (day^{-1}) = \ln W_2 - \ln W_1 / (T_2 - T_1)$$

Where, $W_2$ = dry weight at time $T_2$, $W_1$ = 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 ($HNO_3$, $H_2SO_4$, and $HClO_4$ in 5:1:1 ratio) at 80 °C 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 standard stock solution of Cu.

### *Nitrate and nitrite uptake rate assay*

The uptake of nitrate ($NO_3^-$) and nitrite ($NO_2^-$) 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 °C and a PAR of 75 µmol photons $m^{-2}$ $s^{-1}$. After 10 min of stabilization, the experiments were started by the addition of 100 µM of $KNO_3$ or $KNO_2$ to the cell suspension at zero time for $NO_3^-$ and $NO_2^-$ uptake, respectively. Samples were withdrawn after 4 h of incubation, subjected to centrifugation at 4,000 g for 10 min and the cell–free supernatants analyzed for residual $NO_3^-$ or $NO_2^-$. $NO_3^-$ and $NO_2^-$concentrations 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 alkyltrimethylammonium bromide (MTA) in the reaction mixture according to the method of Herrero et al. (15,16) and Herrero and Guerrero (17), respectively . For NR assay, cell suspension was added to a reaction mixture which contained the following in a final volume of 1 ml: 75 µg of MTA, 100 µM of $NaHCO_3$–$Na_2CO_3$ buffer (pH 10.5), 20 µM of $KNO_3$, 4 µM of methyl viologen, and 10 µM of $Na_2S_2O_4$ in 0.1 ml of 0.3 M $NaHCO_3$. The reaction mixture was incubated for 5 min at 30 °C, and $NO_2^-$ was estimated in corresponding cell free media. For NiR assay, cell suspension 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 $KNO_2$, 5 µM of methyl viologen and 20 µM of $Na_2S_2O_4$ 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 $NO_2^-$ formed $min^{-1}$. 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-

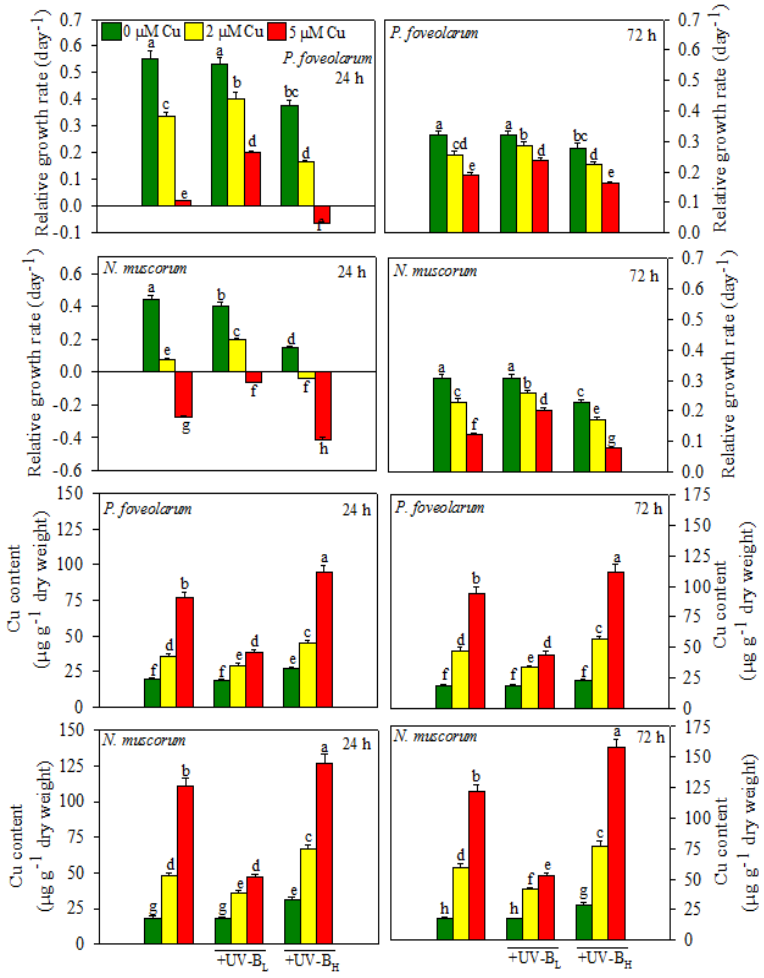


**Figure 1.** Impact of low and high UV-B fluence rates on growth (relative growth rate) and Cu accumulation in *P. foveolarum* and *N. muscorum* under Cu toxicity. Data are means ± standard error of three independent experiments. Bars followed by different letter(s) are significantly different according to the Tukey' HSD test at $P<0.05$ significance level.

rate, 50 mM of $NH_4Cl$, 0.2 mM of NADH and appropriate amount of enzyme. The reaction was started by the addition of $NH_4Cl$ 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 ($UV\text{-}B_L$) and high ($UV\text{-}B_H$) 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 $UV\text{-}B_H$ 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, $UV\text{-}B_H$ 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 $UV\text{-}B_H$ ($P<0.05$; Fig. 1) after 24 h of experiment. Maximum decrease in RGR was registered in *N. muscorum* under 5 µM Cu+$UV\text{-}B_H$ treatment after 24 h of experiment, however, after 72 h a positive RGR was recorded (Fig. 1). Under $UV\text{-}B_L$ 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 $UV\text{-}B_L$ 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

