Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.194

# NITROGEN AND PHOSPHORUS METABOLISM OF *ANABAENA DOLIOLUM* UNDER UV-B AND PESTICIDE STRESS

**Vandana Pandey***
Department of Botany, A.K.A.P.G. College, Bulanala, Varanasi, U.P., India
*Email: vandanapandey.1@rediffmail.com

(Date of Receiving-29-01-2021; Date of Acceptance-12-04-2021)

**ABSTRACT**

The effects of UV-B and pesticides (Butachlor and Carbofuran) on nitrogen and phosphorus metabolism have been studied on filamentous, nitrogen-fixing cyanobacterium, *Anabaena doliolum.* The study showed a decrease in the uptake of NH4+, PO4-3, activities of nitrogenase, glutamine synthetase, and alkaline phosphatase, when the cells were exposed to UV-B and pesticides. The interaction of two stresses showed the additive type of behavior for the studied parameters. In contrast, a significant increase in NO3- uptake and nitrate reductase activity was seen when *Anabaena* cells were exposed to different doses of UV-B.

***Keywords:*** UV-B, Pesticides, Nutrient uptake, Nitrogenase, *Anabaena doliolum.*

## INTRODUCTION

The reduced ozone level in the stratosphere, due to the disintegration of anthropogenic chlorofluorocarbons (CFCs), resulted in increased availability of ultraviolet-B (UV-B) radiation (280-320nm) on the earth' surface. UV-B is a small (<1% of total energy) but the highly active component of the solar spectrum has the potential to cause wide-ranging effects. Ultraviolet –B radiation (UVBR; 290-320 nm) is found to inhibit ammonium and nitrate uptake in natural plankton assemblages collected during a transect from 370 N in the Pacific Ocean (Behrenfeld *et al*., 1995). G. Dohler and co-workers have investigated the inhibition of inorganic nitrogen uptake by UVBR (Dohler, 1994; Dohler *et al.*, 1991). Their results have provided the information that the degree of nitrogen uptake inhibition is dependent upon the nitrogen source (NO3- or NH4+), species composition, and UVBR dose. According to Dohler (1991; 1992), differential responses of NO3- and NH4+ uptake (Behrenfeld *et al.*, 1995) to UVB exposure may be influenced by the availability of ATP and NADPH. Exposure of *Nostoc calcicola* cultures to UV-B (5W/m-2) for 30 min. caused complete inactivation of nitrogenase activity whereas nitrate reductase activity was stimulated twofold. GS activity was also inhibited by UV-B exposure (Kumar *et al*., 1996) in the cyanobacterium. Rai *et al*., (1998) have observed a general decrease in uptake of ammonium, urea, and phosphate, activities of nitrogenase, glutamine synthetase, alkaline phosphatase, and ATPase following UV-B treatment. In contrast, a significant increase in NO3- uptake, nitrate reductase was observed, following the treatment of UV-B. The dominant periphyton species, *Anabaena circinalis* RAB, showed sensitivity to ambient levels of UV-B radiation possibly due to UV inhibition of N2-fixation (Higley *et al.,* 2001). Microbial nutrients such as nitrogen (N) and phosphorus (P) have been shown to increase aspects of algal metabolism and compensate for UVR inhibition (Higley *et al*., 2001). According to Solheim *et al.,* (2002), nitrogen fixation potential by cyanobacteria in vegetation exposed to experimentally enhanced levels of UV-B for three and four years in the arctic was reduced by 50% compared to controls. UV radiation showed decreased cellular nitrogen content in Epilithon (Walkins *et al.*, 2001).

Among various stress factors, wide application of pesticides is also proved to be harmful which is used during the cultivation of food crops. There are few reports regarding the inhibitory effects of pesticides on nutrient uptake and their assimilatory enzymes in the case of cyanobacteria as of Kaushik and Venkataraman (1983), Rath and Adhikary (1995), and Singh and Tiwari (1988).

