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Biological effects
Oil pollution
Scenedesmus quadricauda

Growth responses of *Scenedesmus quadricauda* to oil pollution at different temperatures and light intensities*

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Abstract

The influence of crude oil extracts on the growth of *Scenedesmus quadricauda*, cultivated at a temperature of 12°C and 22°C and a light intensity of 1500 lux or 3000 lux, was investigated. The basal oil extract prepared from 50 cm^3 of oil in 1 dm^3 of the cultivation medium and its 50% and 10% dilutions were examined.

Generally the growth of investigated algae was lower at the temperature of 12°C and higher at the temperature of 22°C. The higher light intensity (3000 lux) applied at 12°C increased significantly the growth inhibiting effect of oil pollution. On the contrary, algae cultivated at the temperature of 22°C showed a lower degree of inhibition, under the influence of oil extracts, in higher intensity than at the lower light intensity.

In summary, it may be stated that the biological consequences of oil-based pollutants in natural water basins are to a large extent determined by existing environmental conditions, a significant part being played by light intensity and temperature.

1. Introduction

The various activities of man in the marine environment have brought about an increase in its pollution. This, in turn, is increasingly affecting the growth and development of living organisms in the sea and phytoplankton is no exception here.

Crude oil and its refinery products are, on a percentage basis, the greatest pollutants of the sea. The physical and chemical properties of waters are altered in various ways by oil pollution. Wave action in the neighbourhood of an oil slick causes the oil and water to mix and form an emulsion. The presence of such an emulsion in the surface layers of water profoundly affects their illumination and decreases the depth to which light may penetrate; furthermore, changes in the spectral properties are induced and the coefficient of the reflection of light from the water surface increases. As a result of these changes gas exchange may be impaired, while oxygen may be used up during the oxidation of the oil [6].

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The chemical changes which could modify the growth of phytoplankton depend on the kind of petroleum derivatives in question, and the solubilities and concentrations of the hydrocarbons they are composed of.

The most drastic changes in the physical properties of sea water take place in in the surface layers, the organisms living in this zone – among them the phytoplankton – are endangered the most [11].

The role played by phytoplankton in the marine environment is an extremely important one, so a number of detailed laboratory tests were carried out to determine the effect of various oil-based pollutants on the growth rates of different phytoplankton organisms. These tests show that 'low' concentrations of such pollutants actually promote the growth of phytoplankton and some associated species. On the other hand, 'high' concentrations of these pollutants do inhibit growth [2, 8, 13].

The studies carried out so far to determine the action of various crude oil fractions on microalgae indicate that the aromatic fractions is the most reactive and produces so-called 'acute' (immediate) effects. Hence, a number of papers dealing with the action on microalgal growth of various aromatic hydrocarbons, contained in crude oil, confirm that both 'low' and 'high' concentrations may modify growth [3, 15].

The biological effects of oil-based pollutants also depend on the kind of oil product tested [9]. Crude oil was found to affect the growth of microalgae less than certain refinery products tested at the same concentration. The way in which phytoplankton reacts towards oil derivatives further depends on the phylogenetic origin of the species in the phytoplankton and also on the sensitivity of various species of microalgae from the same family [1, 7, 14, 16].

The statements made above, about the effect of oil derivatives on microalgal growth, are based mainly on the results of the tests carried out under laboratory conditions. It does seem, however, that the action of these compounds may also be governed to a large extent by changes in environmental factors responsible for the normal growth and development of microalgae.

In the present study, an attempt was made to define the manner in which crude oil affects the growth of one representative of the Baltic phytoplankton, *Scenedesmus quadricauda*, at different light intensities and temperatures.

2. Material and methods

In the experiments, the unicellular alga *Scendesmus quadricauda*, isolated from the natural Baltic phytoplankton, was used.

