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Diesel-fuel-oil induced morphological changes in some *Scenedesmus* species (Chlorococcales)

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With 3 figures and 3 tables in the text

Abstract: Three species of *Scenedesmus* – *S. quadricauda*, *S. armatus* and *S. microspina* – with markedly different growth sensitivity to aqueous fuel oil extract (AFOE) were examined to determine how their morphology was affected by AFOE. The results demonstrated a good correlation only between growth inhibition (expressed as the population density in relation to the control) and increase in mean cell volume. Changes in other morphological characteristics – changes in cell shape, production of unicells, organisation of coenobia and abnormalities induction – seem to be species-dependent. The increase in cell volume in algae exposed to AFOE may be due to the uncoupling of cell growth and reproductive processes. Sporulation is especially affected by AFOE. Daughter cells grow within mother cells but are not released, leading to an increase in mean cell volume in the moderately sensitive *S. armatus*, but especially in the most sensitive *S. microspina*. It appears that cell volume and algal growth can be used interchangeably in toxicological tests.

Key words: *Scenedesmus*, sensitivity, Diesel-oil, morphology, growth inhibition, reproduction, sporulation, toxicological tests.

Introduction

Phenotypic variation in the genus *Scenedesmus* is known to occur both in nature and under artificial conditions. Many morphological forms of *Scenedesmus* defined by Egan & Trainor (1989 b) as ecomorphs (the morphological expression of a physiological state), can be environmentally induced by factors such as: light (Steenbergen 1975), temperature (Komárek & Růžička 1969), medium composition and inoculum size (Egan & Trainor 1989 c). These exceptional relations between the growth of some strains and their morphology have taxonomic implications. Some species, such as *Scenedesmus armatus*, each of whose developmental stages has been classified in its life history by Trainor (1991), has

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sometimes been described as a distinct taxon. On the other hand, under strictly-maintained growth conditions of a well-known strain, some morphological characteristics may be sensitive indicators of the different pollutants influencing algae.

There are many papers describing the considerable morphological changes brought about in *Scenedesmus* strains exposed to toxins such as heavy metals (STARODUB et al. 1987, GREGER et al. 1992, CORRADI & GORBI 1993) or herbicides (EL-DIB et al. 1989). However, the authors of several papers on the physiology of different microalgae treated with crude oils and related compounds have reported their morphological alterations only in passing (BOTT & ROGENMUSER 1978, NORLAND et al. 1978, FABREGAS et al. 1984). One exception is the paper by SOTO et al. (1979) in which the changes in the green flagellate *Chlamydomonas angulosa* induced by crude oil treatment are described.

We have frequently observed evident morphological effects induced by fuel oil extracts in *Scenedesmus*. The preliminary results of these observations regarding *S. armatus* are described elsewhere (TUKAJ et al. 1984, TUKAJ 1989). The present paper reports comparative data obtained for three *Scenedesmus* species cultured with aqueous fuel oil extracts. We chose these species because of their distinct growth sensitivity to fuel oil. The results suggest that some morphological features of the species could supplement the growth parameters used in toxicological studies.

Materials and methods

Three *Scenedesmus* species were used in the experiments. Two of them, i.e. *S. armatus* (CHOD.) G. S. SMITH (formerly *S. quadricauda* B1-76) and *S. microspina* R. CHOD were isolated from southern Baltic Sea water at the Institute of Oceanology, Sopot (Poland) (GĘDZIOROWSKA 1983). The third one, *S. quadricauda* (TURP.) BRÉB. strain GREIFSWALD/15, was obtained from the Institute of Botany Collection in Třeboň (Czech Republic).

The species were stored in test-tubes on agar slants (2 %) containing bactopeptone »Difco« (1 %) and glucose (2 %). They were later removed from the agar slants with BRISTOL's (BBM) medium (NICHOLS & BOLD 1965) and transferred to 125 ml E-flasks, which were several times per day shaken. After an adaptation time of 5–7 days, the microalgae were inoculated into the batch cultures. The initial cell density was 10^5 cells per cm^3 in a 50 cm^3 suspension. Cultures were conducted at 22–24 °C in a 12:12 h light-dark cycle of fluorescence illumination providing a photon flux density of about 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Direct microscopic counts were performed on the cultures using a Biolar, PZO light microscope in a FUCHS-ROSENTHAL counting chamber.

