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EFFECT OF UV RADIATION ON THE GROWTH, PIGMENT AND HORMONE CONTENT OF MICROALGAE

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1. INTRODUCTION

Anthropogenic ozone depletion discovered during the 1970s has caused an increase in the intensity of solar ultraviolet radiation reaching the Earth's surface, generating extensive research programs with the intention of studying its effects on living organisms. In this regard, primary producers are a subject of high concern, since changes in their biomass affects every trophic level of the food web, while light intensity through its direct effect on photosynthesis has an influence on the global carbon cycle.

Algae, as the most ancient and simplest photosynthetic organisms, are considered particularly unique in respect of UV radiation for several reasons. From an ecological aspect, these organisms are the major primary producers of most aquatic environments, moreover, some species are adapted to extreme habitats often exposed to high light intensities. On the cellular level, the consequences of an increase in UV intensity are generally harmful to algae, with photosynthesis and growth as the primary targets. On the other hand, there are many defence mechanisms, such as the synthesis of UV-absorbing mycosporine-like amino acids, as responses given to these harmful effects, the variable occurrence and effectiveness of which suggest broad interspecific differences in UV-resistance.

Most research projects have focused on marine, polar and tropical environments so far, or in habitats characterised by extreme environmental conditions. In temperate freshwater habitats, however, the number of accounts on the subject is relatively limited, despite the fact that UV-effects reaching the communities living there are similarly complex and unknown in detail. In addition to the ecological consequences, changes in the light climate can indirectly affect the biotechnological applicability of algae. Macroalgae used for industrial purposes are harvested in natural habitats,

and microalgae are also often grown in lakes or outdoor pools, thus UV radiation might cause changes in the quantity and quality of algal products.

Objectives

1. UV effects on phytoplankton assemblages have not been studied in the surface waters of Hungary so far. Lake Balaton, with its several unique characteristics, is a convenient site for studying *in situ* UV effects. With this end in view, the author's first objective was to examine the effect of UV radiation on the photosynthesis of Lake Balaton phytoplankton in summer, when light intensity reaches its peak.
2. The research was continued under laboratory conditions, where the experiments were aimed at studying the growth and photosynthetic pigment content of representative green algal and cyanobacterial strains as a function of different intensities of photosynthetically active (PAR) and UV radiation. The next objective of the research was to screen the investigated strains for the ability of synthesizing UV-absorbing mycosporine-like amino acids.
3. The relationship between UV radiation and the plant hormone content of algae has not been studied previously, thus the last part of the work consisted of examining the possible UV influence on hormone content and growth in synchronized cultures of the green alga *Chlorella sp*.

2. MATERIALS AND METHODS

2.1. *In situ* experiments in Lake Balaton

Experiments were conducted on two sites, in the eastern and western basin of the lake. Phytoplankton samples were incubated in quartz tubes suspended horizontally at depths of 0.05, 0.25, 0.50, 1.00, 2.00 and 2.75 m, primary production was measured with the ^{14}C-method (STEEMANN NIELSEN, 1952). Beta radiation of fixed ^{14}C isotope was measured with an LKB 1211-RACKBETA liquid scintillation counter. Each incubation lasted for 4 hours centred around noon.

During the *in situ* and the laboratory experiments the separation of PAR, UV-A and UV-B wavebands was provided with cut-off filters. Unwrapped quartz tubes transmitted both PAR and UV radiation (treatment PAR+UV-A+UV-B). Tubes wrapped with filters transmitted PAR and UV-A radiation (PAR+UV-A), or PAR only (control). Primary production of the incubated samples was characterized by the photosynthetic rate, equivalent with the amount of carbon assimilated in unit volume of the sample within a given time (WETZEL és LIKENS, 1991).Thus, primary production was measured for each incubation depth yielding a vertical profile, from which the maximum photosynthetic rate, surface photoinhibition and areal primary production (in units of $\mu gC \cdot m^{-2} \cdot h^{-1}$) could be determined.

Chlorophyll-a concentration of the incubated samples was determined photometrically from hot methanolic extracts (NÉMETH, 1998) with a SHIMADZU UV-VIS spectrophotometer.

Underwater light intensity (PAR) was measured with a LI-COR LI-185B radiometer equipped with a 2π sensor, from which the extinction

coefficient within the water column can be calculated on the basis of the Lambert-Beer law.