The algae were cultivated on an artificial medium (BBM), after Nichols and Bold [12]. Prior to the experimental cultivation the algae were incubated on an agar medium containing 10 g bactopeptone, 20 g agar and 20 g glucose in 1 dm^3 distilled water. The algae were cultivated in this medium at 25°C and under continuous illumination of 2,000 lux for one week.

After this period of time the algae were washed with 50 cm^3 of the above-mention-

ed BBM liquid medium and incubated for 5 days to adapt them to the conditions of a liquid medium. 5 days later, the algae were transferred to a fresh liquid BBM medium and cultivated at two different temperatures (12° and 22°C) and light intensities (1500 and 3000 lux) for 13 days. The initial number of algal cells taken for experiments in all combinations was 10^5 cells per 1 cm^3 of the cultivation medium. The 12h/12h photoperiodic conditions were applied in all experiments.

Libian crude oil was used throughout the tests. The crude oil sample (50 cm^3) to be tested was thoroughly mixed with 1 dm^3 of nutrient for 24 h with the aid of a magnetic mixer. The mixture so obtained was separated during 1 hour in a separating funnel. The lower layer, designated as a 100% aqueous extract of crude oil, was diluted with suitably pure nutrient to make 50 and 10% v/v solutions.

The cell population growth was characterized by calculating the cell number after 1, 3, 5, 7, 9, 11, and 13 days of cultivation. The cell number was determined by using a Fusch-Rosenthal chamber and evaluated after Guilard [5].

Having found the number of cells in 1 cm^3 of suspension, the coefficient of characteristic growth rate (μ) was calculated using the equation after Guillard [5]:

$$\mu = \frac{1{,}443}{t_2 - t_1} \cdot \ln \frac{N_2}{N_1},$$

where:
N_1 – the cell number in 1 cm^3 at time t_1,
N_2 – the cell number in 1 cm^3 at time t_2,
μ – number of cell divisions per day.

3. Results and discussion

The results of this study into the effects of crude oil extracts on the growth of *Scenedesmus quadricauda* cultivated under varying conditions of temperature and illumination are presented tabularly (Tables 1 and 2) and graphically (Fig. 1A, B and Fig. 2A, B).

In all the experimental variants the oil extracts were found to inhibit the growth of the microalgal populations. The extent of this inhibition depended on the concentration of the extract used. The undiluted extracts (100%) produced the greatest effect, the 50% and 10% extracts correspondingly lesser effects.

There were significant differences in the intensity of growth of the tested cell populations depending on the conditions of cultivation. Reduced light intensity and temperature slow down the growth rate both in algae cultivated in the presence of oil extracts and in the controls where no oil was used. Fact that both these factors affect the action of oil derivatives on the growth of the tested organisms may be inferred from the difference in the degree of growth inhibition with respect to the control plants when one of the factors was changed and the other kept constant. Therefore, the difference which appeared when reducing the light intensity from