It is well known that the two toxicants when are exposed to an organism simultaneously, may affect in a synergistic, antagonistic, or additive way. Rai *et al.,* (1998), have seen the synergistic effect of UV-B and heavy metals for the cyanobacterium. Similarly, natural ecosystems like paddy fields which harbor N2-fixing cyanobacterium, is contaminated with pesticide and exposed to the increasing UV-B as well. These two stress factors which are reported to inhibit nutrient uptake and their assimilatory enzymes (Behrenfeld, 1995; Singh *et al*., 1988) may also affect nutrient uptake and their assimilatory enzymes in one of the above ways. This kind of report is not known earlier and hence prompted to deal with the work.

## MATERIALS AND METHODS

### Growth and Culture of Test Algae

Prokaryotic nitrogen-fixing cyanobacterium *Anabaena doliolum* Bharadwaja was selected for the present study. The organism was grown in modified nitrogen-free Chu-10 medium (Gerloff *et al.*, 1950) at 24 ± 20C under 72 µmol m-2 s-1 PAR photon flux and a photoperiod of 14:10 h. Exponentially grown cultures were taken in triplicate and all the experiments were repeated twice for the confirmation of the results.

### Source and Mode of UV-B Treatment

The UV-B treatment was provided by a UV-B lamp, CAT No. 3-4408, Fotodyne, Inc., USA, giving its maximum output at 310 nm. The desired radiation dose (12.9 mWm-2 nm-1) was obtained by adjusting the distance between the UV-B light source and the cyanobacterial suspension, which was calculated according to Crutzen (1992) and Smith *et al*., (1992). The cyanobacterial cells exposed to UV-B for 0-1 h were withdrawn at regular intervals and plated onto agar (Difco 0560). Percent survival was calculated according to Rai and Raizada (1985). The LD25 (12 min.) and $LD_{50}$ (26 min.) doses were used in the successive experiments.

### Pesticide Treatment

Commercial grades of butachlor, a pre-emergence herbicide (Machete, 50% E.C.), and carbofuran, an insecticide (Furadan 3%G) were used in the study. Their LC25 (5-and 12 ppm) and LC50 (100 and 300 ppm respectively) doses were selected for the study. Stock solutions were prepared in double-distilled water and filtered through Millipore membrane before use.

### Nitrate, Ammonium, and Phosphate Uptake

The uptake of NO3- from the medium was estimated colorimetrically using the brucine sulphuric acid method (Nicholas and Nason, 1957) by measuring the depletion of nitrate from the external medium. The ammonium left after algal consumption was estimated by measuring the amount present in the external medium at different time intervals. Ammonium in culture medium was assayed by Nessler's reagent as described by Herbert *et al.,* (1971). Phosphate uptake was measured by the stannous chloride method (APHA 1985).

### Assay of Enzyme Activity

In vivo nitrate reductase activity was measured following the method of Camm and Stein (1974). The activity is based on the total nitrite formed. In vivo nitrogenase activity was measured by acetylene–ethylene assay (Stewart *et al*., 1968). Glutamine synthetase (transferase) activity was measured by n-glutamyl transferase assay as described by Stacey *et al*., (1977). Alkaline phosphatase activity was assayed by the method of Ihlenfeldt and Gibson (1975).

### Lipid peroxidation and Efflux of Na+ and K+

The lipid peroxidation was measured by the TBA-rm method of De Vos *et al.,* (1989). The peroxidized membranes may show increased cell permeability as the loss of Na+ and K+. The amount of potassium and sodium in the cells was determined following the method of De Filippis (1978).

## RESULTS AND DISCUSSION

### Uptake of Ammonium, Nitrate, and Phosphate

Ammonium, nitrate, and phosphate uptake by *Anabaena doliolum* treated with butachlor, carbofuran, and UV-B was measured after 48h. It showed a concentration and dose-dependent significant inhibition ($p<0.05$, ANOVA) of uptake from the medium (Table-1). Interactive cases also produced appreciable ($p<0.05$, ANOVA) inhibition of ammonium uptake (Table-1). UV-B2+B2 produced a higher inhibition than UV-B2+C2.

