

Effects of petroleum hydrocarbons on the growth of a microalga, *Isochrysis* sp. (Chrysophyta)

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Effects of water soluble fraction of Bombay High crude oil and heavy duty marine diesel on the growth of a microalga were examined and compared. Most concentrations of the oil depressed the growth rate in *Isochrysis* sp. Marine diesel prevented the growth of the alga in a concentration above 10%, while crude oil at a similar concentration had little effect on the growth. Hydrocarbon would cause environmental damage through selective effects on natural biota in the marine environment.

There is a growing concern about the widespread occurrence of oil pollution in the sea. There is considerable evidence that petroleum and its products become toxic in varying degrees to different organisms of the marine food chain^{1,4}. However, a direct comparison due of these results becomes difficult to differences in experimental conditions, complex behaviour of oil-water mixtures and the source of the petroleum hydrocarbon¹.

The composition of the water soluble fraction (WSF) of oil is less complex than the whole crude oil. The WSF is the remaining portion of oil in the water column after weathering and mechanical processes are over. The acute toxicity of petroleum hydrocarbon to marine phytoplankton is generally assessed by comparing growth rates of single species cultures and natural phytoplankton assemblage with uncontaminated controls. The objective of the present study was to assess the relative toxicity of the WSF of Bombay High crude oil and heavy duty marine diesel oil on a microalga, *Isochrysis* sp. cultured under laboratory conditions.

Materials and Methods

The microalga used was *Isochrysis* sp. (Chrysophyta). Pure isolates were obtained from the Marine Corrosion and Materials Research Division of the National Institute of Oceanography. The microalga was maintained in batch cultures in an autoclaved enriched medium

containing filtered seawater⁵. Erlenmeyer flasks of 250 ml capacity were used with a culture medium of 150 ml.

Cultures of the microalgae were added (1 ml in each flask) plugged with sterile cotton and kept in a fluorescent light chamber under artificial light (384 lux) on a 14h : 10 h L: D cycle. All cultures were allowed to grow at room temperature. Growth of the cultures was maintained by transferring 1 ml of the grown culture into the fresh medium. Cells from the freshly inoculated medium were used for the experiments in order to adapt cells to the medium and other laboratory conditions. Extreme care was taken throughout the process to prevent any bacterial or fungal contaminations and the cultures were maintained axenically.

The water soluble fractions (WSF) of test oils were prepared by placing one part of the oil over nine part of filtered autoclaved seawater¹ (1:9, v/v). The flasks were capped with aluminium foil to minimise evaporation during the mixing process. Mixing was done at room temperature on a magnetic stirrer continuously for 20 h at a speed of 250 rpm. After mixing the solution was allowed to stand undisturbed for 8 hours in a separating funnel. The aqueous phase was then drained out. The solution thus obtained was designated as 100%. Fresh WSF solutions were prepared and used for all toxicological studies.

The test solutions were inoculated with cultures of cell concentrations 1×10^6 cell/ml thus giving a

final concentration of 6×10^3 cell/ml. Experiments were set up in 200 ml Erlenmeyer flasks with a culture volume of 150 ml. To acquire different toxicant concentrations WSFs were added to the flasks and the flasks were plugged with sterile cotton. Duplicate cultures for all the test concentrations and a control were maintained for each set of experiment. Growth was assessed on alternate days by counting the cells in a Sedgewick rafter under a microscope. The possible concentrations of different WSFs for growth studies were selected by running trial experiments at varied range of concentrations.

For estimation of petroleum hydrocarbon in WSFs, 150 ml of WSF was extracted with 25 ml double distilled hexane. The hexane fraction was separated using a separating funnel and collected in stoppered test tube. Hexane extracts were then dried using anhydrous sodium sulphate which was cleaned earlier with hexane and oven dried. The final volume of the hexane extract was adjusted to 25 ml and used for spectrofluorometric analysis⁶. Concentration of hydrocarbon was measured at 360nm. Growth rate (K) was calculated as: $K = \ln(N/N_0)/T$, where K=growth rate, N=microalgal population at time T, N_0 =Initial microalgal population at time zero and T=time interval from time zero. Generation time (G) was calculated as $G=T/K$, where K=growth rate, T=time interval from time zero. Percentage inhibition of growth for test cultures was also calculated taking growth (number of cells in control) as 100%.

Results

Effect of crude oil

Growth of *Isochrysis sp.* was affected only at higher concentration (10-50%) (Fig. 1.) At 10% and 20% concentration a slow but steady increase in cell number was observed which, was comparatively slower than the growth in the control. At higher concentrations (30, 40, 50%), fall in initial cell density was observed, suggesting the negative effect of the oil on the microalga. After this initial slack, a recovery was observed with an increase in cell count in each culture.

