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Time-Related Increase of Hydrocarbons in Barnacles in the North-Western Waters of the Arabian Gulf

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The Arabian Gulf is almost a land-locked body of water, with an average depth of 35m and a turnover and a flushing time in the range of 3 - 5 years (Price and Sheppard 1991). Consequently, pollutants entering the Gulf are likely to stay for a considerably long period of time. The Arabian Gulf is drawn into intense petroleum activities and presents an evident risk of contamination of its coastal zones. In addition to this, the 1991 Gulf war and the intentional discharge of an estimated ten million barrels of crude oil into the Gulf, further escalated the environmental risk. Blazing of the oil wells resulted in various types of combustion products together with uncombusted crude oil in the environment, thus threatening the Gulf ecosystem. Polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbons (HCs) in crude oil can produce significant contamination of the marine environment and adversely affect marine life (Clark Jr. and MacLeod 1977). PAHs are known carcinogens for marine organisms and can be transported over long distances adsorbed onto airborne and waterborne particles (Malins et al. 1984).

Many studies have shown that sediments are the major reservoirs for pollutants such as aromatic hydrocarbons, including benzo [a] anthracene and benzo [a] pyrene (Malins et al. 1984). Fish and other organisms are also known to accumulate many of these chemicals from polluted aquatic environment causing a number of pathological conditions such as fin erosion. Thus the highest prevalence of this disease and liver neoplasm have been reported in fishes from areas with highly contaminated bottom sediments (Falkmer et al. 1979; Smith et al. 1979). Bivalve mollusc, found in contaminated sites, also bear proliferative diseases that may be neoplastic (Stegeman and Lech, 1991). Chronic exposure to oil reduces light transmission and oxygen levels and can alter metabolic activity causing tumour growth (McCoy and Brown 1998). Involvement of nitrated polycyclic aromatic hydrocarbons, produced as a result of the combustion of fossil fuel, has caused an alarm due to their potent mutagenic and carcinogenic activities (Enya et al. 1997). Exposure of fish to petroleum hydrocarbons with their aromatic hydrocarbon contents can also induce increased levels of microsomal mono-oxygenases for metabolism of these compounds through induction of cytochrome P-450 mono-oxygenase, which helps in the hydroxylation and metabolism of PAHs (Schnell et al. 1980;

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Stegeman and Lech 1991; Mitchelmore et al. 1998). The co-occurrence of pollutants and neoplastic diseases in animals from these locations suggest that the accumulated pollutants are responsible for these disorders. These pollutants could be transferred to humans through diet.

Barnacles are sensitive to heavy metals, petroleum hydrocarbons and organic compounds and may indicate the level of pollution in their location (Sasi Kumar et al. 1995). In this paper, PAHs and HCs accumulated in barnacles in the North-Western waters of the Gulf were analysed to gauge the level of these pollutants.

MATERIALS AND METHODS

Two species of Barnacles *Balanus tintinabulum* and *Balanus amphitrite* were collected at different time intervals, during June 1996, June 1997 March 1998 and between August - October 1999 at different locations in the north western waters of the Arabian Gulf. The barnacle sample locations and their systematic identification are given in Table 1. The samples were stored in ice immediately after collection and brought to the laboratory. The external hard shells were removed and animals were stored frozen at -80° C until analysed.

Table 1. Sample Locations

S	Scientific Name	Location	Col. Date	Lat. N	Long. E
1	*Balanus tintinabulum*	Al-Durrar	14 06 1996	28^0 58.60’	49^0 08.22’
2	*Balanus tintinabulum*	Al-Durrar	12 06 1997	28^0 57.51’	49^0 08.45’
3	*Balanus amphitrite*	Marina	08 06 1997	29^0 20.50’	48^0 03.17’
4	*Balanus amphitrite*	Marina	20 03 1998	29^0 20.50’	48^0 03.17’
5	*Balanus amphitrite*	Um Al-Maradim	03 09 1999	29^0 04.08’	48^0 39.33’
6	*Balanus amphitrite*	Kuwait Bay	25 09 1999	28^0 40.12’	47^0 59.63’
7	*Balanus amphitrite*	Al-Durrar	20 08 1999	28^0 58.60’	49^0 08.22’
8	*Balanus amphitrite*	Kubbar	24 09 1999	29^0 04.08’	48^0 29.37’
9	*Balanus amphitrite*	Marina	01 10 1999	29^0 20.50’	48^0 03.17’

