

Response of meiofauna to petroleum hydrocarbon of three fuel oils

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Abstract

Oil spills are recurrent problem in marine system. Effects of oil pollution are many. The present paper evaluates the effect of Petroleum Hydrocarbon of three fuel oils on metazoan meiofauna. The results suggest significant variations in the toxic effect of three oils used. The meiofauna responded differently to crude oil, diesel and kerosene. The fauna reduced significantly ($p < 0.05$) in all concentrations after two weeks. The reduction in crude oil was more than 50% in 25g/l, 65% in 50 g/l and 69% in 100g/l. In other cases the reduction was higher. In lower concentrations (25g/l) the more sensitive taxa such as harpacticoids, turbellaria and ostracods were more affected. The meiofauna exposed to PHC showed recovery in the last week, particularly the nematodes showing increase in number indicating their resilient nature. Other groups particularly crustacea appeared more sensitive because they reappeared and recovered much slowly. The abundance of most meiofaunal groups remained low at the end of four weeks exposure to PHC. The effect of oil on the fauna was dose dependent. The toxicity was in the order of kerosene > diesel > crude oil.

Keywords: Meiofauna, response, petroleum hydrocarbon.

Introduction

Oil is a major pollutant in the marine environment. Polyaromatic hydrocarbon (PAH) are ubiquitous contaminants in aquatic environment¹. The fate and effect of petroleum hydrocarbons (PHC) in the marine environment is a major concern for environmentalists and conservationists. The estimated petroleum influx in the marine environment from all sources is about 2 million metric tones per annum². Because of their hydrophobicity, PAH accumulate in the bed sediments and pose a risk to the benthos³. Oil pollution can cause toxic effects on the marine community structure and abundance. Marine benthic organisms are valuable in impact assessment studies due to their ecological importance, numerical abundance and sessile life style functions as integrators of ecological processes and disturbances over long time

सारांश

समुद्री प्रणाली में तेल फैलाव एक पुनरावर्ती समस्या है। तेल प्रदूषण के अनेक प्रभाव हैं। प्रस्तुत लेख बहुकोषीय मियोफाना पर तीन ईंधन तेलों के पेट्रोलियम हाइड्रोकार्बन के प्रभाव का मूल्यांकन करता है। इसके परिणाम प्रयोग किये गये तीनों तेलों के विषैले प्रभाव में महत्वपूर्ण विभिन्नताओं का आभास देते हैं। कच्चे तेल, डीजल और मिट्टी के तेल के प्रति मियोफाना अलग-अलग ढंग से अनुक्रिया प्रदर्शित करता है। दो सप्ताह के बाद सभी सकेन्द्रणों में प्राणि समूह महत्वपूर्ण रूप से घटता (पी. -0.05) है। कच्चे तेल में यह घटत 25 ग्रा/ली में 50% से अधिक, 50 ग्रा/ली में 65/ और 100 ग्रा/ली. में 69% थी। दूसरे मामलों में और अधिक घटत थी। निम्न एकेन्द्रणों (25 ग्रा/ली) में हरपैक्टीक्वायड, टर्बेलेरिया, और ओस्ट्रैकोड जैसे संवेदनशील टैक्सा अधिक प्रभावित थे। अन्तिम सप्ताह में पी.एच सी से अपावरित मियोफाना, विशेषकर निमैटोडा ने अपनी संख्या में बढ़त दर्शा कर अपने लचीलेपन को इंगित कर दिया। दूसरे समूह विशेषकर क्रस्टेशिया अधिक संवेदनशील दिखे क्योंकि ये अधिक धीमी गति से पुनः प्रकट होकर पुनरुत्थानित हुये। पी.एच सी. से चार सप्ताह की समाप्ति तक अपावरित हो कर अधिकांश मियोफाना के समूहों ने निम्न प्रचुरता दर्शाई। प्राणी समूह पर तेल का प्रभाव इसकी अनुपूरक मात्रा पर निर्भर था। विशाक्तता का क्रम मिट्टी का तेल > डीजल > कच्चा तेल था।

सांकेतिक शब्द : मियोफाना, अनुक्रिया, पेट्रोलियम हाइड्रोकार्बन

scales⁴⁻⁷. Effects of PHC on meiofauna have been little studied under experimental conditions. The short life cycle and the resilient nature make them ideal organisms to study the anthropogenic effects. The high numerical abundance makes meiofauna ideal community for pollution monitoring⁸. Demand for more laboratory-oriented research comes from the increasing recognition of meiofauna community structure as a sensitive bioindicator of pollution in the benthic environment.

