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Effects of the Water-Soluble Fraction of Cook Inlet Crude Oil on the Marine Alga, *Dunalliela tertiolecta*

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Among the commonest and most obvious pollutants of marine and estuarine waters are oil and oil byproducts. While there have been numerous investigations on the effects of the water-soluble components of crude oils on fish, crustaceans, molluscs, and other aquatic organisms, few studies have examined the influence of these materials on phytoplankton which are among the most basic and essential components of aquatic food chains. Effects of oil pollution on phytoplankton communities are of primary concern since the existence of all marine fauna ultimately depends on the photosynthetic capacity of phytoplankton populations.

Previous oil bioassays on phytoplankton report the combined effects of oil and oil dispersing agents. This study attempts to determine the effects of the water-soluble components of Cook Inlet crude oil on the marine alga, *Dunalliela tertiolecta*, without the addition of emulsifying agents.

MATERIALS AND METHODS

The marine alga, *D. tertiolecta*, is a ubiquitous, green flagellate which grows extremely well in the laboratory. Tests were conducted in seawater enriched with the Loosanoff and Davis Universal medium (LOOSANOFF and DAVIS 1963). Sterile cultures were set up in replicates for each growth condition in control and WSF media. Innoculates of known equal cell concentrations were always taken from exponentially growing stock cultures and pipetted under sterile conditions into sterilized 500-ml Erlenmeyer flasks with cotton plugs which simulated "open" systems by allowing the more volatile compounds to evaporate and escape the flasks. The algae were dosed with five concentrations of the WSF of Cook Inlet crude oil and incubated for 72 hr under the original growth conditions at 30 ppt salinity, 18^{o}C on a 12-hr light/12-hr dark regime of a known light intensity. The culture flasks were manually shaken several times daily. Subsamples were removed using axenic techniques to determine cell counts with a Coulter Counter Model ZB1.

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The water-soluble fraction of crude oil was obtained by stripping a 4 cm layer of crude oil with seawater in a modified 2.5-gal bottle (Fig. 1). Seawater flows at 1 l/min through a stainless steel diffuser plate which converts a large stream of seawater into many small streams. These streams of seawater flow through the oil layer into the lower seawater layer. Crude oil was pumped through the system at 3 ml/min by a fluid metering pump (Model RPIG50/CSC). Higher concentrations were achieved by continually recirculating the seawater in the bottle using a submersible pump (March Model 1A-MD-1) and collecting the effluent after 24 hr (Fig. 2).

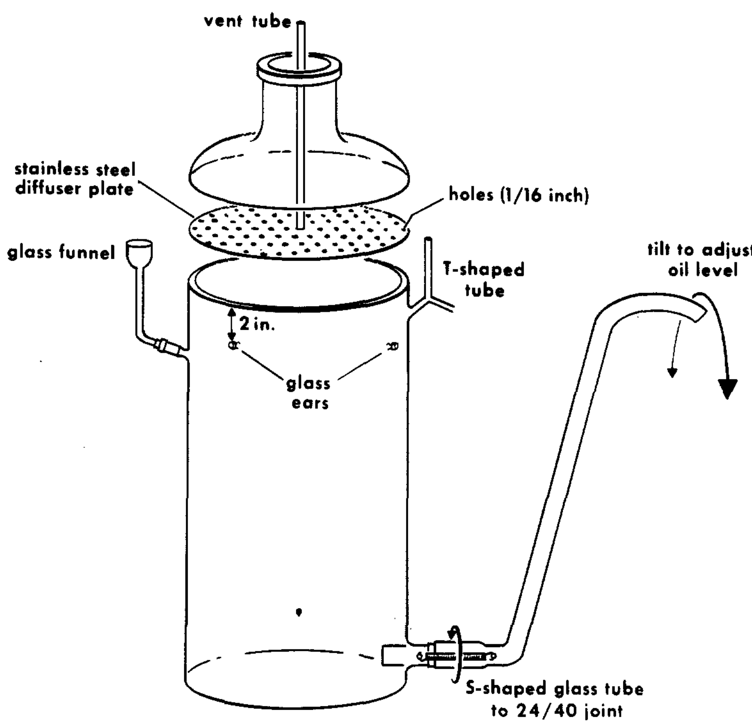

Fig. 1. Solubilizer: A Modified 9.5 l Pyrex Bottle for Dissolving Petroleum Hydrocarbons from Crude Oil into Seawater.

