

Effect of Bombay high crude oil and its water-soluble fraction on growth and metabolism of diatom *Thalassiosira* sp.

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Effect of Bombay high crude oil (BHC) and its water-soluble fraction (WSF) on growth and metabolism of the phytoplankton, *Thalassiosira* sp. was assessed. The study revealed the signs of acute toxicity at higher concentrations of crude oil (0.5%) and WSF (40%), while stimulatory effect was observed at lower concentrations (0.01 and 0.1% of BHC and 5, 10% of WSF). WSF at higher concentrations (20 and 40%) caused reduction in DNA and RNA of the diatom. At lower concentrations it caused increase in protein and RNA content indicating increased metabolism. High concentrations of oil and its fraction had inhibitory effect on growth, protein content and nucleic acid content. This indicates that biosynthesis of these molecules may be probable targets for toxicity of oil.

[**Keywords:** Phytoplankton, diatom, *Thalassiosira* sp., water-soluble fraction, DNA/RNA, protein]

Introduction

Oil pollution is one of the major problems faced by coastal ecosystems. Since phytoplankton is largely responsible for primary production in marine ecosystem it is vital to know effect of oil pollution on them. Many reports suggest the toxicity of crude oils and its water-soluble fractions on marine biota^{1,2}. Pulich *et al.*³ has investigated effects of some crude oils on the growth and photosynthesis of microalgae. It is also reported that Venezuelan crude, No. 2 and No. 6 fuel oils inhibited photosynthesis of natural phytoplankton communities and the degree of inhibition was dependent upon oil type and concentration⁴. The field study attempts are often inconclusive due to complexity of the open system and intricacy of biological processes involved. The acute toxicity of petroleum hydrocarbons to marine phytoplankton is generally assessed by comparing growth rates of single species culture and natural phytoplankton assemblage with uncontaminated controls⁵. Ohwada *et al.*⁶ have studied the effect of water soluble fractions of heavy oil using small scale mesocosm facilities that stimulate the natural

environment. The relative impacts of fuel oil on microalgae were assessed by simulation of spill in microcosm⁷.

There are very few reports regarding effect of Bombay high crude oil on phytoplankton. Ansari *et al.*⁵ investigated the effect of Bombay high crude oil and heavy duty marine diesel oil on the growth and photosynthesis of microalga, *Isochrysis* sp. by comparing their growth rates. In the present study we attempt to shed light on the alterations caused by Bombay high crude oil (BHC) and water-soluble fraction (WSF) in the metabolism of phytoplankton. Investigations regarding biochemical changes taking place in phytoplankton cells due to toxicity of crude oil are scarce. Hence the impact of oil and its fraction on synthesis of macromolecules such as DNA, RNA and proteins was investigated. Pure culture of *Thalassiosira* sp. was used for the purpose as it is a sensitive species observed in non-polluted water^{8,9}.

Materials and Methods

WSF of BHC was prepared by placing 1 part of oil over 9 part of filtered (using Whatman No. 1 filter paper), autoclaved seawater, (1:9, v/v). The flask was capped to minimize evaporation and mixing was done using magnetic stirrer for 24 hr. Further this solution was allowed to stand for 8 hr in the separating funnel.

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The aqueous phase thus obtained was treated as 100% WSF solution³.

The diatom *Thalassiosira* sp. (Division: Chrysophycophyta, Class: Bacillariophyceae), the most abundant species in the seawater, was isolated using brush and fine pipette and cultured separately in Schreiber's medium¹⁰. It was maintained in the batch culture of volume 4 liter at room temperature under white fluorescent light (4000-10,000 Lux at the surface of water) with light cycle 10L:14D. Out of this 200 ml culture having optical density 0.09 was taken into Erlenmeyer flask of 250 ml capacity each. Crude oil (0.01%, 0.1% and 0.5% w/v) and WSF (5%, 10%, 20% and 40% v/v) were added to the flasks to acquire different concentrations. Duplicate cultures for all the test concentrations and a control were maintained for each set of experiment. Further the culture was examined for growth and biochemical changes.

Phytoplankton culture growth was monitored daily turbidimetrically using spectronic model systronic spectrophotometer (model -106). Optical density³ (OD) was measured at 530 nm. The measured OD is proportional to the cell number over the range used. In case of turbidimetry analysis, BHC interference influenced optical density readings. Hence the cell count was adapted to monitor the changes in the cell growth in case of BHC set up. The number of phytoplankton cells were counted using haemocytometer.

Total soluble proteins were extracted by adding 5 ml phosphate buffer (pH 8.0) to pigment free pellet and incubated overnight at 4°C. Proteins from the extract were estimated by adding Bradford reagent and the absorbance¹¹ was measured against blank at 595 nm.