Both cell volume and cell shape were evaluated on photographically docu-

mented cells under light microscopy (Docuval, CARL ZEISS Jena). The length and width of randomly chosen cells were measured under an enlarger (Documator, CARL ZEISS Jena). All mean cell dimensions are based on 100 cell measurements. The shape index represented the ratio of two perpendicular size parameters of the cells - the maximum width and the maximum length. Cell volume was determined using the techniques and formulas described by EDLER (1979); the cross-section of a cell was assumed to be circular.

The frequencies of unicell, 2-, 4- and 8-celled coenobia as well as of degenerated cells within coenobia and of abnormal four-celled coenobia were determined in representative samples of living cells under the microscope; at least 500 coenobia were randomly taken from every sample. Every culture was repeated two or three times in the same experimental series and every series was performed twice.

The significance of the morphological differences was determined by STUDENT's t-test.

No. 2 diesel fuel oil obtained from Gdańsk Refinery was used in this work. The aqueous fuel oil extract (AFOE) was prepared by mixing 50 cm^3 of oil with 1 dm^3 of BBM medium in a bottle tightly sealed with a Teflon-plug. The mixture was agitated for 24 hours by means of a magnetic stirrer and then transferred to a separating funnel for about 2 hours before the algae were cultured or the aqueous layer was sampled for hydrocarbon analysis. The lower aqueous phase was carefully siphoned from below the surface oil slick. Generally, AFOE is a water-soluble fraction of the tested oil with small amounts of oil dispersed. A 100–500 cm^3 aliquot was taken for analyis. Total fuel-oil hydrocarbons in the water samples (carbon tetrachloride extractable oil) were determined by the infrared spectrophotometer method (GRUENFELD 1975). The concentration of hydrocarbons in AFOE measured by the above method is 49.8 ± 9.1 ppm ($n = 5$). A qualitative characterisation of AFOE will be found elsewhere (TUKAJ 1987).

Results

The growth of microalgae exposed to AFOE (expressed as the cell number in relation to the control) is shown in Figure 1. Depending on the species, AFOE can completely suppress growth or induce long lags in growth initiation or lower the growth rate of the organisms. The population density of *S. quadricauda* was reduced only temporarily on days 3 and 5 of culture, after which the growth rate was close to the control. In *S. armatus* fuel oil induced a lag of several days, but after this time (ca. one week) the population density which had been reduced to about 40% of the control, recovered to growth at a rate close to that of the control. By contrast, the growth of *S. microspina* in practice was completely suppressed by AFOE. During 19 days of culture, the population density of this

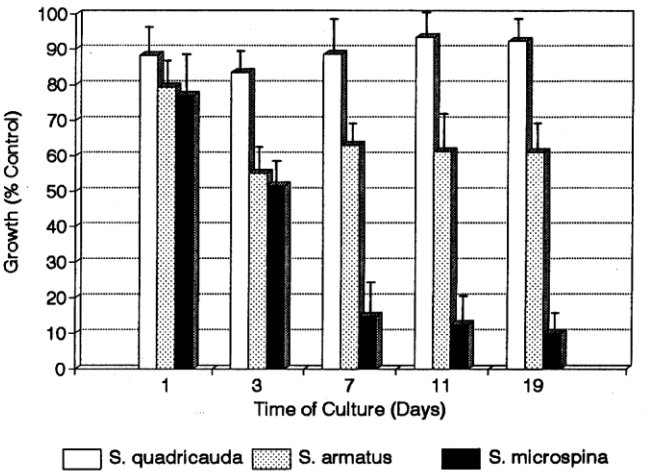

Fig. 1. Growth (% of control growth) of three *Scenedesmus* species exposed to aqueous fuel oil extract (AFOE) at total concentration of hydrocarbons 49.8 ppm. Vertical bars represent the standard error (SE) of the mean of three cultures; each cultur is the mean of at least three repetitions. Inocula 10^5 cells · cm^{-3}, temp. 22–24 °C, light intensity about 50 µmol · $m^{-2} \cdot s^{-1}$, photoperiod 12/12 h.