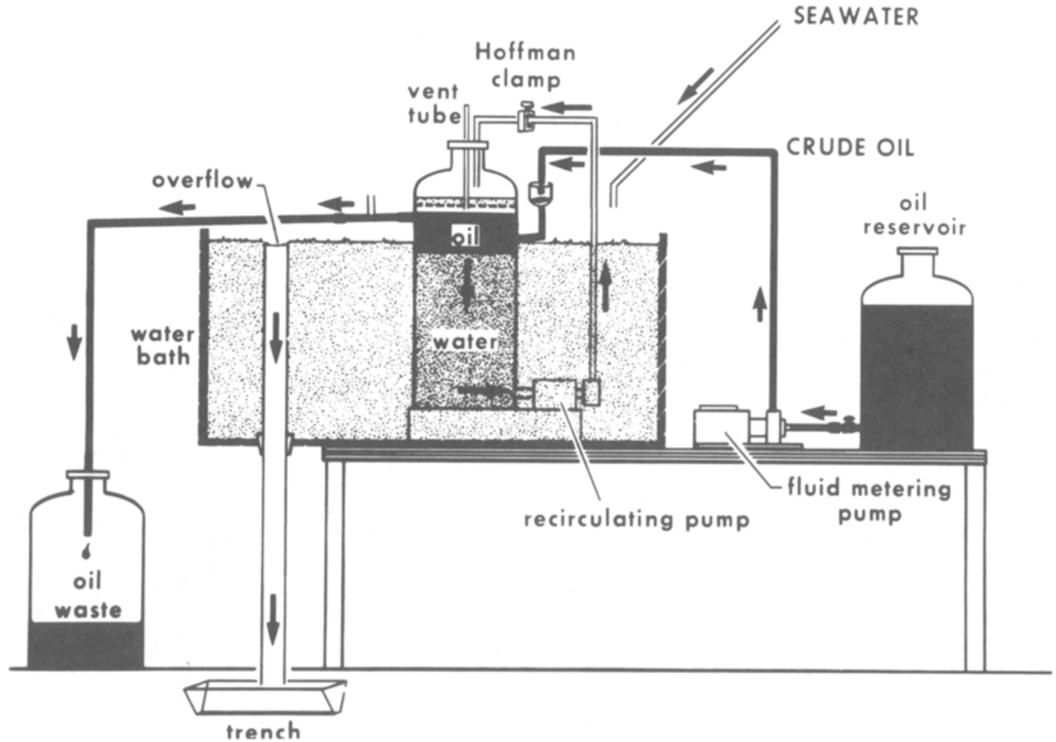

Fig. 2. A Recirculating System Using a Solubilizer.

Concentrations of the water-soluble fraction from the solubilizing apparatus were extracted with 10 ml of TF Freon (trichlorotrifluoroethane) and 4 ml of 6N HCl. A 3.6 μl aliquot of the extract was injected into a Micro-Tek 220 Gas Chromatograph equipped with a flame ionization detector. The separation of the six monocyclic aromatics was performed on a 1.8 m x 4 mm ID column of 5% Bentone 34/5% SP-1200 on Supelcoport. Flow rates of air, helium, and hydrogen were 1.2 SCFH, 40 cc/min, and 60 cc/min, respectively. A 3380A Hewlett-Packard Integrator was used to quantitate the chromatographic curves. Known concentrations of the water-soluble fraction were then diluted with an appropriate volume of sterilized seawater with enriched media to make up the test media.

RESULTS

The effects of the water-soluble fraction of Cook Inlet crude oil on *Dunalliela* sp. can be seen in Table 1. The results of this study indicate the low concentrations of the water-soluble fraction of crude oil has a stimulatory effect on the growth rate of this marine alga. Growth of *Dunalliela* expressed as a percentage of cells in control groups.

DISCUSSION

Crude oil is composed of many hydrocarbons with the low-boiling point aromatics considered to be potentially the most immediately toxic to aquatic life (BLUMER 1969, HOLCOMB 1969). Evidence by STRAUGHAN (1972) indicates that the more highly refined oils are more toxic to the biota than heavier oils perhaps due to their greater percentage of aromatic hydrocarbons which are known to be toxic to a wide variety of marine organisms. Mindful that the aromatic content of an oil is usually indicative of its toxicity, the six monocyclic aromatics, benzene, toluene, ethylbenzene, *p*-xylene, *o*-xylene, and *m*-xylene, were selected for study because of their occurrence in aqueous extracts of crude oils and oil fractions (BOYLAN and TRIPP 1971).

