The toxicity of three dispersed diesel fuel oils and dispersant towards some *Scenedesmus (Chlorococcales)* species

OCEANOLOGIA, No. 36 (2)
pp. 175–186, 1994.
PL ISSN 0078–3234

Fuel oils
Dispersant
Toxicity
Scenedesmus

Zbigniew Tukaj
Department of Plant Physiology,
University of Gdańsk,
Gdynia

Manuscript received September 2 , 1994, in final form December 15, 1994.

Abstract

The growth of *Scenedesmus* species exposed to three diesel fuel oils was examined in batch cultures. Oils were added to BBM medium in the form of aqueous fuel oil extracts (AFOE) and oil-in-water dispersions (OWD). Cell density, chlorophyll *a* and dry matter were used as growth parameters.

The growth of an *S. armatus* population was affected by AFOE and OWD of No. II oil in a similar way, even though the respective total hydrocarbon concentrations of AFOE and OWD were 49.8 and 15.1 ppm. This result indicates the dominant role of oil dispersion in the reduction of algal growth.

The toxicities of dispersant DP–105, mechanical dispersion (md) and chemical dispersion (cd) (using DP–105 plus I LS or I DS fuel oils) were compared. $EC_{50/48}$ values for I DS fuel oil were similar – 301.9 ppm (md) and 308 ppm (cd), whereas they were quite different for I LS fuel oil – 586.7 ppm (md) and 171.5 ppm (cd). $EC_{50/48}$ for dispersant DP–105 was 74.6 ppm. The toxicity of DP–105 and both oils increased after 96 h, especially in relation to I DS oil.

The results indicate significant differences in sensitivity of six *Scenedesmus* species to AFOE of No. II oil. The species can be ordered from the most sensitive to the most tolerant as follows: *S. microspina* $\gg$ *S. obliquus* $>$ *S. armatus* $>$ *S. opoliensis* $>$ *S. acutus* $\gg$ *S. quadricauda* strain G–15.

Analysis of variance (factorial experiment) resulted in a significant F–value for the combined effects of AFOE, light and temperature, suggesting an interaction between them.

1. Introduction

Oil spilled at sea is subjected to the natural turbulence of the sea surface and tends to break up into droplets. Small droplets are permanently incor-

porated into the water column where they later dissolve and are degraded. The larger drops, however, presumably return to the surface and coalesce with the slick. The other process that occurs under an oil slick is hydrocarbon dissolution. This is a very complicated process because the solubility of an oil is not simply the sum of the solubilities of its constituents. Furthermore, the relative importance of dispersion and dissolution varies with the weather and sea conditions, and also with the nature of the spilled oil. From the viewpoint of toxicity towards phytoplankton, it is worth stressing that the dissolved hydrocarbons are predominantly aromatic. This is due to the much greater water solubility of aromatic hydrocarbons as compared to that of alkanes of similar molecular weight (McAuliffe, 1977). The hydrocarbon composition of the dispersed oil is relatively deficient in aromatics since it closely resembles that of the parent oil (Anderson *et al.*, 1974).

For several years, oil dispersants have been proposed as a means of removing oil from the water surface. A dispersant contains surface-active ingredients reducing the oil-water interfacial tension, and thus the energy barrier to the formation of the large interfacial area associated with dispersion. However, the dispersants themselves can be toxic to organisms (Heldal *et al.*, 1978; Bratbak *et al.*, 1982; Singer *et al.*, 1991). Moreover, most studies have found exposure to oil dispersed with dispersants more toxic to organisms than non-dispersed oil or dispersants alone. This has been demonstrated for both micro- and macroalgae studied in the laboratory (Fabregas *et al.*, 1984; Chan and Chiu, 1985) as well as *in situ* (Hsiao *et al.*, 1978; Harrison *et al.*, 1986; Siron *et al.*, 1993).