Table 1. Cell volume (μm^3) of three *Scenedesmus* species treated by aqueous fuel oil extract (AFOE) at a concentration of 49.8 ppm. Values are the means (± SE) of three replicate cultures.

Days of culture	*Scenedesmus quadricauda*		*Scenedesmus armatus*		*Scenedesmus microspina*	
	Control	AFOE	Control	AFOE	Control	AFOE
0	166.4 (4.5)		119.5 (2.8)		37.6 (1.0)	
3	160.7 (10.1)	220.0** (7.8)	117.1 (3.2)	181.6** (6.0)	41.4 (1.5)	68.3** (2.1)
7	132.3 (6.3)	164.4** (5.8)	127.0 (4.2)	235.2** (7.3)	34.5 (1.2)	94.3** (2.2)
11	127.6 (4.4)	157.2** (6.0)	109.9 (3.2)	209.5** (7.5)	31.4 (0.8)	117.6** (2.6)
19	138.4 (4.0)	160.1* (6.1)	75.0 (2.4)	121.7** (4.0)	27.0 (0.6)	140.3** (3.8)

One and two asterisks mean significant differences between treated and control algae at $\alpha = 0.05$ and 0.01 respectively.

strain increased 150 times in the control but only 8 times in the AFOE variant; there no signs of normal growth being restored.

The mean volume changes of the species during the experiments are reported in Table 1. The pattern of these changes is negative in relation to the reproductive pattern of the three *Scenedesmus* species affected by AFOE: cell volume increased during the period of reproductive depression, and decreased again if the reproductive rate increased. In this way, the cell volume of *S. microspina* treated with AFOE increased dramatically from 37.6 μm^3 (inoculum) to 140.3 μm^3 on the 19th day of culture, whereas during the same period, the cell volume of the control slowly decreased to 27.0 μm^3. The mean cell volume of *S. armatus* exposed to AFOE temporary increased with a max. on the 7th and 11th days of culture (twofold increase), but decreased when the population growth rate reached the control rate (see the same relative values of growth inhibition for 11 and 19 day of culture in Fig. 1.) The growth of cell volume of *S. quadricauda* appears to be stimulated by AFOE only about 20 % in comparison to control, except on the 3rd day, when AFOE stimulation was 37 %.

When cultured under control conditions, the cells of tested species, are more or less ellipsoidal (*S. microspina* and *S. armatus*) and cylindrical in shape (*S. quadricauda*). Cells exposed to AFOE generally tend to be more spherical in shape (Tab. 2, Fig. 2). Among the species, *S. armatus* is the one whose shape is the most affected by AFOE - the treated cells are nearly spherical during the entire

Table 2. Cell shape (expressed as quotient of the mean width and length) of three *Scenedesmus* species treated by aqueous fuel oil extract (AFOE) at a concentration of 49.8 ppm. Values are the means (± SD) of three replicate cultures.

Days of culture	*Scenedesmus quadricauda*		*Scenedesmus armatus*		*Scenedesmus microspina*	
	Control	AFOE	Control	AFOE	Control	AFOE
0	0.31 (0.03)		0.50 (0.06)		0.53 (0.05)	
3	0.29 (0.05)	0.30 (0.06)	0.52 (0.07)	0.62** (0.11)	0.52 (0.07)	0.50* (0.08)
7	0.32 (0.05)	0.31 (0.05)	0.52 (0.07)	0.75** (0.12)	0.56 (0.07)	0.55 (0.08)
11	0.34 (0.05)	0.35* (0.04)	0.53 (0.07)	0.70** (0.12)	0.62 (0.08)	0.56** (0.08)
19	0.34 (0.04)	0.36* (0.05)	0.47 (0.07)	0.71** (0.16)	0.66 (0.10)	0.59** (0.10)

One and two asterisks indicate significant differences between variants at $\alpha = 0.05$ and 0.01 respectively.