In the present communication an effort has been made to study the effects of PHC on the metazoan meiofauna under laboratory conditions.

Material and Methods

Sediment samples were collected from a beach. The sediment was randomly taken by scraping the top

10 cm of surface into a bucket and brought to the laboratory. Three different sources of petroleum hydrocarbon, namely, crude oil, diesel and kerosene were used to study the toxicity on meiofauna. The crude oil was obtained from Bombay High (ONGC). Three concentrations were prepared by mixing 25g/l, 50g/l and 100g/l of PHC. After addition of appropriate amount of PHC the sediment was thoroughly mixed and kept in glass tubs. Experiments were set up in triplicate and a control was maintained. Each tub was immersed in a bigger tank containing seawater. A continuous but slow flow of overlying water was maintained.

The observations were made at weekly intervals and the experiments lasted for 4 weeks. From each tub sediment samples were taken with the help of a disposable syringe of 2 cm diameter and 5 cm length. All sediment samples were preserved in 5% buffered Formalin-Rose Bengal solution (1:500). The meiofauna was extracted from the sediment by decantation and sieving⁹. The organisms retained on a 63-micron sieve were counted and identified under binocular microscope. Total petroleum hydrocarbons were estimated in the sediment by fluorescence after extracting with spectrograde hexane. One g sediment samples were placed in an Erlenmeyer flask (50 cm³) with a glass stopper and 4cm³ of water and 10 cm³ n-hexane were added. The samples were mixed on a shaking device for half an hour. The mixture obtained was transferred to a centrifuge tube and spun for 10 mins at 2000 rpm. After centrifugation the n-hexane phase was transferred to a clean glass tube using a glass pipette. Extracts were dried by passing them through a column of Na₂SO₄ (anh.) and evaporated to 5cm³ using a rotatory evaporator. The fluorescence of the extracts was measured in a 1cm quartz cell at 290 nm excitation. The emission was recorded at 350 and 390 nm. The concentrations of petroleum hydrocarbons measured and calculated after extraction of sediment samples and drying and concentrated to appropriate volume. After fluorescence measuring, the extract is concentrated in a water bath at 313K to 1cm³, and applied to 4m i.d. column holding 2 g alumina (neutral, Merck). Elution was performed with 15 cm³ 30% (v/v) benzene in n-pentane. The eluate was then concentrated in a water bath to 5cm³ and fluorescence was measured again. Fluorescence and concentration of petroleum hydrocarbons was calculated after alumina cleaning of the extract.

A known volume (most frequently 50% of the total volume of alumina cleaned extract) is diluted by the addition of n-hexane until the fluorescence of the

sample extract is similar to the fluorescence of standard crude oil with a concentration between 1 and 4µg cm⁻³ in n-hexane.

The evaluation and correction of any quenching was possible by using the standard additions method in which after the measurement of fluorescence of the cleaned extract a known amount of standard crude oil solution was added to it. The obtained values of quenching are presented as the correction factor according to the relationship:

$$\text{Correction Factor} = \frac{F_s \text{ (before addition)}}{F_s \text{ (after addition)} + \frac{F_s}{[(F_a + S)_{\text{measured}} - F_a]}}$$

Where,

F_s = fluorescence of the standard solution of Kuwait crude oil

F_a = fluorescence of the sample after alumina cleaning

$(F_a + S)_{\text{measured}}$ = fluorescence of the sample after the addition of a standard solution of crude oil.