Nitrate uptake was stimulated by 2.0 and 6.0% at LC25 and LC50 doses of UV-B. C2 showed higher inhibition (by 2%) than B2 for nitrate uptake.

Maximum inhibition of phosphate uptake was observed with UV-B2. (Table1). However, combination of UV-B2+C2 generated higher 47.06% and significant ($p<0.05$) inhibition than UV-B2+B2 (38.75%) (Table1).

The studied stress factors (UV-B and pesticide) showed a non-competitive inhibition for ammonia, nitrate, and phosphate uptake. The stimulation of NO3- after UV-B exposure (Table-1) might be due to a change in membrane permeability, which facilitates the entry of NO3- into the cells (Saralabai *et al.*, 1989). Stimulation of NO3- uptake after UV-B exposure has also been observed in diatoms *Lithodesmium cariable* and *Thalassiosira rotula* (Döhler, 1986) and *Anabaena doliolum* (Rai *et al*., 1998).

Inhibition of nutrients uptake by UV-B and pesticide may be due to the reasons that the stresses have reduced photosynthetic production of ATP and NADPH (Vosjan *et al*., 1990; Braune and Döhler, 1996) thus altering the amount of energy available for incorporation of nitrogen and other nutrients through ATP dependent permease. In other words, decreased uptake of these nutrients is due to the UV-B-induced damage of photosynthetic electron transport (Eichern *et al*., 1993), (Rai *et al*., 1998). Ammonia uptake was maximally inhibited due to stresses. The non-competitive inhibition of NH4+ and PO4-3 uptake by the stresses could be due to an alteration in the structure of the enzyme responsible for its uptake or transport (Rai *et al*, 1998). The approximately additive response of the stresses for the inhibition of nutrients uptake may be due to firstly the increased lipid peroxidation and damage

**Table 1:** Effects of UV-B ,butachlor and carbofuran on ammonium, nitrate, phosphate uptake, lipid peroxidation and K+/Na+ efflux of *Anabaena doliolum*