2.2. Studying the effect of UV-A radiation on the green alga *Desmodesmus armatus*

The author's preliminary laboratory study focused on studying the effect of UV-A radiation on the growth, pigment content and morphology of *Desmodesmus armatus*, a green alga also present in the phytoplankton of Lake Balaton. Experiments were carried out in three replicates, using the tubes and filters mentioned above. Cultures grown in BG-11 medium were either exposed to PAR or PAR and 3.75 $mW \cdot cm^{-2}$ UV-A radiation, in a L:D cycle of 14:10 hours at 23°C. PAR intensity applied was 30, 100, 200, 400, and 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Aeration and homogenization of the cultures was maintained with an air pump.

Growth of the cultures was monitored using a HITACHI F-4500 fluorimeter, measuring daily the concentration of chlorophyll-a. The wavelength of excitation and detedtion was 650 and 682 nm, respectively. Chlorophyll-a concentration was determined from the fluorescence values by a calibration curve. The UV-A-induced inhibition of growth was calculated in two ways: based on the areas under the growth curves and on the maximum growth rates characteristic of the exponential phase, respectively.

At the end of the experiments, absorption spectra and chlorohpyll-a concentration were determined from the methanolic extracts of the cultures. Absorption spectra were normalized to chlorophyll-a. Total carotenoid content of the cultures was calculated from the absorption spectra using the equation of WELLBURN (1994).

Finally, samples were fixed in Lugol's solution for microscopy to study morphological changes. Coenobia in the settling chamber were counted with a Zeiss inverted microscope using Utermöhl's method.

2.3. Experiments conducted with laboratory cultures

2.3.1. Studying the growth and pigment content of green algal and cyanobacterial strains exposed to UV-A and UV-B radiation

Experiments were carried out at the Department of Plant Physiology and Plant Biotechnology, University of West Hungary. The selected strains originated from the culture collection of the department (Mosonmagyaróvár Algal Culture Collection – MACC).

Green algae (Chlorophyta):
MACC-203 *Pseudochlorococcum typicum*
MACC-458 *Chlorella sp.*
MACC-469 *Scenedesmus sp.*
MACC-534 *Coenochloris sp.*

Cyanobacteria:
MACC-277 *Cylindrospermopsis raciborskii*
MACC-304 *Anabaena sphaerica*
MACC-541 *Synechococcus elongatus*
Microcystis aeruginosa

150 ml cultures were incubated in 30 cm long quartz tubes (Ø35 mm) with an initial dry mater content of 10 $mg \cdot l^{-1}$. Aeration and homogenization of the cultures was maintained with an air pump. Cultures were grown in the following treatments with a L:D cycle of 14:10h:

PAR intensity	**Treatment**		
85 $\mu mol \cdot m^{-2} \cdot s^{-1}$	Control (PAR)	PAR+UV-A (UV-A=1,00 $mW \cdot cm^{-2}$)	PAR+UV-A+UV-B (UV-A = 1,00 $mW \cdot cm^{-2}$; UV-B = 0,12 $mW \cdot cm^{-2}$)
250 $\mu mol \cdot m^{-2} \cdot s^{-1}$	Control (PAR)	PAR+UV-A (UV-A=1,00 $mW \cdot cm^{-2}$)	–

Growth rates were determined by measuring the optical density (absorption at 750 nm) each day during the incubation with a Cary 50 UV-Vis spectrophotometer. Chlorophyll-a concentration was measured on a two-day basis from the methanolic extracts as described above. Dry matter content was determined at the end of each experiment.

2.3.2. Determination of mycosprine-like amino acids (MAAs)

Extraction and identification of MAAs was performed using the method of SINHA et al. (1999). Incubation for the screening of the strains took 10-14 days. Strains capable of synthesizing MAAs were sampled after 7 and 10 days after the start of incubation for determining dry matter and MAA content.

MAA detection was carried out with a Cary 50 UV-Vis spectrophotometer, identification of the compounds was performed with HPLC consisting of a Waters W2690 separation module and a W996 diode array UV/VIS detector. After a ten-minute-long centrifugation at 1500 g, the residue was redissolved in 20 and 100% methanol, respectively. The extracts were used for determining absorption spectra and for HPLC analysis. During the analysis the extracts were evaporated in a Thermo Scientific Savant SPD 1010 Spedvac evaporator. The residue was redissolved in 0.2% acetic acid, and injected onto a 4x250 mm, 5 μm pore size LiCrospher RP 18 column equipped with a guard column at a flow rate of 1 $ml \cdot min^{-1}$. The wavelength of detection was 330 nm.