Concentrations of petroleum hydrocarbons calculated after the alumina cleaning of extract^{10,11}.

Results and Discussion

Concentration of Petroleum hydrocarbon in sediment

The total petroleum hydrocarbon concentration before and after the experiment was monitored. The estimated concentration of PHC at the beginning of the experiment in the sediment is given in Table-1. Highest value of PHC was observed in sediment containing 100g/l of crude oil (943.55 µg/g sediment) and the lowest in control tub as expected. Tub containing diesel had concentration ranging between 22.85 and 69.77µg/g while the tub with kerosene had the PHC concentration in the range of 8.52 and 15.89 µg/g. At the end of experiment (4 weeks) the concentration of PHC was measured in all tanks and found to be reduced by at least 50%. Reduction may be due to dilution and weathering¹¹. While the hydrocarbons in the water column are diluted and dispersed, sediments act as sink by adsorption and incorporation of oil onto particles that they sink¹³. The benthic organisms from PHC contaminated sediment are exposed to higher PHC due to the slow process of removal of PHC from the sediment¹⁴.

Table-1 Concentration of total petroleum hydrocarbon in the wet sediment

Fuel oil Sample	Initial conc PHC $\mu\text{g/g}$	Final conc. PHC $\mu\text{g/g}$
Control	1.86	1.02
25 g/l kerosene	8.52	3.58
50 g/l kerosene	11.27	4.65
100 g/l kerosene	15.89	5.88
25 g/l diesel	22.85	12.59
50 g/l diesel	29.31	11.33
100 g/l diesel	69.77	22.35
25 g/l crude oil	95.01	56.82
50 g/l crude oil	314.13	115.95
100 g/l crude oil	943.55	462.76

Effect of PHC on meiofauna

The meiofaunal density of the control tank is given in Table-2. The numerical density during the period of observation ranged between 300 and 342/10 cm^2 . The metazoa was dominated by nematoda, harpacticoida, turbellaria, polychaeta and foraminifera. The other groups included the minor phyla such as kinorhyncha, tardigrada, gastrotricha and crustacean nauplii. No significant change was observed in the numerical abundance of major taxa in the 4 week period. The changes in the density of meiofauna in the controlled tank could be related to the short generation time in which they multiply and grow.

Table-2 Meiofaunal density (no./10 cm^2) in experimental tubs after 4 weeks of exposure to PHC.

Faunal Groups	Kerosene			
	Control	25 g/l	50 g/l	100g/l
Nematoda	186	64	46	45
Harpacticoida	64	4	0	0
Turbellaria	15	6	4	2
Polychaeta	20	10	7	4
Foraminifera	16	8	8	7
Ostracoda	11	2	0	0
Others	30	16	12	16
Total	342	110	77	74

Table 2 Contd .

Faunal Groups	Diesel			
	Control	25 g/l	50 g/l	100g/l
Nematoda	180	70	58	55
Harpacticoida	70	6	0	0
Turbellaria	18	8	12	8
Polychaeta	16	10	8	6
Foraminifera	20	12	8	10
Ostracoda	8	2	0	0
Others	30	10	11	18
Total	342	118	97	90

Faunal Groups	Crude Oil			
	Control	25 g/l	50 g/l	100g/l
Nematoda	174	83	67	62
Harpacticoida	58	6	0	0
Turbellaria	18	10	8	6
Polychaeta	23	8	8	4
Foraminifera	22	6	6	8
Ostracoda	17	4	0	0
Others	30	15	17	21
Total	342	132	106	101

The meiofaunal response to different concentrations of PHC is shown in Fig.-1. The response of metazoans was different to the three fuel oils used. The response appeared to be dose dependent and the toxicity increased with increasing concentration of PHC. Reduction in the total number of meiofauna was significant ($p < 0.05$) particularly in the first two weeks, as could be seen in the Figure 1. Maximum reduction of 61% was observed in kerosene, 54% in diesel and 52% in crude oil exposure at a concentration of 100g/l in the first week. The density of meiofauna further reduced in the second and third week but the changes were much slower. No significant changes were discernible in the fourth week by which time reduction in the concentration of PHC was also observed. The toxic effect of PHC was less in the lower (25g/l, 50g/l) concentrations used. The toxicity was in the order of kerosene>diesel>crude oil. The refined products having high aromatic and volatile hydrocarbons act faster. The effect reduces fast due to the evaporation, as can be seen in the present study¹⁵. Another study¹⁶ reported that kerosene was more toxic than diesel and crude oil to the phytoplankton.

