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> Dispersants Chlorella vulgaris Scenedesmus quadricauda Cultivation

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#### Abstract

Studies were carried out on the effect of five dispersants: Corexit 7664, Corexit 9525, Corexit 9600, OSD/LT and BP 1100 WD on growth of two species of planktonic green algae: *Chlorella vulgaris* and *Scenedesmus quadricauda*, isolated from water in the Gulf of Gdańsk. All the dispersants had a more or less similar effect on the growth of both algal species. The lowest limitation of growth was observed for Corexit 7664, while the strongest reaction took place in case of OSD/LT, and especially of BP 1100 WD.

### Introduction

Dispersants are commonly used against oil pollution. In view of this fact it is important to know the reaction of organisms to these substances in the aquatic environment. This problem has been delt with in many papers. They concentrated mainly on testing different animal responses, mostly mortalities, especially in molluscs and fish larvae (Hargrave, Newcombe 1973, Wilson 1976, 1977), and plants, with special attention given to algae (Hsiao *et al* 1978, Thélin 1981, Hatcher, Larkum 1982).

Phytoplanktonic organisms are not frequently delt with in this aspect although the phytoplankton represents the ecologic formation most susceptible to changes in the environmental conditions. In view of this, toxicity of selected dispersants was tested on two species of planktonic green algae, isolated from the phytoplankton of the Gulf of Gdańsk, *ie* on the organisms occurring at low salinities. So far, toxicity of the dispersants was usually tested using the organisms living in waters of high salinity, over  $30^{\circ}/_{00}$ . There are only a few papers dealing with Baltic organisms, mostly animals (Nagell *et al* 1974, Linden 1975, 1976).

Due to specific hydrologic conditions in the Baltic Sea, consisting of constant mixing of fresh and marine waters, the Baltic organisms are subjected to considerable physiological stress caused by the necessity of continuous adaptation to changing conditions of the osmoregulation (Linden 1974). Consequently, the results obtained with ocean organisms cannot be transferred to the Baltic Sea. Thus, it is necessary to obtain the knowledge on biological effects of using the dispersants in local conditions.

### Material and methods

Two planktonic green algae were tested: *Chlorella vulgaris* Beijernick and *Scenedesmus quadricauda* (Turpin) Brébisson. Both species were isolated from the phytoplankton in the Gulf of Gdańsk.

The materials were cultured in cone flasks of 1 l capacity. The flasks were filled with 400 ml of the medium f/2 (Guillard, Ryther 1962), prepared using water from the Gulf of Gdańsk, with  $8^{\circ}/_{00}$  salinity. The flasks were kept in a light thermostat at 20°C, and illumination of about 2000 lx. The source of light consisted of an electric bulb Polam LF 25 W. The photoperiod was 16 h of light and 8 h of darkness.

The test consisted of an analysis of the cell multiplication rate in order to obtain the growth curves. Cell numbers in a unit of the medium volume were determined under a microscope, using Bürker's chamber. The cultures were terminated at the stage of stationary growth.

The following dispersants were tested: Corexit 7664, Corexit 9525, Corexit 9600, OSD/LT and BP 1100 WD. Each dispersant was used in a number of concentrations, from 50, through 150, 500, 1500 to 5000 ppm. Additional concentrations of 15 and 15,000 ppm were used for some dispersants in order to obtain a fuller picture of the reaction.

# Results

The results of the tests revealed noticeably different growth responce in both species under study, depending on the dispersant used. Essentially, degree of the effect of these dispersants on growth was similar for both species (Figs. 1 and 2). Comparison of the growth rates showed that the lowest limitation of algae growth took place in case of Corexit 7664. Corexit 9527 was characterized by a similar effect, while Corexit 9600 affected growth of algae in a slightly more pronounced way. The other two dispersants, *viz* OSD/LT and BP 1100 WD resulted in a much stronger response, especially in case of the latter one.

