J. mar. biol. Ass. India, 2001, **43** (1&2) : 173 - 180

Short-term UV-B radiation induced changes in marine micro algae

C. Poppy Mary Vimalabai and G. Kulandaivelu

School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

Abstract

With the primary objective of comparing the UV-B induced changes in algae having different thylakoid organization, three species of marine microalgae, *Chlorella salina, Dicrateria inornata* and *Isochrysis galbana* in pure cultures were screened for their UV-B sensitivity based on the changes in the rate of photosynthetic O_2 evolution. *Chlorella* was found to be highly sensitive whereas *Dicrateria* was moderately sensitive and *Isochrysis* was resistant.

Under short term UV-B treatment (5 $W.m^{-2}$) there was no significant change in the level of pigments. But the cellular protein content showed drastic changes in both *Chlorella* and *Dicrateria* while *Isochrysis* showed only a marginal change. Absorption spectral studies in *Chlorella* cells revealed only marginal changes in the major chlorophyll absorption bands. In *Dicrateria* and *Isochrysis* cells, the levels of major red and blue peaks had decreased. In addition, a shift towards long wavelength of the red absorption peak was also observed. Flourescence excitation spectra for F-682 in *Chlorella* cells showed no significant change in the excitation characteristics but *Dicrateria* and *Isochrysis* cells showed a progressive reduction in the level of 530 and 590 nm excitation peaks.

Marine organisms in the upper layer of the sea are endangered due to the increased UV flux. It has been shown that ambient or slightly elevated doses of UV-B radiation can decrease the primary production by phytoplankton (Worrest, 1986, USEPA 1987). Several reports (Karentz *et al.*, 1994) suggest that small organisms (bacteria and microalgae) because of their size and short generation times, are likely to be more susceptible to UV stress than the larger organisms. Majority of the plankton algae are enable to protect themselves from the harmful effects of UV-B radiation. There exists a vast body of reference on the study of UV-B effects on marine algae.

The primary objective of this work is to investigate the UV-B induced changes in algae having different thylakoid organization.

Material and methods

Culture conditions : The eukaryotic unicellular microalgae *Dicrateria inornata, Isochrysis galbana* (Chrysophycean flagellates) and *Chlorella salina* (Chlorophyceae) were cultured photoautotrophically at 25°C in liquid culture medium as devised by Walne (1974). Cultures were maintained under white light at an intensity of 20 W m^{-2}. The age of the culture was counted from the day of inoculation. Cells on the required day were harvested by centrifugation at 5,000 g for 10 min., washed twice with the medium and then suspended in a minimum volume of the medium at a final concentration of 100 µg Chl a/ml for immediate use.

UV-B irradiation : A thermostated plexiglass irradiation chamber placed on a magnetic stirrer was used for UV-B treatment. Irradiation at the sample surface was 5 W m^{-2}. The Chl *a* content was determined following the method of Jeffrey and Humphrey (1975). Chl *a* and Chl *b* contents of *Chlorella* were determined according to Arnon (1949). Total carotenoid content was estimated following the method of Metzner *et al.* (1965). Protein content was estimated following the method of Lowry *et al.* (1951). Photosynthetic O_2 evolution was continuously monitored at 25°C using a oxygen electrode (Hansatech, UK). $^{14}CO_2$ fixation by the whole cells was carried out by the modified method of Peschek (1978). Cell free chloroplast particles were prepared following the modified method of Senger and Mell (1977).

Measurements : Room temperature fluorescence excitation and emission spectra were recorded using a Hitachi MPF4 spectrofluorimeter. Electrophoretic separation of proteins in PAGE was performed according to the method of Laemmli (1970).

Results and discussion

Screening of micro algae for UV-B sensitivity : To find out the UV-B induced changes in algae having different thylakoid organization, three species of marine micro algae in pure cultures were screened for their UV-B sensitivity. The criterion for UV-B sensitivity was based on the changes in the rate of photosynthetic O_2 evolution. Among the three species tested, *Chlorella salina* was found to be highly sensitive in exhibiting less than 50% activity even after 30 min of UV-B treatment. The activity declined sharply to about 13% after 60 min of UV-B treatment (Fig. 1). The other two species, *Dicrateria inornata* and *Isochrysis galbana* exhibited differential response to UV-B sensitivity. *Dicrateria* was found to be moderately sensitive while *Isochrysis* was found to be resistant showing nearly 55% activity even after 60 min of UV-B treatment. Thus the extent of inhibition was species-specific. This implies the presence of genetic limits to UV photoadaptation among species as shown by Calkins and Thordardotier (1980). In the present study, *Isochrysis* showed higher resistance to UV-B radiation than the other two organisms. This agrees with the report of Jokiel and York (1984). There were sev-