Previous results indicate that mechanically-dispersed oils decrease *Scenedesmus* growth, according to the proportions of soluble and dispersed hydrocarbons occurring in the culture medium (Tukaj, 1987). This paper reports on the toxicity of mechanically and chemically dispersed diesel fuel oils towards some *Scenedesmus* species. The importance of light and temperature in this toxicity has also been taken into account. The use of an algal inhibition test demonstrates significant differences in sensitivity among the species tested.

2. Materials and methods

Organisms

Six *Scenedesmus* species were used in the experiments. Two of them, *i.e. S. armatus* (formerly *S. quadricauda*) and *S. microspina* were isolated from southern Baltic Sea water at the Institute of Oceanology, Sopot, Poland. The

other four species, *i.e. S. obliquus*, *S. opoliensis*, *S. acutus* and *S. quadricauda* strain G–15 were obtained from the Collection of the Institute of Botany, Treboń, Czech Republic.

Algal cultures

The species were kept in test-tubes on agar slants (2%) containing 'Difco' bactopeptone (1%) and glucose (2%). The species were removed from the slants with Bristol's (BBM) medium (Nichols and Bold, 1965) to 125 ml E–flasks. 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 ml in a 50 ml suspension. Cultures were conducted at 22°C in a 12:12 h light-dark cycle of fluorescence illumination providing a photon flux density of about 50 μmol m^{-2} s^{-1}.

Growth parameters

Direct microscopic counts were performed on the cultures with a light microscope in a Fuchs-Rosenthal counting chamber.

The quantity of algal dry matter was determined by weighing. The algal suspension centrifuged at 1000 g was rinsed twice with BBM medium and then collected under slight vacuum on Sartorius 0.45 μm pore membrane filters. The algae were dried at 105°C to constant weight and stored in a desiccator in the presence of P_2O_5.

The chlorophyll *a* of the algae collected on the glass filters (Whatman GF/C) was extracted with a 4:6 mixture of dimethyl sulphoxide (DMSO) and 90% acetone at 65°C. Absorbance measurements were performed on a VSU2–P (Carl Zeiss, Jena) spectrophotometer. The chlorophyll *a* concentration was determined according to the formula given by Jeffrey and Humphrey (1975).

Preparation of the tested oils

Three diesel fuel oils, *i.e.* No. II, I DS and I LS (Polish Norm: PN–67, C–96048), obtained from the Gdańsk Refinery, were used in this work.

The dispersant (DP–105), a mixture of a nonionic surfactant (10%) in a hydrocarbon solvent (90%), was prepared at the Technical University of Gdańsk (Dobrzyński *et al.*, 1990). The surfactant compounds include polyoxyethylene oleyl (55%) and lauryl (25%) alcohols and polyoxyethylene castor oil (20%). The main component of the hydrocarbon solvent is mineral oil (76%), fatty acids of tall oil (8%), ethanol (2%), n-octanol (2%) and water (2%).

The oil-in-water dispersion (OWD) was prepared in two ways:

- 1 cm^3 of oil was dispersed in 1 dm^3 of BBM medium by means of an ultrasonic vibrator (UM–1, Unitra) at 25 kHz for 0.5 h. The mixture was transferred to a separating funnel, where it was kept for 24 h.