The stimulatory effects of low concentrations of the water-soluble fraction of crude oil on the growth rate of *D. tertiolecta* observed in this study (Table 1) suggest that this species of marine algae is able to assimilate and utilize certain non-toxic water-soluble components of crude oil as a carbon source. These non-toxic fractions may have been initially present in the test medium or could have been breakdown products as a result of autoxidation or microbial degradation of oil compounds. (Although axenic techniques were

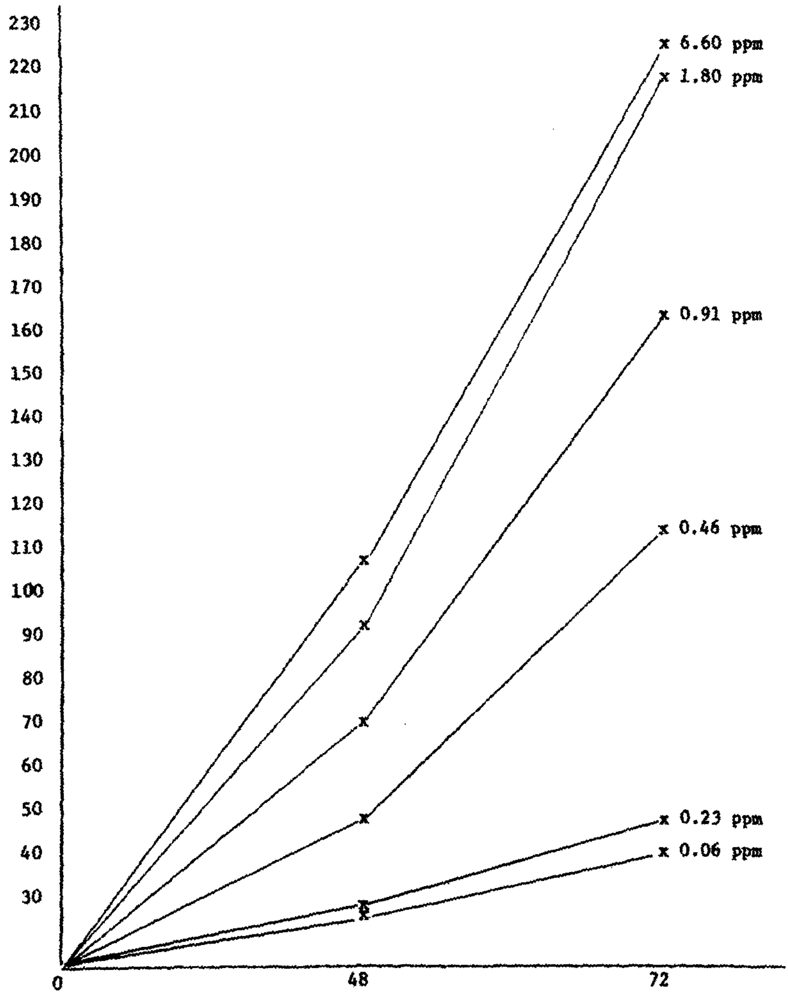

Table 1. Effects of Different Concentrations of the Water-Soluble Components on the Growth Rates of the Marine Alga, *Dunalliela tertiolecta*.

used throughout the period of study, bacterial contamination may have occurred during sampling for cell determinations.) Similar stimulatory effects of the water-soluble components of oil on the fresh water algae, *Chlorella vulgaris*, *Anabaena flosaquae* and *Ankistrodesmus convultus* and to a lesser degree on *Tabellaria fenestrata* have been reported by KAUSS *et al.* (1973).

Due to the simplified nature of algal bioassays, care must be exercised in extrapolating the results of laboratory experimentation to field conditions. The fact that Dunalliela was not adversely affected by the oil concentrations in this study should not be used to draw the general conclusion that oil pollution in marine waters causes no ecological or physiological damage. Furthermore, the observed favorable response of Dunalliela to the water-soluble fraction of Cook Inlet crude oil is not likely to be representative of all marine algae. Instead, Dunalliela may well be an oil-tolerant species. However, these results suggest that the consequences of oil pollution may not be as adverse on certain organisms under certain conditions as it has been widely speculated.

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