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Accumulation and depuration of petroleum hydrocarbons from WSFS of Bombay High crude oil by the marine bivalve *Gafrarium divaricatum* (Gmelin)

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Abstract

Medium sized intertidal hard clams *G afrarium divaricatum* (Gmelin), were exposed to the water soluble fractions (Wsfs) of Bombay High (BH) crude oil at concentrations ranging from 583.6 ppb (10% Wsf stock) to 5.8 ppb (1% Wsf dilution). During 28 days of exposure period and 14 days of depuration period, hydrocarbon retention and release were recorded. PHC concentrations in water samples were measured quantitatively by UV flourescence spectrometry (excitation 310 nm, emmision 360 nm) IR spectrophotometer studies showed that petroleum derived hydrocarbons were incorporated into the tissues of *G. divaricatum*. Clams surviving exposure to high sublethal levels of crude oil Wsfs were adversely affected both during exposure and depuration.

The low boiling fractions of oil are toxic and readily soluble in seawater causing biological damage at the very moment of the accident or oil spill (Whittle and Blummer, 1968). Due to increased bulk oil traffic, oil pipeline terminals, increased production at Bombay High rig, there has been considerable increase in oil pollution. The solubility of oil is increased in the shore waters by agitation and thus toxic fractions dissolve more rapidly and reach higher concentrations (Blumer, 1971).

Majority of the research on Wsfs of oil involves acute toxicity studies with adult and juvenile forms and the chronic effects of sub-lethal quantities of Wsfs are relatively unknown (Anderson *et al.*, 1974, Cucci and Epitanio, 1979). The information concerning the rate of recovery from the oil exposure and loss of hydrocarbons from the body tissues is very less (Stainken, 1976, Widdows *et al.* 1987).

The bivalve *G. divaricatum* known to survive in highly polluted waters was used to gain an insight into the accumulation and depuration of the pollutant Wsfs of Bombay High (BH) crude oil. We are ignorant of long term and low level effects of oil pollution. In this study, filter feeding mollusc G *divaricatum* is used to determine the relative PHC uptake during 4 weeks exposure period to various dilutions of stock Wsfs of Bombay High crude oil.

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Material and methods

The Wsfs were prepared by vigorously mixing Bombay High crude oil and sea water at a constant temperature (Neff and Anderson, 1975). Filtered sea water was used for further dilutions to produce the desired concentrations. The Wsfs were measured routinely by UV flourescensce analysis of hexane extracts using an excitation wavelength of 310nm and an emission wavelength of 360nm. The subsamples extracted were scanned for absorbance at 2930 cm^{-1} with a perkin Elmer Model 700 Infrared spectrophotometer. Hydrocarbon concentrations was determined by comparison with standard curves prepared for BH crude oil used in this study. n-Hexane was used as a blank. The rubber stoppers or aluminium foil were used to reduce the loss of volatiles that could occur.

The animals used in these static tests were chosen because of their wide availability and occurrence throughout the year. The static tests were conducted as per the method of Rice *et al.*, (1979). The sessile and polysyringic characters of clams make them important biomonitor and bioindicators of pollutants (Mason, 1987, Burns and Knapp 1989). *G.divaricatum* of length 30-35 mm and weight 20-21g (with shell)collected from unpolluted source around Bombay were acclimatized for a week. The lots which had less than 10% mortality during acclimatization were used for the experiment. Tissues were pooled for biochemical assay.

All the samples were analysed five times and all values are averages of five extractions of clams. Following preliminary tests to establish a close range of concentrations for exposure, a series of stock Wsfs were prepared (10%, 20%, and 30%) by proportional oil and seawater mixing, followed by preparing various dilutions of the respective Wsf stock. 10% Wsf stock was chosen for chronic studies as all clams in 20% and 30% Wsfs dilutions were found dead within a week period.

Results

Weights of hydrocarbon residues in the test solutions ranged from 5.8 ppb to 583.6 ppb. The present study revealed the accumulation of PAH in the tissues of clams. The average PAH accumulated are shown in Table 1. Length of exposure was major factor in the comparative toxicity to test animals (Moles, 1998). Clams exposed to higher dilutions of the Wsf stock continued to respond normally for several days. None died during the two weeks period in any of the dilution concentrations. All clams in pure concentration of Wsf stock were found dead by third week. In 75% dilution concentration all clams died by the end of exposure period. By the end of fourth week only 50% of clams were found dead in 50% dilution concentration. In 1%, 10% and 25% dilution concentrations all clams survived till the end of exposure period and thus only these concentrations were used for depuration studies. The clams generally showed high accumula-

tion in higher concentrations upto third week of exposure, there after the levels dropped by the end of experiment. In the lower dilution concentrations there was a gradual increase in hydrocarbon concentrations.