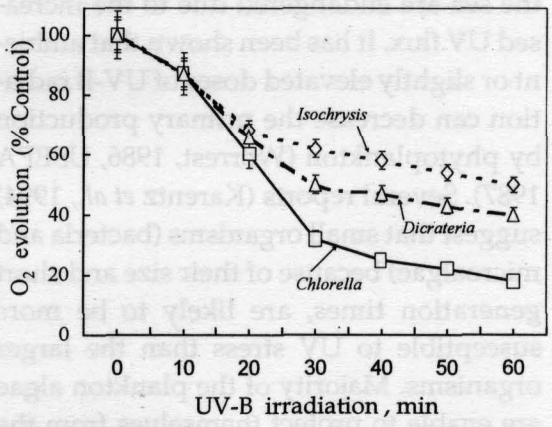

Fig. 1. *Changes in the rate of O_2 evolution in whole cells of Chlorella, Dicrateria and Isochrysis as a function of time of UV-B irradiation (5 W m^{-2}). The 100% rates are 390, 295 and 300 nmol (O_2) Kg^{-1}(Chl).s^{-1} respectively for Chlorella, Dicrateria and Isochrysis. Values represent average of 5 independent measurements and are significant at ± 5% level.*

eral studies which have examined the sensitivity of phytoplankton to UV radiation (Lorenzen 1979, Hobson and Hartley 1983, Buhlmann *et al.* 1987). Increase in UV-radiation could depress the photosynthetic activity by bleaching or altering the composition of photosynthetic pigments (Häder and Häder 1988 b). However, phytoplanktons show great variation in their sensitivity to UV radiation which is due to the presence of different mechanisms to escape or protect from the damaging effect of UV-B radiation (Karentz *et al.* 1991 b, Smith *et al.* 1992).

Changes in pigment protein content: Table 1 shows that there was no significant change in the level of Chl *a* and carotenoids. When biochemical changes were followed, the total cellular protein content showed drastic changes in both *Chlorella* and *Dicrateria* and only a marginal change in *Isochrysis.* This could be due to the fact that green algae are highly sensitive to UV-B radiation.

Changes in energy transfer: *Chlorella* cells irradiated for a short periods did not reveal much change in the level of the excitation peak (Fig. 2). The excitation spectra of *Dicrateria* cells for F-682 had four major peaks, of which, the peak at 466 nm emanating from Chl *c* was high compared to 530 and 590 nm excitation peaks (Fig. 3). Under short-term UV-B treatment, a reduction in the long wavelength peaks was noticed which lead to the conclusion that UV-B affects primarily the Chl *c*-fucoxanthin complex. Similar excitation peaks were obtained from chloroplasts isolated from marine brown algae (Caron *et al.* 1984). The appearance