It was found that all dispersants limited the growth, the lower concentration being at most indifferent. Limiting effect of the dispersant was manifested by prolongation of the phase of growth stagnation compared to the control culture, less sloping curve in the phase of exponential growth, and lowering of the maximal cell numbers in the culture. At different concentration of the dispersant, their limiting effect was more pronounced in *Chlorella*, pointing to higher susceptibility of this species compared to *Scenedesmus*. In case of the latter species, lower dispersant concentrations resulted in the growth pattern similar to the control one.

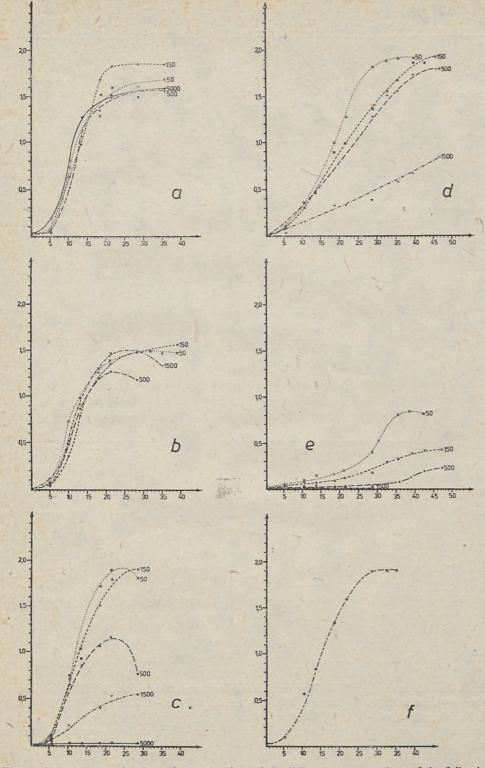


Fig. 1. Growth curves for Scenedesmus quadricauda in different concentrations of the following dispersants:  $a - \text{Corexi}_{1}$  7664, b - Corexit 9527, c - Corexit 9600, d - OSD/LT, e - BP 1100 WD, f-the control culture

Concentrations are given in ppm, at the curves. Abscissa-days, ordinate-number of cells in million  $ml^{-1}$ 

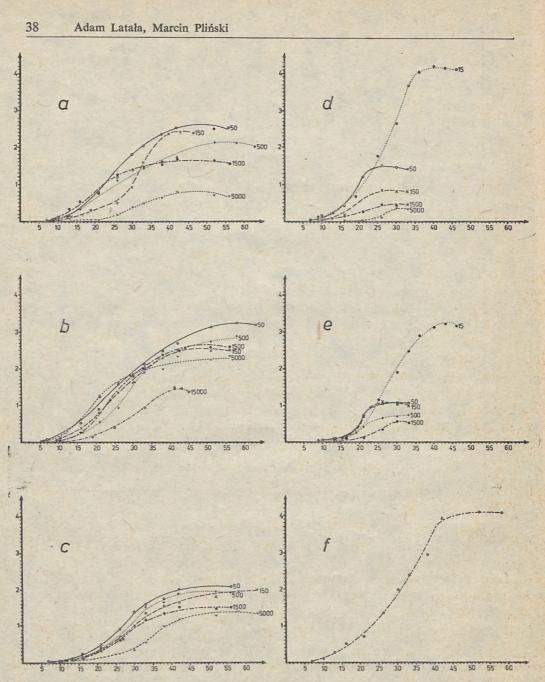


Fig. 2. Growth curves for *Chlorella vulgaris* in different concentrations of the following dispersants: a-Corexit 9527, b-Corexit 7664, c-Corexit 9600, d-OSD/LT, e-BP 1100 WD, f-the control culture

Concentrations are given in ppm, at the curves. Abscissa-days, ordinate-number of cells in million  $\cdot$  ml<sup>-1</sup>

