

Interactive effects of UV-B and Cu on photosynthesis, uptake and metabolism of nutrients in a green alga *Chlorella vulgaris* under simulated ozone column

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(Received September 1, 1997; Accepted October 23, 1997)

This study demonstrated a general reduction in photosynthesis (carbon fixation, O₂-evolution and photochemical electron transport chain), the uptake of NH₄⁺, NO₃⁻, urea and PO₄³⁻, and activities of nitrate reductase, urease, acid phosphatase and ATPase following UV-B and copper exposure of *Chlorella vulgaris* in the absence or presence of 1 and 2 ppm concentrations of a 4-inch-thick ozone layer. Though the effect of stressors used in combination was very detrimental to the above processes, selected concentrations of ozone not only counteracted the UV-B-induced inhibition of the above processes, but also stimulated O₂-evolution and the photochemical electron transport chain. Kinetics of nutrient uptake and enzyme activities demonstrated that UV-B causes structural change(s) in the enzymes/carriers responsible for the uptake of NH₄⁺, NO₃⁻, urea and PO₄³⁻ as well as their assimilatory enzymes. Except for nitrate reductase, copper was found to compete for the binding sites of all the above enzymes. Synergistic inhibition of photosynthetic activity, nutrient (except NH₄⁺) uptake, and enzyme activities by UV-B+Cu seems to be due to increased Cu uptake as a consequence of altered membrane permeability brought about by the peroxidation of membrane lipids in UV-B-exposed cells.

Key Words—acid phosphatase and ATPase; *Chlorella vulgaris*; copper; nitrate reductase; nutrient uptake; ozone layer; urease; UV-B irradiation

The increased anthropogenic emission of chlorofluorocarbons (CFCs) and other ozone-eating chemicals has resulted in a significant depletion of stratospheric ozone. Meteorological data have revealed a 4–5% decrease in stratospheric ozone at mid-latitude since 1979; the depletion being maximum during late winter and early spring (Barnes et al., 1996; Niu et al., 1992). An increased depletion of stratospheric ozone is expected to result in a substantial increase in UV-B (280–320 nm) irradiation that reaches the earth's surface. Variations in available UV-B radiation are also influenced by latitude, longitude, solar angle, temperature and other factors. UV-B is a very small (<1% of total energy) but very energetic component of the solar electromagnetic spectrum endowed with the potential to cause deleterious effects on living organisms (Madronich, 1992) including algae.

Algal responses to UV-B include the inhibition of

phototaxis, motility, orientation, photosynthetic carbon fixation, electron transport chain (ETC), nitrogen and phosphorus metabolism, enzyme activities (Braune and Döhler, 1996; Murthy and Rajagopal, 1995; Schofield et al., 1995) and change in species diversity (Häder and Häder, 1989; Häder and Worrest, 1991). Since the availability of nitrogen, phosphorus and photosynthetically-fixed carbon is extremely essential for metabolic processes of algae, any adverse effect of UV-B to these processes is likely to be mirrored through a reduction in growth or change in community structure. It is befitting to mention that much is known about the mechanisms of UV-B toxicity to different physiological processes, while comparatively, very little is known about the uptake and metabolism of nitrogen and phosphorus (Braune and Döhler, 1996; Döhler, 1994) and the kinetics of enzymes associated in their assimilation in green algae.

Besides UV-B, the accelerated input of heavy metals also causes serious threat to aquatic biota. Though several metals, in traces, are essential for different organisms including algae (De Filippis and Pallaghy, 1994; Rai et al., 1981), their high concentrations ad-

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versely affect photosynthesis, nutrient uptake, enzyme activities and other metabolic processes (De Filippis and Pallaghy, 1994; Mallick and Rai, 1994).

Algae constitute the first link in the primary production of freshwater and marine ecosystems. Any change(s) in their structure and/or function would seriously affect the entire food chain. In view of the meager information on the effects of UV-B and metals separately as well as in combination, and total lack of such information in the presence of an ozone layer, this study was undertaken to investigate (i) the impact of UV-B and Cu separately as well as in combination, in the absence and presence of ozone, on photosynthetic carbon fixation, O_2 -evolution, electron transport chain of the green algae *Chlorella vulgaris*, and (ii) the effect of these stressors on the uptake and inhibition kinetics of NH_4^+ , NO_3^- , urea and PO_4^{3-} and their assimilatory enzymes of the above alga.

Materials and Methods

The unicellular green alga *C. vulgaris* was grown axenically in modified Chu-10 medium (Gerloff et al., 1950) under $14.4 W m^{-2}$ photosynthetically available radiation (PAR) light intensity and a 14 : 10 h light/dark cycle at $24 \pm 2^\circ C$. The alga was subcultured twice a week into fresh medium and cultures from the logarithmic phase only were used for the toxicity test. A stock solution ($1.0 g l^{-1}$) of $CuCl_2 \cdot 2H_2O$ was prepared in Milli Q grade water and sterilized by passing through a Millipore membrane filter ($0.22 \mu m$) before supplementation to the culture media. All experiments were conducted in triplicate and repeated at least twice to confirm the reproducibility of the results.