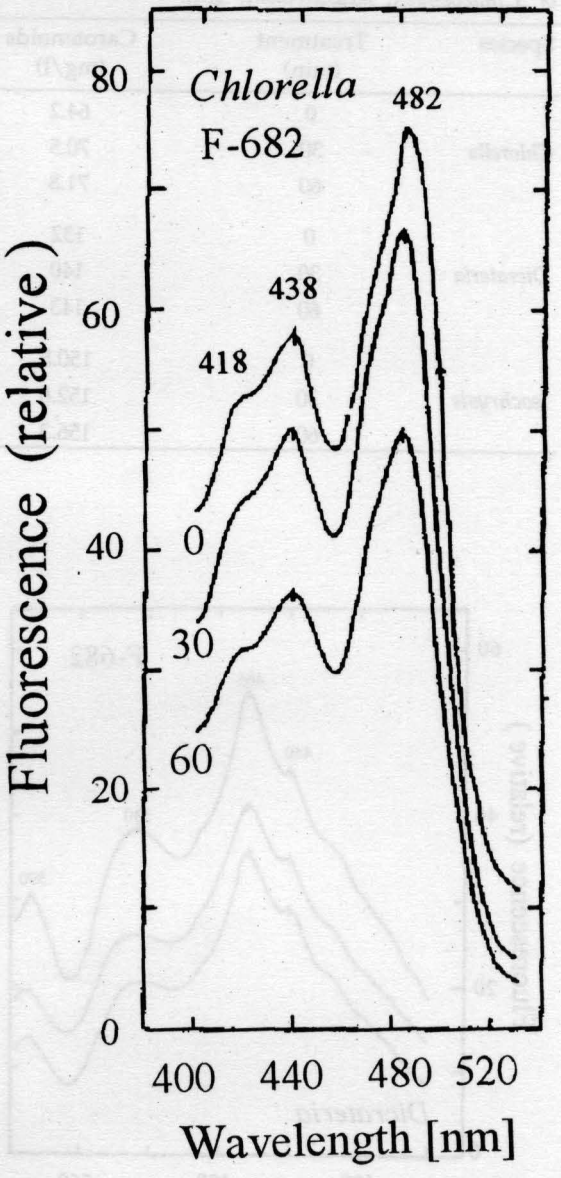

Fig. 2. *Room temperature excitation spectra for F-682 fluorescence of control and UV-B irradiated cells of Chlorella. Cells were suspended at a final Chl concentration of 2 ug/ml. The spectra are arbitrarily shifted to avoid overlapping. Numbers along the traces indicate the time of UV-B treatment. For other details see Materials and methods.*

Table 1. *Effect of short-term UV-B irradiation on the biochemical composition of marine micro algae. The UV-B irradiance at the cell surface was 5 W m^{-2}. The samples were irradiated at 20 ± 1°C. Each value is an average of 5 independent measurement. ±SE.*

Species	Treatment (min)	Carotenoids (mg/l)	Chl *a* (mg/l)	Protein (mg/l)
Chlorella	0	64.2	3.00 ± 0.15	14.00 ± 0.7
	30	70.5	3.31 ± 0.20	11.75 ± 0.5
	60	71.8	3.34 ± 0.21	8.96 ± 0.4
Dicrateria	0	132	3.30 ± 0.27	27.43 ± 1.5
	30	140	3.50 ± 0.18	23.50 ± 1.1
	60	143	3.50 ± 0.18	20.17 ± 1.0
Isochrysis	0	150.5	3.22 ± 0.15	41.04 ± 2.0
	30	152.6	3.23 ± 0.16	38.06 ± 1.9
	60	156.3	3.25 ± 0.17	36.57 ± 1.7

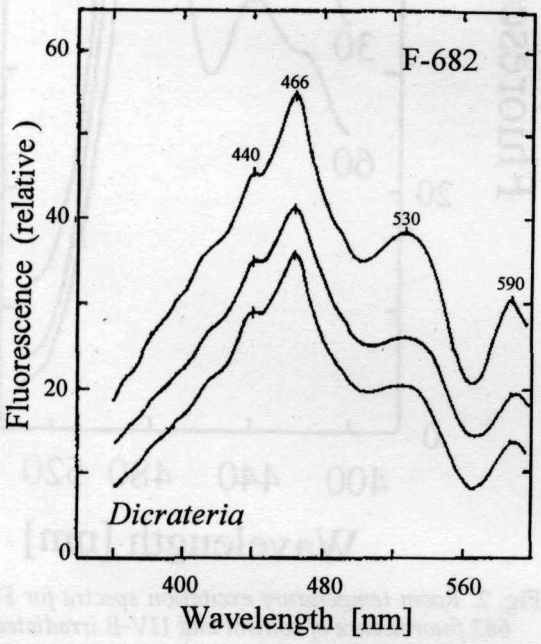

Fig. 3. *Room temperature excitation spectra for F-682 fluorescence of control and UV-B irradiated cells of Dicrateria. Cells were suspended at a final Chl concentration of 2 ug/ml. Other details are as in Fig. 2.*

of excitation peaks at 530 and 590 nm for F-682 in chrysophytes and diatoms was shown to be due to Chl cl + c2-fucoxanthin complex (Boczar and Prezelin, 1989). In the emission spectrum of *Dicrateria*, among the excitation wavelengths, only the 585 nm excitation revealed the association of Chl *c* (Fig. 4). Excitation at 535 nm is considered to be due to *in vivo* absorption of fucoxanthin. The presence of this pigment allows the Chl *c* containing algae to collect good amount of green radiation (Caron *et al.* 1984). Moreover, analysis of 466/590 nm ratio, reveals a 42% increase in UV-B irradiated *Dicrateria* cells which could be due to either loss of fucoxanthin or increase in the level of Chl *a* (Table 2). The absorption spectra as well as excitation spectra support the former. In contrast to *Dicrateria*, *Isochrysis* was found to be UV-B resistant. Absence of any significant change in the fluorescence spectra supports this conclusion (Fig. 5).