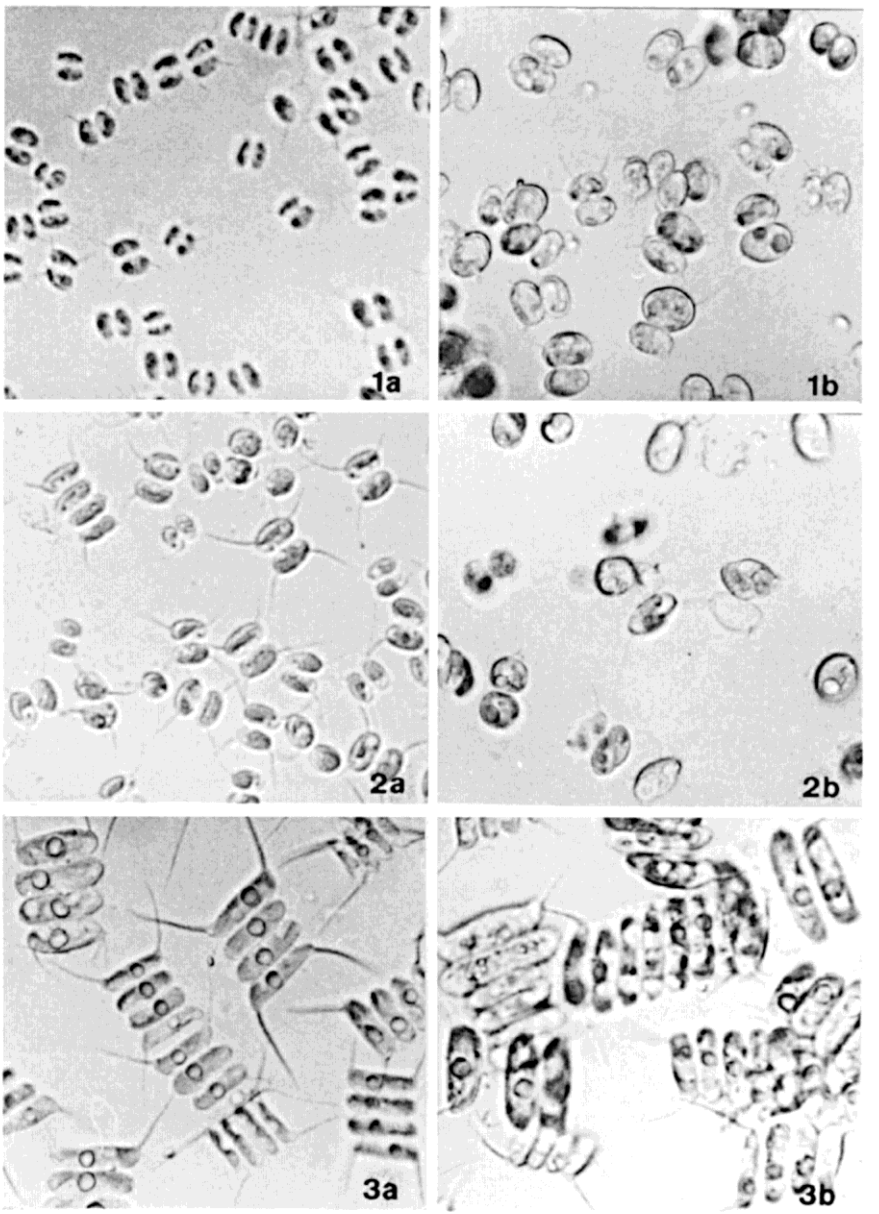

Fig. 2. Light microscopy of *S. microspina* (**1**), *S. armatus* (**2**) and *S. quadricauda* (**3**). Control cells (**a**); 7 day cells cultured with AFOE (**b**). (Magn. 710 ×)