2.3.3. Studying the effect of UV-A radiation on growth and hormone content in synchronized cultures of Chlorella sp.

Synchronization of strain MACC-458 (*Chlorella sp.*) was attained by multiple inoculation under a 14:10 h light/dark cycle. Synchronized cultures

were incubated in three replicates treated with 85 μmol·m^{-2}·s^{-1} PAR and PAR+UV-A radiation. Sampling took place every two hours from the dark phase following the last inoculation till the end of the next light phase. Samples for microscopic analysis were fixed in Lugol's solution, while another set of samples was stored at -24°C for measuring hormone content.

Microscopic analysis was performed with an Olympus BX60 light microscope and a SIS View FireWire ColorViewII digital camera. Cells were counted and cell size was measured in a Burker chamber, images were evaluated with analySIS image processing software.

Plant hormone content of the samples taken for analysis was determined at Palacký University, Laboratory of Growth Regulators in Olomouc, Check Republic. Analysis was performed with ELISA (Enzyme-linked immunosorbent assay), using the modified method of WEILER et al. (1981).

Each well of the ELISA plates was coated with 150 μl mouse anti-hormone antibody solution. After storing at 4°C overnight plates were rinsed with distilled water, then filled with 200 mg·l^{-1} bovine serum albumin dissolved in TBS buffer (pH 7,5) and incubated for 1 h at 25°C. Next, after repeated rinsing, plates were filled with 50 μl TBS buffer, 50 μl sample or standard and 50 μl tracer solution.

After 1 h incubation at 25°C and rinsing the wells were filled with 150 μl p-nitrophenil phosphate. The reaction was stopped after 1 h with 50 μl NaOH, the absorbance of the solution was measured at 405 nm with a Titertek Multiscan PLUS microplate reader. Per cent binding of the standard solutions determined from the absorbance and plotted against hormone concentration yields a calibration curve, from which the hormone content of the samples can be determined.

2.3.4. Statistical analysis

Statistical analysis of the data was carried out with SPSS 13 program. Average values and standard deviation were measured for each data set. Data obtained from different treatments were compared using two- and three-way ANOVA and Tukey HSD test.

3. RESULTS AND CONCLUSION

3.1. *In situ* experiments in Lake Balaton

On both incubation sites there was a near-surface inhibition of phytoplankton photosynthesis caused by light oversaturation. In the eastern basin with its greater transparency the zone of light saturation was deeper resulting in surface photoinhibition nearly twice as much as that in the western basin. The vertical profiles of photosynthesis showed a considerable effect of UV radiation near the surface, causing higher inhibition as compared to PAR. However, the extent of UV-induced inhibition in terms of areal primary production turned out to be smaller.

The results suggest that the inhibition of phytoplankton photosynthesis was mainly caused by UV-A radiation. Taking the inhibition measured at treatment PAR+UV-A+UV-B to be 100%, 75 and 79% of surface photoinhibition, and 76 and 74% of the inhibition of areal primary production was induced by UV-A radiation in the eastern and western basin, respectively.

Regression analysis of the inhibition values, global radiation data and the extinction coefficients clearly showed that the extent of inhibition is related to both extinction within the water column and global radiation.

3.2. Effect of UV radiation on *Desmodesmus armatus* (Chlorophyceae)

The studies on *Desmodesmus armatus* showed significant UV-A effects. Control cultures reached maximum growth around 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, beyond which the decrease in growth was presumably related to photosynthetic inhibition. On the other hand, significantly lower growth rates were found in the PAR+UV-A cultures, with increasing inhibition towards higher PAR intensities.

Cellular carotenoid content of the green alga increased when exposed to UV-A radiation, whereas a decrease was observed with increasing PAR intensity. Despite that, carotenoid/chlorophyll-a ratios showed an increase toward higher PAR intensities, moreover, the slope of the curves differed between the two treatments. The observed changes were caused by the decrease of cellular chlorophyll-a content with increasing PAR levels, which surpassed the decline in the carotenoid content.

UV-A radiation can influence coenobium development, as demonstrated by the microscopic analysis of the samples. 4-celled coenobia dominated in the control cultures, with a decrease in the relative abundance of 2-celled coenobia and an increase of 8-celled coenobia toward higher PAR levels. UV-A radiation, on the other hand, caused an increasing relative abundance of 2-celled and teratological coenobia accompanied by the decline of 4- and 8-celled forms. UV-A induced changes in coenobium composition can have numerous consequences (eg. changes in sinking rate or grazing pressure). Previous studies on *Desmodesmus* morphology were conducted without taking into account the effects of UV radiation, thus, extrapolating these studies to *in situ* conditions needs reconsideration.