| ***Treatment*** | $NH_4^+$ uptake (µmol $NH_4^+$ $\mu g^{-1}$ protein) | $NO_3^-$ uptake (µmol $NO_3^-$ $\mu g^{-1}$ protein) | $PO_4^{-3}$uptake (µmol $PO_4^{3-}\mu g^{-1}$ protein) | Lipid peroxidation $E_{532\text{-}600}$ $mg^{-1}$ protein | $K^+$ (µm $K^+$/ µg protein) | $Na^+$ efflux (µm $Na^+$/µg protein) |
|---|---|---|---|---|---|---|
| Control | 23.27 ± .14 | 10.0±.6 | 16.0±.11 | 0.29±0.02 | | |
| $UV\text{-}B_1$ | 19.3±.14 (17.06) | 10.2±0.5 (-2) | 15.0±0.12 (6.25) | 0.36±0.03 | 1.05±0.02 (30) | 1.2±0.01 (19.7) |
| $UV\text{-}B_2$ | 16.5±0.11 (28.79) | 10.6±0.5 (-6.0) | 8.0±0.16 (50) | 0.59±0.03 | 1.42±0.01 (41) | 2.28±0.02 (37.4) |
| $B_1$ | 17.80±.13 (23.51) | 9.8±0.2 (2.0) | 14.5±0.13 (9.38) | 0.52±0.04 | 1.32±0.02 (38) | 1.74±0.03 (28.52) |
| $B_2$ | 10.90±.13 (53.16) | 7.8± (22) | 9.1±0.14 (43.13) | 0.90±0.06 | 1.65±0.02 (48) | 2.25±0.01 (41.31) |
| $C_1$ | 17.3±.12 (25.66) | 9.6±0.6 (4) | 14.0±0.5 (17.65) | 0.39±0.03 | 1.23±0.05 (35) | 1.61±0.01 (25.97) |
| $C_2$ | 10.50±.13 (54.88) | 7.6±0.5 (24) | 9.0±0.4 (47.06) | 0.75±0.05 | 1.54±0.02 (44) | 2.39±0.02 (38.6) |
| $UV\text{-}B_1 + B_1$ | 13.9±.13 (40.27) | 8.4±0.24 (16.0) | 13.6±0.13 (15) | 0.69±0.05 | 1.58±0.02 (456.5) | 2.16±0.01 (36) |
| $UV\text{-}B_2 + B_1$ | 13.0±.12 (44.13) | 7.9±0.2 (21.0) | 12.70±0.15 (20.63) | 0.70±0.03 | 2.03±0.03 (59.7) | 2.80±0.02 (47) |
| $UV\text{-}B_1 + B_2$ | 10.0±.13 (57.03) | 6.9±0.17 (31.0) | 11.90±0.16 (11.88) | 0.85±0.04 | 2.28±0.02 (67.05) | 3.18±0.01 (53) |
| $UV\text{-}B_2 + B_2$ | 9.0±.13 (61.32) | 5.8±0.24 (42) | 9.20±0.16 (38.75) | 1.28±0.06 | 2.70±0.03 (79.4) | 3.48±0.02 (58) |
| $UV\text{-}B_1 + C_1$ | 13.7±.12 (41.13) | 8.1±0.6 (19) | 12.1±0.3 (29.41) | 0.45±0.04 | 1.33±0.4 (38) | 1.97±0.01 (31.8) |
| $UV\text{-}B_2 + C_1$ | 13.6±.13 (41.56) | 7.0±0.5 (30) | 12.00±0.2 (28.82) | 0.49±0.05 | 1.79±0.03 (51) | 2.50±0.01 (40.32) |
| $UV\text{-}B_1 + C_2$ | 11.60±.12 (50.15) | 6.3±0.6 (37) | 11.6±0.19 (31.76) | 0.63±0.03 | 2.11±0.02 (60.28) | 3.04±0.02 (49.03) |
| $UV\text{-}B_2 + C_2$ | 9.10±.12 (60.89) | 5.8±0.4 (42) | 9.0±0.25 (47.06) | 0.88±0.03 | 2.43±0.03 (69.43) | 3.30±0.01 (53.22) |

All the values are ± SD.
Data in parentheses denote percent inhibition.
All treatments are significantly different ($p<0.05$) from the control according to ANOVA test

of the membranes and also due to the altered enzyme structure as evidenced by Table-1 and 2. The damaged membrane facilitates the leakage of Na+ and K+ in turn (Table=1). UV-B-induced lipid peroxidation and leakage of Na+ and K+ ions are known in *Anabaena doliolum* (Rai *et al*., 1998).

**Enzyme Activities**

Alkaline phosphatase was found to be highly sensitive to UV-B and pesticides (Table- 2). Carbofuran (LC50) individually caused the highest i.e. 71% inhibition of this enzyme. The percent inhibition for alkaline phosphatase produced by UV-B2+C2 (65.59%) was the highest (Table-2). Interestingly, the nitrate reductase activity was stimulated by 7.5 and 10 % with the LC25 and LC50 dose of UV-B respectively. The interaction of UV-B2 and C2 caused greater inhibition (55%) of NR than the UV-B2+B2 (51 %) (Table-2).

C2 produced approximately 37% inhibition of glutamine synthetase (GS) activity. UV-B with carbofuran produced significantly ($p<0.05$, ANOVA) higher inhibition of GS activity than with butachlor (Table-2) except the case UV-B2+C2. Interactive cases of UV-B and butachlor always produced higher significant inhibition ($p<0.05$, ANOVA) than with carbofuran (Table-2) for the nitrogenase activity.