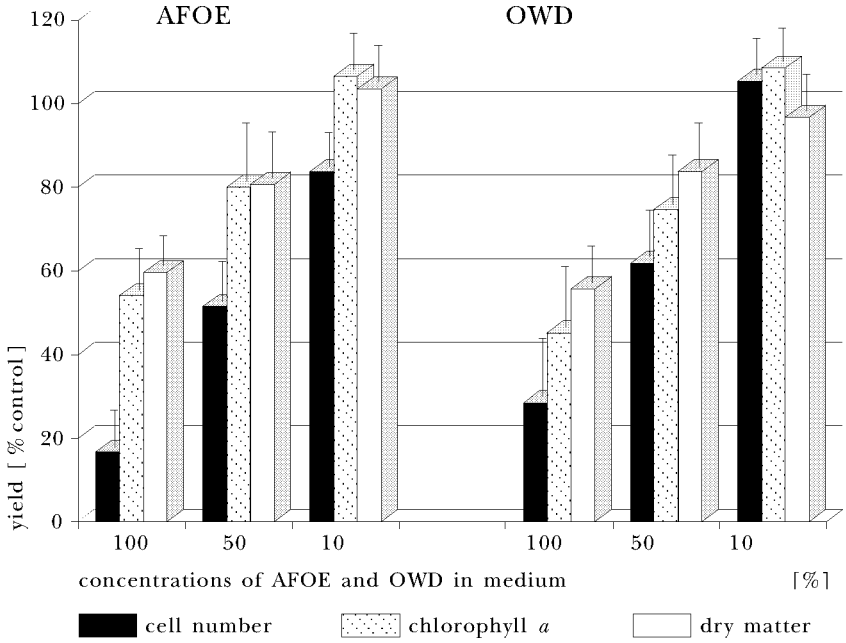

Fig. 1. Growth of the *Scenedesmus armatus* population exposed to No. II diesel fuel oil added to the culture medium in the form of aqueous fuel oil extract (AFOE) and oil–in–water dispersion (OWD) after 7 days of culture. AFOE and OWD, prepared from 50 and 1 cm^3 of oil in 1 dm^3 of medium, contain hydrocarbons at a concentration of 49.8 ppm and 15.1 ppm respectively. The vertical bars indicate the standard error of the mean

Table 1. Results of the algal growth inhibition test of dispersant DP–105 and two diesel fuel oils (I DS and I LS) for the alga *Scenedesmus armatus*

Substances	$EC_{50/48}$ [ppm]	(A)	$EC_{50/96}$ [ppm]	(B)	(A) / (B)
1. Dispersant DP–105	74.6	(12.4)	28.0	(11.0)	2.7
2. Oil I DS	301.9	(43.5)	51.3	(8.1)	5.9
3. Oil I DS + DP–105	308.0	(40.0)	29.4	(9.2)	10.5
2/3	0.98		1.74		
4. Oil I LS	586.7	(55.2)	308.8	(46.1)	1.9
5. Oil I LS + DP–105	171.5	(45.7)	77.8	(37.8)	2.2
4/5	3.42		3.97		

EC_{50}: effective concentration inhibiting algal growth by 50% in 48 h and 96 h respectively. Values in parentheses indicate the standard error of the mean.

The lower layer, containing the oil-in-water dispersion, was designated as 100% (vol./vol.) concentration, and its 50% and 10% dilutions were used. These OWDs were tested in the experiments, the results of which are presented in Fig. 1.

Table 2. Analysis of variance (factorial experiment) to study the combined effects of light, temperature and AFOE of No. II diesel fuel oil on the growth of *Scenedesmus armatus*

Source of variation			Cell number in cm^3 ($\times 10^5$)[c] Day of culture		
A	B	C	3	7	14
1500	12	0	5.91	15.63	36.51
3000	12	0	5.79	15.60	45.24
1500	22	0	7.22	17.49	44.76
3000	22	0	8.41	24.66	60.01
1500	12	49.8	3.12	5.67	12.39
3000	12	49.8	3.69	6.36	14.70
1500	22	49.8	3.99	9.28	20.22
3000	22	49.8	5.41	14.14	34.61

Analysis of variance

Treatment	Main effects and interactions Day of culture			F–test Day of culture		
	3	7	14	3	7	14
A	3.06	12.69	40.68	11.65[a]	6099.82[b]	794.38[b]
B	6.52	22.31	50.76	52.87[a]	18853.64[b]	1236.84[b]
AB	2.16	11.37	18.60	5.80	4896.82[b]	166.07[b]
C	–11.12	–37.93	–104.60	153.80[b]	54495.64[b]	5252.09[b]
AC	0.92	–1.59	–7.28	1.05	95.73[b]	25.44[b]
BC	1.34	0.47	4.72	2.23	8.36[a]	10.69[b]
ABC	0.46	–3.03	5.56	+	347.73[b]	14.84[b]

