

## Effect of Oil and Dispersant on Growth and Chlorophyll *a* Content of the Marine Microalga *Tetraselmis suecica*

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Low hydrocarbon concentrations stimulated the growth of *Tetraselmis suecica*, whereas higher concentrations (200 ppm) inhibited growth. The content of chlorophyll *a* in this microalga was affected in a similar way. Crude oil had the most marked effects. Dispersant SEAKLIN-101-NT and mixtures of oil and SEAKLIN-101-NT did not show selective toxicity for the microalga, although inhibitory effects could be observed at high concentrations.

The accumulation of hydrocarbons in seas and oceans has become a source of considerable environmental concern, especially in recent decades. In the last century, human activities have resulted in substantial additional hydrocarbon inputs to the oceans (17). The growing demand for oil has been accompanied by an increase in oil pollution due to spillage in both freshwater and marine ecosystems. Spills occur at a rate of about 10,000 incidents per year (1). When an oil spill occurs in the sea, the oil spreads on the water surface and drifts by wind and currents, the low-boiling fraction evaporates, and the low-boiling aromatic fraction dissolves easily in the water (7).

The damage caused by an oil spill depends on a number of factors: quantity and type of oil, oceanographic and meteorological conditions, ecosystem, methods used in cleaning operations, etc. (2). In addition, damage to the marine environment is caused by dispersants (type and concentration), exposure time, temperature, stage, and life cycle. Crude oils vary greatly in their composition and toxicity. The more volatile constituents of crude oil, such as the low-boiling aromatic compounds, are the most toxic to plant life (16). The toxicity is influenced by viscosity and surface tension (2).

The effect of crude oil and other contaminants on marine microalgae can be assayed by growth measurements (1, 19) or metabolic or photosynthetic activities (6, 12, 18). We report herein on the effects of crude oil, of a water-soluble fraction of crude oil, of SEAKLIN-101-NT (a nonionic dispersant), and of oil dispersed with SEAKLIN-101-NT on the growth and chlorophyll *a* content of microalgae.

The microalga used was *Tetraselmis suecica*, isolated from Ria de Arosa waters (J. Fabregas, Ph.D. thesis, University of Santiago, Spain, 1982) and cultured in seawater filtered through a 0.5- $\mu$ m filter (Millipore Corp.), autoclaved at 120°C for 20 min, and enriched with: NaNO<sub>3</sub>, 2 mM; NaH<sub>2</sub>PO<sub>4</sub>, 100  $\mu$ M; ZnCl<sub>2</sub>, 1  $\mu$ M; MnCl<sub>2</sub>, 1  $\mu$ M; Na<sub>2</sub>MoO<sub>4</sub>, 1  $\mu$ M; CoCl<sub>3</sub>, 0.1  $\mu$ M; CuSO<sub>4</sub>, 0.1  $\mu$ M; ferric citrate, 20  $\mu$ M; thiamine, 35  $\mu$ g; biotin, 5  $\mu$ g; vitamin B<sub>12</sub>, 3  $\mu$ g; Tris, 15 mM; and EDTA, 26.4  $\mu$ M.

Crude oil and dispersant SEAKLIN-101-NT from the *Urquiola* spillage were used. The wreck of the *Urquiola* on the Spanish coast in April 1983 caused massive oil pollution. Weathered crude oil was obtained 5 days after the *Urquiola* spillage, so most of the volatile compounds had disappeared.

Concentrations of 1, 2, 4, 8, 12, 20, 40, 80, 100, 200, and 400 ppm of crude oil and 1, 2, 4, 8, 12, 20, 40, 80, 100, 200, 400, and 800 ppm of SEAKLIN-101-NT were used. Water-soluble fractions were prepared by placing weathered oil in a bottle with filtered and autoclaved (120°C for 20 min) seawater (1:1, vol/vol). The bottles were stirred slowly at 4°C with a magnetic stirrer for 48 h. This solution was filtered through a 0.45- $\mu$ m Millipore filter and used to prepare concentrations of 100, 200, 400, 800, 1,000, 1,100, 1,200, 1,600, 2,200, and 4,800 ppm. SEAKLIN-101-NT and weathered oil (1:1, vol/vol) were thoroughly mixed in sterile distilled water (1 volume). This solution was diluted 100-fold and used to prepare concentrations of 1, 2, 4, 8, 12, 20, 40, 80, 100, 200, and 400 ppm of oil dispersed with SEAKLIN-101-NT.