The UV-B irradiation was provided by a UV-B lamp (CAT No. 3-4408, Fotodyne Inc., U.S.A.) with a maximum output at 310 nm. The desired radiation dose ($12.9 mW m^{-2}$) was obtained by adjusting the distance between the UV-B source and the alga. The above UV-B dose was selected keeping in mind the latitude of our work station ($25^\circ N$) and the mean percent depletion of ozone layer as calculated by Crutzen (1992) and Smith et al. (1992).

Different concentrations of ozone were prepared by an ozone layer simulator (Ozonex System, Standard Appliances, Varanasi, India) and passed through a 4-inch-thick perspex chamber having quartz windows on both lower and upper sides. During treatment of alga with UV-B, in the presence of the ozone layer, the perspex chamber was placed in-between the test alga and UV-B source.

The algal cells exposed to UV-B for a 0–1 h duration were withdrawn at regular intervals, plated onto agar plates and kept in the dark for 24 h. For measuring survival against Cu, algal cells were subjected to dif-

ferent concentrations ($0-5 mg l^{-1}$) of Cu. The LC_{50} dose of UV-B and Cu was determined by the plate colony count method (Rai and Raizada, 1985). Approximately 25 (LC_{25}) and 50% (LC_{50}) survival of the test alga was observed after 6 and 15 min of UV-B exposure, respectively. However, the LC_{25} and LC_{50} concentrations for Cu were 0.7 and $2.0 mg l^{-1}$, respectively. The doses of UV-B and Cu selected for further study were: (i) LC_{25} (denoted as UV-B₁ and Cu₁), and (ii) LC_{50} (denoted as UV-B₂ and Cu₂). The synergistic, additive and antagonistic effects were analyzed as given in Mallick and Rai (1989).

Carbon fixation was measured according to the method described in Rai and Raizada (1985). Oxygen evolution was measured at $25 \pm 2^\circ C$ at an illumination of $60 W m^{-2}$ PAR light intensity using a Clark O_2 electrode (Digital Oxygen System, Model 10, Rank Brothers, U.K.). The activity of the electron transport chain was measured as per the method of Lien (1978).

The uptake of NH_4^+ , NO_3^- , urea and PO_4^{3-} from the medium was estimated colorimetrically by Nessler's reagent (Herbert et al., 1971), brucine-sulphuric acid (Nicholas and Nason, 1957), diacetyl mono oxime-thiosemicarbazide (Wootton, 1974) and stannous chloride (American Public Health Association, 1985) methods, respectively, by measuring the depletion of these nutrients from the culture medium. Inhibition kinetics of the above nutrients were studied at their different concentrations.

In vivo nitrate reductase activity was measured by the method of Camm and Stein (1974). Whole cell urease activity of the test alga was assayed following the method of Mackerras and Smith (1986). Acid phosphatase activity was measured following the method of Juma and Tabatabai (1977). For extraction of ATPase, exponentially grown alga was harvested by centrifugation ($5,000 \times g$ for 3 min), washed and re-suspended in Tris HCl buffer (30 mM; pH 8.1) and sonicated (Ultrasonicator, Heat System, SL 2020, New York, U.S.A.) for 5 min. The suspension was centrifuged in a refrigerated centrifuge (C-24, Remi, Bombay, India) at $10,000 \times g$ for 30 min and supernatant so obtained was dialyzed for 3 h against Tris HCl buffer (10 mM; pH 8.1). The resulting preparation was used as the crude enzyme extract. All the preparations for ATPase measurement were done at $4^\circ C$. Mg^{2+} -dependent ATPase activity was measured as described by Lockau and Pfeffer (1982). To measure ATPase activity, the assay mixture (2.0 ml) containing Tris HCl (30 mM; pH 8.1), ATP (6 mM), $MgCl_2$ (6 mM) and crude enzyme was kept at $37^\circ C$ for 30 min and reaction was terminated by adding 0.25 ml of TCA solution (40%, w/v). The amount of inorganic phosphate, thus, liberated was measured as per the method of APHA (1985). Inhibition kinetics of enzymes were studied at

different substrate concentrations (0–125 $\mu\text{mol l}^{-1}$). Results were analyzed by Chi-square (χ^2) and Student's *t* test.

Results

Table 1 demonstrates the impact of LC₂₅ and LC₅₀ doses of UV-B and Cu separately as well as in combination in the presence and absence of an ozone layer on carbon fixation, O₂-evolution and the photochemical electron transport chain of *C. vulgaris*. Exposure of the alga to LC₂₅ and LC₅₀ doses of UV-B caused, respectively, 21 and 36% inhibition of carbon fixation. In contrast to this, the inhibition produced by the above doses of Cu was, respectively, 28 and 40%. However, UV-B and Cu together inhibited carbon fixation in a synergistic manner; a combination of LC₅₀ dose each of UV-B and Cu was approximately 100% inhibitory to carbon fixation. Though both the concentrations (i.e., 1 and 2 ppm) of ozone were effective in decreasing the inhibitory effect of UV-B, 2 ppm was more effective ($p < 0.01$, Student's *t* test).

