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ABSTRACT

This paper embodies the preliminary results of experimental studies with different hydrocarbons such as crude oil, furnace oil, high speed diesel oil, benzene, hexane and xylene about their toxicity on phytoplankters. The studies with these hydrocarbons on natural populations and selected species of phytoplankton cultures showed that there is sometimes enhancement of photosynthesis with lesser concentrations of aqueous extract. The rate of production varies with different hydrocarbons depending on the species composition and it is found that the more toxic portions are the volatile fractions with low boiling point and the response is a function of species composition.

INTRODUCTION

In recent years, there has been an increasing awareness to the problem or uncontrolled discharge of oil into both fresh water and marine environments. Disastrous accidents such as the wreck of the "Torrey Canyon" off Cornwall in Britain and the blow out of an oil well off Santa Barbara, California, have sparked off an interest in oil pollution. Oil spills from accidental losses in production, utilisation and transportation have been steadily increasing.

The world petroleum production has increased steadily from 278 million tonnes in 1938 to about 2500 million tonnes. The contribution made by Middle East Oil Fields has grown from 4% in 1937 to 30%. As a result of increasing demand for oil, the number and size of oil tankers have greatly increased and discharge of oil intentionally or due to accidents has been on the increase.

It has been estimated that annually 5 to 10 million tonnes of oil reaches the marine environment (Blumer, 1970). Once the oil reaches the sea certain damages take place irrespective of any counter measures. At first the oil spreads rapidly forming a thin homogeneous slick. The volatile fractions evaporate within 2 to 3 days. The residue often forms viscous compounds in which the rates of spreading and evaporation decrease. Dependiag on the type of oil and roughness of the sea, some emulsification also takes place. Subsequent changes depend on the properties of the oil converted into water-in-oil or oil-in-water suspension. The highest fraction of the n-paraffin in the oil are those having the greatest solubility. Together with the aromatic compounds they are regarded as the most harmful.

Though several authors have worked on the ecological and biological effects of oil pollution (Mironov, 1968; 1969 and 1970; Blumer, 1969; Carthy and Arthur, 1968; Mitchel *et. al.*, 1970), almost all of them deal with the zoological aspects. Recently Hutchinsen *et. al.*, (1972) have shown the effect of crude oil on selected components of freshwater phytoplankton both in a field situation and in the laboratory. Menzel *et. al.*, (1970) have observed that photosynthesis and growth in cultures of marine phytoplankters were affected by chlorinated hydrocarbons to varying extent ranging from complete insensitivity to toxicity. The purpose of this study was to determine the effect of various hydrocarbons on the rate of photosynthesis measured by ^{14}C uptake on different populations of phytoplankton. The experiments were designed to measure the immediate response of the pollutant on the natural plankton and cultures. Long-term effects of the pollutant were not studied.

METHODOLOGY

In vitro experiments were conducted using aqueous extracts of 6 hydrocarbons - crude oil, diesel oil, furnace oil, benzene, hexane and xylene. 25/10 ml of the oil was shaken with 500 ml of the medium for 12 to 24 hours. The aqueous extracts were separated and diluted with the medium to various concentrations. After incubation of the natural plankton (predominantly with *Coscinodiscus* spp.) or culture (*Tetraselmis gracilis*) with 5 μc of $NaH^{14}CO_3$, the samples were filtered on Millipore HA filters and the activity (cps) was plotted against the percentage concentration (Fig. 1).

RESULTS AND DISCUSSION

As may be seen from Fig. 1, there is a general reduction in the rate of photosynthesis with all the hydrocarbons except crude oil. Crude oil extract at low concentrations seems to enhance the rate of photosynthesis. It has been observed by Hutchinsen *et al.*, (1972) that certain species are inhibited in their growth while certain species do not appear to be affected and a number of species are stimulated. Differences in response therefore are the effect of the actual planktonic composition of the water body. It is

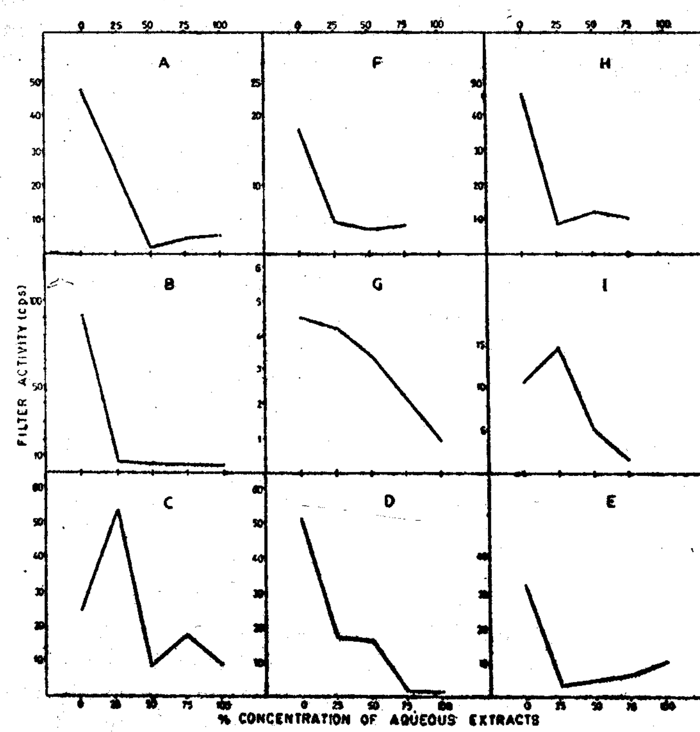

Fig. 1. A-I. Rate of ^{14}C uptake as a function of hydrocarbon concentration. A, Hexane; B, Xylene; C, Crude oil; D, Benzene; E, Diesel oil; F, Furnace oil residue; G, Furnace oil. All these are treated with natural population of phytoplankton. H and I, Furnace oil residue and oil treated with culture of phytoplankton.