3.3. UV effects on laboratory cultures of green algae and cyanobacteria

Data derived from the experiments performed with selected MACC strains both confirm and supplement the conclusions of related papers. Generally speaking, UV radiation can have a considerable contribution to the inhibited growth of green algae and cyanobacteria, which greatly depends on the sensitivity of the strains, the composition of UV radiation and the UV:PAR intensity ratio.

Cultures of green algae showed generally higher UV-resistance as compared to cyanobacteria. Growth curves of the green algae had an

elongated lag phase in the presence of UV-A radiation, which increased under additional UV-B radiation, but the cultures were able to acclimate to the applied UV environment. Interspecific differences in the responses also occurred in the growth rates. The effect on cyanobacteria, however, appeared to be more variable. The two filamentous strains (MACC-277: *Cylindrospermopsis raciborskii* and MACC-304: *Anabaena sphaerica*) exhibited high sensitivity in response to UV radiation, especially in the case of *C. raciborskii*, where growth was insignificant. The unicellular *Synechococcus elongatus* showed significant sensitivity in response to UV radiation at 85 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR, whereas at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ high UV-resistance was observed. Similar results were obtained in the case of *Microcystis aeruginosa*. Cultures exposed to PAR+UV-A radiation at 85 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR demonstrated intensive growth after an elongated lag phase, however, the addition of UV-B induced strong inhibition.

The generally lower rate of resistance found in the cyanobacterial isolates raises several questions from an ecological point of view, particularly in the case of potentially bloom-forming strains (*C. raciborskii*, *M. aeruginosa*). Considering this high rate of sensitivity, the ecophysiological background of their ability to form massive blooms in lakes such as Lake Balaton remains unsolved, although vertical migration or wind mixing may serve as potential ways to avoid harmful UV intensities.

In respect of chlorophyll-a content green algae provided a more or less uniform response to UV radiation, while cyanobacteria responded more diversely. UV-A induced decreases in the chlorophyll-a content of the green algae were significantly higher at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as compared to 85 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Concerning the effect UV-B radiation, no significant additional decreases were observed, except *Pseudochlorococcum typicum*,

whose chlorophyll-a content was 43% lower in treatment PAR+UV-A+UV-B than in treatment PAR+UV-A. This dramatic decline, in addition to the decreased growth rate, can imply a low UV-B resistance.

The abovementioned tendencies in the green algal cultures were not observed in the cyanobacterial strains. The dramatic decrease in the chlorophyll-a content of *C. raciborskii* also occurred in *A. sphaerica*, although UV-B caused no further decline. Unicellular strains reacted differently, namely, no decrease was found in the presence of UV-A, however, with the addition of UV-B radiation the cultures showed a strong decrease in chlorophyll-a content.

3.4. Induction of mycosporine-like amino acids (MAAs)

The author managed to detect MAAs in the filamentous green alga *Klebsormidium sp.* (strain MACC-426). Based on the HPLC analysis, the isolated MAA with a retention time of 4,3 min and maximum absorbance at 320 nm is presumably palythine. No MAAs have been detected among charophycean algae so far, consequently, the presence of palythine in *Klebsormidium sp.* is a novel finding, raising further questions in connection with the occurrence and synthesis of MAAs.

Spectrophotometric analysis of the methanolic extracts revealed that the MAA was present only in the cultures treated with UV radiation. Comparison of the different treatments showed that MAA accumulation was primarily induced by UV-B radiation, however, an accurate exploration of the action spectrum would need further adjustment of the UV treatments, i.e. dividing up the UV-A and UV-B wavebands. Dry weight of the cultures treated with UV radiation was lower as compared to the controls, with a notable difference between the two days of sampling. The increase in dry weight from day 7 to day 10 was highest in treatments PAR+UV-A and

PAR+UV-A+UV-B, while in treatments PAR and PAR+UV-B no significant change was observed.

Algae are supposed to have numerous defence mechanisms against the harmful effects of UV radiation, which may act in a simultaneous manner. These findings appear in strain MACC-426 (*Klebsormidium sp.*) as well, at least as regards MAA and total carotenoid content. In treatments PAR+UV-B and PAR+UV-A+UV-B, where the cultures had lower total carotenoid content, the relative amount of palythine surpassed that of treatments PAR and PAR+UV-A. This leads to the assumption that under UV-B stress, when carotenoid synthesis cannot counteract UV-induced damage, *Klebsormidium sp.* gains further resistance by the accumulation of MAAs.