Although UV-B and Cu separately as well as in combination inhibited O₂-evolution in the same way as

carbon fixation, the level of inhibition was slightly lower. A very interesting point that emerged from this study was the stimulation of O₂-evolution over control in the alga treated with UV-B in the presence of ozone layer. The test alga treated with a LC₂₅ dose of UV-B in the presence of 1 and 2 ppm ozone showed, respectively, 61 and 80% stimulation of O₂-evolution over the control. However, the above concentrations of ozone produced respectively 16 and 49% stimulation of O₂-evolution in the alga treated with the LC₅₀ dose of UV-B. A combination of UV-B and Cu was less inhibitory to O₂-evolution at selected doses in the presence of two concentrations of ozone.

Though all the components of the photochemical electron transport chain were inhibited by UV-B and Cu, PS II activity was comparatively more sensitive to the stressors used. The exposure of alga to LC₂₅ and LC₅₀ doses of UV-B caused, respectively, 19 and 25% inhibition of PS II activity. However, the activities of PS I and redox coupling between the two photosystems were inhibited only by 16, 22, and 14 and 18%, respectively, at the above doses of UV-B. Interestingly Cu produced a similar (like UV-B) but more severe inhibition of photochemical electron transport chain.

Table 1. Interactive effects of UV-B and Cu on photochemical electron transport chain, O₂-evolution and carbon fixation of *C. vulgaris* in the absence or presence of O₃ layer.

Treatment	Photochemical electron transport chain			O ₂ -evolution ($\mu\text{mol O}_2\text{-evolved}$ $\text{mg}^{-1}\text{ protein h}^{-1}$)	Carbon fixation ($\text{CPM} \times 10^5 \text{mg}^{-1}$ protein)
	PS-I ($\mu\text{mol O}_2\text{-consumed}$ $\text{mg}^{-1}\text{ protein h}^{-1}$)	PS-II ($\mu\text{mol O}_2\text{-evolved}$ $\text{mg}^{-1}\text{ protein h}^{-1}$)	Whole chain ($\mu\text{mol O}_2\text{-evolved}$ $\text{mg}^{-1}\text{ protein h}^{-1}$)		
Control	61.4	57.9	46.4	40.0	3.74
UV-B ₁	51.5 (16)	46.7 (19)	39.9 (14)	36.0 (10)	2.95 (21)
UV-B ₂	48.0 (22)	43.4 (25)	37.9 (18)	32.0 (20)	2.41 (36)
Cu ₁	50.3 (18)	45.0 (22)	39.0 (16)	33.8 (15)	2.70 (28)
Cu ₂	36.8 (28)	7.2 (32)	22.7 (24)	24.9 (38)	2.24 (40)
UV-B ₁ +Cu ₁	32.7 (47)*	16.1 (72)*	27.3 (41)*	15.9 (60)*	1.33 (65)*
UV-B ₁ +Cu ₂	29.9 (51)*	-12.9 (122)**	25.1 (46)*	-2.6 (106)**	0.22 (94)**
UV-B ₂ +Cu ₁	27.9 (55)*	10.9 (81)*	27.7 (49)*	8.2 (80)*	0.08 (98)**
UV-B ₂ +Cu ₂	21.1 (66)*	-23.6 (141)**	19.4 (58)*	-3.7 (109)**	0.01 (100)**
#UV-B ₁	81.0 (+32) NS	80.0 (+38) NS	59.4 (+28) NS	64.6 (+61) NS	3.09 (17) NS
#UV-B ₂	68.7 (+12) NS	68.6 (+19) NS	42.8 (8) NS	46.3 (+16) NS	2.65 (29) NS
#UV-B ₁ +Cu ₁	44.0 (28) NS	37.4 (35) NS	34.7 (25) NS	19.4 (52) NS	1.71 (54) NS
#UV-B ₁ +Cu ₂	41.1 (33) NS	34.8 (40) NS	36.4 (28) NS	15.7 (61) NS	1.04 (72) NS
#UV-B ₂ +Cu ₁	31.2 (49) NS	21.9 (62) NS	26.4 (43) NS	12.9 (68) NS	0.83 (78) NS
#UV-B ₂ +Cu ₂	22.7 (63) NS	18.6 (68) NS	24.2 (48) NS	11.1 (72) NS	0.48 (87) NS
##UV-B ₁	104.3 (+70) NS	107.0 (+85) NS	80.9 (+74) NS	83.5 (+80) NS	3.24 (13) NS
##UV-B ₂	88.4 (+44) NS	87.8 (+52) NS	65.8 (+42) NS	69.1 (+49) NS	2.81 (25) NS
##UV-B ₁ +Cu ₁	47.8 (22) NS	41.8 (28) NS	38.4 (17) NS	25.9 (35) NS	2.10 (44) NS
##UV-B ₁ +Cu ₂	45.4 (26) NS	39.3 (32) NS	36.2 (22) NS	23.2 (42) NS	1.90 (49) NS
##UV-B ₂ +Cu ₁	41.9 (32) NS	37.7 (35) NS	34.5 (26) NS	22.4 (44) NS	1.67 (55) NS
##UV-B ₂ +Cu ₂	38.0 (38) NS	33.6 (42) NS	32.1 (31) NS	19.3 (52) NS	1.41 (62) NS
Standard error	0.32–0.52	0.32–0.50	0.32–0.50	0.36–0.59	0.22–0.54