A – light intensity (lx)
B – temperature (°C)
C – concentration of fuel oil (ppm)
\+ – F° value <1
a – $P \leq 0.05$
b – $P \leq 0.01$
c – every result is the sum of three separate measurements

- Oil, dispersant and oil plus dispersant (10:1) were added to the medium and shaken for five min at 2800 rpm using a shaker (Type 327, Premed). At least five different concentration series in each experiment were prepared. The mixture was prepared directly in Erlenmeyer culture flasks and tested immediately after preparation. These forms of dispersion were tested in experiments, the results of which are presented in Tab. 1.

Aqueous fuel oil extracts (AFOE) were prepared by vigorously stirring the oil samples with the culture medium (1:20 vol./vol.) for 20 h, so that the vortex reached the bottom of a vessel. The mixture was transferred to a separating funnel for about 4 h. The aqueous phase below the oil layer, containing dissolved hydrocarbons and a 'small' amount of dispersed oil, was collected. Further dilutions with BBM medium were made from this 100% AFOE for a nominal concentration exposure test. These AFOEs were used in the experiments, the results of which are presented in Fig. 1 and Tabs. 2 and 3.

Table 3. Growth of *Scenedesmus* species in AFOE of No. II diesel fuel oil. The inhibition of cell growth J_A* is calculated as the difference between the control area A_C and the area below the curve of treated algae A_T, expressed as a percentage of the control A_C (time culture 14 days, initial cell density 10^4 cells per ml BBM medium)

Species	Time of culture (days)	J_A values [%]
S. microspina	1–8	90
S. obliquus	1–8	45
S. armatus	3–8	43
S. opoliensis	3–12	38
S. acutus	1–5	31
S. quadricauda (G–15)	0–5	11

* $J_A = [(A_C - A_T) : A_C] \times 100\%$.

Selected samples of 100% AFOE and 100% OWD of oil No. II were chemically analysed. The oil hydrocarbons of both were extracted using carbon tetrachloride and determined by infrared spectrophotometry (Gruenfeld, 1975). The concentrations of hydrocarbons measured by the above method in AFOE and OWD are 49.8 ± 9.1 ppm (n = 5) and 15.1 ± 1.4 ppm (n = 8) respectively.

All preparations were carried out at room temperature in vessels tightly closed with Teflon plugs. The tested mixtures were not sterilized. Single

bacteria visible under the light microscope did not multiply during the culture.

Statistics

The significance of the growth differences of all tests were determined by T–test.

The variance-factorial experiment (Oktaba, 1980) was analysed to evaluate the combined effects of AFOE, light and temperature on the growth of *S. armatus* (Tab. 2).

To compare the sensitivities of the species tested to AFOE (Tab. 3), the International Standard formula (ISO, 1989) was used, enabling the area under the linear growth curve to be calculated for each test organism, both treated and untreated with AFOE. The inhibition of cell growth J_A was estimated as the difference between the control area A_C and the area below the curve for the toxically inhibited growth A_T, expressed as a percentage of the control area A_C:

$$J_A = [(A_C - A_T) : A_C] \times 100\%.$$

EC_{50} values in Tab. 1 (EC–the effective concentration causing a 50% reduction in algal growth with respect to the control) were calculated by a regression analysis technique known as probit analysis.

3. Results and discussion

The effects of AFOE and OWD produced from 50 and 1 cm^3 of No. II diesel fuel oil respectively on the growth of the 7–day *S. armatus* cultures are presented in Fig. 1.