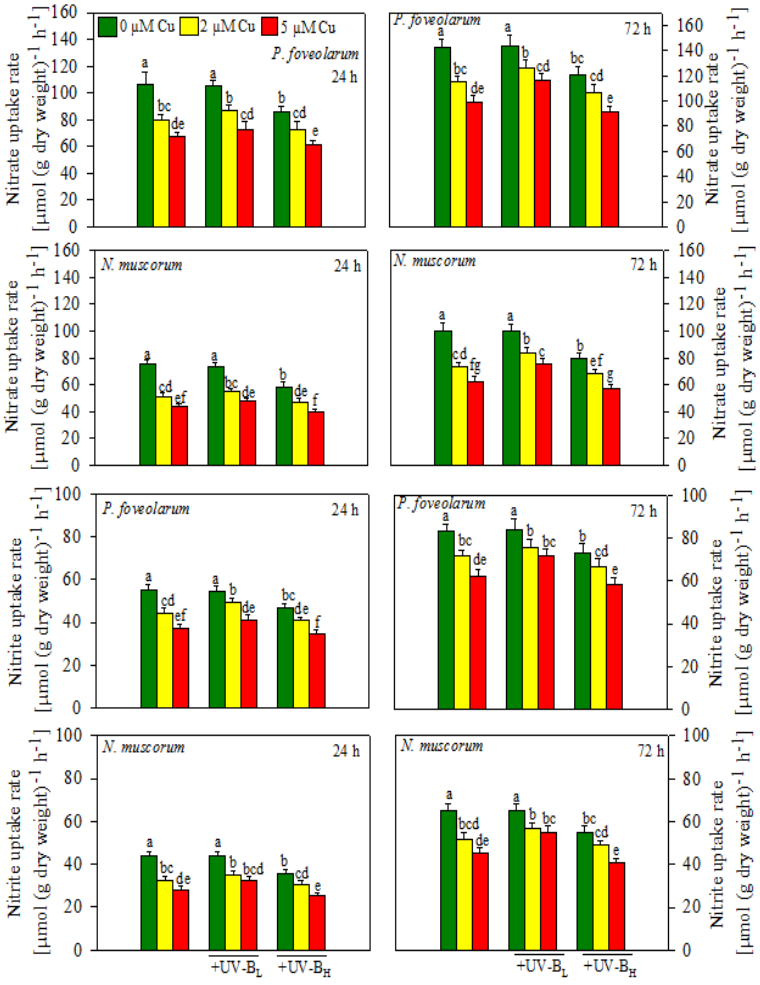


**Figure 2.** Impact of low and high UV-B fluence rates on nitrate ($NO_3^-$) and nitrite ($NO_2^-$) uptake rate in *P. foveolarum* and *N. muscorum* under Cu toxicity. Data are means ± standard error of three independent experiments. Bars followed by different letter(s) are significantly different according to the Tukey' HSD test at $P<0.05$ significance level.

experiment (P<0.05; Fig. 1). Under similar condition after 72 h of experiment the cellular Cu was 59.4 and 121.8 µg $g^{-1}$ dry weight in *N. muscorum* and 47.5 and 94.6 µg $g^{-1}$ dry weight in *P. foveolarum*, respectively. Low fluence rate ($UV\text{-}B_L$) of UV-B together with 2 and 5 µM of Cu exhibited similar pattern in Cu accumulation in both the cyanobacteria, however, it was significantly (P<0.05) lower than those recorded in presence of Cu alone (Fig. 1). High fluence rate ($UV\text{-}B_H$) of UV-B, on the other hand, exhibited an enhancement (P<0.05) in Cu accumulation in both the cyanobacteria under Cu and $UV\text{-}B_H$ treatments over the value obtained only with Cu treatment (Fig. 1).

### *Nitrate and nitrite uptake*

Copper and $UV\text{-}B_H$ 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 $UV\text{-}B_H$) further exacerbated inhibitory (P<0.05) effect on $NO_3^-$ and $NO_2^-$ uptake in both the cyanobacteria. The $UV\text{-}B_L$ exposure did not show significant (P<0.05) effect on $NO_3^-$ and $NO_2^-$ uptake in both the cyanobacteria but $UV\text{-}B_L$ 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