All the values are means \pm SE. Data in parentheses show percent inhibition. Data with negative sign show O₂ consumption. A positive sign shows percent stimulation over control; # shows presence of 1 ppm (4-inch-thick) and ## 2 ppm O₃ layer; *t* significant at $p < 0.01$. χ^2 -test confirmed that the interactive effects of UV-B and metals were significantly higher (* $p < 0.025$, ** $p < 0.05$) than their additive values. NS, not significant.

Table 2. Impact of O₃ layer on interactive effects of UV-B and Cu on ammonium, nitrate, urea and phosphate uptake of *C. vulgaris*.

Treatment	NH ₄ ⁺ uptake (μmol NH ₄ ⁺ mg ⁻¹ protein)	NO ₃ ⁻ uptake (μmol NO ₃ ⁻ mg ⁻¹ protein)	Urea uptake (μmol urea mg ⁻¹ protein)	PO ₄ ³⁻ uptake (μmol PO ₄ ³⁻ mg ⁻¹ protein)
Control	56.0	10.2	32.4	38.5
UV-B ₁	43.7 (22)	8.0 (22)	25.0 (23)	31.5 (18)
UV-B ₂	38.6 (31)	7.3 (29)	22.1 (32)	28.7 (25)
Cu ₁	43.1 (23)	8.2 (20)	26.0 (20)	32.6 (15)
Cu ₂	36.8 (34)	7.2 (30)	22.7 (30)	29.8 (23)
UV-B ₁ +Cu ₁	29.3 (48) NS	5.3 (48)	16.3 (49) NS	22.2 (42)
UV-B ₁ +Cu ₂	23.0 (59) NS	3.9 (62)	14.2 (56) NS	18.4 (52)
UV-B ₂ +Cu ₁	21.8 (61) NS	4.2 (59) NS	12.9 (60)	17.7 (54)
UV-B ₂ +Cu ₂	18.0 (68) NS	3.1 (70)	10.8 (67)	12.0 (69)
#UV-B ₁	45.8 (18) NS	8.4 (18) NS	26.4 (18) NS	32.7 (15) NS
#UV-B ₂	41.6 (26) NS	8.0 (21) NS	24.4 (25) NS	30.8 (20) NS
#UV-B ₁ +Cu ₁	35.4 (37) NS	6.0 (42) NS	18.9 (42) NS	24.8 (36) NS
#UV-B ₁ +Cu ₂	30.9 (45) NS	5.0 (51) NS	16.5 (49) NS	21.0 (45) NS
#UV-B ₂ +Cu ₁	30.7 (45) NS	5.2 (49) NS	17.3 (47) NS	22.2 (42) NS
#UV-B ₂ +Cu ₂	25.9 (54) NS	4.1 (60) NS	15.4 (52) NS	16.5 (57) NS
##UV-B ₁	49.8 (11) NS	8.7 (14) NS	27.6 (15) NS	34.2 (11) NS
##UV-B ₂	44.0 (21) NS	8.6 (16) NS	26.2 (19) NS	32.8 (15) NS
##UV-B ₁ +Cu ₁	38.7 (31) NS	6.9 (32) NS	21.9 (32) NS	27.2 (29) NS
##UV-B ₁ +Cu ₂	36.4 (35) NS	6.2 (40) NS	19.3 (40) NS	23.7 (38) NS
##UV-B ₂ +Cu ₁	33.8 (40) NS	6.0 (41) NS	19.1 (41) NS	24.6 (36) NS
##UV-B ₂ +Cu ₂	29.9 (47) NS	5.3 (48) NS	17.5 (46) NS	22.0 (43) NS
Standard error	0.32–0.52	0.02–0.04	0.32–0.50	0.44–0.60

All the values are means ± SE. Data in parentheses show percent inhibition. # 1 ppm and ## 2 ppm (4-inch-thick) O₃ layer. *t* significant at $p < 0.01$. χ^2 -test confirmed that the interactive effects of UV-B and metals are significantly higher ($p < 0.05$) than their additive values. NS, not significant.

Like other variables, the UV-B-induced inhibition of the activity of photochemical electron transport was not only counteracted but stimulated by both concentrations of ozone; the stimulation was more pronounced for UV-B₁ than UV-B₂. The stimulation produced by 2 ppm of ozone was more than that by the 1 ppm concentration.