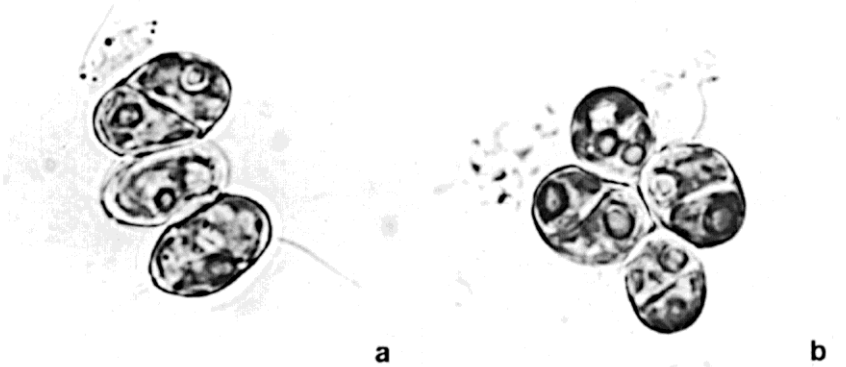

Fig. 3. Cultures of *S. armatus* treated for 7 days with AFOE. **a** – 4-celled coenobium containing one teminally degenerated cell; **b** – tetragonal coenobium. Note that the cells of both coenobia contain autospores able to growth within mother cells. (Magn. 1450 ×)

culture. The same tendency occurs in *S. quadricauda* but to a lesser extent. *S. microspina* has the largest cell volume after AFOE treatment, but the shape of the cells does not alter during the first week of culture. Moreover, in the second week the mean cell width of this species decreases as compared to the control.

The morphological abnormalities of the cells within 4-celled coenobia, i.e. 4-celled coenobia containing degenerated (dead?) cells and so-called tetragonal coenobia, are presented in Figure 3. The degenerated cells are smaller in size compared with the other ones in the coenobium (Fig. 3a). The surface, especially of the middle cells, is shrunk and deformed, one of the probable reasons for this being growth of adjacent cells. The degenerated cells are colourless and have a higher granule content. In control cultures the occurrence of these cells is very rare (below 0.5 %, irrespective of species). The longer the time of exposure to AFOE, the higher the frequency of these cells. In an abnormal coenobium, there is most frequently one degenerated cell. The strain GREIFSWALD/15 the most susceptible, as regards the formation of degenerated cells, contains 3.3 %, 5.6 %, 6.7 % and 7.0 % of such coenobia after 3, 7, 11 and 19 days treatment with AFOE, respectively, whereas at the end of culture with *S. armatus* and *S. microspina* 2.8 % and 5.9 % of such coenobia were found. There were two or three degenerated cells within 4-celled coenobia only in *S. quadricauda* and rarely in *S. armatus*.

The second type of morphological abnormalities involves changes in coenobium configuration from a linear to a tetragonal form (Fig. 3b). Worst affected is *S. armatus* and to a smaller extent *S. quadricauda*; these changes are closely related to the time of culture. Hardly any of these forms are observed by the end of culture, regardless of species. The cells become oval and are larger than in the linear forms. The tetragonal forms were not observed in *S. microspina*.

The changes in the development of coenobia organisation (unicell, 2-, 4-, 8-celled coenobia) of species cultured with or without AFOE are presented in Table 3. In *S. armatus* unicell formation decreased distinctly with the time of control culture, whereas in the AFOE variant the percentage of unicells was still very high. At the same time there are more then twice as many 2-celled coenobia in the control as in the treated population of this species. Another way of unicell formation is observed in *S. microspina*. In the control these forms increased continuously reaching about 50 % on the 19th day of culture, whereas in medium containing AFOE they persisted (about 10 %) throughout the whole growth period. Contrary to the above are 4-celled coenobia formed within this species. In the control, these forms decrease to about 1 % after one week of culture; this process is accompanied by a constant level (about 50 %) of 4-celled coenobia in the AFOE-treated population, regardless of the culture time. Unicells are never produced by *S. quadricauda*. The 2-, 4- and 8-celled coenobia of this species are affected only to a small extent by AFOE in comparison with the control (with the exception of 2-celled coenobia formation stimulated by AFOE on day 3 of culture).

Table 3. Mean per cent occurrence of morphological forms in population of three *Scenedesmus* species exposed to aqueous fuel oil extract (AFOE). The initial cell densities of the batch cultures were 10^5 cells per ml. Values are means from twoplicate cultures, in each at least 500 forms were counted.