Despite the difference in hydrocarbon concentration, both neat extracts and the 50% and 10% dilutions of the three extracts influenced the growth of algae to a similar extent. This means that the degree of inhibition is dependent not only on the final concentration of hydrocarbons but also on the quantity of oil introduced to the culture medium in dispersed form. The increase in dispersed oil in the overall quantity of hydrocarbons present in the medium abruptly retarded algal growth. Therefore, OWD and AFOE of No. II oil at respective hydrocarbon concentrations of 15.1 ppm and 49.8 ppm revealed similar growth effects. This result confirmed and extended previous observations on the toxicity of mechanically dispersed samples of this oil (Tukaj, 1987). 10% concentrations of AFOE and OWD did not significantly modify algal growth in relation to the control. This indicates that hydrocarbon concentrations in southern Baltic water ranging from 0.2 to 200 μg dm^{-3} (Law and Andrulewicz, 1983) do not seem to be toxic towards *S. armatus*.

It was also found that the growth parameters were reduced to different extents as a result of oil activity (Fig. 1). The cell density of algae was the most seriously affected, the chlorophyll *a* content and dry matter production of cells less so. It was observed earlier that in synchronically-cultured *S. armatus*, the inhibitory action of this oil affects principally the reproductive processes, and to a lesser extent macromolecular synthesis, including that of chlorophylls (Zachleder and Tukaj, 1993). Dry matter production of algae in batch cultures, however, is relatively weakly inhibited, because autospore growth within mother cells is only slightly affected by oil (Tukaj *et al.*, 1984).

The dispersion of crude oil and petroleum products by chemical dispersants has long been applied as a means of controlling oil spills, and extensive studies have been carried out to assess the effectiveness of oil spill dispersants and their toxicity (Heldal *et al.*, 1978; Daling and Lichtenthaler, 1986/87). The results of the toxicity test of the new dispersant (DP–105) alone, oil/dispersant mixtures and mechanically dispersed oils are presented in Tab. 1. The toxicities of I DS oil dispersed chemically and mechanically are the same after 48 h of exposure (EC_{50} about 300 ppm), but after 96 h, chemical dispersion is far more toxic than mechanical dispersion. By contrast, I LS oil is 3–4 times more toxic when chemically dispersed than when mechanically dispersed, irrespective of the exposure time. The toxicity of all dispersions to algae increases with the culture time. Dispersant DP–105 and both dispersions of I LS oil are about twice as toxic after 96 h than after 48 h, whereas the toxicity of both I DS oil dispersions increased dramatically after 96 h. It is known that dispersants generate more small droplets, which release more toxicants into the water. Thus, there is an increase in the toxicity of a medium containing dissolved hydrocarbons following chemical dispersion as compared with mechanical dispersion. Additional studies on the interrelationships of dispersant efficiency, droplet size and toxicity using test organisms from the food web should be done before DP–105 can be applied in practice.

Interaction between substances in seawater has important ecological implications, as organisms are not separately affected by pollutants. There are only a few papers, however, that have reported interaction effects between extracellular products and hydrocarbons (Karydis and Fogg, 1980), crude oil and nutrient limitation (Karydis, 1981), and growth substances and AFOE (Kentzer and Tukaj, 1990). Moreover, in aquatic ecosystems algae are exposed to chemicals under dynamic conditions, so it would be logical to take this important parameter into account in an ecotoxicity assessment. Thus, the possible interaction between AFOE of No. II oil and light and temperature was examined by setting up the ANOVA–factorial experiment.

The results are shown in Tab. 2. The analysis of variance resulted in a significant F value for the main and combined treatment, suggesting interaction between oil, temperature and light after 7 and 14 days of culture. After 3 days of culture only the main factors, *i.e.* AFOE, light and temperature, alone brought about significant effects on the growth of *S. armatus*. Previously it had been found (Kentzer and Tukaj, 1985) that the AFOE of No. II oil was the most effective in retarding algal growth at a light intensity of 1500 lx and a temperature of 12°C, whereas at 3000 lx and 22°C the effect was less marked. The photooxidation of some hydrocarbons at the photosynthetically active radiation (PAR) light intensities used in this work can probably be ignored. The results obtained relating to temperature therefore suggest that the waters of the strongly polluted southern Baltic Sea (where the species was isolated) seem to be more toxic to chlorococcal algae in winter and spring when they are cold than in summer and autumn when they are warm.