Anhydrous sodium sulfate and potassium hydroxide were of analytical grade, and were purchased from Fluka, Switzerland. All solvents used in this study were of HPLC grade and were purchased from either Fluka or BDH, England. A modified procedure of Kristiina et al. (1986) was followed for the extraction of PAHs and

HCs from barnacles. Barnacle animals (5 gm., wet weight) were weighed accurately and placed in a mortar and pestle and finely crushed after the addition of anhydrous sodium sulfate (10 gms.). The crushed sample was taken in a thimble, pre-eluted with water:methanol mixture (1:9 V/V) and soxhlet extracted with 100 mL of the same solvent mixture containing 3.5 gm. of potassium hydroxide, for 4 hours. The extract was cooled and mixed with 100 mL of water:methanol mixture (2:8 V/V), and extracted twice with cyclohexane (100 mL). The organic extract was first washed with 100 mL of water:methanol mixture (1:1 V/V) and then twice with 100 mL of water. The cyclohexane extract was dried over anhydrous sodium sulfate and concentrated to 1 mL at 30° C on a rotary evaporator. A chromatographic column (40 cm x 0.8 cm ID), packed with 6.5 g. of florisil (60-100 mesh) deactivated with 5% water, was eluted with 80 mL of toluene and then the cyclohexane concentrated extract was loaded on top of the column. Elution of the column was made with 30 mL toluene. Toluene was evaporated to dryness on a rotavapor at 35 - 40° C. The residue was dissolved in 10 mL hexane and extracted twice with 10 mL of DMSO (Dimethyl Sulphoxide). The combined DMSO extracts were diluted with 40 mL water and extracted with cyclohexane (2 x 20 mL). The combined cyclohexane extracts were washed twice with water (40 mL) and dried over anhydrous sodium sulfate. Cyclohexane was evaporated to dryness at 30° C and the residue was redissolved in 50 µL of dichloromethane containing the internal standard viz. d-10 acenaphthene and analysed by Gas Chromatography/Mass Spectrometry (GC/MS) for PAHs. The residual hexane solution, after the extraction with DMSO, was then dried with anhydrous sodium sulfate and the solvent was evaporated to dryness. The residue was redissolved in dichloromethane containing eicosene, as an internal standard and analysed for HCs by GC/MS.

RESULTS AND DISCUSSION

The samples for our study were collected from five different locations in the Gulf. The sampling locations were selected so as to reflect the environmental situation in the North Western part of the Gulf, from Kuwait Bay in the north (where Kuwait city is located), to Um Al-Maradim Island in the Southern part of the Kuwaiti territorial waters, and from Kuwait Bay in the West to Al-Durrar oil field area in the East (50 nautical miles east of the Kuwaiti coast). Kuwait Bay, Marina site and Um Al-Maradim island were heavily polluted with crude oil during the 1991 Gulf war, while Kubbar island was much less impacted by the crude oil. Analytical results for PAHs in barnacles are given in Table 2. Barnacles (*Balanus tintinabulum*) collected from Al-Durrar in June 1996 had accumulated a total of 3.71 $\mu g\ g^{-1}$ (wet wt.) of PAHs, while the same species of barnacles collected from the same site during June 1997 showed a total of 6.93 $\mu g\ g^{-1}$ (wet wt.) PAHs, which is almost double the level of PAHs as compared to the samples collected one year earlier in 1996. In August 1999 our sample collection effort for *Balanus tintinabulum* from the same site failed, because only shells of dead animals were found on the oil rig, while a new colony of a different species viz. *Balanus*

Table 2. Levels of PAHs in Barnacles in µg/g (wet wt.)

S.No.	Location	Na	Acn	Acna	Fl	Ph	An	Flu	Py	BaA	Chr	BbF	BkF	BaP	Ind	Dib	Bper	Total
1	Al-Durrar	0.13	ND	ND	0.21	0.37	0.39	0.64	0.64	0.64	0.52	0.17	ND	ND	ND	ND	ND	3.71
2	Al-Durrar	0.42	ND	ND	ND	0.78	ND	1.18	1.25	3.3	ND	ND	ND	ND	ND	ND	ND	6.93
3	Marina	ND	ND	ND	0.325	0.764	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.09
4	Marina	0.022	ND	0.144	0.075	0.186	0.165	0.625	0.77	0.863	0.63	0.269	0.62	0.65	0.28	ND	ND	5.3
5	Um Al-Maradi	0.86	ND	0.122	0.092	0.312	0.018	0.48	0.61	0.312	0.44	0.281	0.61	1.165	0.92	0.79	0.72	7.73
6	Kuwait Bay	0.003	ND	ND	ND	0.006	ND	ND	ND	ND	ND	ND	ND	0.6	ND	ND	ND	0.61
7	Durrar	Tr	0.01	Tr	Tr	0.01	Tr	0.033	0.19	Tr	Tr	ND	ND	1.2	ND	ND	Tr	1.44
8	Kubber	Tr	ND	Tr	ND	0.01	Tr	0.037	0.032	0.04	0.017	0.015	ND	0.01	ND	ND	ND	0.16
9	Marina	0.023	0.005	0.09	0.04	0.27	0.073	0.616	0.42	0.57	0.45	3.274	0.043	0.87	0.484	0.79	0.19	8.21