Morphological forms	Day of culture								
	0	3		7		11		19	
	Cont.	Cont.	AFOE	Cont.	AFOE	Cont.	AFOE	Cont.	AFOE
Scenedesmus quadricauda coenobia:									
2-celled	1.4	1.9	13.2	2.6	2.9	6.1	12.1	11.6	22.3
4-celled	83.7	93.3	81.6	94.9	95.8	93.5	87.4	88.1	77.3
8-celled	14.9	4.8	5.2	2.5	1.3	0.4	0.5	0.3	0.4
Scenedesmus armatus coenobia:									
2-celled	64.0	46.3	25.0	64.9	24.6	77.4	23.8	79.7	44.9
4-celled	30.8	26.1	15.8	18.8	9.9	15.4	10.6	18.8	22.5
unicells	5.2	27.6	59.2	16.3	65.5	7.2	65.6	1.5	32.6
Scenedesmus microspina coenobia:									
2-celled	70.7	66.6	39.0	68.3	38.6	63.7	44.4	45.1	39.4
4-celled	19.2	11.3	53.2	4.4	53.2	1.1	47.7	1.3	48.1
unicells	10.1	22.1	7.8	27.3	8.2	35.2	7.9	53.6	12.5

Discussion

The aqueous fuel oil extract (AFOE) completely suppressed growth (*S. microspina*), induced long lags in growth initiation (*S. armatus*) or lowered the population growth rate (*S. quadricauda*). However, the mean cell volume increased during the period of reproductive depression, but decreased again if the reproductive rate increased. Then there is good correlation between growth inhibition and stimulation of a cell volume increase in cells affected by AFOE. This seems to demonstrate that by decreasing algal reproduction processes, the most prominent targets for its inhibitory action (ZACHLEDER & TUKAJ 1993), the AFOE indirectly caused an increase in cell volume. Additionally, in both AFOE sensitive species, i.e. *S. microspina* and, rarely, *S. armatus* mother cells with autospores are recorded most frequently. This means that autospore formation and their growth within mother cells generally took place. However, because autospore release is inhibited, the mother cells become bigger than the control ones, especially when AFOE exerts its algistatic effect. Sporangia in the control cultures were not observed, because the sporulation process is short and, with a photoperiod such as in our study, proceeds virtually in the dark (ŠETLÍK et al. 1972).

AFOE treatment significantly changes the shape of *S. armatus* cells, which become more rounded. Similar tendency is observed in *S. quadricauda* cells treated by AFOE after the first week of culture. But at the same time the mean width of *S. microspina* cells decreased in comparison to the control. No adequate physiological explanation can be given for this observation. The ability of crude oil and some hydrocarbons to alter cell osmotic stability has been demonstrated in algae (HUTCHINSON et al. 1981). This can only partially explain the results obtained in this work, because the cells of the three AFOE-treated species changed shape in different ways. These differences appear to be more closely related to the predominant ecomorphs in a given population or/and the cell cycle stage fixed by AFOE. AFOE treatment of *S. armatus* leads to the formation of unicells (the most strongly affected forms), whereas in the *S. microspina* population 4-celled coenobia are formed. Unicells of both organisms are nearly spherical in shape. Then the relative number of these »giant« forms in the populations of both species seem to be one of the main reason for the differences in cell shape between them.

Different kinds of morphological abnormalities in *S. quadricauda* have been described in detail by NEČAS & SULEK (1977, 1982). They found that under growth conditions close to the optimum, the occurrence of such forms is very low. Their frequency increases considerably under stress growth conditions, for instance, in unsuitable light and temperature combination (LUKAVSKÝ 1982). Our results also indicate that both degenerated cells within coenobia and abnormal coenobia increase in all of three populations studied, but to different extents. It follows that the abnormalities may not only be an indicator of the heavy metal

toxicity microalgae (THOMAS et al. 1980) but to some extent also of oil pollution. Oddly enough, however, the abnormalities occurred especially in the species most resistant (*S. quadricauda*) to fuel oil.