**Lipid Peroxidation**

**Table 2:** Effects of UV-B, butachlor and carbofuran on nitrate reductase, alkaline phosphatase, glutamine synthatase and nitrogenase activities of *Anabaena doliolum* after 48 hrs.

| ***Treatment*** | Nitrate reductase ($\mu g\ NO_2^-/\mu g$ protein x $10^{-2}$) | Alkaline phosphatase ($\mu mol\ PO_4^{3-}$ released $\mu g^{-1}$ protein) | Glutamine synthetase ( mmol y-glutamylhydroxamate $\mu g$-1 protein $x10^{-2}$) | Nitrogenase ($\mu$ mol $C_2H_4$ released $\mu g^{-1}$ protein $h^{-1}$) |
|---|---|---|---|---|
| Control | 8.0 ± 0.4 | 4.65 ± 0.5 | 8.7 ± 0.3 | 6.65 ±0.4 |
| $UV\text{-}B_1$ | 8.6 ± 0.3 (-7.5) | 3.51 ± 0.24 (24.52) | 7.5 ± 0.2 (13.76) | 5.19 ±0.3 (21.95) |
| $UV\text{-}B_2$ | 8.8 ± 0.4 (-10) | 1.91 ± .03 (59) | 6.4 ± 0.25 (26.44) | 4.16 ±0.12 (37.44) |
| $B_1$ | 7.4 ± 0.42 (7.5) | 2.91 ± 0.23 (62.58) | 6.9 ± 0.31 (20.69) | 4.35 ±.14 (34.49) |
| $B_2$ | 5.4 ± 0.4 (32.5) | 1.57 ± 0.3 (70.43) | 5.9 ± 0.4 (32.18) | 3.25 ±.15 (51.13) |
| $C_1$ | 7.0 ± 0.5 (12.5) | 2.65 ± 0.21 (43.01) | 6.71 ± .0.3 (22.99) | 4.73 ±.12 (28.87) |
| $C_2$ | 5.1 ± 0.3 (36.25) | 1.35 ± 0.32 (71) | 5.5 ± 0.41 (36.78) | 3.76 ±.13 (43.46) |
| $UV_1 + B_1$ | 6.5 ± 0.4 (18.75) | 2.60 ± 0.3 (44) | 6.7 ± 0.3 (22.99) | 3.6 ± .1 (44.51) |
| $UV_2 + B_1$ | 5.9 ± 0.3 (26.25) | 2.42 ± 0.36 (48) | 6.1 ± 0.25 (29.89) | 2.74 ± .14 (58.74) |
| $UV_1 + B_2$ | 5.8 ± 0.5 (27.5) | 2.28 ± 0.3 (51) | 5.0 ± 0.36 (42.53) | 1.86 ± .15 (72.03) |
| $UV_2 + B_2$ | 3.9 ± 0.43 (51.25) | 1.93 ± 0.29 (58.49) | 3.9 ± 0.45 (55.17) | 1.01 ± .13 (84.81) |
| $UV_1 + C_1$ | 6.0 ± 0.42 (25) | 2.46 ± 0.28 (47) | 6.5 ± 0.46 (25.29) | 3.9 ± .14 (40.30) |
| $UV_2 + C_1$ | 5.6 ± 0.35 (30) | 2.33 ± 0.26 (50) | 5.9 ± 0.43 (32.18) | 2.82 ± .13 (57.59) |
| $UV_1 + C_2$ | 4.6 ± 0.4 42.5 | 2.19 ± 0.29 (53) | 4.9 ± 0.45 (43.68) | 2.60 ± .15 (60.90) |
| $UV_2 + C_2$ | 3.6 ± 0.4 (55) | 1.60 ± 0.28 (65.59) | 3.9 ± 0.43 (55.17) | 2.32 ± .6 (65.11) |

All the values are ± SD.
Data in parentheses denote percent inhibition All treatments are significantly different ($p<0.05$) from the control according to ANOVA test.