Na=Naphthalene; Acn = Acenaphthylene; Acna = Acenaphthene; Fl= Fluorene; Ph = Phenanthrene; An = Anthracene; Flu = Fluoranthene; Py= Pyrene; BaA = Benzo [a] anthracene; Chr = Chrysene; BbF = Benzo [b] fluoranthene; BkF = Benzo [k] fluoranthene; BaP = Benzo [a] pyrene; Ind = Indeno [123-cd] pyrene; Dib = Dibenzo [ah] anthracene; Bper = Benzo [ghi] perylene ND = Not Detected

Table 3 Levels of Alkanes in Barnacle µg/g (wet wt.)

S.No.	Location	C_{14}	C_{15}	C_{16}	C_{17}	Pr	C_{18}	Py	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	Total
1	Al-Durrar	ND	ND	ND	0.08	ND	0.13	ND	0.18	0.12	0.96	0.71	0.29	0.16	0.18	ND	ND	ND	ND	ND	2.81
2	Al-Durrar	0.16	0.23	0.28	0.76	ND	0.03	ND	0.66	0.48	ND	ND	0.13	0.15	0.16	0.21	ND	ND	ND	ND	3.25
3	Marina	0.12	0.12	0.01	0.13	ND	Tr	ND	ND	ND	ND	ND	0.01	ND	0.01	ND	ND	ND	ND	ND	0.4
4	Marina	0.44	0.48	0.37	0.09	ND	0.41	ND	0.08	0.16	2.95	4.36	0.67	0.86	0.97	0.9	ND	0.2	ND	0.35	13.28
5	Um Al-Maradim	0.04	0.25	0.13	0.14	1.51	0.06	0.05	0.69	0.22	0.89	0.28	0.49	0.2	0.17	0.37	0.06	ND	ND	ND	5.56
6	Kuwait Bay	0.01	0.01	0.01	0.02	0.02	Tr	Tr	0.04	0.03	ND	0.12	0.11	0.1	ND	0.2	ND	ND	ND	ND	0.66
7	Durrar	ND	Tr	Tr	Tr	0.2	Tr	Tr	Tr	0.05	0.02	0.02	0.02	0.01	0.01	0.01	ND	ND	ND	ND	0.34
8	Kubber	ND	0.06	0.02	0.06	0.04	Tr	Tr	0.18	0.05	0.55	0.02	0.07	0.01	0.03	ND	0.18	ND	0.08	0.07	1.43
9	Marina	0.02	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.04	0.06	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.21