About 100 mg (wet weight) of phytoplankton was crushed with 4 ml tris-EDTA buffer (pH 8.0), 2 ml of 1% sodium dodecyl sulphate and 1 ml sodium saline citrate (pH 7.0). Equal volume of chloroform / isoamyl alcohol (24:1, v/v) was added. The mixture was shaken for 20 min on a rocker and centrifuged at 3000 rpm for 10 min. The clear upper aqueous phase containing nucleic acids was used for estimation of DNA and RNA. Amount of DNA was determined quantitatively using diphenylamine reagent by standard procedure. The absorbance¹² was measured at 595 nm. RNA content was estimated¹² by using orcinol solution and measured at 670 nm. Amounts of

DNA and RNA were calculated from standard graph and expressed in mg g⁻¹ of wet weight of cells. Students' *t*-test was used to compare the results of treated cultures with that of control.

Results and Discussion

Effect on growth of *Thalassiosira* sp.

Data presented in Fig. 1 shows that the low concentrations (0.01, 0.1%) stimulate growth of *Thalassiosira* sp. whereas, high concentration (0.5%) caused 40% reduction in the growth compared to control. Further there was no increase in cell number during the period of six days. These results are consistent with the findings of Nayar *et al.*¹³ who observed stimulatory effect on phytoplankton growth at lower concentrations of diesel oil.

WSF showed toxic effect on growth at initial period of the experiment (Fig. 2A). For 5% concentration there was an extended lag phase up to 24 hr and then gradual increase in cell density was observed. At 10 and 20% concentrations, WSF caused decrease in cell number after 24 hr but there was recovery in the cell culture after 48 hr. At higher concentration, i.e. 40%, there was significant ($P < 0.05$) reduction in growth and *Thalassiosira* sp. failed to grow throughout the experiment.

Effect of WSF on growth was also studied in relation to growth rate (*k*) (Fig. 2B). Negative growth rate at conc. 10, 20 and 40% at 24 hr indicates toxic effect of WSF. But after 24 hr there was gradual increase in growth rate of conc. 10 and 20%. Growth rate for 40% remained low for all six days.

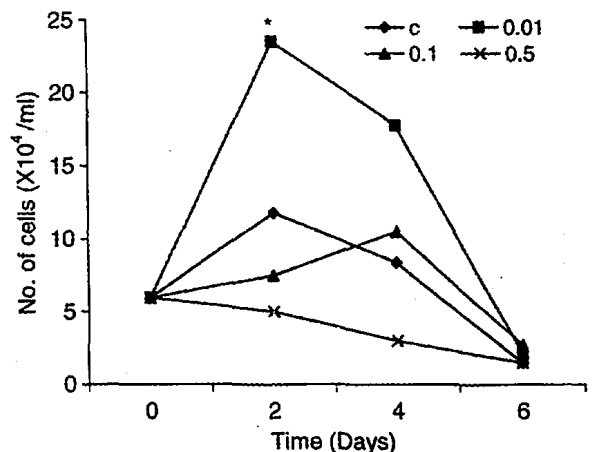


Fig. 1—Effect of Bombay High crude oil on cell count of *Thalassiosira* sp. (*t*-test, * represents significance at $P < 0.05$)

In mesocosm study, acute toxicity to phytoplankton was not detected at lower concentrations of water-soluble fraction of heavy oil⁶. Our data suggests that *Thalassiosira* sp. culture showed growth stimulation at lower oil concentration. This stimulation may be attributed to utilization of oil as a source of carbon by the phytoplankton cells.

Effect on protein content

The total soluble protein content from all experimental flasks treated with BHC was lower than that of the control (Fig. 3A). Eventhough, amount of protein in cultures treated with 0.01 and 0.1% BHC increased with time but it did not exceed control values. However BHC at higher conc. (0.5%) showed maximum reduction in the protein content after 4th

day. There was no significant effect of WSF on protein content at concentrations 5%, 10%, 20%. All these cultures showed increase in protein content after 2nd day. In contrast, 40% WSF treatment caused pronounced decrease in total proteins (Fig 3B). Thus low concentrations increased protein percentage.

Rate of change of protein content was according to the change in growth rate for all the treatments. The increase in protein contents in cultures treated with 10% and 20% WSF after 2nd day was accompanied by increased growth rate of *Thalassiosira* sp. As Mustafa *et al.*¹⁴ suggested this accumulation of protein may be due to mechanism through which phytoplankton can abolish the toxic effect of soluble fraction or may be due to increased respiration leading to utilization of carbohydrates.