Toxicity of WSF was further studied in relation to growth rate (K) parameter. The daily variation in growth rate clearly depicted different growth

phases of the microalga (Fig. 2). Growth rate in control showed a sharp increase and exponential growth in cell count. After this there was no further growth in the control which indicated the stationary phase. In contrast, the test cultures showed no exponential increase in the cell count at

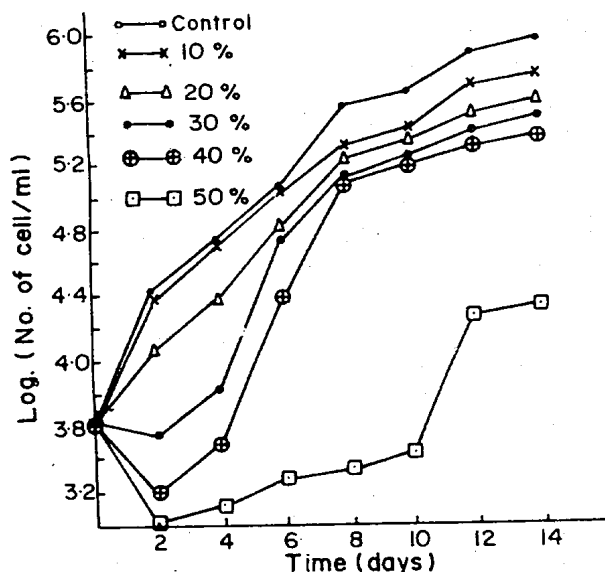


Fig. 1—Growth of *Isochrysis sp.* in cultures having different concentrations of WSF of crude oil.

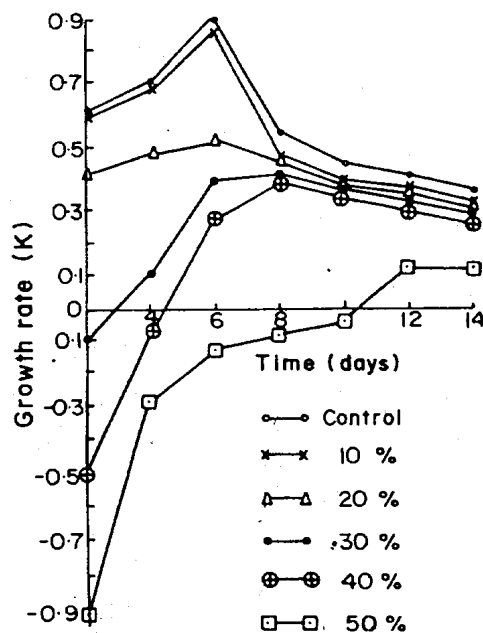


Fig. 2—Growth rates of cultures of *Isochrysis sp.* at different concentrations of WSF of crude oil.

the beginning. A slack in growth in the initial stage, showing negative growth and toxic effect of WSF of crude oil particularly at higher concentration was evident.

Effect of heavy duty marine diesel (HDMD)

The WSF of heavy duty marine diesel was more active in suppressing the growth of *Isochrysis* sp. even at a very low concentration of 5-10%. Therefore 5-20% concentrations were used to study the effect on the growth (Fig. 3). The growth of the control and 5% test solution showed some lag and then recovery in the beginning. The recovery was inconsistent in the 5% solution as compared to normal control culture. At concentrations of >5%, the cell density was found to decline sharply from the initial level, resulting in the complete death of the culture after a few days. This was in contrast to the increasing cell count in the control. At higher concentration (above 20%) all cultures died within 4-5 days.

Comparative toxicity of two oils

It has been reported that most species of phytoplankton respond differently to different petroleum hydrocarbons^{8,9}. The comparative average growth rate estimated under different concentrations of WSFs of crude and marine diesel oil is given in Table 1. HDMD was found to be comparatively more toxic than the crude oil. This was clear from the comparison of data at 10% WSF concentration for both the oils. Retardation of growth was found to be much higher in HDMD

than the Bombay high crude oil. This was further supported by the total mortality of culture at higher WSF concentration in HDMD while no sign of retardation of growth and culture mortality was recorded due to WSF concentration of crude oil. Attempt was also made to study the generation time in each culture (Table 2). In crude oil the growth occurred up to 40% concentration. While only a decline in cell counts was recorded in samples treated with WSF of HDMD. The overall picture showed significant reduction in cell multiplication with increase in concentration of WSF of refined oil. The cultures were generally found to recover after the initial retardation in culture growth of phytoplankton.