Pr = Pristane; Py = Phytane; ND = Not Detected; Tr = Traces *amphitrite* was found. Analysis of young *Balanus amphitrite* showed only 1.44 μg g^{-1}, which is only 39% of the concentration detected in *Balanus tintinabulum* samples of 1996. The decrease in PAHs concentration may be species and age related. Barnacles (*Balanus amphitrite*) collected from the yacht club marina, Salmiyah, Kuwait city in June 1997 showed a total concentration of 1.09 μg g^{-1}. (wet wt.) of PAHs, while samples collected in March 1998 showed a highly increased concentration of 5.30 μg g^{-1}. Furthermore, samples collected in October 1999 showed yet further increase in concentration of PAHs to 8.21 μg g^{-1} (Table 2). This indicated a higher level of marine pollution in the marina in Kuwait city which may be due to the combined effect of slow release of these hydrocarbons from the sediment as well as from marina boating activities. In 1999, in order to confirm the level of hydrocarbon pollution in Kuwaiti territorial waters, samples were collected from three more locations namely Kuwait Bay, Kubbar island and Um Al-Maradim island. Barnacles (*Balanus amphitrite)* collected from Um Al-Maradim showed a total value of 7.73 μg g^{-1} (wet wt.). Kuwait bay site had only young *Balanus amphitrite* as living animals and showed a concentration of 0.61 μg g^{-1}. Kubbar island which used to harbour numerous quantities of barnacles had only a small number of young living barnacles. This site showed a concentration of 0.16 μg g^{-1} (wet wt.). It is interesting to note that the highest concentration of the most potent individual PAHs were found in all barnacle samples of marina, Al-Durrar and Um Al-Maradim (Table 2). The low concentration of PAHs were associated with young barnacles collected in 1999 from Kuwait Bay, Kubbar island and Al-Durrar. The massive barnacle kill observed in Kuwait bay, Kubbar island and recently at Al-Durrar might be attributed to hydrocarbon toxicity as well as other factors. In these barnacle samples Ph/An and Flu/Py ratios were calculated in order to define their origin. In this study all the samples, except those from Um Al-Maradim, exhibited Ph/An ratios lower than 10 and Flu/Py ratios ≈ 1 or more suggesting their strong pyrolytic origin (Yang 2000). This could well be related to the oil discharge and oil fires of the 1991 Gulf war origin.

Analysis of alkanes is given in Table 3. Barnacle samples (*Balanus amphitrite*) collected from the marina during 1997 showed a total of 0.40 μg g^{-1} (wet wt.) alkanes, while samples collected in 1998 showed a total of 13.28 μg g^{-1} (wet wt.). However, the concentration of these compounds in samples collected in 1999 dropped to 0.21 μg g^{-1} (wet weight). This drop in alkane accumulation to 50% of its original level observed in 1997 might be due to microbial degradation of alkanes during this period of time, aided by increased human activities in the marina since Kuwait was liberated. In the 1997 marina samples, n-C_{17} had the highest concentration, while in 1998, n-C_{22} alkane was predominant. This indicated an increased marine contamination by crude oil derived pollutants. The Al-Durrar samples of *Balanus tintinabulum* collected in June 1996 showed a total of 2.81μg g^{-1} (wet wt.) alkanes, while during June 1997 it showed an increased level to 3.25 μg g^{-1} (wet wt.) HCs. Um Al-Maradim, Kubbar, Kuwait Bay and Al-

Durrar barnacle samples collected in 1999 (*Balanus amphitrite*) showed 5.56 μg g^{-1}, 1.43 μg g^{-1}, 0.66 and 0.34 μg g^{-1} (wet wt) concentration of alkanes respectively. The dominance of the n-alkanes C_{18} - C_{24} in these samples suggested a relatively recent oil input (Readman et al. 1986). This was verified by calculating the carbon preference index (CPI) for n-alkane odd/even between n-C_{18} - C_{25}. The CPI values of 0.81, 0.56 and 0.54 for marina (1998), Kuwait Bay (1999) and Al-Durrar (1999) samples respectively indicated a recent oil contamination (Al-Lihaibi and Ghazi 1997). The CPI values for other samples was >1, suggesting no recent oil input .

Our results confirm increasing trend of petroleum hydrocarbons in the Arabian Gulf. There are reports to show that the concentration of total petroleum hydrocarbons in the Gulf coastal and offshore sediments and fishes are increasing with time (Al-Lihaibi and Ghazi 1997; Talat et al. 1995). The time dependent increase of PAH and HC concentrations in barnacles may be due to the persistent presence of hydrocarbons in the Gulf waters that could lead to contamination of food chain.

Accumulation of pyrene, benzo [a] anthracene and benzo [a] pyrene in our samples is alarming, since these PAHs are potent carcinogens to mankind. Their presence in high concentrations is a marker for the Gulf marine environmental pollution, potentially affecting edible fish in this region. The amount of oil may not be important when evaluating the possible biological effect of hydrocarbons in the environment, but the type and concentration of the pollutants are of prime importance (Lee and Page 1997). Transportation of crude oil and oil production related activities have not increased steeply, nor was there a major oil spill in the Gulf marine environment since the 1991 Gulf war to justify the large increase of PAHs and HCs, suggesting that the increased concentration due to some other factors such as crude oil deposit in the sediment from the oil spill during the 1991 Gulf war slowly becoming bioavailable. It is our intention to extend this study into the future, in order to gauge the alarming increase of these harmful hydrocarbons in the marine waters, as their presence and continued increase can have serious implications on marine animals and human health as well as on fisheries in the Gulf.

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