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Short Term Studies on Effect of Water Soluble Fractions of Diesel on Growth of *Chaetoceros calcitrans*, Paulsen

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Abstract: Effects of the Water Soluble Fractions (WSF) of diesel on the growth responses of a marine microalgae, *Chaetoceros calcitrans* were studied in the controlled laboratory conditions for a short-term period of 96 h. Differential growth responses were observed at comparatively lower diesel concentrations (5, 10, 20 and 40%), though higher concentration (80%) was found to suppress the growth effectively. The actual concentration of the WSFs were analyzed using a spectrofluorometer and a 96 h-EC_{50} value was found to be 36.56-38.02 mg of diesel per liter of sea water. It has been concluded that diesel spills are not very much suppressive to the microalga, if not a major spillage accident.

Key words: Diesel, Water Soluble Fraction (WSF), toxicity, *Chaetoceros calcitrans*, growth, 96 h-EC_{50}

INTRODUCTION

The chemistry and biology of coastal waters are very vulnerable to additions of biodegradable and stable compounds from land. The fate of petroleum hydrocarbons (PHC) in the marine environment has been studied extensively both qualitatively and quantitatively over past decade. Though massive oil spills are the most visible form of oil pollution in marine environment less dramatic forms occurring due to loading and unloading operations, refinery waste, urban runoff, or atmospheric deposition are more important in most areas. Surface dwelling populations of microalgae are often the most conspicuous victims of oil spills.

Ecotoxicology of hydrocarbons are highly variable, depending on their type and concentration, exposure time, state, environmental condition and the sensitivity of affected species. The effect of contaminants on marine microalgae can be assayed by growth measurements. A decrease in algal density and species as primary producers in food web affects the aquatic ecosystem directly by reducing their biodiversity and primary products. Therefore algae are frequently used in various bioassays. Many studies have reported a reduction in photosynthesis and changes in growth properties of microalgae as well as other effects of PHCs (Lee and Takahashi, 1977). Thompson and Eglintan (1979) documented accumulation of PHC in benthic diatoms collected from oil contaminated site. Others have also reported accumulation and production of different metabolites as a result of exposure of unicellular blue green algae to oil (Prouse *et al*., 1976; Corniglia *et al*., 1980). Exposure to high concentrations of oil resulted in reduced photosynthesis among macroalgae (Shiels *et al*., 1973) whereas exposure to low concentrations appeared to enhance the photosynthetic rate in microalgae (Prouse *et al*., 1976). Diesel is one among the most extensively used PHC. Phatarpekar and Ansari (2000) found that at higher concentrations (10-50%) of diesel oil, the toxic effects were manifested several times than that of crude oil when it was tested against *Tetraselmis gracilis*. Carman *et al*. (2000) reported high mortality among benthic crustaceans and blooms of benthic microalgae in diesel

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fuel-contaminated salt marsh sediments. Wolfe *et al*. (1998) carried out an investigation on bioavailability of naphthalene from crude oil to the unicellular golden-brown algae, *Isochrysis galbana*. However, a large increase microalgal biomass was observed in high diesel treatments as reported by Carman *et al*. (1997) which was thought to be attributed by reduced meofaunal grazing. Siron *et al*. (1996) observed a marked decline in the diversity of centric diatoms in tanks with dispersed crude oil. Roseth *et al*. (1996) made a comparative study on the toxicity of petroleum hydrocarbons under the trade name Nalco. on *Chaetoceros gracilis*, *Isochrysis galbana* and Zebra fish.

The objective of the present study is to investigate the growth suppressive effects of various water soluble fractions of diesel oil on microalga, *Chaetoceros calcitrans* a commonly available coastal species of diatom. The degree of toxicity in terms of median effective concentration (EC_{50}), was determined by graphically interpolating the data at the end of 96 h of exposure.

MATERIALS AND METHODS

Microalgal Culture

An unialgal *Chaetoceros calcitrans* culture was developed in the marine biology laboratory of CAS in Marine Biology (Annamalai University, Parangipettai 608502, Tamil Nadu, India) from a mixed phytoplankton soup, collected during the post-monsoon period of the year 2005, from Muttukadu backwaters, Chennai, Tamil Nadu, India. Identification was done using the keys by Subramanian (1946) while isolation and subsequent unialgal culture was then raised by following the techniques of serial dilution and agar plating. The cultures were grown in batches using filtered autoclaved habitat seawater enriched with F/2 medium (Guillard and Ryther, 1962). All the cultures were maintained in a culture chamber with fluorescent light (2400±200 Lux intensity) on a 16:8; L:D (Light: Dark) cycle at 23°C. Table 1 shows the various physicochemical parameters maintained during the experiment.