Literature data on the effect of oils on algal growth are difficult to compare because not only different evaluation methods and test conditions but also different strains of algae have been used. In many cases a distinct sensitivity of microalgae to oils has been found among algae of different phylogenetic origins (Winters *et al.*, 1976), as well as in species of the same genus (Kauss and Hutchinson, 1975) and in clones of the same species (Mahoney and Haskin, 1980). These results also indicate the different growth sensitivity of six *Scenedesmus* species exposed to AFOE of No. II oil (Tab. 3). The extent of the response observed in this experiment varied with the species tested, ranging from the quite tolerant *S. quadricauda* (11% inhibition) to the extremely susceptible *S. microspina* (90% inhibition). *S. obliquus*, *S. opoliensis*, *S. armatus* and *S. acutus* were moderately sensitive to both extracts. At present there is no physiological explanation(s) for these differences. It is, however, possible that the same experimental conditions are favourable to the growth of all species, though to different extents. As the data in Tab. 2 indicate, the response of the algae to oils depends on their growth conditions. It is also known that the cell walls of *Scenedesmus* differ considerably among themselves, which seems to have some significance as a mechanical barrier to oil in dispersed form (unpublished data).

Finally it could be stated that little is still known about the toxic mechanism of oil droplet-microalgae interaction. There may be classes of toxic compounds, such as some PAHs, that are leached from oil droplets into the water. For these compounds, the algae may act as a sink and thus the compounds could be continuously transferred to and accumulated by an algal cell. Of course, this process should be faster when a dispersant is used because it generates more small droplets, resulting in the stabilization of the

dispersed oils. On the other hand, the toxicity data of static tests seem to yield much more optimistic results than *in situ* tests. Then, algae treated only once at the beginning of the test in the static culture system, even if mixed several times daily, are deposited, as a result of which they are not directly affected by the dispersed oils. In nature, however, several toxicants may be present simultaneously so interaction may occur. In addition, there exists a natural variation in temperature, illumination and nutrient level, which is not usually taken into consideration in the laboratory.

Nevertheless, the results lead to the cautious suggestion that an increase at sea of the hydrocarbons used in this work may have serious ecological implications. This is especially related to the different sensitivity of chlorococcal algae as well as to the toxicity of dispersed oil stabilized with dispersants.

References

Anderson J. W., Neff J. M., Cox B. A., Tatem H. E., Hightower G. M., 1974, *Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish*, Mar. Biol., 27, 75–88.

Bratbak G., Heldal M., Knutsen G., Lien T., Norland S., 1982, *Correlation of dispersant effectiveness and toxicity of oil dispersants towards the alga Chlamydomonas reinhardti*, Mar. Pollut. Bull., 10, 351–353.

Dobrzyński J., Pączkowski P., Zwierzykowski W., 1990, *A dispersant composition to remove spills of petroleum products from water surfaces*, Patent nr 146958, Urząd patentowy PRL.

Chan K. Y., Chiu S. Y., 1985, *The effects of diesel oil and oil dispersants on growth, photosynthesis, and respiration of Chlorella salina*, Arch. Environ. Contam. Toxicol., 14, 325–331.

Daling P. S., Lichtenthaler R. G., 1986/87, *Chemical dispersion of oil. Comparison of effectiveness results obtained in laboratory and small-scale field tests*, Oil Chem. Pollut., 3, 19–35.

Fabregas J., Herrero C., Veiga M., 1984, *Effect of oil and dispersant on growth and chlorophyll a content of the marine microalga Tetraselmis suecica*, Appl. Environ. Microbiol., 47, 445–447.

Gruenfeld M., 1975, *Quantitative analysis of petroleum oil pollutants by infrared spectrophotometry*, Water Quality Parameters, ASTM STP 573, American Society for Testing and Materials, 290–308.

Harrison P. J., Cochlan W. P., Acreman J. C., Parsons T. R., Thompson P. A., Dovey H. M., 1986, *The effects of crude oil and Corexit 9527 on marine phytoplankton in an experimental enclosure*, Mar. Environ. Res., 18, 93–109.