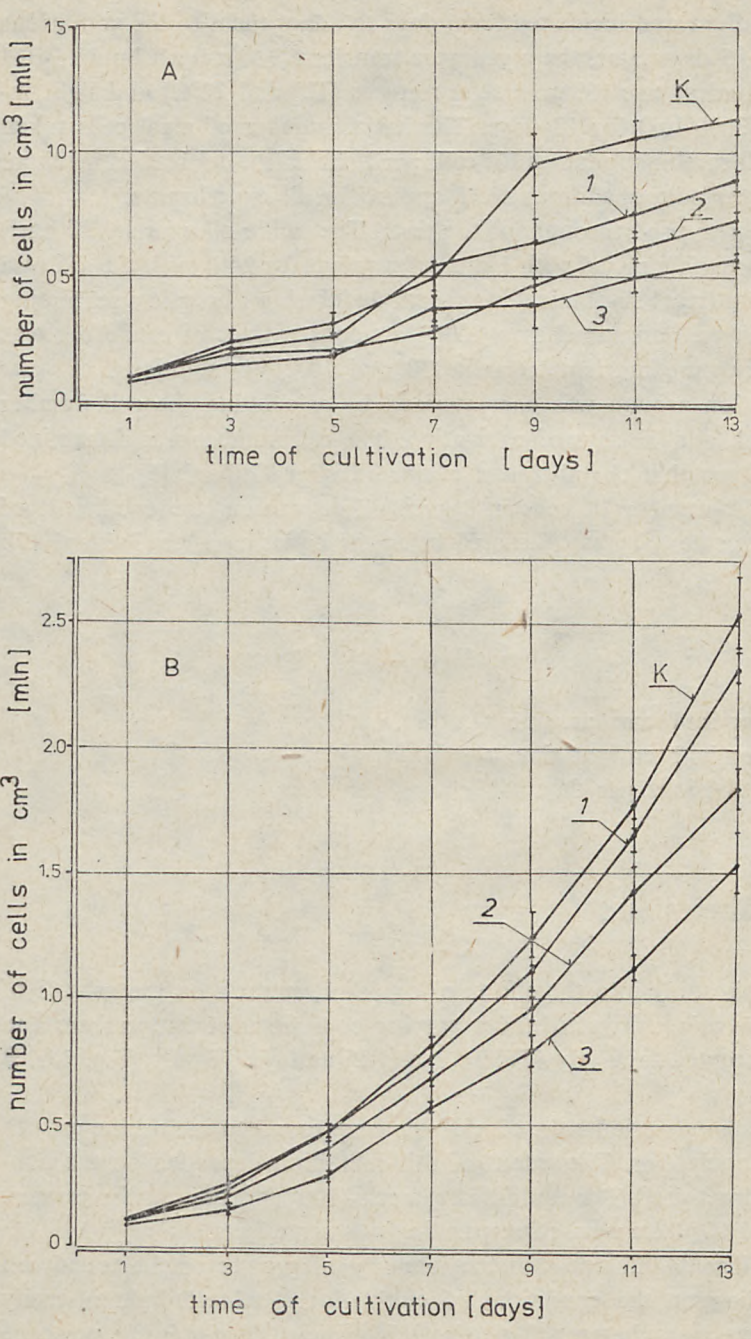

Fig. 1. The influence of crude oil extracts on the growth of *Scenedesmus quadricauda* cultivated at a constant temperature of 12°C and different light intensity: 1500 lux (A) and 3000 lux (B). The basal extract was prepared from 50 cm^3 of oil in 1 dm^3 of the cultivation medium and designated as 100% extract
K – control; the oil extract: *1* – 10%; *2* – 50%; *3* – 100%