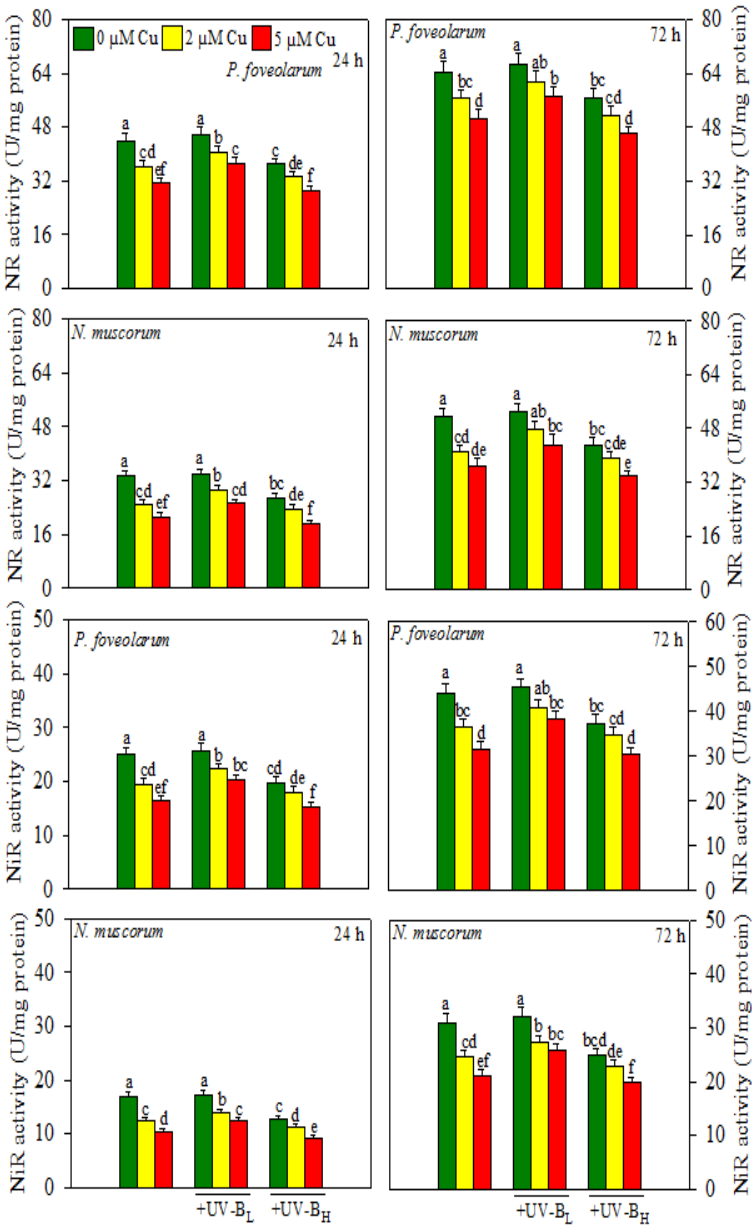


**Figure 3.** Impact of low and high UV-B fluence rates on NR and NiR activity in *P. foveolarum* and *N. muscorum* under Cu toxicity. Data are means ± standard error of three independent experiments. Bars followed by different letter(s) are significantly different according to the Tukey' HSD test at P<0.05 significance level.

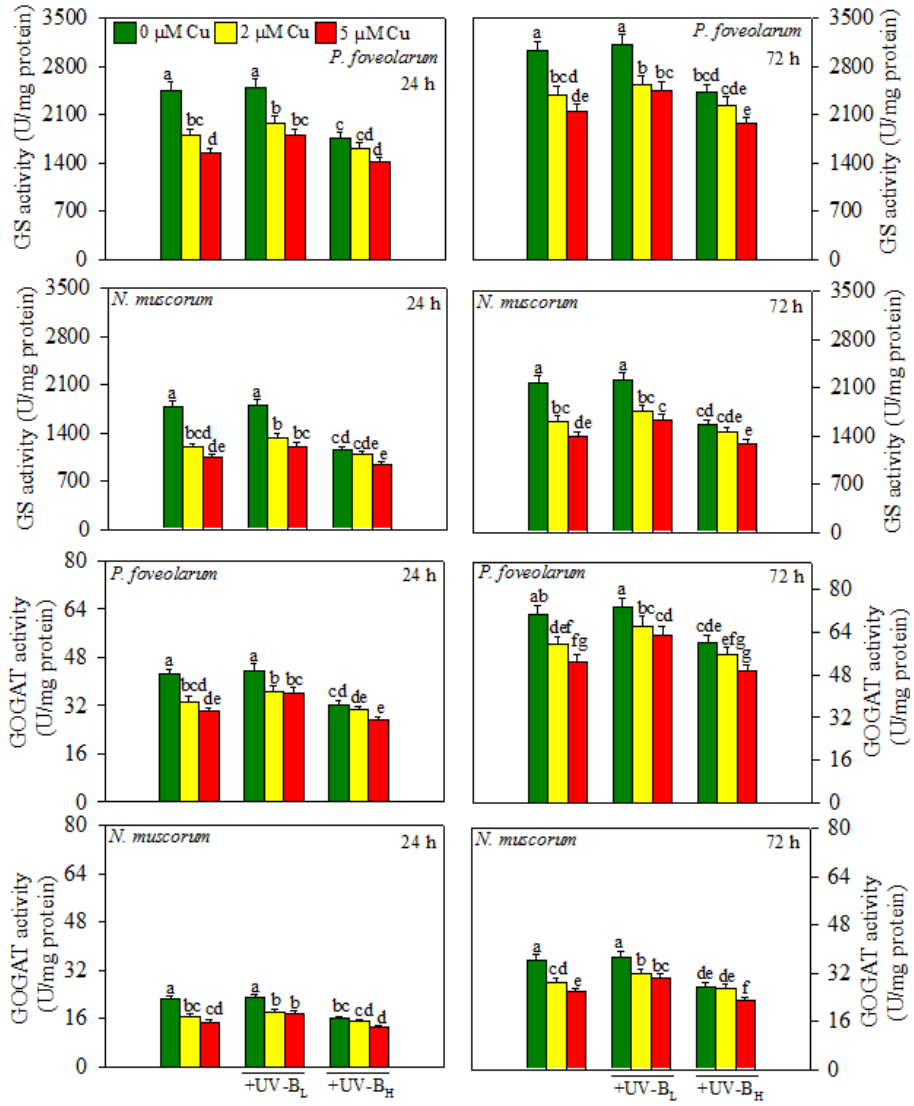


**Figure 4.** Impact of low and high UV-B fluence rates on GS and GOGAT activity in *P. foveolarum* and *N. muscorum* under Cu toxicity. Data are means ± standard error of three independent experiments. Bars followed by different letter(s) are significantly different according to the Tukey' HSD test at P<0.05 significance level.