Table 2 shows the impact of LC₂₅ and LC₅₀ doses of UV-B and Cu separately as well as in combination on NH₄⁺, NO₃⁻, urea and PO₄³⁻ uptake of *C. vulgaris* in the presence and absence of 1 and 2 ppm concentrations of ozone layer. UV-B at LC₂₅ and LC₅₀ dosages produced 22 and 31% inhibition of NH₄⁺ uptake, respectively. The application of LC₂₅ and LC₅₀ doses (i.e., 0.7 and 2.0 mg l⁻¹) of Cu caused a decrease in NH₄⁺ uptake by 23 and 34%, respectively. A combination of LC₂₅ and LC₅₀ doses of UV-B+Cu proved more inhibitory (additive effect) to NH₄⁺ uptake than their (UV-B or Cu) individual effects. Though both the concentrations of ozone could counteract the inhibition caused by UV-B alone or in combination with Cu, 2 ppm ozone was more effective. Application of 1 and 2 ppm ozone produced significant ($p < 0.01$, Student's *t* test) amelioration over the control. The inhibition produced by UV-B and Cu alone or in combination on uptakes of NO₃⁻, urea and PO₄³⁻ was very low but quite similar to NH₄⁺ uptake either in the absence or pres-

ence of two dosages of ozone. The combination of UV-B and Cu inhibited the uptake of these nutrients synergistically ($p < 0.025$, χ^2 test). Contrary to this, PO₄³⁻ uptake was least affected by the two stressors, either separately or in combination.

Figure 1A provides information on the inhibition kinetics of NH₄⁺ uptake of *C. vulgaris* following supplementation of LC₅₀ doses of UV-B and Cu. The apparent decrease in V_{max} and constant K_m of NH₄⁺ uptake at the selected dose of UV-B alone or in combination with Cu clearly indicated a non-competitive inhibition of NH₄⁺ uptake. In contrast to this, supplementation of a LC₅₀ dose of Cu failed to produce any significant change ($p > 0.05$, Student's *t* test) in V_{max} but increased the K_m , thereby suggesting a competitive inhibition of NH₄⁺ uptake of *C. vulgaris* by Cu. The inhibition kinetics of urea and PO₄³⁻ uptake also showed trends similar to NH₄⁺ uptake (data not shown).

The kinetics of NO₃⁻ uptake of *C. vulgaris*, subjected to LC₅₀ doses of UV-B and Cu can be found in Fig. 1B. It is apparent from this figure that the V_{max} for NO₃⁻ uptake decreased at all the doses of UV-B and Cu when applied to alga either singly or jointly. However, no change in the apparent K_m of NO₃⁻ uptake was observed. This clearly indicated a non-competitive inhibition of NO₃⁻ uptake by UV-B and Cu.

The data of the inhibitory effects of UV-B and Cu on

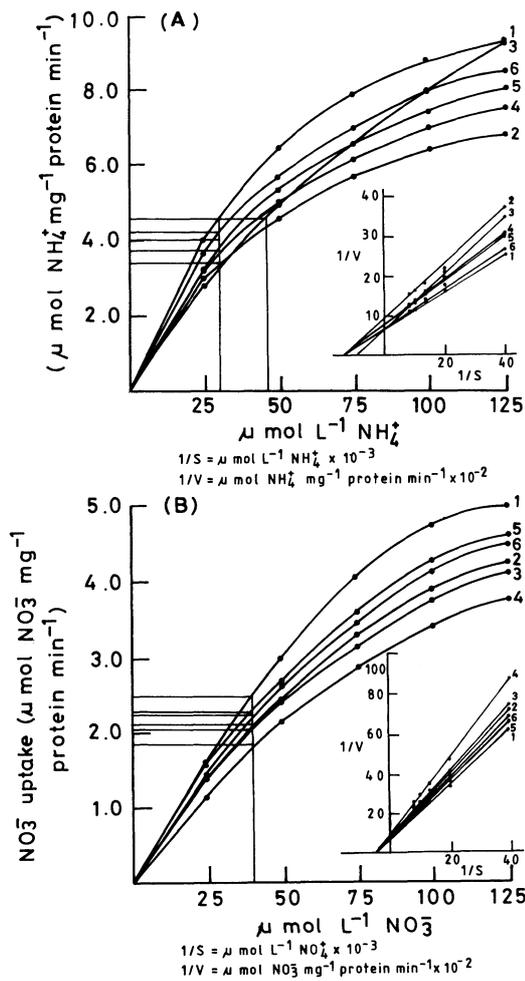


Fig. 1. Michaelis-Menten and Lineweaver-Burk (in inset) plots showing UV-B- and Cu-induced inhibition of NH_4^+ (A) and NO_3^- (B) uptake of *C. vulgaris*.