Net photosynthetic reactions : The rate

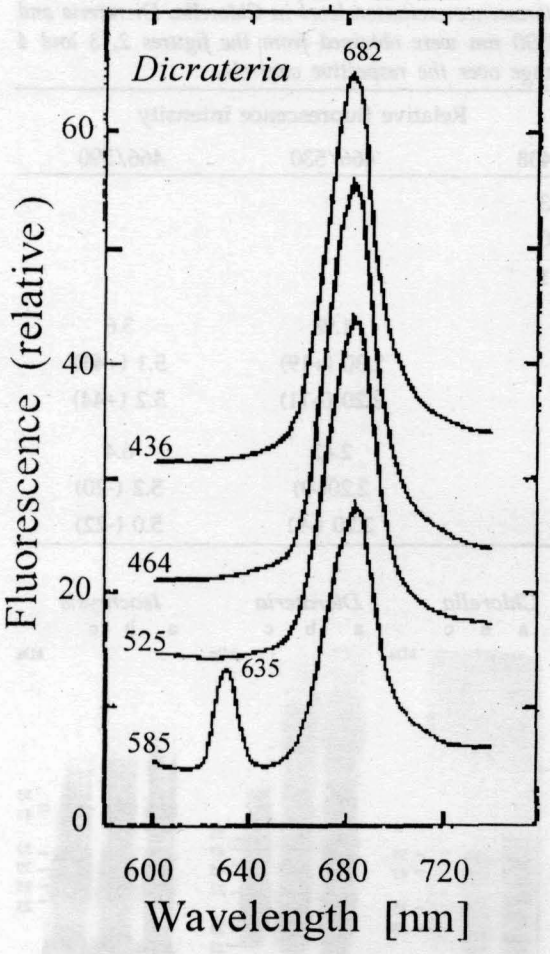

Fig. 4. *Room temperature fluorescence emission spectra of Dicrateria cells excited at 436, 464, 525 and 585 nm. The energy level at all excitation wavelengths was adjusted with reference to differences in the pigment absorption. The spectra are arbitrarily shifted to avoid overlapping.*

of CO_2 fixation has decreased in all the three species of algae after UV-B treatment. Maximum decrease in the rate was observed in *Chlorella* and *Dicrateria* (Fig. 6). This is in agreement to the observation by Lorenzen (1979) who have shown that $^{14}CO_2$ assimilation of natural polyplankton populations in the upper part of the euphotic zone has been reduced by solar UV-B stress. Depression of CO_2 fixation by UV-B radiation may be explained by diminution of supply of ATP and $NADPH_2$ and also by a change in the carboxylase activity.

Changes in thylakoid proteins : To study the effect of UV-B irradiation on thylakoid proteins, SDS-PAGE was carried out using the protein samples prepared from control and UV-B treated cells of *Chlorella, Dicrateria and Isochrysis.* UV-

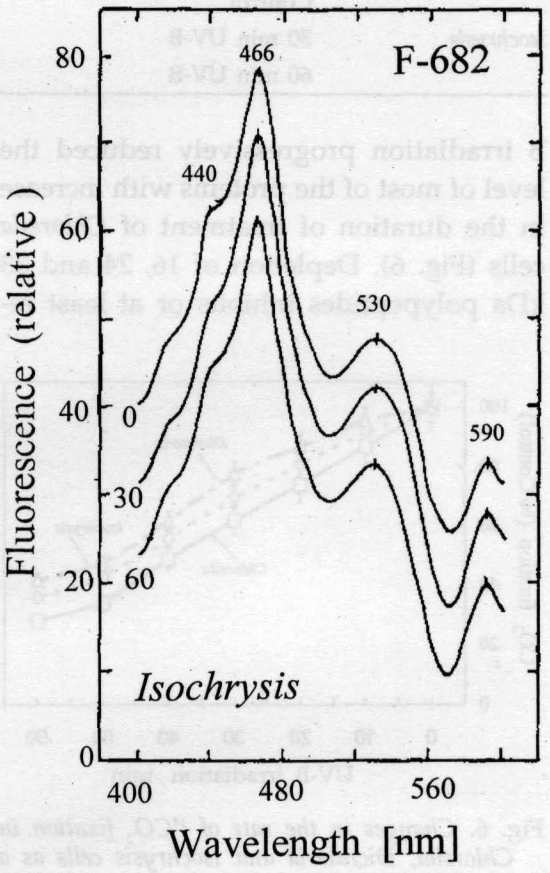

Fig. 5. *Room temperature excitation spectra for F-682 fluorescence of control and UV-B irradiated cells of Isochrysis. Cells were suspended at a final Chl concentration of 2 µg/ml. The spectra are arbitrarily shifted to avoid overlapping.*