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-$B_H$ 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–GOGAT 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-$B_H$ 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-$B_H$ 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-$B_H$ 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

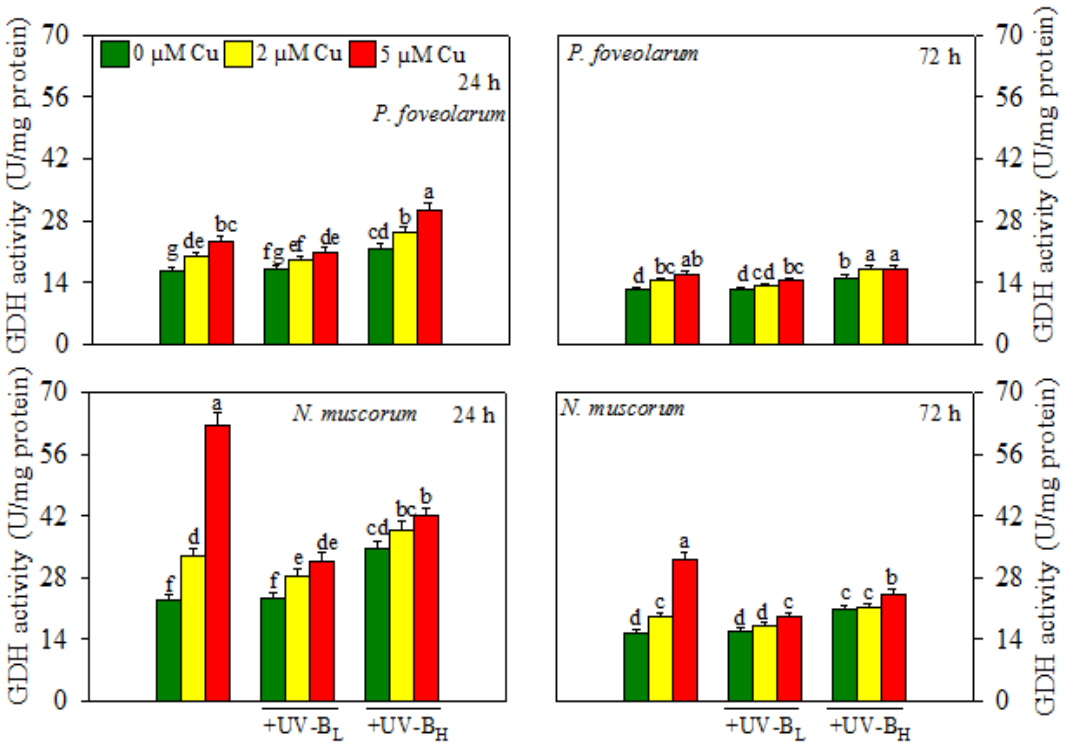


**Figure 5.** Impact of low and high UV-B fluence rates on GDH activity in *P. foveolarum* and *N. muscorum* under Cu toxicity. Data are means ± standard error of three independent experiments. Bars followed by different letter(s) are significantly different according to the Tukey' HSD test at P<0.05 significance level.

in both the cyanobacteria after 72 h of experiment; however, it was less than that observed after 24 h of experiment. The GDH activity was not influenced (P<0.05) in both the cyanobacteria by UV-B$_L$ exposure after 24 and 72 h of experiments. Further, UV-B$_L$ dose when combined with Cu (2 and 5 µM), stimulation in GDH activity was observed but the increase in enzyme activity was significantly (P<0.05) less than the activity observed with Cu treatment alone (Fig. 5).

## 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 doliolum* (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 from17.8–18.4 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*a and allowed more Cu to enter into the cells. Other stress UV-B (UV-B$_H$) 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-B$_H$ 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-B$_H$ 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-$B_L$ combinations (data not shown) may also regulate the level of free Cu in cells and thus protected cells against Cu stress.

Results revealed that exposure of both organisms to Cu and UV-$B_H$ significantly ($P<0.05$) decreased $NO_3^-$ and $NO_2^-$uptake compared to their respective controls (Fig. 2). Flores and Herrero (10) showed that entry of $NO_3^-$ and $NO_2^-$ in cells of cyanobacteria is facilitated by ABC-type transporter that likely uses ATP as a source of energy. Thus, Cu and $UVB_H$ induced decrease in $NO_3^-$ and $NO_2^-$ 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 $UVB_H$ induced severe damage to photosynthetic electron transport chain in both the organisms was also observed (data not shown). On the contrary, UV-$B_L$ alone did not significantly ($P<0.05$) influence $NO_3^-$ and $NO_2^-$ uptake in both the organisms compared to their respective controls (Fig. 2). Furthermore, UV-$B_L$ together with both doses of Cu appreciably alleviated Cu–induced inhibition in $NO_3^-$ and $NO_2^-$ uptake (Fig. 2). Under Cu+UV-$B_L$ combinations, less damage to photosynthetic electron transport chain than Cu treatments alone (data not shown) could provide better ATP pool thus an improvement in $NO_3^-$ and $NO_2^-$ uptake in both the organisms was noticed.