An inoculum of 10<sup>4</sup> logarithmic-phase cells per ml was used. All cultures were maintained in a controlled-environment incubator (New Brunswick Scientific Co., Inc.) at 15°C and 3,900 lx from fluorescent lamps (Phillips model TL 20W/55). A 12-h light-12-h dark period was maintained. Optical density of the cultures was determined by using a Coleman model II 6/20 spectrophotometer reading at 530 nm. Chlorophylls were extracted in acetone-methanol (2:1) at 4°C for 48 h from cells in the stationary phase (11 days of culturing). The extracts were filtered through a Fluoropore filter for clarification, and absorbances of the pigment extract at specific wavelengths were recorded. The concentration of chlorophyll *a* was determined by the following formula (14): chlorophyll *a* (mg/liter) = 11.64  $D_{663}$  - 2.16  $D_{645}$  - 0.1  $D_{630}(U/V)$ , where  $D_{630}$ ,  $D_{645}$ , and  $D_{663}$  are the absorbances at 630, 645, and 663 nm, respectively, read in a 1.0-cm cell,  $V$  is the sample volume, and  $U$  is the final methanol volume.

Results showed that low crude oil concentrations stimulated growth. *T. suecica* was affected at 200 ppm (a nonparametric Kruskal-Wallis test indicates significant differences with  $H = 32.6$ ; the multiple comparisons test indicates significant differences with  $P \leq 0.05$ ) (Fig. 1). There was also an increase in the chlorophyll *a* content of the cells up to 8 ppm; this content decreased quickly at 200 ppm (Fig. 2). The water-soluble fraction had no apparent effect on the growth and chlorophyll *a* content of *T. suecica*. Thus, the water-soluble fraction of the crude oil cannot account for much of the toxicity of crude oil. The presence of SEAKLIN-101-NT resulted in an increase in growth and chlorophyll *a* content of *T. suecica* at concentrations up to 8 ppm. Higher concentrations resulted in inhibitory effects on growth and

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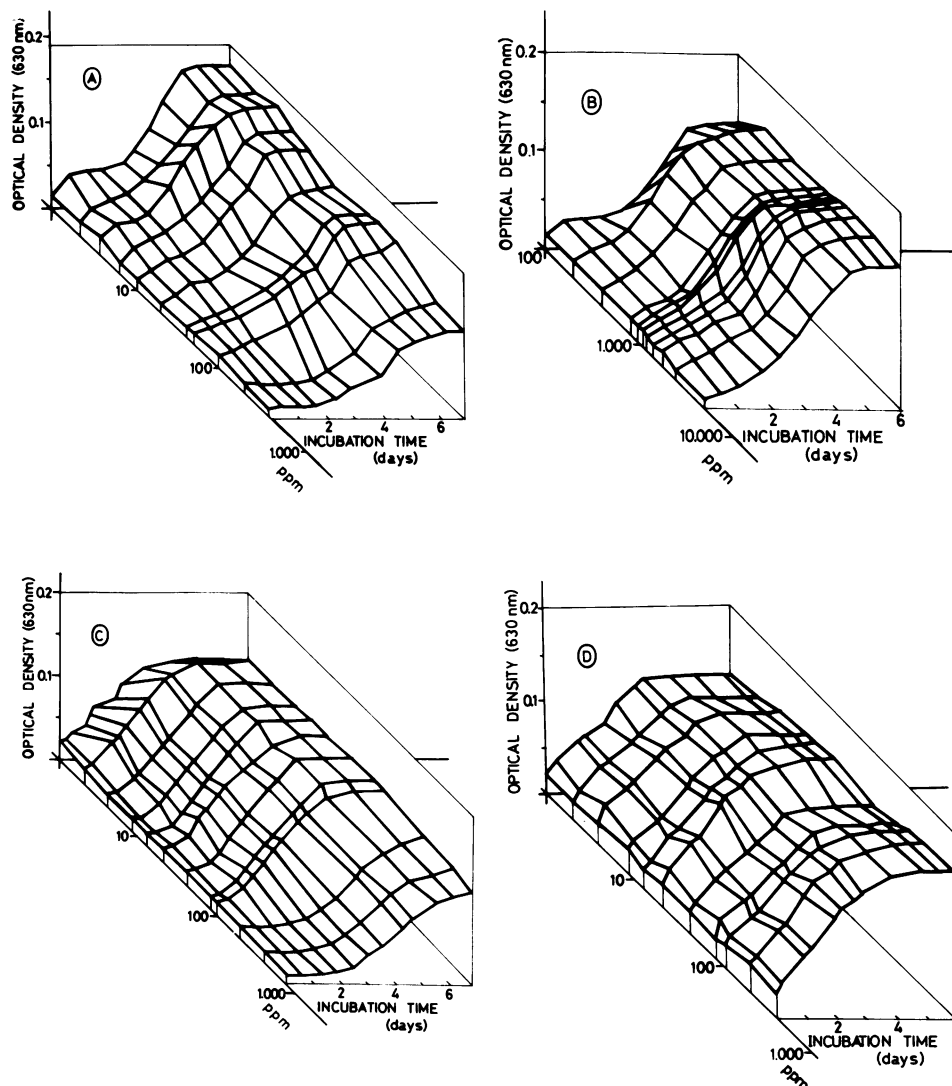