In this study *Scenedesmus* exists as unicells, 2-, 4- and 8-celled coenobia, depending on the species. The relative occurrence of these forms in a given population change with the time of culture. The pattern of these changes is strongly affected by AFOE, and manifested especially by the stimulation of unicells (*S. armatus*) and 4-celled coenobia (*S. microspina*) formation. Many data provide evidence that unicell formation may in particular be affected by environmental factors (TRAINOR & EGAN 1990). EGAN & TRAINOR (1989 a) found a very low cell density (10^3 cells per cm^3) in the culture to be the unifying principle for unicell development in *Scenedesmus*. Our initial density was 10^5 cells per ml and the cultures were not diluted daily as in the above mentioned experiments. SIVIER & TRAINOR (1983) found that a soil extract solution induced the production of unicells, and an extensive unicells population of *Scenedesmus* in a sewage oxidation pond was reported by MATTONI et al. (1965). These data suggested that organic matter may be a unicells formation inducer in these media. But recently HESSEN & VAN DONK (1993) have reported that substances released by *Daphnia* or water filtered from algal cultures with *Daphnia* present, promoted the formation of large 4- and 8-celled coenobia in *S. subspicatus*. Some conflicting data remain, and it could be, as our results suggest, that the formation pattern of unicells and other ecomorphs is a species-dependent phenomenon.

In summary it can be stated that there is good correlation between growth sensitivity of algae to AFOE and their morphology. The extent of morphological changes in cells treated with AFOE may be due in general to the uncoupling of cell growth and reproductive processes. Cell volume seems to be the best indicator among morphological characteristics of such changes. It appears that cell volume and growth of algae can be used interexchangeably in toxicological tests.

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References

BOTT, T. L. & ROGENMUSER, K. (1978): Effects of no. 2 fuel oil, Nigerian crude oil, and used crankcase oil on attached algal communities: Acute and chronic toxicity of water-soluble constituents. - Applied Environ. Microbiol. **36**: 673–682.

CORRADI, G. M. & GORBI, G. (1993): Chromium toxicity on two linked trophic levels II. Morphophysiological effects on *Scenedesmus acutus*. - Ecotoxicol. Environ. Saf. **25**: 72–78.

EDLER, L. (1979): Recommendations of methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. - The Baltic Marine Biologists (BMB) **5**: 1-38.

EGAN, P. F. & TRAINOR, F. R. (1989 a): Low cell density: Principle of unicell development in *Scenedesmus* (Chlorophyceae). - Br. phycol. J. **24**: 271-283.

- (1989 b): The role of unicells in the polymorphic *Scenedesmus armatus* (Chlorophyceae). - J. Phycol. **25**: 65-70.

- (1989 c): The effect of media and inoculum size on the growth and morphological development of *Scenedesmus communis* HEGEW. (Chlorophyceae) in culture. - Arch. Hydrobiol. **117**: 77-95.

EL-DIB, M. A.; SALWA, S. A. & ABOU WALY, H. F. (1989): Response of freshwater alga *Scenedesmus* to triazine herbicides. - Water Air Soil Pollut. **48**: 307-316.

FABREGAS, J.; HERRERO, C. & VEIGA, M. (1984): Effect of oil and dispersant on chlorophyll a content of the marine microalga *Tetraselmis suecica*. - Appl. Environ. Microbiol. **47**: 445-447.

GĘDZIOROWSKA, D. (1983): Izolacja bałtyckich glonów jednokomórkowych i uzyskanie kultur aksenicznych dla badań fizjologiczno-biochemicznych. Isolation of Baltic unicellular algae and obtaining of axenic cultures for physiological-biochemical studies. - Stud. Mat. Oceanol. **41**: 209-226.

GREGER, M.; TILLBERG, J.-M. & JOHANSSON, M. (1992): Aluminium effects on *Scenedesmus obtusiusculus* with different phosphorus status. II. Growth, photosynthesis and pH. - Physiol. Plant. **84**: 202-208.

GRUENFELD, M. (1975): Quantitative analysis of petroleum oil pollutants by infrared spectrophotometry. - Water Quality Parameters, ASTM STP 573, p. 290-308, American Society for Testing and Materials.

HESSEN, D. O. & VAN DONK, E. (1993): Morphological changes in *Scenedesmus* induced by substances released from *Daphnia*. - Arch. Hydrobiol. **127**: 129-140.

HUTCHINSON, T. C.; HELLEBUST, J. A. & SOTO, C. (1981): Effect of naphthalene and aqueous crude oil extract on the green flagellate *Chlamydomonas angulosa*. IV. Decrease in cellular manganese and potassium. - Can. J. Bot. **59**: 742-749.