Control (1), UV-B₂ (2), Cu₂ (3), UV-B₂+Cu₂ (4), UV-B₂ in the presence of 2 ppm concentration of ozone (5) and UV-B₂+Cu₂ in the presence of 2 ppm concentration of ozone (6).

the activities of nitrate reductase, urease, acid phosphatase and ATPase of *Chlorella* in the absence or presence of ozone are incorporated in Table 3. LC₂₅ and LC₅₀ doses of UV-B reduced the nitrate reductase activity of test alga from 19.06 to 11.98 and 9.96 $\mu\text{mol NO}_2^- \text{mg}^{-1} \text{protein}$, respectively. The LC₅₀ dose of Cu produced a complete inhibition of NR activity when combined with both the doses of UV-B. All the combinations of UV-B with Cu showed synergistic inhibition of NR activity. UV-B₁-induced inhibition of NR activity was ameliorated by 25 and 29% respectively at 1 and 2 ppm concentrations of ozone. This inhibition was, however, reduced by 10 and 26%, respectively, after exposure of the test alga to a LC₅₀ dose of UV-B in the presence of the above concentrations of ozone. The inhibitory effect of UV-B+Cu was also decreased significantly ($p < 0.01$; t test) in the presence of the two

concentrations of ozone.

Exposure of *Chlorella* to LC₂₅ and LC₅₀ doses of UV-B resulted in 22 and 29% inhibition of urease activity, respectively. Likewise, Cu at the above doses inhibited urease activity by 20 and 30%, respectively. A combination of UV-B+Cu was more detrimental to urease activity than the two stressors used separately. Urease activity was also ameliorated by both concentrations of ozone.

The interactive effect of UV-B and Cu on the inhibition kinetics of nitrate reductase activity of *C. vulgaris* can be found in Fig. 2A. A consistent decrease in V_{max} and no change in apparent K_m of the NR activity of the test alga following exposure to different doses of UV-B and Cu, either alone or in combination, clearly suggests non-competitive inhibition of NR activity. Like NR, a decrease in V_{max} of urease activity (see Fig. 2B) after exposure of the alga to a LC₅₀ dose of UV-B, either alone or in combination with Cu, and a constant K_m clearly indicate non-competitive inhibition. In contrast to this, supplementation of the LC₅₀ dose of Cu did not produce any significant change ($p > 0.05$, Student's t test) in V_{max} but did produce an increase in K_m , thereby suggesting a competitive inhibition of urease activity of *C. vulgaris*.

Approximately 23 and 32% inhibition of acid phosphatase activity of the test alga was observed following exposure to LC₂₅ and LC₅₀ doses of UV-B irradiation, respectively. In the same way, Cu at the above dosage inhibited the acid phosphatase activity by 20 and 30%, respectively. A combination of a LC₅₀ dose of UV-B with two doses of Cu caused synergistic inhibition ($p < 0.025$, χ^2 test) of acid phosphatase activity. Like other variables, the inhibition of acid phosphatase was also ameliorated by selected concentrations of ozone.

The ATPase activity of *C. vulgaris* was inhibited in the same fashion as other enzymes by the two stressors used. UV-B irradiation was found to be more deleterious to ATPase activity in comparison to Cu. A combination of both doses of these stressors inhibited the ATPase activity in a synergistic manner ($p < 0.025$, 0.05 , χ^2 test). Though ozone was found to counteract the toxicity produced by UV-B and Cu, the level of amelioration was not very significant ($p > 0.05$).

Discussion

The loss of photosynthetic O_2 -evolution following exposure of the test alga to UV-B (Table 1) could be due to photoinhibition or photodamage (Gerber and Häder, 1995) of the photosynthetic apparatus. The complete inhibition of PS II and a significant decrease in PS I activity and redox coupling between the two photosystems revealed that the photochemical elec-

Table 3. UV-B and Cu-induced toxicity on nitrate reductase, acid phosphatase, urease and ATPase activities of *C. vulgaris*: effect of O₃ layer.