Significant lipid peroxidation was observed when the test organism was exposed to UV-B and pesticides. Butachlor either alone or in combination with UV-B generated significantly ($p<0.05$) higher lipid peroxidation than carbofuran (Table-1).

The enzymes also showed various responses to the stresses. UV-B exposure showed the stimulation in nitrate reductase activity of *Anabaena doliolum* and this seems to be due to the accelerated NO3- uptake which is favored by a positive correlation between NO3- uptake, and nitrate reductase activity. This kind of stimulation of NR is reported earlier in several plants including angiosperms *Crotalaria juncea* (Saralabai *et al.,* 1989), fungi *Neurospora crassa* (Roldan and Butler, 1980), and cyanobacteria *Nostoc linckia* (Tyagi *et al*., 1995) *Anabaena doliolum* (Rai *et al.*, 1998).

UV-B exposure inhibited the enzyme glutamine synthetase which incorporates nitrogen into the carbon skeleton and produces amino acids (Döhler, 1991). This kind of inhibition is supported by the earlier finding of Döhler (1986) in *Thalassiosira rotula* and Rai *et al.*, (1998) in *Anabaena doliolum*.

Nitrogenase showed significantly inhibited activity after UV-B exposure. Nitrogenase activity following UV-B exposure may be inhibited due to loss of reductant and ATP, complete inactivation of nitrogenase polypeptide, conformational changes in the enzyme complex, loss of oxygen protection in the heterocyst. UV-B is already known to inhibit nitrogenase activity in *Nostoc linckia*

(Tyagi *et al*., 1992), *Anabaena doliolum* (Rai *et al.,* 1998) and *Anabaena circinalis* RAB (Higley *et al.,* 2001). The inhibited nitrogenase activity due to pesticides in cyanobacteria has been reported by different workers (Da Silva *et al.*, 1975; Rahwer and Flockiger, 1979; Singh and Tiwari, 1988a; Rath and Adhikary, 1995; Pandey and Rai, 2002). Singh and Tiwari (1988) observed inhibition of nitrogenase, nitrate reductase, and glutamine synthetase activities of *Nostoc muscorum* by butachlor and fluchloralin.

Besides these, the alkaline phosphatase activity of the cyanobacterial cells was also inhibited after UV-B exposure. Rai *et al*., (1998) has documented inhibition of alkaline phosphatase after UV-B exposure in the cyanobacterium *Anabaena doliolum*. The activity of the enzymes nitrate reductase, glutamine synthetase, nitrogenase, and alkaline phosphatase was not only affected by UV-B but also by pesticide butachlor and carbofuran both individually and in combination with UV-B. Inhibition in the activity of nitrate reductase and glutamine synthetase, in turn, maybe due to a decrease in nitrate uptake by the pesticides as evidenced by a positive correlation of these enzymes with nitrate uptake.

Thus above findings show that the exposure of UV-B and pesticides to the cyanobacterium caused inhibition of nutrients uptake and their assimilatory enzymes (Table-1 and 2) but the system, already exposed to UV-B when contaminated with pesticides, shows additive or less than the additive type of inhibition. This kind of behavior of pesticides may be due to their partial degradation when exposed to UV-B and the daughter products may be less toxic than the parental compound.

## REFERENCES


American Public Health Association (1985). Standard Methods for the Examination of Water and WasteWater (16th ed.). *APHA*, Washington, D.C.

Behrenfield, M.J., D.R.S. Leen and H. Lee (1995). Ultraviolet B radiation effects on inorganic nitrogen uptake by natural assemblages of Oceanic Plankton. *J. Phycol*. 31: 25-36.

Braune, W. and G. Döhler (1996). Impact of UV-B radiation on 15N-ammonium and 15N-nitrate uptake by *Haematococcus lacustris* (Volvocales) II. The influence of recovery. *J.Plant Physiol.* 149: 348-357.