Table 2. *Effect of short-term UV-B irradiation on F-682 fluorescence excitation level in Chlorella, Dicrateria and Isochrysis cells. Values of 482/438 nm, 466/530 and 466/590 nm were obtained from the figures 2, 3 and 4 respectively. Figures in parenthesis indicate percentage change over the respective controls.*

Organism	Treatment	Relative fluorescence intensity		
		482/438	466/530	466/590
Chlorella	Control	1.43		
	30 min UV-B	1.40		
	60 min UV-B	1.41		
Dicrateria	Control		1.98	3.6
	30 min UV-B		2.30 (+19)	5.1 (+41)
	60 min UV-B		2.20 (+11)	5.2 (+44)
Isochrysis	Control		2.42	6.4
	30 min UV-B		2.20(-9)	5.2 (-20)
	60 min UV-B		2.20 (-9)	5.0 (-22)

B irradiation progressively reduced the level of most of the proteins with increase in the duration of treatment of *Chlorella* cells (Fig. 6). Depletion of 16, 24 and 33 kDa polypeptides inhibits or at least re-

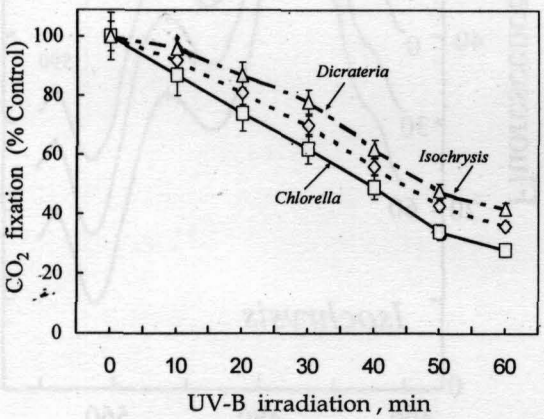

Fig. 6. *Changes in the rate of $^{14}CO_2$ fixation in Chlorella, Dicrateria and Isochrysis cells as a function of time of UV-B irradiation (5 W m^{-2}). $NaH^{14}CO_3$ was added at a final concentration of 1.5 mBq ml^{-2}. The 100% rates of CO_2 fixation were 52, 46 and 43 nmol $(CO_2)kg^{-1}$ (protein)s^{-1}. Values represent average of 6 independent measurements.*

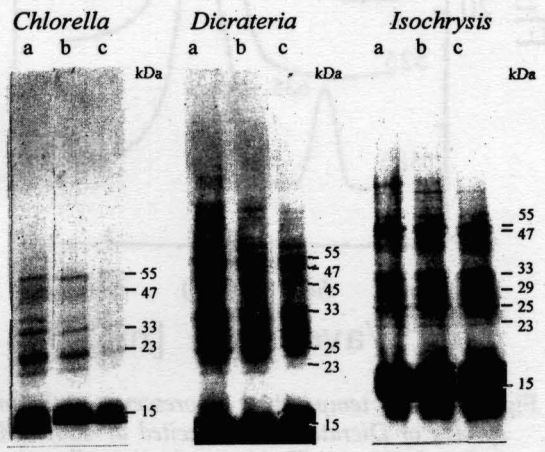

Fig. 7. *SDS-PAGE polypeptide profiles of thylakoid proteins of a) control b) 30 min and c) 60 min UV-B irradiated (5 W m^{-2}) cells of Chlorella, Dicrateria and Isochrysis.*

tards the S3-S4/S0 transition *i. e.*, O_2 releasing step. It is likely that a 60 min UV-B treatment might have led to the breakage of the electrostatic forces binding the 24 and 17 kDa to 33 kDa polypeptide, thereby affecting the overall electron transport.

The main accessory pigments of chromophytes are Chl *c*, Chl *c2* and fucoxanthin. The principal LHC has 1-3 polypeptides of 17-24 kDa size and they are probably encoded by nuclear multigene families, although definite evidence for this is available only for diatoms (Grossman *et al.* 1990). Chl *a* and Chl *c*-fucoxanthin complexes isolated from diatoms, Chrysophytes and brown macroalgae have typically possessed 16-20 kDa polypeptides (Hiller *et al.* 1988). The >64 kDa fucoxanthin containing complexes of *Nitzchia, Closterium* and *Isochrysis galbana* had Chl *a*, Chl *c* and fucoxanthin and a polypeptide composition dominated by three presumed apoproteins of around 18-22, 37-48 and 55-56 kDa size (Boczar and Prezelin 1989).