Treatment	NR activity ($\mu\text{mol NO}_2^-$ released mg^{-1} protein)	Urease activity ($\mu\text{mol NH}_4^+$ released mg^{-1} protein)	Acid phosphatase activity ($\mu\text{mol pNP}$ released mg^{-1} protein)	ATPase activity ($\mu\text{mol PO}_4^{3-}$ released mg^{-1} protein)
Control	19.1	10.2	32.4	38.5
UV-B ₁	12.0 (37)	8.0 (22)	25.0 (23)	31.5 (18)
UV-B ₂	10.0 (48)	7.3 (29)	22.1 (32)	28.7 (25)
Cu ₁	12.9 (32)	8.2 (20)	26.0 (20)	32.6 (15)
Cu ₂	10.5 (45)	7.2 (30)	22.7 (30)	29.8 (23)
UV-B ₁ +Cu ₁	2.9 (85)*	5.4 (48)*	16.5 (49) NS	22.2 (42)**
UV-B ₁ +Cu ₂	ND (100)*	3.9 (62)*	14.2 (56) NS	18.4 (52)**
UV-B ₂ +Cu ₁	0.9 (95)*	4.2 (59)*	12.9 (60)*	17.7 (54)*
UV-B ₂ +Cu ₂	ND (100)*	3.1 (70)**	10.8 (67)*	12.0 (69)*
#UV-B ₁	16.7 (12) NS	8.4 (18) NS	26.4 (18) NS	32.7 (15) NS
#UV-B ₂	11.8 (38) NS	8.0 (21) NS	24.4 (25) NS	30.8 (20) NS
#UV-B ₁ +Cu ₁	7.1 (62) NS	6.0 (42) NS	18.9 (42) NS	24.8 (36) NS
#UV-B ₁ +Cu ₂	5.4 (72) NS	5.0 (51) NS	16.5 (49) NS	21.0 (45) NS
#UV-B ₂ +Cu ₁	6.1 (68) NS	5.2 (49) NS	17.3 (47) NS	22.2 (42) NS
#UV-B ₂ +Cu ₂	4.9 (74) NS	4.1 (60) NS	15.4 (52) NS	16.5 (57) NS
##UV-B ₁	18.8 (8) NS	8.7 (14) NS	27.6 (15) NS	34.2 (11) NS
##UV-B ₂	14.8 (22) NS	8.6 (16) NS	26.2 (19) NS	32.8 (15) NS
##UV-B ₁ +Cu ₁	9.9 (48) NS	6.9 (32) NS	21.9 (32) NS	27.2 (29) NS
##UV-B ₁ +Cu ₂	1.2 (41) NS	6.2 (40) NS	19.3 (40) NS	23.7 (38) NS
##UV-B ₂ +Cu ₁	9.3 (51) NS	6.0 (41) NS	19.1 (41) NS	24.6 (36) NS
##UV-B ₂ +Cu ₂	8.0 (58) NS	5.3 (48) NS	17.5 (46) NS	22.0 (43) NS
Standard error	0.32–0.52	0.02–0.04	0.32–0.50	0.44–0.60

All the values are means \pm SE. Data in parentheses show percent inhibition. # 1 ppm (4-inch-thick) and ## 2 ppm O₃ layer. *t* significant at $p < 0.05$. χ^2 -test revealed that the interactive effects of UV-B and metals are significantly higher (* $p < 0.025$, ** $p < 0.05$) than their additive values. NS, not significant; ND, not detectable.

tron transport chain is the primary target of UV-B. The observed high sensitivity of PS II to UV-B agrees well with Murthy and Rajagopal (1995) and Rajagopal and Murthy (1996) who reported UV-B-induced loss of Mn²⁺ and structural alteration in the D1 and D2 polypeptides, which are considered responsible for the inhibition of PS II activity. The sensitivity of PS II to Cu agrees well with the findings of Rai et al. (1991), where heavy metals were found to inhibit electron transport at the oxidizing side of PS II or destroy the photosynthetic membranes, consequently inactivating the PS II reaction center. The consumption of O₂, instead of evolution, by PS II after exposure to both the doses of UV-B and a LC₅₀ dose of Cu might be due to the arrest of O₂-evolution and increase in respiration rate of the test alga as reported by Goyal and Tolbert (1991) and Rai et al. (1995) for *Chlamydomonas reinhardtii* and *Anabaena doliolum*, respectively. It is pertinent to mention that algal cells treated with UV-B in the presence of two dosages of ozone depicted higher O₂-evolution and increased activity of ETC than the control. This could be due to the selective absorption of UV-B radiation by the ozone layer, thereby ensuring the availability of useful radiation for algal photosynthesis.

A reduction in photosynthetic carbon fixation of the

test alga after exposure to UV-B might be the result of either a reduced supply of ATP and reduced form of ferredoxin due to the decreased activity of ETC or loss of the carboxylating enzyme RUBP carboxylase (Schofield et al., 1995). The later is known to be sensitive to H₂O₂ produced in large quantities within the chloroplast during photooxidation by UV-B (Asada and Takahashi, 1987) or alterations in mRNA levels and polypeptide subunits of RUBP carboxylase (Jordan, 1996).

The inhibition of nutrient uptake (Table 2) by UV-B irradiation supports the earlier findings of Döhler (1992, 1994) and Braune and Döhler (1996), who demonstrated that the high energetic cost of nitrogen uptake results in tight coupling between nitrogen metabolism and photosynthetic production of ATP and NADPH (Vosjan et al., 1990). This is likely to alter the amount of energy required for the incorporation of nitrogen and other nutrients since the transport of nitrogenous compounds is effected through ATP-dependent permease. In other words, a decrease in the uptake of these nutrients constitutes the secondary response to the primary UV-B radiation damage of ETC (Rai et al., 1995). The non-competitive inhibition of these nutrients by UV-B (Fig. 1) suggests an alteration in the structure of the enzyme(s) responsible for their