$NO_3^-$ is the available form of nitrogen which is most widely used by cyanobacteria. Like other $NO_3^-$–utilizing organisms, in cyanobacteria reduction of $NO_3^-$ occurs in two successive steps. In first step, $NO_3^-$ is reduced to $NO_2^-$ in a two electron reaction catalyzed by NR; in second step, resulting $NO_2^-$ is reduced to ammonium ($NH_4^+$) in a six electron reaction catalyzed by NiR (12,17). Exposure of both the organisms to Cu and $UVB_H$ decreased NR and NiR activities but the effect was more in *N. muscorum* (Fig. 3). It has been shown that sulfhydryl (–SH) groups are required for catalytic activity of NR (43), thus it is possible that Cu with and without UV-$B_H$ 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 $UVB_H$ on the enzyme synthesis and/or alteration in enzyme. Appenroth et al. (1) have shown that UV-B exposure decreases NR and NiR in *Spirodela 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-$B_L$ that could be correlated with less availability of free cellular Cu.

Ammonium ions produced from $NO_3^-$ by successive action of NR and NiR are toxic to cell so they must rapidly be assimilated into organic compounds. Like other $NH_4^+$–assimilating organisms, in cyanobacteria $NH_4^+$ 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-$B_H$ 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 $NH_4^+$–assimilation and is a primary route of $NH_4^+$–assimilation in cyanobacteria. Thus, under Cu and UV-$B_H$ 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-$B_H$ treatments (Fig. 4) could be attributed to oxidative modifications. It has been reported that cadmium stress oxidatively modified GOGAT and GS activities and alters $NH_4^+$–assimilation in soybean plants (2). Reverse to this, under Cu+UV-$B_L$ 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 $NH_4^+$–assimilation than those of Cu alone treated cells hence; improved RGR was recorded under Cu + UV-$B_L$ 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-$B_H$ 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-$B_H$ exposure, greater increase in GDH activity suggested that it might have partially compensated $NH_4^+$–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 (aminating 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 $NH_4^+$–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-$B_H$ exposure, 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-$B_L$ alone did not significantly ($P<0.05$) influence GDH activity (Fig. 5). Moreover, exposure of cells to UV-$B_L$ 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-$B_L$ 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-$B_H$ alone affected growth, $NO_3^-$ and $NO_2^-$ uptake and nitrogen metabolism in both the organisms. Besides, we observed that when UV-$B_H$ 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-$B_H$. In contrast, UV-$B_L$ alone did not influence growth, nutrient uptake and nitrogen metabolism in both the organisms. Besides, UV-$B_L$ exposure alleviated toxic effects of Cu on growth, nutrient uptake and nitrogen metabolism. The UV-$B_L$-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-$B_H$ 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.

Other articles in this theme issue include references (46-73).

## REFERENCES

1. Appenroth, K.J., Augsten, H., Mattner, A., Teller, S. and Döhler, G., Effect of UVB irradiation on enzymes of nitrogen metabolism in turions of *Spirodela polyrhiza* (L.) Schleiden. *J. Photochem. Photobiol. B.* 1993, **18**:215–220.

2. Balestrasse, K.B., Gallego, S.M. and Tomaro, M.L., Oxidation of the enzymes involved in ammonium assimilation plays an important role in the cadmium-induced toxicity in soybean plants. *Plant Soil* 2006, **284**:187-194.

3. Brown, B.A. and Jenkins, G.I., UV-B signaling pathways with different fluence-rate response profiles are distinguished in mature *Arabidopsis* leaf tissue by requirement for UVR8, HY5, and HYH. *Plant Physiol.* 2008, **146**:576–588.

4. Burkhead, J.L., Reynolds, K.A.G., Abdel-Ghany, S.E., Cohu, C.M. and Pilon, M., Copper homeostasis. *New Phytol.* 2009, **182**:799–716.

5. Cawse, P.A., The determination of nitrate in soil solution by ultraviolet spectrophotometry. *Analyst* 1967, **92**:311–315.

6. Chávez, S. and Candau, P., An NAD-specific glutamate dehydrogenase from cyanobacteria identification and properties. *FEBS Lett.* 1991, **285**:35–38.

7. Chávez, S., Lucena, J.M., Reyes, J.C., Florencio, F.J. and Candau, P., The presence of glutamate dehydrogenase is a selective advantage for the cyanobacterium *Synechocystis* sp. strain PCC 6803 under nonexponential growth conditions. *J. Becteriol.* 1999, **181**:808–813.

8. Christie, J.M. and Jenkins, G.I., Distinct UV-B and UV-A blue light signal transduction pathways induce chalcone synthase gene expression in *Arabidopsis* cells. *Plant Cell* 1996, **8**:1555-1567.

9. Dietz, K.J., Baier, M. and Kramer, U., Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. In: *Heavy Metal Stress in Plants-from Molecules to Ecosystems*, Prasad, M.N.V., Hagemeyer, J. (eds.), Springer-Verlag, Berlin, Heidelberg, New York, 1999, pp. 73–97.

10. Flores, E. and Herrero, A., Assimilatory nitrogen metabolism and its regulation. In: *The Molecular Biology of Cyanobacteria*, Bryant, D.A. (ed.), Kluwer Academic Publishers, Dordrecht, The Netherlands, 1994, pp. 487–517.