Crutzen, P.J. (1992). Ultraviolet on the increase. *Nature*, 356: 104-105.

Camm, E. L. and J. R. Stein (1974). Some aspects of nitrogen metabolism of *Nodularia spumigena* (Cyanophyceae). *Can. J. Bot*. 52: 119-126.

Da Silva E.J., L.E. Heuriksson and E. Heuriksson (1975). Effects of pesticides on blue green algae. *Microbios Letters* 4: 99-102.

De Filippis, L. F. (1979). The effect of heavy metal compounds on the permeability of *Chlorella* cells. *Z. Pflanzenphysiol* 92: 39-49.

De Vos, C.H.R., H. Schat, R. Vooijs and W.H.O. Ernst (1989). Copper–induced damage to the permeability barrier in roots of *Silene cucubalus*. *J. Plant Physiol*. 135, 164-169.

Döhler, G. (1985). Effects of UV-B radiation (290-320 nm) on the nitrogen metabolism of several marine diatoms. *J. Plant Physiol.*, 118: 391-400.

Döhler, G. (1991). Uptake of 15N-ammoniaium and 15N-nitrate by Antarctic diatoms: Dependence on the daytime and effect of UV-B radiation. *Biochem Physiol Pflanzen* 187: 347-355.

Döhler, G. (1992). Impact of UV-B radiation on uptake of 15N-ammonia and 15N-nitrate by phytoplankton of the Wadden Sea. *Mar. Biol*. 112: 485-489.

Eichern, M., Döhler G. and Audsten, H. (1993): Impact of UV-B radiation on photosynthetic electron transport of *Wolfia arrinisa*. *Wimm Photosynthetica*, 29: 613-618.

Gerloff, G.C., G. P. Fitzerald and F. Skoog (1950). The isolation, purification and culture of blue-green algae. *Am. J. Bot*. 27: 216-218.

Herbert D., Phipps, P.J. and Strange, R.E. (1971): Chemical analysis of microbial cells. In: *Methods in Microbiology* VB (eds.) Norris, J.R., and Ribbon, D.W., Academic Press, London, pp. 209-344.

Higley, B., H. G. Carrick, M. T. Brett, C. Luecke and C. R. Goldman (2001). The effects of ultraviolet radiation and nutrient additions on periphyton biomass and composition in a sub-alpine lake (Castle lake, USA) Internat*. Rev. Hydrobiol.* 86 (2): 147-163.

Ihlenfeldt, M. J. A. and, J. Gibson (1975). Phosphate utilization and alkaline phosphatase activity in *Anacystis nidulans* (*Synechococcus*). *Arch. Microbiol*. 102: 23-28.

Kaushik, B. D. and G. S. Vekatraman (1983). Response of cyanobacterial nitrogen fixation to insecticides. *Curr. Sci*. 52: 321-323.

Kumar, A., R. P. Sinha and D. P. Hader (1996). Effect of UV-B on enzymes of nitrogen metabolism in the cyanobacterium *Nostoc calcicola*. *J. Plant Physiol*. 148: 86-91.

Nicholas, D.J.D. and A. Nason (1957). Determination of nitrate and nitrite. In: Colowik S.P., Kaplan, N. O. (Eds.), *Methods in Enzymology*, 111. Academic Press, New York, pp. 981-984.

Panday,V. and L.C. Rai (2002). Interactive effects of UV-B and Pesticides on Photosynthesis and Nitrogen Fixation of *Anabaena doliolum*. *Journal of Microbiol. and Biotechnol.* 12(3): 423-430.

Rahwer, S. and W. Flockiger (1979), Effect of atrazine on growth, nitrogen fixation and photosynthetic rate of *Anabaena doliolum. Angew Botanik* 55: 59-64.

Rai, L. C. and M., Raizada (1985). Effect of nickel and silver ions on survival, growth, carbon fixation and nitrogenase activity in *Nostoc muscorum*: Regulation of toxicity by EDTA and calcium. *J. Gen. Appl. Microbiol*. 31: 329-337.