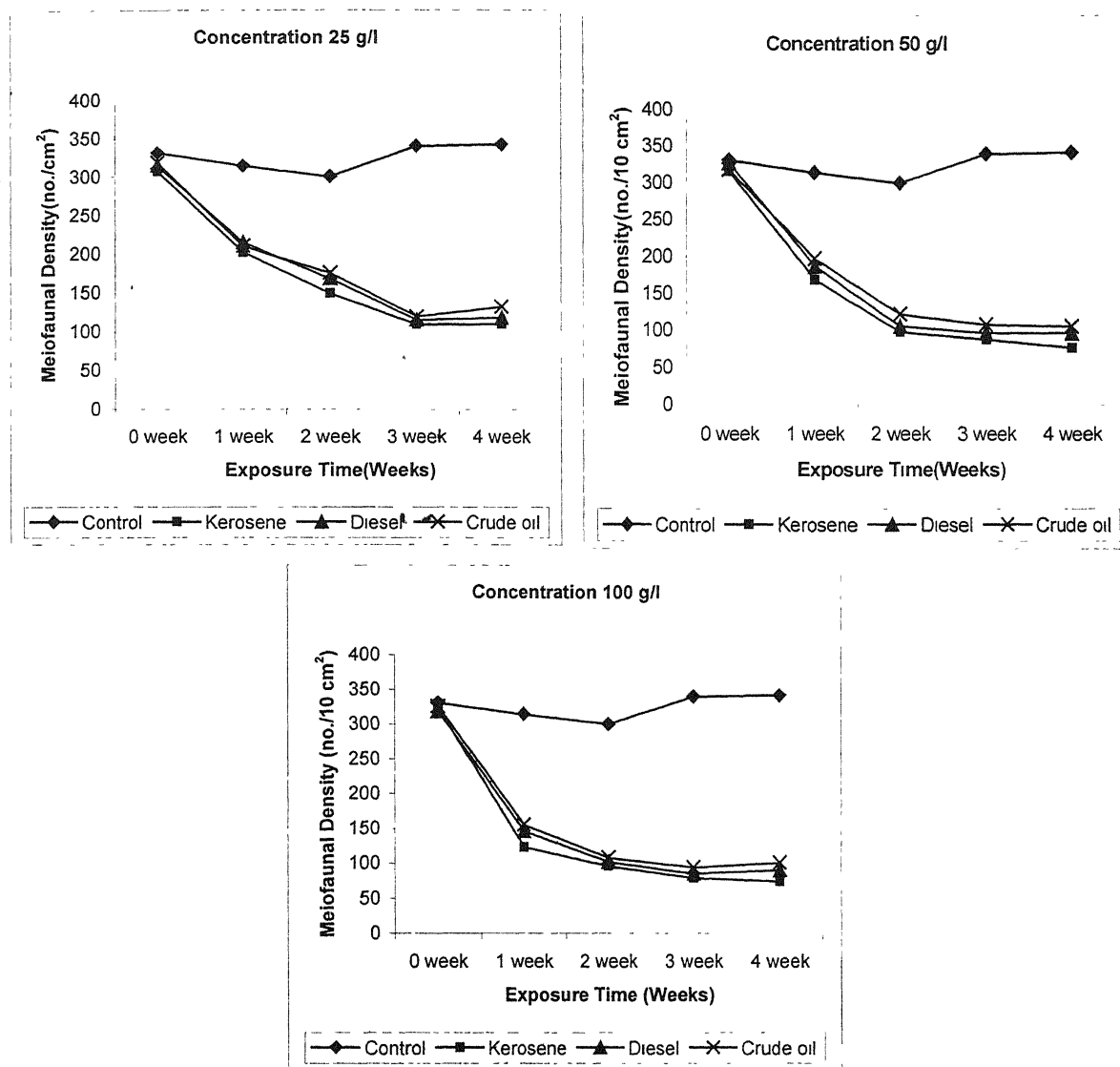


Fig.1. Effect of petroleum hydrocarbon on meiofauna

A reduction in the total number and species abundance of meiofauna after contamination with hydrocarbons has been demonstrated both in the natural habitat and in the laboratory experiments^{5, 17-19}. The response of different taxa varied and was species specific. Of all the metazoans, the nematodes appeared to be more resilient and showed sign of quick recovery after initial exposure to PHC. The crustaceans were more sensitive and no sign of recovery was noticed after four weeks. The recovery response of other groups was also slow. Effects of PHC on benthic communities have been shown to depend strongly on the proportion of hydrocarbon-sensitive species^{20, 21}. Nematodes have previously been shown

to be tolerant to PHC, so their high abundance could explain the lack of response to treatments²². Similar observations have been made earlier²³ in the studies on colonization of meiobenthos in oil contaminated subtidal sands in the lower Chesapeake Bay. In an experimental study²⁴ low abundance of metazoa was recorded in oiled tubs than in the control. They suggested that the addition of marine diesel can alter dramatically the structure and dynamics of benthic meiofaunal communities.

The meiofaunal density at the end of the experiment (4 weeks) was recorded and is presented in Table-3. Changes in nematode abundance were far more