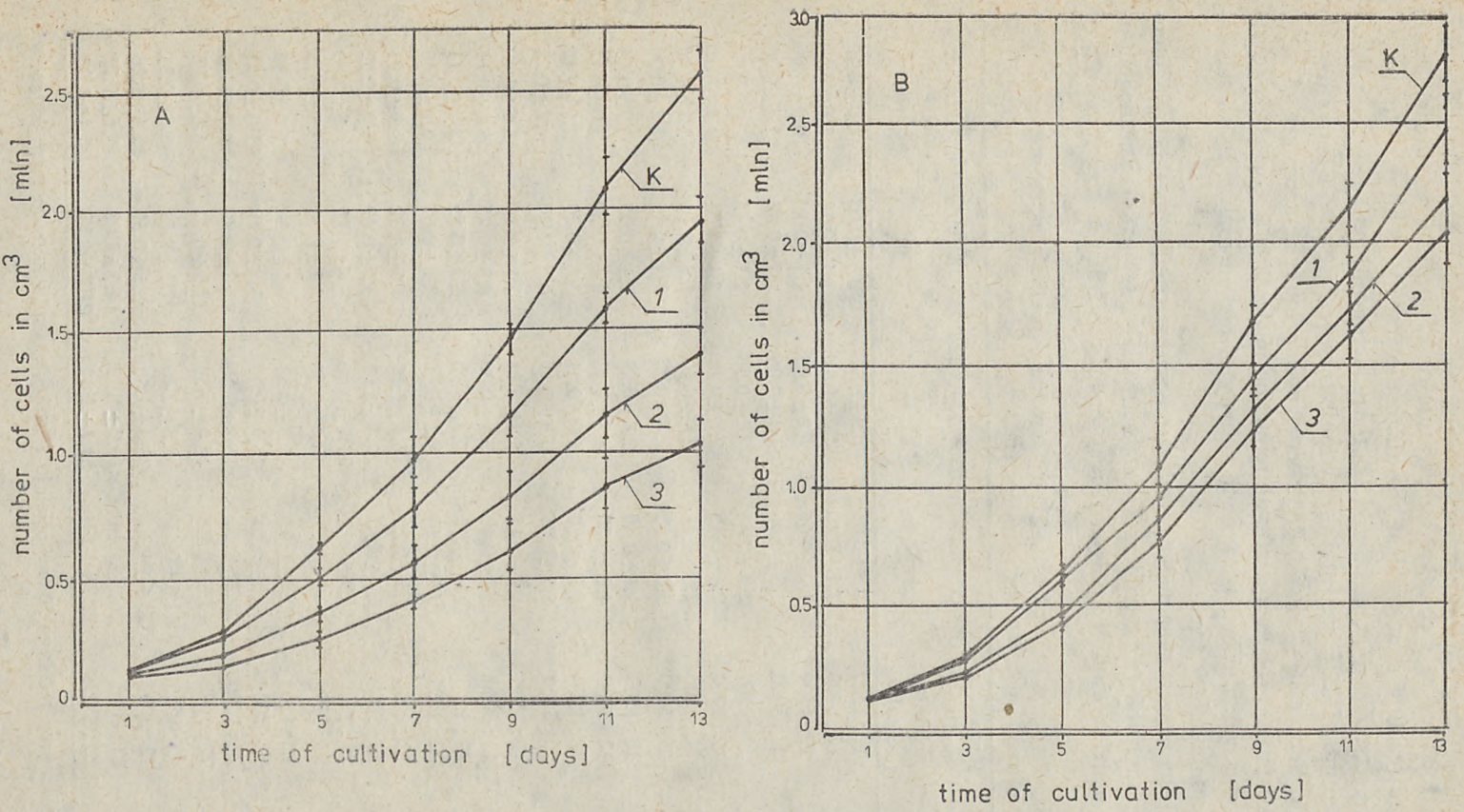

Fig.2. The influence of crude oil extracts on thegrowth of *Scenedesmus quadricauda* cultivated at a constant temperature of 22°C and different light intensity: 1500 lux (A) and 3000 lux (B). The basal extract was prepared from 50 cm^3 of oil in 1 dm^3 of the cultivation medium and designated as 100% extract K – control; the oil extract: *1* – 10%; *2* – 50%; *3* – 100%

3000 lux to 1500 lux at a constant temperature of 22° or 12°C are an indication of the altered reaction of microalgal growth to the activity of oil derivatives.

As can be seen from Figures 2C and 2D, the reduction of light intensity at a constant temperature of 22°C potentiates the inhibitory activity of oil derivatives on algal growth. Such activity was not observed at the lower temperature of 12°C (Figs. 1A and 1B). At this temperature a reduction in the light intensity from 3000 to 1500 lux much diminished the growth activity of the microalgae in all the experimental combinations tried.