11. Frohnmeyer, H. and Staiger, D., Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. *Plant Physiol.* 2003, **133**:1420–1428.

12. Guerrero, M.G., Vega, J.M. and Losada, M., The assimilatory nitrate-reducing system and its regulation. *Annu. Rev. Plant Physiol.* 1981, **32**:169–204.

13. Häder, D.P., Kumar, H.D., Smith, R.C. and Worrest, R.C., Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochem. Photobiol. Sci.* 2007, **6**:267–285.

14. He, Y.Y. and Häder, D.P., UV-B-induced formation of reactive oxygen species and oxidative damage of the cyanobacterium *Anabaena* sp.: protective effects of ascorbic acid and *N*-acetyl-L-cysteine. *J. Photochem. Photobiol. B.* 2002, **66**:115–124.

15. Herrero, A., Flores, E. and Guerrero, M.G., Regulation of nitrate reductase levels in the cyanobacteria *Anacystis nidulans*, *Anabaena* sp. strain 7119, and *Nostoc* sp. strain 6719. *J. Bacteriol.* 1981, **145**:175–180.

16. Herrero, A., Flores, E. and Guerrero, M.G., Regulation of the nitrate reductase level in *Anacystis nidulans*: activity decay under nitrogen stress. *Arch. Biochem. Biophys.* 1984, **234:**454–459.

17. Herrero, A. and Guerrero, M.G., Regulation of nitrite reductase in the cyanobacterium *Anacystis nidulans*. *J. Gen. Microbiol.* 1986, **132**:2463–2468.

18. Hurtubise, R.D. and Havel, J.E., The effects of ultraviolet-B radiation on freshwater invertebrates: experiments with a solar simulator. *Limnol. Oceanogr.* 1998, **43**:1082-1088.

19. Imbrie, C.W. and Murphy, T.M., UV-action spectrum (254-405 nm) for inhibition of a $K^+$-stimulated adenosine triphosphatase from the plasma membrane of *Rosa damascena*. *Photochem. Photobiol.* 1982, **36**:537–554.

20. Kanoun-Boulé, M., Vicente, J.A.F., Nabais, C., Prasad, M.N.V. and Freitas, H., Ecophysiological tolerance of duckweeds to copper. *Aquat. Toxicol.* 2009, **91**:1–9.

21. Karentz, S., Cleaver, J.E. and Mitchell, D.L., DNA damage in the Antarctic. *Nature* 1991, **350**: 28.

22. Lohscheider, J.N., Strittmatter, M., Küpper, H. and Adamska, I., Vertical distribution of epibenthic freshwater cyanobacterial *Synechococcus* spp. strains depends on their ability for photoprotection. *PLoS ONE* 2011, **6(5)**: e20134. doi:10.1371/journal.pone.0020134.

23. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 1951, **193**:265–275.

24. Ma, M., Zhu, W., Wang, Z. and Witkamp, G.J., Accumulation, assimilation and growth inhibition of copper on fresh water alga (*Scenedesmus subspicatus* 86.81 SAG) in the presence of EDTA and fulvic acid. *Aquat. Toxicol.* 2003, **63**:221–228.