Effect on nucleic acids

Both DNA and RNA were affected by different concentrations of BHC as well as WSF (Figs 4, 5). Low concentration of BHC (0.01%) has not shown any significant change in DNA and RNA; whereas soluble fraction caused increase in RNA content at 10% concentration after 2nd day. Reduction in DNA was observed in all treated cultures initially, but at lower concentrations sudden increase was observed

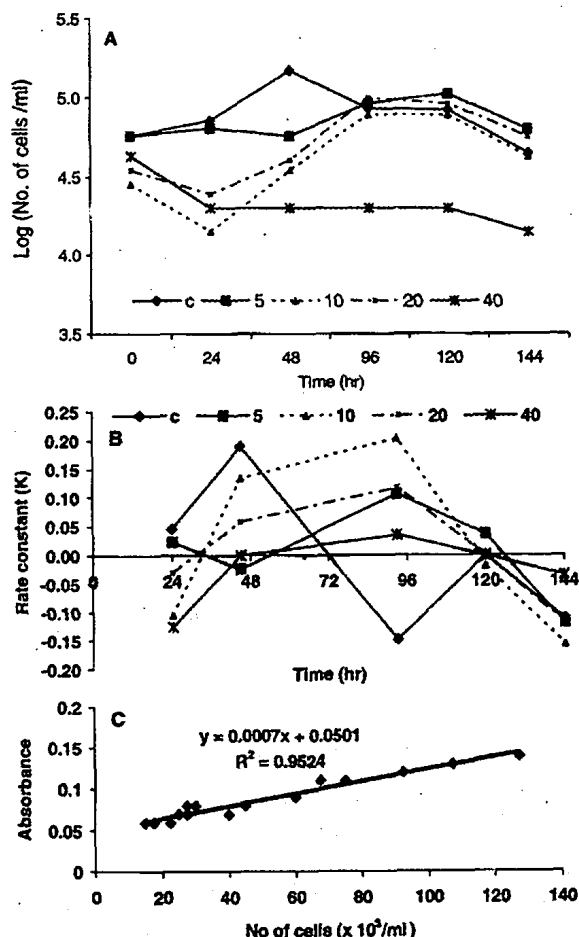


Fig. 2—Effect of WSF on growth of *Thalassiosira* sp. expressed in (A) log of number of cells/ml (B) growth rate (C) linear regression showing relation between optical density and cell count of the diatom.

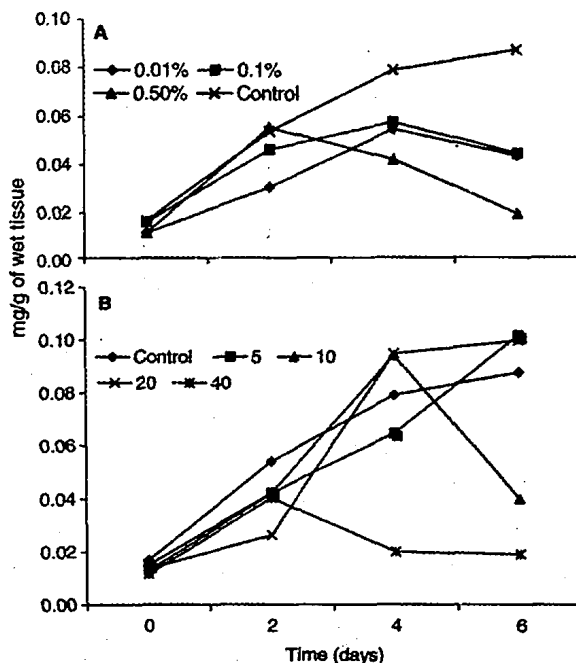


Fig. 3—Effect of (A) Bombay High crude oil and (B) WSF on protein content of *Thalassiosira* sp. Culture.

on day six of the experiment. WSF (5%) initially showed increase in RNA content, which was followed by reduction.

Zachleder and Tukaj¹⁵ have reported that inhibition of DNA synthesis in response to high concentrations of oil is accompanied by slightly delayed cessation of RNA and protein synthesis. This explains the probable nucleic acid variation pattern in above data. Both RNA and DNA vary in similar manner for all the treatments showing their interrelation. In case of BHC, DNA content decreased at the end of 2nd day, whereas reduction in RNA content was observed at the end of 4th day. Although WSF showed similar changes, they were not significant. Results demonstrated that DNA, RNA and protein synthesis are inhibited at higher concentration indicating that the biosynthesis of these compounds are probable targets of oil toxicity.