Tests with Corexit 7664 revealed that character of the growth curves was similar both for *Scenedesmus* (Fig. 1a) and *Chlorella* (Fig. 2b) in a broad range of concentrations, from 50 to 5,000 ppm. Only very high concentration of this dispersant (15,000 ppm) significantly limited the growth of *Chlorella* (Fig. 2b). Corexit 9527 affected the growth of algae similarly as Corexit 7664, but maximal cell numbers in particular concentrations of this dispersant were lower (Fig. 1b, 2a). It was also observed that lower concentrations of Corexit 9527 compared to Corexit 7664 (5000 ppm) resulted in stronger limitation of Chlorella growth (Fig. 2a). From among the three dispersants of Corexit type, Corexit 9600 resulted in the strongest limitation of algae growth. Response of Chlorella was similar for all the concentrations used (Fig. 2c), whereas considerable differences were noted in case of Scenedesmus (Fig. 1c). In the latter case, character of the growth curves for the concentrations of 50 and 150 ppm was similar as in the control, *ie* there was no significant limitation of the growth. Gradually increasing effect was observed only in concentrations of from 500 ppm, while at 5,000 ppm the growth was almost totally inhibited (Fig. 1c). In case of OSD/ /LT relationship between the dispersant concentration and limitation of the growth was more noticeable than in case of Corexits, the response being stronger in case of Chlorella (Fig. 1d). The strongest effect on growth of the algae under study was observed in case of BP 1100 WD, noticeable already in low concentrations of the dispersant (Figs. 1e and 2e). Considerable prolongation of the phase of growth stagnation was a most characteristic feature of this effect.

The tests made it possible to determine the dispersant concentrations resulting in significant limitation of the growth of planktonic algae. And thus, for Corexit 7664 this concentration amounted to 15,000 ppm, for Corexit 9527-to 5000 ppm, for Corexit 9600-to 500 ppm, and for OSD/LT and BP 1100 WD-to 50 ppm.

## Discussion

The results of tests on the limiting effect of selected dispersants on the growth of planktonic algae are of cognitive value only, as they were obtained in laboratory studies and cannot be directly related to natural environment. Nevertheless, they can be used for comparative determination of the effect of particular dispersants, provided that the external conditions of the experiments are similar, as was the case in the present study. The results allowed for a general assessment of the effect of dispersants on planktonic organisms, reflected in this case by changes in the growth rate. It seems that such an approach is quite sufficient, the more so that mechanisms of the effect of dispersants on living organisms are not clear as yet, the opinions on this problem being fairly different (Wilson 1977). They usually refer to animal organisms; the most frequently mentioned hypothesis is that the dispersants inhibit enzymatic activity of the organism. This hypothesis seems probable also for plants. As noted by Boney (1970) during the tests with a green algae Prasinocladus marinus, the dispersant BP 1002 inhibited cell division, limited chloroplast formation, and deformed external cell structure. It was also found that the dispersants of Corexit type limited photosynthetic production of oxygen and increased anaerobic respiration of a benthonic species Posidonia australis (Hatcher, Larkum 1982), as also increased mortality of the zygotes and new growths in Fucus serratus (Thélin 1981). These responses suggest inhibition of some biological processes. With respect to the unicellular planktonic plants, inhibition of growth is equally important and this effect was observed in our studies.

It is difficult to compare directly our results with those by other authors, due to the lack of similar studies. Data presented in the literature suggest differentiated effect of the dispersants on living organisms, depending to a large extent of the dispersant type. Relatively low response is observed in case of the dispersants from Corexit group. Many studies showed that the lowest negative effect on various biological objects was observed in case of Corexit 7664 (Nagell *et al* 1974, Wilson 1976, 1977, Ozelsel 1981). Our studies confirmed these observations, also in case of the planktonic green algae under study.

Literature data on the negative effect of the dispersants point to considerable differences in the observed responses, depending on the group of organisms under study (Nagell *et al* 1974). Consequently, our results should be related to biologic specificity of the tested species and to definite conditions of the brackish water environment.

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