25. Macnicol, R.D. and Beckett, P.H.T., Critical tissue concentrations

of potentially toxic elements. *Plant Soil* 1985, **85**:107–129.
26. Meeks, J.C., Wolk, C.P., Lockau, W., Schilling, W.N., Shaffer, P.W. and Chien, W.S., Pathways of assimilation of [$^{13}$N] $N_2$ and $^{13}NH_4^+$ by cyanobacteria with and without heterocysts. *J. Bacteriol.* 1978, **134**:125–130.
27. Meeks, J.C., Wolk, C.P., Thomas, J., Lockau, W., Shaffer, P.W., Austin, S.M., Chien, W.S. and Galonsky, A., The pathways of assimilation of $^{13}NH_4^+$ by the cyanobacterium *Anabaena cylindrica*. *J. Biol. Chem.* 1977, **252**:7894–7900.
28. Meers, J.L. and Tempest, D.W. and Brown, C.M., Glutamine (amide): 2-oxoglutarate amino transferase oxido-reductase (NADP) an enzyme involved in the synthesis of glutamate by some bacteria. *J. Gen. Microbiol.* 1970, **64**:187–194.
29. Mérida, A., Candau, P. and Florencio, F.J., Regulation of glutamine synthetase activity in the unicellular cyanobacterium *Synechocystis* sp. Strain PCC 6803 by the nitrogen source: effect of ammonium. *J. Bacteriol.* 1991, **173**:4095–4100.
30. Morris, D.P., Zagarese, H., Williamson, C.E., Balserio, E.G., Hargreaves, B.R., Modenutti, B., Moeller, R. and Queimalinos, C., The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. *Limnol. Oceanogr.* 1995, **40**:1381-1391.
31. Muro-Pastor, M.I. and Florencio, F.J., Regulation of ammonium assimilation in cyanobacteria. *Plant Physiol. Biochem.* 2003, **41**:595–603.
32. Navarro, F., Chávez, S., Candau, P. and Florencio, F.J., Existence of two ferredoxin-glutamate synthases in the cyanobacterium *Synechocystis* sp. PCC 6803: isolation and insertional inactivation of *gltB* and *gltS* genes. *Plant Mol. Biol.* 1995, **27**:753–767.
33. Okuhara, H., Matsumura, T., Fujita, Y. and Hase, T., Cloning and inactivation of genes encoding ferredoxin- and NADH-dependent glutamate synthase in the cyanobacterium *Plectonema boryanum*. Imbalance in nitrogen and carbon assimilation caused by deficiency of the ferredoxin-dependent enzyme. *Plant Physiol.* 1999, **120**:33–41.
34. Phung, L.T., Ajlani, G. and Haselkorn, R., P-type ATPase from the cyanobacterium *Synechococcus* 7942 related to the human Menkes and Wilson disease gene products. *Proc. Natl. Acad. Sci. USA* 1994, **91**:9651–9654.
35. Rai, L.C., Tyagi, B., Mallick, N. and Rai, P.K., Interactive effects of UV-B and copper on photosynthetic activity of the cyanobacterium *Anabaena doliolum*. *Environ. Exp. Bot.* 1995, **35**:177–185.
36. Ramos, J.L., Guerrero, M.G. and Losada, M., Photoproduction of ammonia from nitrate by *Anacystis nidulans* cells. *Biochim. Biophys. Acta* 1982, **679**:323–330.
37. Sheeba, Singh, V.P., Srivastava, P.K. and Prasad, S.M., Differential physiological and biochemical responses of two cyanobacteria *Nostoc muscorum* and *Phormidium foveolarum* against oxyfluorfen and UV-B radiation. *Ecotoxicol. Environ. Saf.* 2011, **74**:1981-1993.
38. Singh, S.P., Hader, D.P. and Sinha, R.P., Cyanobacteria and ultraviolet radiation (UVR) stress: Mitigation strategies. *Age. Res. Rev.* 2010, **9**:79–90.
39. Singh, V. and Chandel, C.P.S., Analytical study of heavy metals of industrial effluents at Jaipur, Rajasthan, India. *J. Environ. Sci. Engg.* 2006, **48**:103–108.
40. Skopelitis, D.S., Paranychianakis, N.V. and Paschalidis, K.A., Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. *Plant Cell* 2006, **18**:2767–2781.
41. Smith, R.C. and Baker, K.S., Penetration of UV-B and biologically effective dose-rates in natural water. *Photochem. Photobiol.* 1979, **50**:311-323.
42. Snell, F.D. and Snell, C.T., Colorimetric Methods of Analysis, vol. 3. Van Nostrand, New York, 1949, pp. 804–805.
43. Solomonson, L.P. and Barber, M.J., Assimilatory nitrate reductase; functional properties and regulation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 1990, **41**:225–253.
44. Tottey, S., Rich, P.R., Rondet, S.A. and Robinson, N.J., Two Menkes-type ATPases supply copper for photosynthesis in *Synechocystis* PCC 6803. *J. Biol. Chem.* 2001, **276**:19999–20004.
45. Zeeshan, M. and Prasad, S.M., Differential response of growth, photosynthesis, antioxidant enzymes and lipid peroxidation to UV-B radiation in three cyanobacteria. *S. Afr. J. Bot.* 2009, **75**:466–474.
46. Singh, M. P., and Kumar, V., Biodegradation of vegetable and agrowastes by *Pleurotus sapidus*: A noble strategy to produce mushroom with enhanced yield and nutrition. *Cell. Mol. Biol.* 2012, **58** (1): 1-7.
47. Pandey, V. K., Singh, M.P., Srivastava, A. K., Vishwakarma S. K., and Takshak, S., Biodegradation of sugarcane bagasse by white rot fungus *Pleurotus citrinopileatus. Cell. Mol. Biol.* 2012, **58** (1): 8-14.
48. Ruhal, A., Rana, J. S., Kumar S., and Kumar, A., Immobilization of malate dehydrogenase on carbon nanotubes for development of malate biosensor. *Cell. Mol. Biol.* 2012, **58** (1): 15-20.
49. Vishwakarma, S. K., Singh, M. P., Srivastava A.K. and Pandey, V. K., Azo dye (direct blue) decolorization by immobilized extracellular enzymes of *Pleurotus* species. *Cell. Mol. Biol.* 2012, **58** (1): 21-25.
50. Dash, S. K., Sharma, M., Khare, S. and Kumar, A., *rmpM* gene as a genetic marker for human bacterial meningitis. *Cell. Mol. Biol.* 2012, **58** (1): 26-30.
51. Bertoletti, F., Crespan, E. and Maga, G., Tyrosine kinases as essential cellular cofactors and potential therapeutic targets for human immunodeficiency virus infection. *Cell. Mol. Biol.* 2012, **58** (1): 31-43.
52. Sandalli, C., Singh, K., and Modak, M. J., Characterization of catalytic carboxylate triad in non-replicative DNA polymerase III (pol E) of *Geobacillus kaustophilus* HTA. *Cell. Mol. Biol.* 2012, **58** (1): 44-49.
53. Kaushal, A., Kumar, D., Khare, S. and Kumar, A., *speB* gene as a specific genetic marker for early detection of rheumatic heart disease in human. *Cell. Mol. Biol.* 2012, **58** (1): 50-54.
54. Datta, J. and Lal, N., Application of molecular markers for genetic discrimination of *fusarium* wilt pathogen races affecting chickpea and pigeonpea in major regions of India. *Cell. Mol. Biol.* 2012, **58** (1): 55-65.
55. Siddiqi, N. J., Alhomida, A. S., Khan, A. H. and Onga, W.Y., Study on the distribution of different carnitine fractions in various tissues of bovine eye. *Cell. Mol. Biol.* 2012, **58** (1): 66-70.
56. Ong, Y. T., Kirby, K. A., Hachiya, A., Chiang, L. A., Marchand, B., Yoshimura, K., Murakami, T., Singh, K., Matsushita, S. and Sarafianos, S. G., Preparation of biologically active single-chain variable antibody fragments that target the HIV-1 GP120 v3 loop. *Cell. Mol. Biol.* 2012, **58** (1): 71-79.
57. Singh, J., Gautam, S. and Bhushan Pant, A., Effect of UV-B radiation on UV absorbing compounds and pigments of moss and lichen of Schirmacher Oasis region, East Antarctica. *Cell. Mol. Biol.* 2012, **58** (1): 80-84.
58. Datta, J. and Lal, N., Temporal and spatial changes in phenolic compounds in response *Fusarium* wilt in chickpea and pigeonpea. *Cell. Mol. Biol.* 2012, **58** (1): 96-102.
59. Sharma, R. K., JAISWAL, S. K., Siddiqi, N. J., and Sharma, B., Effect of carbofuran on some biochemical indices of human erythrocytes *in vitro. Cell. Mol. Biol.* 2012, **58** (1): 103-109.
60. Singh, A. K., Singh, S. and Singh, M. P., Bioethics A new frontier of biological Science. *Cell. Mol. Biol.* 2012, **58** (1): 110-114.
61. Adedeji, A. O., Singh, K. and Sarafianos, S. G., Structural and biochemical basis for the difference in the helicase activity of two different constructs of SARS-CoV helicase. *Cell. Mol. Biol.* 2012, **58** (1): 115-121.
62. Singh, S., Choudhuri, G., Kumar, R. and Agarwal, S., Association of 5, 10-methylenetetrahydrofolate reductase C677T polymorphism in susceptibility to tropical chronic pancreatitis in North Indian population. *Cell. Mol. Biol.* 2012, **58** (1): 122-127.
63. Sharma, R. K., Rai, K. D. and Sharma, B., *In* vitro carbofuran in-