pronounced than other groups. The polychaetes, turbellaria and foraminifera showed lesser change. Several investigations have also documented the initial reduction in meiofauna and their recovery within one year after an oil spill, depending on the type of the oil^{14, 18, 21}. Some other studies showed that while high concentrations of petroleum hydrocarbons initially reduce infaunal densities due to toxic effects, intermediate hydrocarbon concentrations stimulate infaunal densities due to organic enrichment^{25, 26}. Many of the meiofaunal groups affected by PHC do not recover to normal abundance after a short recovery period as the concentration of PHC declines slowly in the sediment. The delayed recovery may be caused due to sublethal effect of PHCs and its byproducts on the fauna. It was observed from these laboratory experiments that many of the meiofaunal groups affected by PHC do not recover to normal conditions (abundance) after a short recovery period and the concentration of PHC in the sediment declines slowly.

Conclusions

Exposure to PHC generally has only temporary effect on metazoan meiofauna. Different PHC from fuel oils have different effects on different groups of meiofauna. The toxic effect can be seen initially within minutes or hours. The recovery back to equilibrium or normal condition is quite rapid among meiofauna in the subtidal region of natural environment²⁷. This is attributed to the resilient nature of meiobenthos. The nematodes appeared to be more resistant to PHC compared to other groups.