Preparation of Water Soluble Fractions (WSF) of Diesel Oil

Commercial grade diesel oil was brought from the market. Following the standard procedure of WSF preparation by Phatarpekar and Ansari (2000), a 100% WSF stock solution was prepared freshly.

Test Cultures

From the 100% stock solution a range of various WSFs were prepared. The concentration range with a lower concentration of 5%, followed by 10, 20 and 40 a maximum of 80% by adding 5, 10, 20, 40 and 80 mL of WSF respectively and made up to 100 mL with filtered, autoclaved and enriched seawater (F/2 medium). A control was also maintained in which no WSF was added. All concentrations

Table 1: Water quality parameters maintained during the exposure treatment (Strickland and Parsons, 1968)

Parameters	Water quality
Salinity (ppt)	33.04±0.5
pH	8.17
Dissolved oxygen (mg L^{-1})	6.9±0.2
Calcium (mg L^{-1})	280
Magnesium (mg L^{-1})	1608
Total hardness (mg L^{-1})	1888
Carbonate (mg L^{-1})	24
Bicarbonate (mg L^{-1})	156
Total alkalinity (mg L^{-1})	180
Inorganic phosphate (μmol L^{-1})	7.5
Nitrite (μmol L^{-1})	0.6
Nitrate (μmol L^{-1})	117.6
Temperature (°C)	23±1

Table 2: Concentrations of diesel oil in WSFs

WSF (%)	Concentration (mg L^{-1})
5	2.020
10	3.900
20	10.000
40	17.010
80	39.000
Background	0.004

were run in duplicates. Test media were seeded with an exponentially growing subculture so that an initial cell density of 10^3 cells (approximately) per mL per test chamber was achieved. Growth was assessed at the end of 96 h exposure period by counting the microalgal cell densities using a modified Fuchs Rosenthal haemocytometer.

Estimation of PHC in WSFs

To estimate the PHC, 150 mL of WSF was extracted with 25 mL double distilled hexane. The hexane fraction was then collected using a separating funnel and collected in stoppered test tubes. Finally the hexane fraction was dried using oven dried anhydrous sodium sulfate. The final volume of hexane extract was adjusted to 25 mL and analyzed using a spectrofluorometer. Double distilled hexane was used as blank. Diesel oil standards were prepared following the standard procedure by Phatarpekar and Ansari (2000). Fluorescence emission of the blank, sample as well as the standards was measured at 360 nm when excitation was at 310 nm (Gordon *et al*., 1974). The concentration of the PHC in WSF, corrected for blanks, was expressed in terms of standards (Table 2). All the extractions were done in duplicate.

RESULTS AND DISCUSSION

Figure 1 shows the effect of WSFs of diesel oil on the growth of *C. calcitrans*. The data revealed that growth in control, 20 and 40% of WSF was almost similar at the end of 96 h (Fig. 2). While at a lower concentration of 5% of WSF growth observed was more than the control cultures. Further increase in diesel concentrations decreased the growth. Drastic suppression of growth was observed at a concentration of 80% of WSF of diesel. A graphical interpolation of the data shows that the EC_{50} of WSF of diesel oil is around 75-78% at the end of 96 h. Hence a diesel oil concentration of approximately 36.56-38.02 mg L^{-1} is the 96 h EC_{50} value. A condition observed at a lower concentration of 5% may be explained as a hormatic response by the microalgae.

In nature, large and little-understood variations frequently occur in ecologically important parameters like population density and species composition. It is thus difficult to detect long term effects of environmental stress in the field. Much of our knowledge of the influence of individual pollutants on the marine ecosystem comes from actual dumping practices and from tanker and other disasters.

The effects of diesel on the growth of *C. calcitrans* was investigated in this present study in terms of its growth suppression activities. The water soluble fractions did not seem to be toxic at lower concentrations, i.e., from 5-40%, however an expected growth suppression has occurred at a much higher concentration of 80%. Furthermore, a slight growth stimulation appeared in some cases. Several authors (Soto *et al*., 1975a) have observed in laboratory studies that oil extracts stimulate algal growth and photosynthesis after the evaporation of toxic substances. Our results support these findings at a lower to moderate diesel concentrations (upto 40%).