FIG. 1. Three-dimensional representation of *T. suecica* growth. (A) Effects of different concentrations of crude oil. (B) Effects of different concentrations of water-soluble fraction of crude oil. (C) Effects of dispersant SEAKLIN-101-NT. (D) Effects of oil dispersed with SEAKLIN-101-NT.

depressed the chlorophyll *a* content. At 200 ppm of SEAKLIN-101-NT, a longer extension of the lag phase of *T. suecica* occurred, and the differences were significant (non-parametric Kruskal-Wallis test,  $H = 9.88$ ; multiple comparisons test,  $P = 0.1$ ). Crude oil dispersed with SEAKLIN-101-NT stimulated growth at concentrations up to 20 ppm, and higher concentrations inhibited growth. Chlorophyll *a* content was not affected by this fraction. In this case, differences were not significant.

Stimulation of growth and photosynthesis by microalgae exposed to low concentrations of hydrocarbons has been noted (1, 13). Our results are in general agreement with the work of these authors. Growth stimulation or inhibition depends on oil concentration. Nevertheless, it must be taken into account that some crude oils have been shown to be nontoxic to algae (3), whereas others are toxic (11) to various degrees depending on the species studied and experimental conditions. Crude oil at up to 40 ppm had no toxic effects, although cell density in the postexponential phase was lower in the test than in the control culture, but not at significant

levels. Toxicity was increased with higher concentrations, and longer extension of the lag phase and lower cellular density in the stationary phase occurred. Microscopic examination of *T. suecica* cells indicated abnormal cellular morphology. However, oil toxicity level for microalgae may not be a reliable indication of what may happen in the natural environment. When an oil spill occurs in the sea, there are losses by evaporation and by dissolution, so that the toxic effect of the volatile constituents of crude oil decreases with respect to laboratory assays. We carried out our experiment in screw-capped test tubes, so cells were exposed to constant concentrations of oil throughout culturing; this exposure did not occur in the sea.

The water-soluble fraction did not seem to be toxic at any concentration assayed. Furthermore, a slight growth stimulation appeared in some cases. Several authors (3, 4, 10, 18) have observed in laboratory studies that oil extracts stimulate algal growth and photosynthesis after the evaporation of toxic substances. Our results support these findings.

It has been shown that the majority of the dispersants

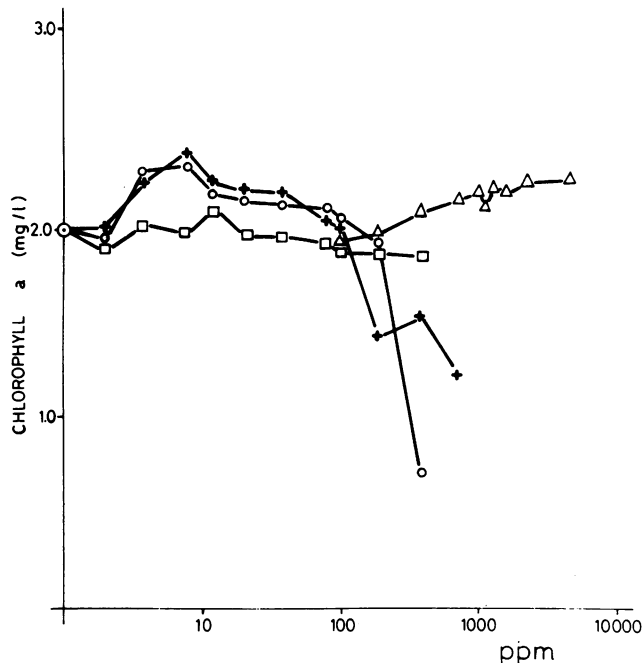


FIG. 2. Chlorophyll *a* content in stationary-phase cells of *T. suecica*. Symbols: ○, crude oil; △, water-soluble fraction; +, dispersant SEAKLIN-101-NT; □, oil dispersed with SEAKLIN-101-NT.

which have been tested with both systems, microalgae and animals, are by and large more toxic to microalgae than to animals (8, 9). We carried out our investigations by testing concentrations of dispersant and mixtures of oil and dispersant. Similar effects were noted with both. Low concentrations stimulated growth, whereas higher concentrations inhibited it. Chlorophyll *a* content was practically unaffected by the mixture, whereas it was enhanced or depressed by the dispersant depending on concentration.

None of the fractions assayed resulted in a high toxicity on the prasinophycete *T. suecica*. Crude oil has the most marked effects on the growth and chlorophyll *a* content of this marine microalga. The fraction of oil dispersed with SEAKLIN-101-NT did not demonstrate selective toxicity for this microalga.

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