After the end of exposure period all live clams were transferred to pollutant free clean sea water. Initially hydrocarbons were released very quickly (48 hrs) irrespective of the concentrations during first week followed by lag phase (showed down). Negligible amounts of hydrocarbons were retained in the tissues at the time of termination of depuration period. Mainly the depuration was more rapid.

Discussion

Delayed responses were generally observed in the test animals which indicate that the clams can tolerate prolonged oil exposure (Moles, 1998). These results contradict the previous short term studies which show intertidal and subtidal invertebrates to tolerate oil exposure (Rice *et al.*, 1979). Lee *et al.*,(1972), Stegeman and Teal (1973), Neff and Burns (1996) and Mohan and Prakash (1998) have also reported the occurrence and uptake of petroleum hydrocarbon by bivalve molluscs. Bivalves are known to accumulate pollutants in their body from surrounding medium either passively or by active absorption.

PAHs are ubiquitous priority pollutants that occur naturally in crude oil. PAHs with high molecular weight are less readily biodegraded by indigenous microorganisms in some regions and given marked hydrophobic characteristic, may persist in aqueous environment, thus contaminating the food chain by accumulating in aquatic species like fish and mussels. The experimental animals were observed to minimize hydrocaron stress by burrowing (Pearson, 1981), and closing their shells tightly (DeZwaan and Zandee, 1971). If their intake of pollutant is reduced, animals require much longer to accumulate sufficient amount of hydrocarbon in their tissues to be adversely affected by oil exposure (Moles, 1998). Some alteration of behaviour (Galtsoff *et al.*, 1935, Moles, 1998) and tissue structure (Stainken, 1976) by acumulation of PAH were recorded. Accumulation was determined to increase with increasing exposure period in the exposure media (Menon and Menon, 1999). The temporal trends in PAH accumulation pattern in *G. divaricatum* reflects selective retention and depuration of different hydrocarbons (Shaw *et al* 1986).Presumably the precise site of these aromatic hydrocarbons includes the lipid stores of the tissues because of their lipophilic nature (Malins, 1997, Farrington *et al.*,1982, Metcalf and Carlton, 1990). Chronic oil exposure appears to minimize the differences in hydrocarbon sensitivity between species (Moles, 1998). Intertidal organisms are vulnerable to chronic pollution through oil spills on beaches and such coated habitat may retain oil for long periods. The medium sized experimental animals were maintained during the experimental period as size related differences in uptake, depuration and bioaccumulation has been reported earlier (Gilek *et al.*, 1996).

Table1. *Uptake and release of PHC in G. divaricatum to dilutions of 10% WSF stock of BH crude oil (µg/g) (Mean values ± SD of 5 determinations)*

%	Exposure Time (Days)				Depuration Time (Days)		
Dilution	7	14	21	28	3	7	14
1	15.10 ± 0.24	16 ± 0.19	23.52 ± 0.11	21.18 ± 0.19	9.03 ± 0.09	2.98 ±0.08	0.65 ± 0.012
10	16.54 ± 0.14	20.2 ±0.08	24.13 ±0.012	26.83 ± 0.2	5.92 ±0.19	2.75 ±0.03	1.3 ±0.05
25	19.90 ± 0.017	22.51 ±0.011	28.09 ±0.04	37.40 ±0.06	10.55 ±0.018	6.85 ±0.24	3.1 ±0.021
50	24.56 ± 0.024	32.84 ± 0.016	36.07 ±0.018	48.74 ±0.021	b	b	a
75	33.40 ±0.23	39.7 ±0.19	51.17 ±0.014	58.87 ±0.2	b	a	a
100	39.71 ± 0.22	43.25 ±0.2	54.79 ±0.13	a	a	a	a

pH = 7.5 ± 1.6; Temperature = 28±2°C; Dissolved Oxygen = 5.6+ 1.0 ml/l; Salinity = 30±2‰
a - all dead; b - moribund

It is well established that bivalve molluscs are not passive acumulators of petroleum but actively depurate those compounds (Shaw *et al.*, 1986).

Mainly depuration was faster in test organisms. The loss of hydrocarbons by metabolism and depuration has been reported earlier (Anderson *et al.*, 1974). The initial rapid drop in tissue hydrocarbon during first week of depuration is probably due to loss of hydrocarbon adhering to exposed epithelial surface such as the mantle and gills and excretion of oiled debris already in the gut (Neff *et al.*, 1976). Among the other factors influencing depuration rate are firstly, lower molecular weight and more water soluble compounds discharged rapidly and secondly animals shortly exposed to petroleum depurate rapidly to low residual concentrations while chronically exposed animals depurate slowly and have higher residual concentrations which are lost very slowly if at all (Farrington *et al.*, 1982). It can be concluded that while crude oil may not be actually toxic to intertidal hard clam *G. divaricatum*, exposure to high sublethal Wsf concentrations may result in long term deleterious effects.

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