These results suggest that under certain temperature conditions the light intensity may be responsible to a considerable extent for the growth reaction of the microalgae to the activity of oil derivatives. It is to be expected that at a temperature of

Table 1. The cell number ($\times 10^5$ *per* 1 $cm^3 \pm$SE) of *Scenedesmus quadricauda* cultivated at different concentrations of crude oil extracts, temperature, and light intensity

Days of cultivation	Temperature and light conditions [° C]; [lux]	Concentration of oil extracts in the cultivation medium [%]			
		0	10	50	100*
1	12 ; 1500	0.9±0.05	0.9±0.08	0.9±0.05	0.9±0.10
	12 ; 3000	1.2±0.10	1.2±0.10	1.1±0.10	1.0±0.10
	22 ; 1500	1.2±0.06	1.2±0.06	1.1±0.10	0.9±0.06
	22 ; 3000	1.3±0.06	1.3±0.06	1.2±0.06	1.1±0.06
3	12 ; 1500	2.4±0.43	2.1±0.36	2.1±0.45	1.6±0.14
	12 ; 3000	2.4±0.15	2.6±0.15	2.1±0.11	1.6±0.10
	22 ; 1500	2.8±0.11	2.6±0.10	1.9±0.10	1.4±0.10
	22 ; 3000	3.0±0.15	2.8±0.17	2.4±0.15	2.2±0.06
5	12 ; 1500	3.1±0.46	2.6±0.29	2.1±0.35	1.9±0.24
	12 ; 3000	4.8±0.30	4.8±0.21	4.1±0.30	3.0±0.15
	22 ; 1500	6.3±0.30	5.0±0.21	3.6±0.29	2.5±0.15
	22 ; 3000	6.5±0.26	5.9±0.15	4.8±0.20	4.4±0.21
7	12 ; 1500	4.9±0.45	5.4±0.21	2.8±0.17	3.4±0.49
	12 ; 3000	8.2±0.40	7.8±0.30	6.9±0.25	5.8±0.15
	22 ; 1500	10.0±0.92	7.9±0.90	5.7±0.55	4.2±0.11
	22 ; 3000	10.8±0.84	9.5±0.60	8.6±0.65	7.7±0.60
9	12 ; 1500	9.5±1.21	6.4±1.10	4.7±0.32	3.9±0.93
	12 ; 3000	12.7±0.90	11.4±1.02	9.7±0.49	8.0±0.62
	22 ; 1500	14.9±0.60	11.7±0.86	8.4±1.15	6.3±0.90
	22 ; 3000	16.7±0.58	14.5±0.50	13.1±0.74	12.3±0.75
11	12 ; 1500	10.6±0.63	7.5±0.69	6.2±0.38	5.0±0.60
	12 ; 3000	18.0±0.60	16.9±0.90	14.5±0.89	11.4±0.55
	22 ; 1500	21.1±1.10	16.2±0.56	11.8±0.99	9.0±1.10
	22 ; 3000	21.6±0.92	18.9±0.56	17.3±0.70	16.2±0.96
13	12 ; 1500	11.3±0.61	8.8±0.40	7.3±0.41	5.7±0.35
	12 ; 3000	25.6±1.36	23.4±0.60	18.6±0.85	15.6±1.26
	22 ; 1500	26.0±1.46	19.9±1.15	14.3±1.08	10.6±1.15
	22 ; 3000	27.7±1.19	24.7±1.44	21.7±1.11	20.4±1.30

* basal extract prepared from 50 cm^3 of oil in 1 dm^3 of the cultivation medium designated as 100% extract

Table 2. Comparative data concerning the influence of crude oil extracts on the cell number and the characteristic growth rate of *Scenedesmus quadricauda*