An attempt was made to study the inhibition of growth in the cultures influenced by different WSF

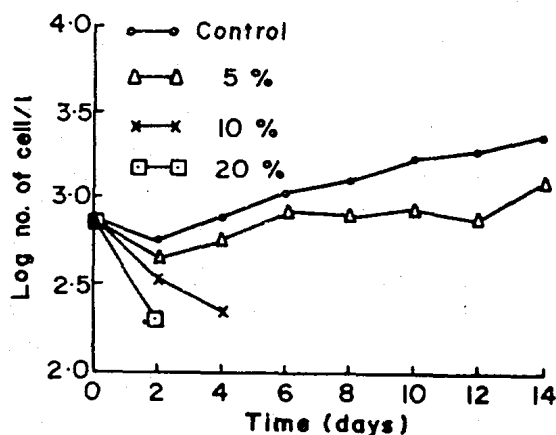


Fig. 3—Growth of *Isochrysis* sp. in cultures of different concentrations of WSF of HDMD oil.

Table 1—Average growth rate of cultures of *Isochrysis* sp.

Oil	Average growth rate (per day) \pm I.S.D.					
	Control	10%	20%	30%	40%	50%
Crude	0.582 \pm 0.169	0.545 \pm 0.176	0.427 \pm 0.071	0.266 \pm 0.183	0.158 \pm 0.305	-0.179 \pm 0.334
HDMD	0.565 \pm 0.202	0.236 \pm 0.302	*	*	*	*

*Cultures died after 4 days and no growth rate calculated

Table 2—Generation time of cultures of *Isochrysis* sp.

Oil	Generation time (in days)					
	Control	10%	20%	30%	40%	50%
Crude	1.718	1.835	2.342	3.759	6.329	*
HDMD	1.770	4.237	*	*	*	*

* Due to negative average growth rate generation time could not be calculated.

concentrations (Fig. 4). The total mortality in cultures above 10% WSF of HDMD and negative growth rate was indicative of high rate of growth inhibition and mortality as compared to the Bombay High crude oil. Inhibition in growth was showed in HDMD even at lower concentrations

Discussion

The occurrence of oil in oceanic waters has a measurable effect on the growth and photosynthesis of microalgae and the ecological significance of this can be assessed by studying the oil concentration in the water itself^{2, 8,9}. The results of the present study support the above inferences. The varying degree of toxic effect under different concentrations depend on the physico-chemical properties of the oils used. The WSF of crude oil has higher total hydrocarbon concentration,¹ but its toxic effect on growth is less. This is because of the lower level of polyaromatic hydrocarbons in such WSF which are more toxic¹⁰ than non aromatic. A few light aromatics are also present but these are short lived and get evaporated rapidly, leaving polycyclic compounds which have less effect on the microalga. Thus the effect of crude oil can be short lived and a total termination of growth does not occur. This is supported by present observations.

Higher concentrations of the toxic aromatics are found in refined oils and hence HDMD was found to be more toxic than crude. Immediate toxic effects of such oil on microalga have been reported by many authors^{9,11}. This explains the enrichment of fuel oils with toxic aromatic compounds such as naphthalene, biphenyl and also the possibility of the presence of soluble aromatic derivatives, which have lethal effects on marine organism².

During the exposure period there is also a significant loss of hydrocarbons due to the weathering effect. A freshly prepared WSF of crude oil gave an initial concentration of 16.5 ppm. After three weeks the same solution yielded a value of 1.53 ppm. It has been reported that at the end of 12 days most aromatics and alkanes are rapidly lost and that the WSF changes qualitatively^{12,13}. The rapid loss of aromatics explain the faster recovery of microalgae in cultures containing WSF of crude oil. Similar effects on algal growth are reported of coloured

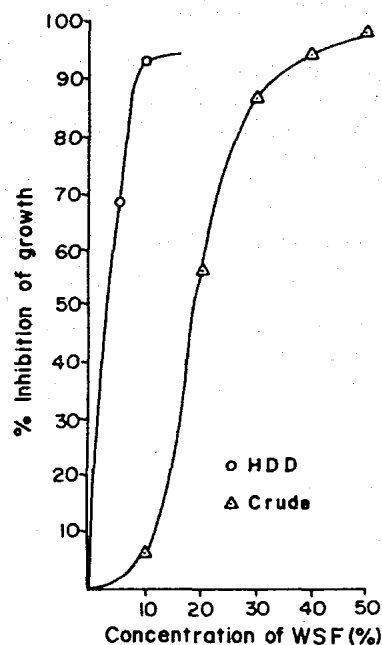


Fig. 4—Percentage inhibition of growth of *Isochrysis sp.* by WSF of crude and HDD oil.

substances and organic solvents^{14, 15}.

Although, the toxicity of WSFs are comparatively lower than the oil itself, its invariable occurrence in the water column of an oil spilled area makes this study important. The growth of microalgae (phytoplankton) is severely inhibited by the introduction of petroleum hydrocarbon even at very low concentration. Such a condition if developed in the coastal waters, will adversely effect the marine food chain since phytoplankton form the first level in the biological productivity. This study has also demonstrated that microalgal species can be quite variable in their sensitivity and susceptibility to different petroleum hydrocarbons.

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