Rai L. C., B. Tyagi, P. K. Rai and Mallick, N. (1998). Interactive effects of UV-B and heavy metals (Cu and Pb) on nitrogen and phosphorus metabolism of a N2-fixing cyanobacterim *Anabaena doliolum Environ. Experimen. Bot*. 39: 221-231.

Rath, B. and S.P. Adhikary (1995). Growth response of two species of *Anabaena* to commercial and analytical grade Furadan *Proc. Internatl. Sympos.* In:

"Impact of modern Agriculture on Environment". Soc. Sust. Agri. and Max. Muller Bhawan, New Delhi, pp. 151-155.

Roldan, J.M. and W.L. Butler (1980). Photoactivation of nitrate reductase from *Neurospora crassa*. *Photochem. Photobiol*. 32: 375-381.

Saralabai V.C., P. Thamizhche Ivan and K. Shanta Gom (1989). Influence of Ultraviolet - B radiation on fixation and assimilation of nitrogen in *Crotolaria - juncea*.

Linn. *Indian J. Plant Physiol.* 32: 65-67.

Singh L. J. and D.N. Tiwari (1988a). Some important parameters in the evaluation of herbicide toxicity in diazotrophic cyanobacteria. *J. Appl. Bacteriol*. 64: 365-376.

Singh, L.J., D.N. Tiwari and H.N. Singh (1986). Evidence for genetic control of herbicide resistance in a rice-field isolate of *Glococapsa* sp. capable of aerobic diazotrophy under autotrophic conditions. *J. Gen. Appl. Microbiol*. 32: 81.

Singh, L. J. and D.N. Tiwari (1988). Effects of selected rice-field herbicides on photosynthesis, respiration and nitrogen assimilating enzyme systems of paddy soil diazotrophic cyanobacteria. *Pesticide Biochem. and Physiol*. 31: 120-128.

Smith, R.C., B.B. Prezelin, K.S. Baker, R.R. Bidigare, N.P. Boucher, T. Coley, D. Karentz, S. Maclntyre, S. Maintyre, H.A. Matiick, D. Menzier, M. Ondrusek, Z. Wan and K.J. Waters (1992). Ozone depletion: Ultraviolet radiation and phytoplankon biology in Antarctic waters. *Science* 255: 952-959.

Solheim, B., Zielke, M., Schutte, U., Olsen, R. A., and Spjelkavik, S. (2002). Environmental stress and nitrogen fixation in the arctic. *ESF-Cyanofix Summer School 2002* –Longyearbyen, Svalbard.

Stacey, G., F. R. Tabita and C. Van Baalen (1977). Nitrogen and ammonia assimilation in a cyanobacterium: Purification of glutamine synthetase from *Anabaena* sp. Strain CA. *J. Bacteriol*. 132: 596-603.

Stewart, W. D. P., G. P. Fitzgerald and R. H. Burris (1968). Acetylene reduction by nitrogen-fixing blue- green algae. *Arch. Microbiol*. 62: 336-348.

Tyagi, R., G. Srinivas, D. Vyas, A. Kumar and H.D. Kumar (1992). Differential effect of ultraviolet-B radiation on certain metabolic processes in chromatically adopting *Nostoc. Photo Chem. Photobiol*. 55: 401-407.

Vosjan, J.H., G. Döhler and G. Nieuwland (1990). Effect of UV-B irradiance on the ATP content of microorganism of the Weedell Sea (Antarctica). *Netherlands J. Sea. Res.* 25: 391-393.

Watkins, E. M., D. W. Schindler, M. A. Turner and D. Findlay (2001). Effect of solar ultra violet radiation on epilithic metabolism, & nutrient & community composition in a clear water boreal lake. *Can. J. fish.Aqat. Sci.halieut. Aquat* 58 (10): 2059-2070.