3.5. The effect of UV-A radiation on the synchronized cultures of *Chlorella sp.* (MACC-458)

Control and PAR+UV-A treatments of the synchronized cultures of *Chlorella sp.* (MACC-458) yielded clear differences from the 8th hour of the incubation. Cell division took place between the 2nd and 4th hour of incubation, as the relative abundance of small cells showed a considerable increase. From the beginning of the light phase there was a growing difference in cell size between the two treatments. 18 hours after the beginning of incubation the relative abundance of 10-15 μm^2 cells in the controls was significantly higher as compared to those treated with UV-A, leading to more discrepancies as incubation time elapsed.

Hormone concentration between the cultures treated with UV-A and the controls differed at certain points of the incubation time, however, no relationship could be found for the observed differences and those experienced in terms of cell size. At the end of the experiment cultures exposed to UV-A radiation exhibited significantly lower dry-matter-based

content of isopentenyl riboside, indole-3-acetic acid and abscisic acid. Despite the apparent effects manifested in the experiment, determining the underlying mechanisms triggering the influence of UV-A radiation on cell growth would necessitate more in-depth studies.

4. NEW SCIENTIFIC RESULTS

1. The author was the first one to detect the effect of UV radiation on phytoplankton photosynthesis in a Hungarian lake. As shown by the results of the experiments conducted in Lake Balaton, an average of 75% of surface UV photoinhibition was caused by UV-A radiation. As for areal primary production, the effect of UV radiation resulted in a considerably lower inhibition of 8-14%.

2. Growth inhibition, changes in cellular carotenoid content and carotenoid/chlorophyll-a ratio, and particularly morphological changes of the green alga *Desmodesmus armatus* induced by UV radiation have not been studied before. In the presence of UV-A radiation the relative abundance of 4- and 8-celled coenobia decreased, while that of the 2-celled and teratological forms showed an increase as compared to the controls.

3. Results of the experiments conducted on microalgal cultures of the Mosonmagyaróvár Algal Culture Collection suggest that growth inhibition and the decline in chlorophyll-a content showed similar trends among the green algae studied, on the other hand, there was considerable variability among the cyanobacterial strains. Opposite to the green algae with higher UV resistance, cyanobacteria showed high sensitivity in respect of growth in response to the UV-A and UV-B radiation applied in the experiments.

4. The author successfully detected UV-absorbing mycosporine-like amino acid in the green alga *Klebsormidium sp.* (strain MACC-426). The compound indentified by HPLC is palythine, its increasing concentration was induced by UV radiation, with UV-B having a greater effect.

5. That was the first occasion of studying the effect of UV radiation on the hormone content of a microalgal culture. The applied UV-A intensity slightly inhibited the growth of the green alga *Chlorella sp.* (strain MACC-458), and caused a significant decrease in the dry-matter-related amount of isopentenil-ribozide, indole-3-acetic acid and abscisic acid. No direct relationship was found between the temporal changes of cell size and hormone content.

5. RELATED PUBLICATIONS

Publications in international journals:

Pálffy, K. & L. Vörös (2003): Effect of ultraviolet radiation on phytoplankton primary production in Lake Balaton. Hydrobiologia 506-509: 289-295.

Pálffy, K. & L. Vörös (2006): Effects of UV-A radiation on *Desmodesmus armatus*: changes in growth rate, pigment content and morphological appearance. International Review of Hydrobiology 91/5: 451-465.

Publications in Hungarian journals:

Pálffy K., Ördög V. & Vörös L. (2004): Az ultraibolya sugárzás hatása zöldalga és cianobaktérium fajok laboratóriumi tenyészeteire. Hidrológiai Közlöny 84/5-6: 115-117.

Oral presentations held on international conferences:

Pálffy, K. & L. Vörös: Effect of ultraviolet radiation on phytoplankton photosynthesis in Lake Balaton. International Conference on Limnology of Shallow Lakes. Balatonfüred, 2002. május 25-30.

Pálffy K, Vörös L & V. Ördög: The effect of UV stress on soil microalgae. 3rd Symposium on Microalgae and Seaweed Products in Agriculture. Mosonmagyaróvár, 2006. június 21-23.

Poster presentations on international conferences:

Pálffy K., Ördög V. & Vörös L.: Effects of UV radiation on some axenic microalgal strains.
5th European Workshop „Biotechnology of Microalgae”
Potsdam, Németország, 2003. június 23-24.

Pálffy K, Szalai G, Ördög V & Vörös L: Synthesis of a UV-absorbing compound by a filamentous green alga.
6th European Workshop "Biotechnology of Microalgae".
Potsdam, Németország, 2005. május 23-25.