Another parameter for studying metabolism of the cell is RNA/DNA ratio. Higher RNA/DNA ratio indicates growth of the cell or organism. After treatment of BHC there was gradual increase in RNA/DNA ratio showing maximum values on day 4 (Fig. 4C). This increase in ratio was also followed by

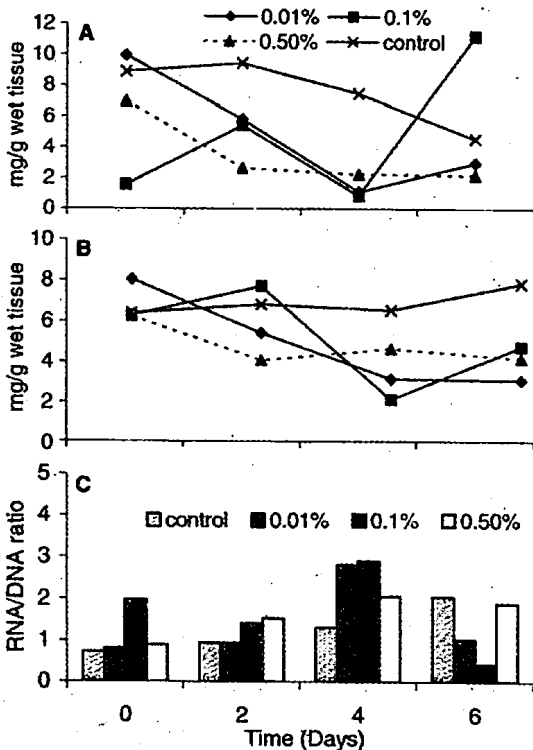


Fig. 4—Effect of Bombay High crude oil on (A) DNA, (B) RNA and (C) RNA/DNA ratio of *Thalassiosira* sp. culture.

the increase in protein content on day 4 for all the concentrations except 0.5%. In case of WSF treated cultures (Fig. 5C), 10 and 20% concentrations showed maximum ratio on day 2. This high ratio explains recovery of the cultures from toxic effects of WSF, which can be further supported by increase in protein content as well as cell number on day 4.

Comparative studies on growth and metabolic activities of two species of green algae *Chlorella* with respect to oil pollution have been reported¹⁴. The crude oil or its product in the culture media of phytoplankton influence cell growth, protein and nucleic acid content and the toxicity of oil is concentration dependent¹⁴.

The present study showed that the different incidences of oil pollution might have different effects in marine waters and may cause ecological damage as composition of petroleum products vary considerably. The oil at high concentrations used for present study, may have serious detrimental effects on other ecological processes such as food webs and energy pyramids. The results also indicate increase in cell number at low concentrations of oil, with rise in amounts of RNA and proteins. From this we can

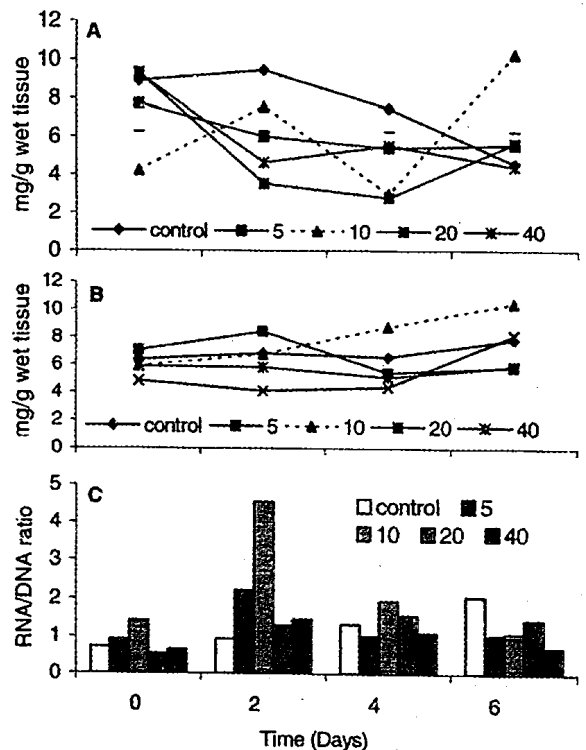


Fig. 5—Effect of WSF on (A) DNA, (B) RNA and (C) RNA/DNA ratio of *Thalassiosira* sp. culture.

interpret that one has to consider this either as stimulatory effect or mutagenic or carcinogenic effect of polycyclic aromatic hydrocarbons (PAHs) present in crude oil. PAHs are known for their mutagenic or carcinogenic activity¹⁶ and these two processes are normally associated with abnormal cell proliferation. Hence further investigations in this direction can lead to the exact mechanism underlying this stimulatory effect of crude oil.

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