Concentration of oil extracts in cultivation medium	Days of cultivation	Temperature and light conditions [° C; lux] 12 ; 1500			12 ; 3000			22 ; 1500			22 ; 3000		
		n	K [%]	μ	*n*	K [%]	μ	*n*	K [%]	μ	*n*	K [%]	μ
0	1	0.9	100		1.2	100		1.2	100		1.3	100	
	3	2.4	100	0.68	2.4	100	0.50	2.8	100	0.61	3.0	100	0.60
	5	3.1	100	0.18	4.8	100	0.50	6.3	100	0.59	6.5	100	0.56
	7	4.9	100	0.33	8.2	100	0.39	10.0	100	0.33	10.8	100	0.37
	9	9.5	100	0.48	12.7	100	0.32	14.9	100	0.29	16.7	100	0.31
	11	10.6	100	0.08	18.0	100	0.25	21.1	100	0.25	21.6	100	0.19
	13	11.3	100	0.06	25.6	100	0.25	26.0	100	0.15	27.7	100	0.18
100*	1	0.9	100		1.0	83		0.9	100		1.1	85	
	3	1.6	67	0.39	1.6	67	0.34	1.4	50	0.32	2.2	73	0.50
	5	1.9	59	0.12	3.0	63	0.45	2.5	38	0.42	4.4	68	0.50
	7	3.4	69	0.42	5.8	70	0.48	4.2	42	0.37	7.7	71	0.40
	9	3.9	41	0.10	8.0	63	0.23	6.3	42	0.29	12.3	74	0.34
	11	5.0	47	0.18	11.4	63	0.26	9.0	43	0.26	16.2	75	0.20
	13	5.7	50	0.09	15.6	61	0.23	10.6	41	0.12	20.4	74	0.17

* see Table 1; *n*-cell number $\times 10^5$ in 1 cm^3 of the cultivation medium; μ-coefficient of characteristic growth rate of the cell population; K-controls

22°C the extent of microalgal growth inhibition resulting from the activity of these compounds will be greater at lower intensities of light and smaller at higher intensities of light. Certain data in the literature also point to such a relationship. Kusk [10] for example tested the influence of different hydrocarbons on *Phaeodactylum tricornutum* and stated that the growth was most depressed at the lowest light intensity and less depressed at the highest.

A significant difference can also be seen in the growth reaction of *Scenedesmus quadricauda* cells to the activity of oil derivatives by comparison the results at varying temperature and constant light intensity. At temperatures of 12° and 22°C and a constant light intensity of 1500 lux (Figs. 1A and 2C) the growth inhibitory effect of the oil derivatives was decidedly greater at the higher temperature, especially in the presence of 100% and 50% extracts. When the light intensity was higher, 3000 lux, these differences were much smaller (Figs. 1B and 2D), which would indicate that at high light intensities, the temperature does not greatly affect the degree of growth inhibition.

So, reducing the temperature of the algal culture by 10°C at a light intensity of 3000 lux brings about quite a different growth reaction in *Sc. quadricauda* to the

activity of oil derivatives than does reducing the temperature of a culture which is less strongly illuminated (1500 lux). Fontaine *et al* (1975) [10] obtained also different results when testing the influence of hydrocarbons on the intensity of photosynthesis in *Phaeodactylum tricornutum* at different temperatures. It was shown that the effects of hydrocarbons on photosynthesis of the given organism were greater at low temperatures than at higher temperatures. Such phenomenon was also observed in our own investigations but only at the higher light intensity (3000 lux). The greater toxicity of crude oil extracts at lower temperatures is probably due to the slower rate of evaporation of the light fractions of the aromatic hydrocarbons contained in the oil. Such conclusion suggests the experiments of Gordon *et al* [4], carried out under laboratory conditions, on the behaviour of given concentrations of crude oil in sea water at different temperatures. The results of the present study indicate, however, that the effect of temperature is largely determined by the actual conditions of illumination.

Evident relationships with respect to the effect of varying illumination and temperature are observed when comparing the growth of microalgae in undiluted crude oil extract (100%) with the control plants (Tables 1, 2). A comparison of the values of the growth coefficient μ shows that differences in the growth reaction in the 100% extract and in the control are especially clear during the first 5 days of cultivation. An exception to this is the culture at 12°C/1500 lux where the differences in growth can be observed for as long as 9 days.

In summary, it may be said that the biological consequences of oil-based pollutants in natural water basins may be to a large extent, also determined by existing environmental conditions; a significant part being played by light intensity and temperature.

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