duced micronucleus formation in human blood lymphocytes. *Cell. Mol. Biol.* 2012, **58** (1): 128-133.
64. Naraian, R., Ram, S., Kaistha S. D. and Srivastava J., Occurrence of plasmid linked multiple drug resistance in bacterial isolates of tannery effluent. *Cell. Mol. Biol.* 2012, **58** (1): 134-141.
65. Pandey, A. K., Mishra, A. K., And Mishra, A., Antifungal and antioxidative potential of oil and extracts, respectively derived from leaves of Indian spice plant *Cinnamomum tamala. Cell. Mol. Biol.* 2012, **58** (1): 142-147.
66. Mishra, N., and Rizvi, S. I., Quercetin modulates na/k atpase and sodium hydrogen exchanger in type 2 diabetic erythrocytes. *Cell. Mol. Biol.* 2012, **58** (1): 148-152.
67. Kumar, A., Sharma, B. and Pandey, R. S., Assessment of stress in effect to pyrethroid insecticides, λ-cyhalothrin and cypermethrin in a freshwater fish, *Channa punctatus* (Bloch). *Cell. Mol. Biol.* 2012, **58** (1): 153-159.
68. Srivastava N., Sharma, R. K., Singh, N. and Sharma, B., Acetylcholinesterase from human erythrocytes membrane: a screen for evaluating the activity of some traditional plant extracts. *Cell. Mol. Biol.* 2012, **58** (1): 160-169.
69. Singh, M.P., Pandey, A. K., Vishwakarma S. K., Srivastava, A. K. and Pandey, V. K., Extracellular Xylanase Production by *Pleurotus* species on Lignocellulosic Wastes under *in vivo* Condition using Novel Pretreatment. *Cell. Mol. Biol.* 2012, **58** (1): 170-173.
70. Kumar, S., Sharma, U. K., Sharma, A. K., Pandey, A. K., Protective efficacy of *Solanum xanthocarpum* root extracts against free radical damage: phytochemical analysis and antioxidant effect. *Cell. Mol. Biol.* 2012, **58** (1): 174-181.
71. Shukla, A., Singh, A., Singh, A., Pathak, L.P., Shrivastava, N., Tripathi, P. K., Singh, K. and Singh, M.P., Inhibition of *P. falciparum* pfATP6 by curcumin and its derivatives: A bioinformatic Study. *Cell. Mol. Biol.* 2012, **58** (1): 182-186.
72. Michailidis, E., Singh, K., Ryan, E. M., Hachiya, A., Ong, Y. T., Kirby, K. A., Marchand, B., Kodama, E. N., Mitsuya, H., Parniak, M.A. and Sarafianos, S.G., Effect of translocation defective reverse transcriptase inhibitors on the activity of n348i, a connection subdomain drug resistant HIV-1 reverse transcriptase mutant. *Cell. Mol. Biol.* 2012, **58** (1): 187-195.
73. Parveen, A., Rizvi, S. H. M., Gupta, A., Singh, R., Ahmad, I., Mahdi, F., and Mahdi, A. A., NMR-based metabonomics study of sub-acute hepatotoxicity induced by silica nanoparticles in rats after intranasal exposure. *Cell. Mol. Biol.* 2012, **58** (1): 196-203.