KOMÁREK, J. & RŮŽIČKA, J. (1969): Effect of temperature on the growth and variability of *Scenedesmus quadricauda* (TURP.) BRÉB. - In: FOTT, B. (ed.): Studies in Phycology: 262-292, Academia, Prague.

LUKAVSKÝ, J. (1982): Cultivation of chlorococcal algae in crossed gradients of temperature and light. - Arch. Hydrobiol./Suppl., Algological Studies **29**: 517-528.

MATTONI, R. H. T.; KELLER, E. C. & MYRICK, H. N. (1965): Industrial photosynthesis. A means to a beginning. - BioScience **15**: 403-407.

NEČAS, J. & SULEK, J. (1977): Developmentally stopped and stunted cells in the culture of the alga *Scenedesmus quadricauda*. - Arch. Protistenkde. **19**: 100-120.

- (1982): Comparison of several characteristics of the chlorococcal alga *Scenedesmus quadricauda* and its complex mutation. - Arch. Hydrobiol./Suppl., Algological Studies **29**: 439-469.

NICHOLS, W. H. & BOLD, H. C. (1965): *Trichosarcina polymorpha* Gen. et Sp. Nov. - J. Phycol. **1**: 34-38.

NORLAND, S.; HELDAL, M.; LIEN, T. & KNUTSEN, G. (1978): Toxicity testing with synchronized cultures of the green alga *Chlamydomonas*. - Chemosphere **3**: 231-245.

ŠETLÍK, I.; BERKOVÁ, E.; DOUCHA, J.; KUBÍN, Š.; VENDLOVÁ, J. & ZACHLEDER, V. (1972): The coupling and synthetic and reproduction processes in *Scenedesmus quadricauda*. - Arch. Hydrobiol./Suppl., Algological Studies **7**: 172-213.

SIVER, P. & TRAINOR, F. R. (1983). Effect of growth rate on unicell productin in two strains of *Scenedesmus* (Chlorophyta). - Phycologia **22**: 127-131.

SOTO, C.; HUTCHINSON, T. C.; HELLEBUST, J. A. & SHEATH, R. G. (1979): The effect of crude oil on the morphology of the flagellate *Chlamydomonas angulosa*. - Can. J. Bot. **57**: 2717-2728.

STARODUB, M. E.; WONG, P. T. S. & MAYFIELD, C. I. (1987): Short term and long term studies on individual and combined toxicities of copper, zinc and lead to *Scenedesmus quadricauda*. - Sci. Total Environ. **63**: 101-110.

STEENBERGEN, C. L. M. (1975): Light-dependent morphogenesis of unicellular stages in synchronized cultures of *Scenedesmus quadricauda* (TURP.) BRÉB. (Chlorophyceae). - Acta Bot. Neerl. **24**: 391-396.

THOMAS, W. J.; HOLLIBAUGH, J. T. & SIEBERT, D. L. R. (1980): Effects of heavy metals on the morphology of some marine phytoplankton. - Phycologia **19**: 202-209.

TRAINOR, F. R. (1991): The format for a *Scenedesmus* monograph. - Algological Studies **61**: 47-53.

TRAINOR, F. R. & EGAN, P. F. (1990): Phenotypic plasticity in *Scenedesmus* (Chlorophyta) with special reference to *S. armatus* unicells. - Phycologia **29**: 461-469.

TUKAJ, Z. (1987): The effects of crude and fuel oils on the growth, chlorophyll α content and dry matter production of a green alga *Scenedesmus quadricauda* (TURP.) BRÉB. - Environ. Pollut. **47**: 9-24.

TUKAJ, Z.; KENTZER, T. & BOHDANOWICZ, J. (1984): The influence of fuel oil on the growth and cell morphology of *Scenedesmus quadricauda* (TURP.) BRÉB. - Proc. XIV Conf. Baltic Oceanographers **2**: 830-844.

- (1989): The effect of fuel oil on the ultrastructure of the chlorococcal alga *Scenedesmus armatus*. - Protoplasma **151**: 47-56.

ZACHLEDER, V. & TUKAJ, Z. (1993): Effect of fuel oil and dispersant on cell cycle and macromolecular synthesis in the chlorococcal alga *Scenedesmus armatus*. - Mar. Biol. **117**: 347-353.

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