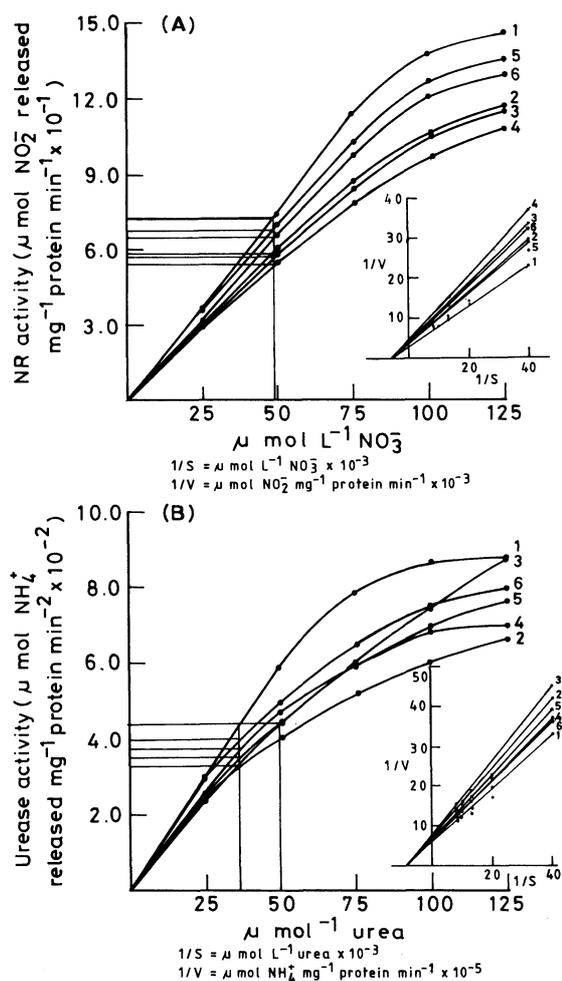


Fig. 2. Interactive effect of UV-B with Cu on kinetics of nitrate reductase (A) and urease (B) activities of *C. vulgaris*.

Control (1), UV-B₂ (2), Cu₂ (3), UV-B₂+Cu₂ (4), UV-B₂ in the presence of 2 ppm concentration of ozone (5) and UV-B₂+Cu₂ in the presence of 2 ppm concentration of ozone (6).

uptake/transport. In contrast to this, Cu was found to exert competitive inhibition on NH₄⁺ (Fig. 1A), urea and PO₄³⁻ uptake (data not shown). This clearly indicates direct competition between Cu and nutrients (NH₄⁺, urea and PO₄³⁻) for the catalytic sites of the enzyme(s) and carrier(s). Contrary to this, the non-competitive inhibition of NO₃⁻ uptake (Fig. 1B) by Cu points toward an alteration in the structure of the enzyme responsible for its uptake/transport.

UV-B-induced inhibition of NR activity agrees well with the findings of Döhler (1992). Some plants including algae and cyanobacteria, however, show the stimulation of nitrate reductase activity following UV-B irradiation (Saralabai et al., 1989). The greater sensitivity of nitrate reductase to UV-B might be due to the reduced energy status of the UV-B-exposed cells. Nevertheless, urease, acid phosphatase and ATPase activities of the alga were not only inhibited by UV-B but

also by Cu exposure. The inhibition of ATPase activity after UV-B exposure was also reported by Murphy (1983). Since ATPase activity is primarily dependent on the ATP pool, reduction of the cellular ATP pool size following UV-B and metal exposure (Rai et al., 1995) may inhibit the enzyme activity, leading to a decrease of nutrient uptake. The synergistic inhibition of nutrient uptake ($p < 0.05$, χ^2 -test) (particularly NO₃⁻, urea and PO₄³⁻; Table 2) under the combined stress of UV-B+Cu could be directly related to the reduced ATPase activity (Table 3), as uptake of nutrients is an energy-dependent process (Oelmüller et al., 1988). The reduction in ATPase activity may not be the sole cause of the inhibition of nutrient uptake. Alteration in the structure of the enzyme(s) following UV-B exposure, as reflected from the non-competitive inhibition pattern, seems to play a significant role ($p < 0.01$, Student's *t* test) in the reduction of nutrient uptake. The synergism produced by UV-B and Cu could be due to an altered membrane permeability brought about by the peroxidation of membrane lipids following UV-B exposure, thereby allowing a facilitated uptake of Cu, hence increased toxicity. A UV-B-induced increase in cell permeability has been clearly demonstrated by Häder et al. (1986) and Häder and Worrest (1991). These workers suggested that the cellular constituents absorbing UV-B radiation are destroyed, thus, causing further damage to protein and glycolipids. This study, therefore, confirms that the environmental hazards of UV-B radiation would be intensified far greater than expected in systems already contaminated with heavy metals. The ozone layer in the stratosphere not only protects the organisms of the earth by absorbing harmful UV-B radiation but also relieves some physiological processes caused by UV-B irradiation from suppression or inhibition.

This study was sponsored by a grant from the Ministry of Environment and Forests, Govt. of India, New Delhi, sanctioned to L. C. Rai.

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