The toxic effects of oil on algae fall into two categories: those associated with the coating of the organism and those due to uptake of hydrocarbons and the subsequent disruption of cellular metabolism (Lobban and Harrison, 1997). Coating reduces carbondioxide diffusion and light

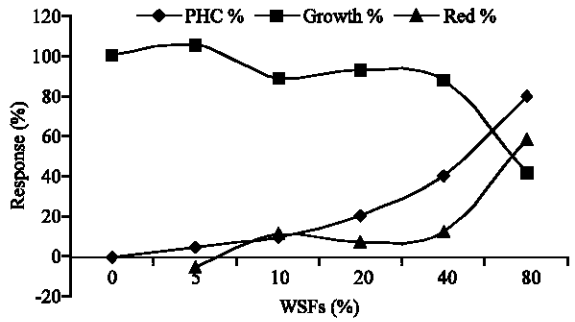

Fig. 1: Responses of *Chaetoceros calcitrans* towards various WSFs of diesel oil during an exposure period of 96 h

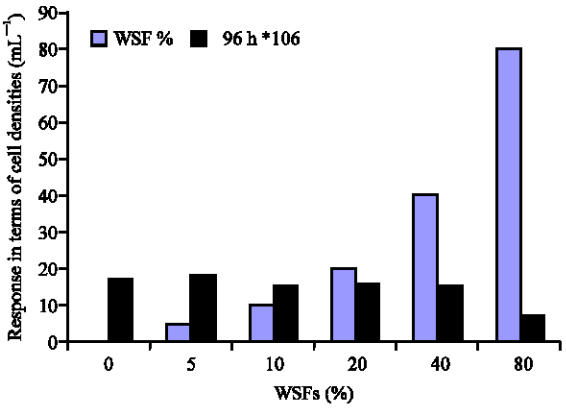

Fig. 2: Variation in final cell densities as a function of increase in the PHC concentrations

penetration. Oil may interfere with any of the various steps in respiration, like gas diffusion, glycolysis and oxidative phosphorylation. Mechanical blockage of gas-diffusion is thought to be less pronounced for oxygen than for carbondioxide (Schramm, 1972). Lipid soluble pigments such as chlorophylls may be leached out of cells by oil (O' Brien and Dixon, 1976). On the other hand low molecular weight, lipophilic compounds including aromatic compounds can easily penetrate the cells.

Diesel oil contains higher levels of aromatic hydrocarbons than that of crude oil and so the toxicity is higher for the diesel oil (Lysyj and Russel, 1974). Aromatic hydrocarbons are more water soluble than alkanes of similar molecular weight and disappear more slowly from solution compared to alkanes (Anderson *et al*., 1974). They are accumulated by organisms in greater extent and retained longer than alkanes (Neff *et al*., 1976). These are the main factors contributing to high toxicity of diesel oil. Diesel also contains hydrocarbons like benzenes and alkylbenzenes. It has been demonstrated that exposure to lower molecular weight aromatics can prolong the lag phase (Mommaerts-Billiet, 1973; Soto *et al*., 1975b; Winters *et al*., 1976; Armstrong *et al*., 1981).

Although laboratory experiments are indispensable and yield useful information, extrapolation of their results to field condition is at present much difficult. Chemical modifications of hydrocarbons can also increase their toxicity. Photo-oxidation is an important mechanism in this respect. Aromatic compounds greatly photo-oxidize and compounds like hydroperoxides are formed which further undergo photochemical transformation forming highly alkylated phenols and substituted benzoic and napthonic acid. Incorporation of oxygen makes them more soluble and hence they are retained in

solution for a longer time. Because of the polar and non-polar nature, they are concentrated more by the cells and have a deleterious effect on the cell membrane which can lead to death of the cell (Larson *et al*., 1979).

It has been shown that the majority of the hydrocarbons which have been tested with both systems, microalgae and animals, are by and large more toxic to microalgae than to animals. A review of literature also shows that such studies are very much lacking in the recent times though a lot of studies have been made during 1970s and 1980s. the present study satiates the recent need, from tropical Indian marine water.

CONCLUSION

From this study it can be concluded that though the coastal waters are very vulnerable to diesel oil pollution due to unwise handling practices, yet it is not much suppressive to the coastal microalgal populations, in general, unless and until its a massive spillage. The water currents along with the cumulative actions of waves and tides disperse the oil drops over a large surface area. Adsorption of these drops to other particulate matters make them even less bioavailable to the microalgae. However, in order to assess the value of experiments in the laboratory, there is a need for experiments with more complex systems that can be regarded as approximating field conditions more closely.

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