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References

1. Smith JE. The control of Oil Pollution. Bristol UK. Arrwosmith Ltd, 1983
2. Anonymous. Petroleum in the marine environment. Proceedings of Workshop on Inputs, Fates and the Effects of Petroleum in the Marine Environment. 1973, 21-25 May, Washington.
3. Rice SD, Short JW, Darmin JF Comparative toxicity and comparative animal sensitivity. In: Wolfe DA, editor. Fate and effect of petroleum hydrocarbon in marine ecosystem and organisms. New York: Pergaman Press, 1976, pp. 78-84.
4. Pearson TH, Rosenberg R. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment *Oceanogr and Mar Biol Ann Rev* 1978, **16**: 229-311
5. Elmgren E, Hansson S, Larsson U, Sundelin B, Boehm PD The Tseis oil spill acute and longterm impact on the benthos *Mar Biol* 1983, **73**: 51-65
6. Kroncke I, Dippner JW, Heyden H, Zeiss B Long-term changes in macrofaunal communities off Norderney (East Frisia, Germany) in relation to climate variability *Mar Ecol Prog Ser* 1998, **167**: 25-36
7. Beuchel F, Gulliksen B, Carroll ML Long-term patterns of rocky bottom macrobenthic community structure in an Arctic fjord (Kongsfjorden, Svalbard) in relation to climate variability (1980-2003). *J Mar Sys* 2006, **63** 1-2.
8. Vinex M, Heip C The use of meiobenthos in pollution monitoring studies A Review *ICES CM* 1987, **33** 1-18
9. Uhlig G, Theil H, Gray JS The Quantitative separation of meiofauna-A comparison of methods. *Helgolander wiss Meeresunters* 1973, **25**:173-195.
10. Anonymous The determination of Petroleum Hydrocarbons in sediment Paris UNESCO, 1982
11. Picer M, Picer N, Ahel M Chlorinated insecticide and PCB residues in fish and mussels of east coastal waters of the Middle and North Adriatic Sea, 1974-1975 *Pest Monit J* 1978, **12**:102-112
12. Hon WM, Moochhala S, Khoo HO, Lee RF, Page DS. Petroleum hydrocarbon and their effect in subtidal regions after major oil spill *Mar Poll Bull* 1997, **34**:928-940
13. Gearing P, Gearing NJ. Behaviour of No 2 Fuel oil in the water column of controlled ecosystem. *Mar Environ Res* 1982; **6** 115-132.
14. Anton M, Beryl H. Effects of crude oil on the supralittoral meiofauna of sandy beach *Mar Env Res* 1982; **7**(1):71-79.
15. Anderson JW, Neff JM, Cox B, Tatem HE. Hightower GM characteristics of dispersions and water soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Mar Biol* 1974, **27**:75-88.
16. Phatharpekar PV, Ansari ZA. Comparative toxicity of water soluble fractions of four oils on the growth of a microalga. *Botanica Marina* 2000; **43**:367-375.
17. Giere O The impact of oil pollution on intertidal meiofauna Field studies after the La Coruna spill, May 1976. *Cah Biol Mar* 1979; **20**:231-251.
18. Boucher G. Impacts of Amoco Cadiz oil spill on the intertidal and sub littoral meiofauna. *Mar Poll Bull* 1980, **11**: 95-101.
19. Donavaro R, Fabiano M, Vinex M. Meiofauna response to the Agip Abruzzo oil spill in subtidal sediments of the Ligurian Sea. *Mar Poll Bull* 1995; **30**: 133-143
20. Dauvin JC. The fine sand *Abra alba* community of the Bay of Morlaix twenty years after the Amoco Cadiz oil spill. *Mar Poll Bull* 1998; **36**: 669-676

- 21 Ansari ZA, Ingole B. Effect of an oil spill from M V Sea Transporter on intertidal meiofauna at Goa, India *Mar Poll Bull* 2002, **44** 396 - 402
- 22 Gyedu-Ababio TK, Baird D. Response of meiofauna and nematode communities to increased levels of contaminant in a laboratory microcosm experiment. *Ecotox Environ Saf* 2006; **63**:443-450
- 23 Alongi DM, Boesch DF, Diaz RJ Colonization of meiobenthos in oil contaminated sub tidal sands in the lower Chesapeake Bay *Mar Biol* 1983; **71**: 325-335.
- 24 Frithsen JB, Elmgren R, Rudnick DT. Responses of benthic meiofauna to long term, low level additions of No 2 fuel oil *Mar Eco Prog Ser* 1985, **23** 1-14.
- 25 Spies RB, Hardin, Des Marias DJ Natural isotopa study of tropic enrichment of marine benthic communities by petroleum seepage *Mar Biol* 1983, **73**: 67-71
- 26 Steichen DJ, Holbrook SJ, Osenberg CW. Distribution and abundance of benthic and demersal macrofauna within a natural hydrocarbon seep *Mar Ecol Prog Ser* 1996, **138**: 71-82
27. Donavaro R Benthic microbial loop and meiofaunal response to oil induced disturbance in coastal sediment – a review *Intern J Environ Poll* 2000; **13** 21-34.