probable that the flagellates are slightly stimulated by oil spills since *Tetraselmis gracilis*, a flagellate in culture showed slightly increased activity with furnace oil obtained during the grounding of the oil tanker "Trans-Huron" at Kiltan Island (Fig. 1 H and I). In higher concentrations of crude oil and furnace oil there is a significant deterioration in photosynthetic activity. The water soluble components of the crude oil generally reduce the pH (even in 1% solution by 0.7). The increase in the acidity of the water by itself could reduce algal growth (Hutchinsen *et al.*, loc. cit).

Experimental work conducted both in the field and in the laboratories elsewhere suggests that the low boiling aromatics have the greater toxicity (Kuhnhold, 1969; Griffith, 1972). Besides, crude and furnace oil, experiments were conducted with benzene, hexane and xylene. Of these, hexane and xylene showed greatest inhibition at lower concentrations only. The observations of Hutchinsen *et al.*, (1972) also showed that benzene has relatively little effect up to concentration of 500 ppm but the higher levels are inhibitory. Xylene showed the greatest phyto-toxicity of all.

The long term effect of an oil spill has not been studied. However, experiments with the residue of the furnace oil spill at Kiltan island showed decreased rate of photosynthesis both with culture and natural plankton. As the volatile fractions have already evaporated, the toxicity is not so apparent. As mentioned before the different phytoplankters respond differently with different hydrocarbons, Diatoms such as *Ditylum brightwelli* and *Coscinodiscus granii* are killed in concentration of kerosene less than 0.01% while *Melosira moniliformis* and *Grammatophora marina* tolerate up to 1%. Very low concentrations (0.1 μl/1) affect more sensitive species. Mirnov and Lanskaya (1967), Mirnov (1970) and Lacaze (1967) reported a depresion in the growth of cultures of *Phaeodactylum tricornutum* exposed to 1% extract of curde oil. But for *Nitzschia closterium* retardation was observed only when the oil concentration exceeds 25% while lower levels have a slight stimulating effect.

Even higher algae such as *Macrocystis pylifera* exhibit decreased rate of photosynthesis in presence of refinery waste and untreated oil brine (North *et al.*, 1972). Oil spillages may affect algae less severally than animals but laboratory studies indicate that hydrocarbons can affect both attached algae and phytoplankton depending upon the composition, degree of refinement, origin of materials and nature of additives. Toxic thresholds of organic compounds tend to be higher than the inorganic compounds and the nature and degree of response of the pollutionary effects is primarily a function of species composition. The results presented here may be taken typically for a natural population of *Coscinodiscus* spp. and *Tetraselmis gracilis* in culture.

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REFERENCES

Blumer, M. 1969 Oil pollution of the Oceans. *Oceanus* 15 (2), 2-7.

Blumer, M. 1970 Colloquim on the pollution of the sea by oil spills. Brussels.

Carthy, J. D. and Arthur, D. R. (Eds.) 1968 The biological effects of oil pollution on littoral communities. *Field Studies Council* (Brit.), 2 (suppl.), 99.

Griffith, D. de G. 1972 Toxicity of Crude oil and detergents to two species of edible molluscs under artificial tidal conditions. *Marine Pollution and Sea Life*, FAO, 224–229.

Hutchinsen, T. C., Kauss, P. and Marta Griffiths 1972 The phyto-toxicity of crude oil spills in freshwater. *Water Pollution Res. in Canada*, 7, 52-58.

Kuhnhold, W. W. 1969 Effect of water soluble substances of crude oil on eggs and larvae of cod and herring. I.C.E.S. CM 1969/E: 18 (mimeo).

Lacaze, J. C. 1967 E'tude de la croissance d'une algue planctonique en presence d'un detergent utilise pour la destruction des nappes de petrole en mer. *C. r. hebd. Seances. Acad. Sci. Paris*, 265 (Ser. D), 489.

Menzel, D.W., Anderson, J. and Ann Randtke 1970 Marine phytoplankton vary in their response to chlorinated hydrocarbons. *Science, 167* (3926), 1724-1726.

Mirnov, O. G. 1968 Hydrocarbon pollution of the sea and its influence on marine organisms. *Helgolander Wiss. Meeresunters, 17* (1-4), 335-339.

Mirnov, O. G. 1969 Development of some Black sea fishes in oil polluted sea water. *Vop. Ikhtiol.*, *9* (6), 1136-1139. (in Russian).

Mirnov, O. G. 1970 Biological aspects of the pollution of the seas with oil and oil productions. Synopsis of the thesis, Moscow State University, 26 p. (in Russian).

Mirnov, O. G. and Lanskaya, L. A. 1967 Razvitie nekotorykh diatomovykh vodoroslei v morskoi vode, Zagriaznennoi refteprodu-ktami. Biologiia i raspredelenii planktona iuzhnykh morei. Kiev.

Mitchell, C. T., Anderson, E. K., Jones, L. G. and North, W. J. 1970 *J. Water Pollution Control Fed.*, 42 (5, pt. 1), 812-818.

North, W. J., Stephens, G. C. and North, B. B. 1972 Marine algae and their relation to pollution problems. *Marine Pollution and Sea Life*, FAO, 330-340.