In the case of *Dicrateria* UV-B irradiation brought about drastic reduction in the level of 58, 47, 45 and 25 kDa polypeptides. Some large MW polypeptides (>75 kDa) also showed reduction in their levels. But the major accessory pigment-protein band (15 kDa) did not show any reduction when compared on total protein basis. This implies that UV-B irradiation has affected only the core PS2 proteins and not the large pool of accessory chromophore.

In contrast to *Dicrateria, Isochrysis* cells showed increase in the level of 47, 33, 29 and those in the range of 75-80 kDa polypeptides while 55, 25 and 23 kDa had decreased. This supports the observed resistance to UV-B damage in the cells of *Isochrysis*.

References

Arnon, D. I. *Plant Physiol.* **24** : 1-15

Boczar, B. A. and B. B. Prezelin. 1989 *Plant Cell Physiol.* **30** : 1047-1056

Buhlmann, B., P. Bossard and U. Vehlinger. 1987. *J. Plankton Res.* **9** : 935-943.

Calkins, J. and T. Thordardottier. 1980 *Nature.* **282** : 563-566

Caron, L., H. Jupin and C. Berkaloff. 1984. In : Advances in photosynthesis Research, (Sybesma, C.ed.) Vol 1. Martinus Nijhoff/Dr. W. Junk Publishers. The Hague/Boston/ Lancaster, Netherlands.

Grossman, A. R., A. Manoclori and D. Snyder. 1990. *Mol. Gen. Genet.* **224** : 91-100.

Häder, D. P. and M. A. Häder. 1988 b *Current Microbiol.* **17** : 215-220.

Hiller, R. S., A. W. D. Larkum and D. M. Wrench. 1988. *Biochim. biophys. Acta* **932** : 233-231.

Hobson, L. A. and F. A. Hartley 1983. *J. Plankton. Res.* **5** : 325-331.

Jeffrey, S. W. and G. F. Humphrey. 1975. *Biochem. Physiol pflanzen* **167** : 191-194.

Jokiel, P. L. and J. R. H. York. 1984. *Limnol. Oceanogr.* **29** : 192-199.

Karentz, D., F. S. Mc Even, M. C. Land and W. C. Dunlop. 1991b. *Mar. Biol.* **108** : 157-166

Karentz, D., M. L. Bothwell, R. B. Coffin, A. Hanson G. J. Herndl, S. S. Kilham, M. P. Lesser, M. Lindall, R. E. Noeller, D. P. Morris, P. J. Neale, R. W. Sander, C. S. Weiler and R. G. Wetzel. 1994. *Arch.Hydrobiol. Beiheft.* **43** : 31-69.

Laemmli, V. K. 1970 *Nature* **227** : 680-685.

Lorenzen, C. J. 1979. *Limnol. Oceanogr.* **24** : 1117-1120.

Lowry, O. H. N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. *J. biol. Chem.* **193** : 265-275.

Metzner, H., H. Rau and H. Senger. 1965 *Planta* : 186-194.

Peschek, G. A. 1978. *Arch. Microbiol.* **119** : 313-322.

Senger, H. and V. Mell. 1977. In Methods in Cell Biology (Prescott, D. M. ed.,), Vol. XXV pp. 201-219. Academic Press, New York

Smith, R. C., B. B. Prezelin, K. S. Baker, R. R. Bidigare, N. P. Boucher, T. Coley, D. Karentz, S. Macintyre, H. A. Matlick, D. Menzies, M. Ondrusek, Z. Wan and K. J. Waters. 1992 *Science* **255** : 952-959.

USEPA (US Environmental Protection Agency) USA 1987. In Assessing the Risks of Trace Gases that can modify the Stratosphere (J. Hoffman, ed.), USA XXXII pp. 632.

Walne, P. R. 1974. Culture of bivalve molluscs. 50 years experience at Conway. Fishing News (Books) Ltd, 1-173.

Worrest R. C. 1986. In effect of changes in Stratospheric Ozone and Global Climatic Vol. I overview (Titus, J. g. ed.), USEPA, Nairobi, Kenya and USEPA, Washington, D. C. USA VII. Pp. 379.