Heldal M., Norland S., Lien T., Knutsen G., 1978, *Acute toxicity of several oil dispersants towards the green algae Chlamydomonas and Dunaliella*, Chemosphere, 3, 247–255.

Hsiao S. I. C., Kittle D. W., Foy M. G., 1978, *Effects of crude oils and oil dispersant Corexit on primary production of arctic marine phytoplankton and seaweed*, Environ. Pollut., 15, 209–221.

ISO 8692, 1989E, *Water quality – fresh water algal growth inhibition test with Scenedesmus subspicatus and Selenastrum capricornutum*, International Organisation for Standardization (Geneva), 1–6.

Jeffrey S. W., Humphrey G. F., 1975, *New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton*, Biochem. Pflanzen (BPP), 167, 191–194.

Karydis M., 1981, *The toxicity of crude oil for the marine alga Skeletonema costatum (Greville) Cleve in relation to nutrient limitation*, Hydrobiologia, 85, 137–143.

Karydis M., Fogg G. E., 1980, *Physiological effects of hydrocarbons on the marine diatom Cyclotella cryptica*, Microb. Ecol., 6, 281–290.

Kauss P. B., Hutchinson T. C., 1975, *Studies on the susceptibility of Ankistrodesmus species to crude oil components*, Verh. Internat. Verein. Limnol., 19, 2155–2164.

Kentzer T., Tukaj Z., 1985, *Growth responses of Scenedesmus quadricauda to oil pollution at different temperatures and light intensities*, Oceanologia, 22, 41–49.

Kentzer T., Tukaj Z., 1990, *The interaction effects of crude oil-growth regulator combinations on Scenedesmus armatus growth*, Acta Physiol. Plant., 4, 293–301.

Law R., Andrulewicz E., 1983, *Hydrocarbons in water, sediment and mussels from the Southern Baltic Sea*, Mar. Pollut. Bull., 14, 289–293.

Mahoney B. M., Haskin H. H., 1980, *The effects of petroleum hydrocarbons on the growth of phytoplankton recognized as food forms for the eastern oyster, Crassostrea virginica Gmelin*, Environ. Pollut., 22, 123–132.

McAuliffe C. D., 1977, *Dispersal and alteration of oil discharged on a water surface*, [in:] *Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms*, D. A. Wolfe (ed.), Pergamon Press, Oxford–New York–Toronto–Sydney–Paris, Frankfurt, 19–35.

Nichols W. H., Bold H. C., 1965, *Trichosarcina polymorpha Gen. et Sp. New*, J. Phycol., 1, 34–38.

Oktaba W., 1980, *Introduction to mathematical statistics and experimentation methodology*, PWN, Warszawa, (in Polish).

Singer M. M., Smalheer D. L., Tjeerdema R. S., Martin M., 1991, *Effects of spiked exposure to an oil dispersant on the early life stages of four marine species*, Environ. Toxicol. Chem., 1367–1374.

Siron R., Pelletier E., Dellile D., Roy S., 1993, *Fate and effects of dispersed crude oil under icy conditions simulated in mesocosms*, Mar. Environ. Res., 35, 273–302.

Tukaj Z., 1987, *The effects of crude and fuel oils on the growth, chlorophyll a content and dry matter production of a green alga Scenedesmus quadricauda (Turp.) Breb.*, 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.) Breb.*, Proc. 14 Conf. Baltic Oceanographers (Gdynia, Poland), 2, 830–844.

Winters K., O'Donnell R., Batterton J. C., Van Baalen C., 1976, *Water-soluble components of four fuel oils: Chemical characterization and effects on growth of microalgae*, Mar. Biol., 36, 269–276.

Zachleder V., Tukaj Z., 1993, *Effects of fuel oil and dispersant on cell cycle and macromolecular synthesis in the chlorococcal alga Scenedesmus armatus*, Mar. Biol., 117, 347–353.