Polycyclic Aromatic Hydrocarbons and Aliphatics in the Coral Reef Skeleton of the Egyptian Red Sea Coast

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Polycyclic Aromatic Hydrocarbons (PAHs) are natural constituents of crude oil and are a mixture of organic compounds of fossil and biogenic origin. They account for about 20% of total hydrocarbons in crude oil and are the most biologically toxic of all the petroleum compounds (Neff 1990). Generally, a mixed petroleum product containing a broad spectrum of hydrocarbon classes is released to the marine environment, where it may affect a variety of biological processes and be a potent cell mutagen and carcinogen (Capone and Bauer 1992). On the other hand, the highest concentrations of PAHs are generally found around urban centers (Meador et al. 1995). The higher-molecular weight PAHs can be acutely toxic which, when ingested by marine animals, can form metabolites that are active carcinogens. The PAHs are formed as a consequence of incomplete combustion (e.g., from fossil fuels) and they reach the marine environment via effluent discharges, urban run-off, atmospheric transport, and the spillage or disposal of oil and petroleum products. As they are hydrophobic (log K_{ow} = 3-8), PAHs tend to adsorb to particulate material, be deposited in the underlying sediments, and enter the food chain.

It is estimated that approximately 6.1 million metric tons of petroleum products are released to global oceans annually, the majority of which is derived from anthropogenic sources and which pass through the coastal zone before being carried out to sea (Capone and Bauer 1992). Worldwide, major inputs of petroleum into the marine environment occur via industrial discharge and urban run-off (37%), vessel operations (33%), tanker accidents (12%), atmospheric deposition (9%), natural resources (8%) and exploration production (2%).

The Egyptian Red Sea coast has been developed for industrial, tourist, and residential uses. As a result, the concentration of pollutants in the Red Sea may increase day by day if steps are not taken for their protection. However, coral reefs which are present on the Egyptian coast of the Red Sea, are among the most deteriorated ecosystems and considered to be in critical status in many places (Wilkinson 1992). Various effects of pollution on coral reef organisms and communities have been documented (Loya and Rinkevich 1980; Hatcher et al. 1989; Rogers 1989; Hughes 1994).
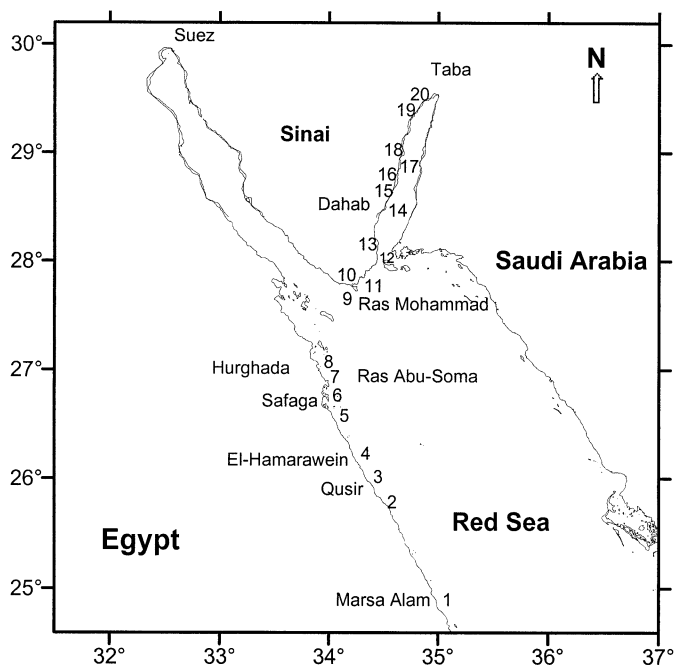
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Figure 1. Sampling locations in the Egyptian Red Sea.

No systematic studies have been conducted on coral reefs in the Egyptian part of the Red Sea (DANIDA 1996; EIMP 1996). Therefore, our aim was to determine the levels of hydrocarbon pollutants in the coral reef skeleton of the Egyptian Red Sea coast.

MATERIALS AND METHODS

Coral reef sampling stations were located along the Red Sea coast starting from Marsa Alam to Taba (about 900 km, Fig. 1). Coral reef samples (*Acropora* sp.) were collected at 20 sites within a period of two weeks during April 1999. At each site, the samples were collected within a reef area of 1 km² with collection depths ranging from 2-5 m. Coral samples were sun-dried, placed in pre-cleaned aluminum bags, and transported to the laboratory. Coral samples were washed with tap water and then with distilled water, and oven dried at 45°C for 48 hr. Sub-samples (100-150 g) of coral skeleton were crushed and homogenized before extraction with organic solvents.

Each coral reef sample (30 g) was Soxhlet extracted for 8 h into 200 ml of 1:1 hexane-dichloromethane. The extracted solvent was concentrated down to a few milliliters using rotary evaporation followed by gentle nitrogen "blow down".

Clean-up and fractionation was performed by passing the extract through a silica/alumina column (silica and alumina were activated at 200 °C for 6 h and

then partially deactivated with 5% water as described by Ehrhardt 1987). The chromatography column was prepared by slurry packing 10 ml of silica, followed by 10 ml of alumina and finally 1 g of sodium sulphate. Elution was performed using 35 ml of hexane to yield the first fraction (which contained the aliphatic hydrocarbons), then 50 ml of hexane/dichloromethane (90:10) (which contained the polycyclic aromatic hydrocarbons).

Fractions were then analyzed by gas chromatography (GC) using a Hewlett-Packard HP5890 series II with flame ionization detector (FID), split/splitless injector and a fused silica capillary column (HP-1, 30m length, 0.32mm i.d., 0.17μm film thickness) 100% dimethylpolysiloxane. The GC temperature was programmed from 60 °C to 290 °C at 3 °C min^{-1} and was then maintained at 290 °C for 25 min. A 2μL splitless injection was used, and the injection port was maintained at 290 °C. Helium was used as a carrier gas at a flow of 2ml min^{-1}.

A stock solution containing the following PAHs was used for quantitation: naphthalene, acenaphthylene, acenaphthane, fluorine, phenathrene, anthracene, fluoranthene, Benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k) fluoranthene, pyrene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(ghi) perylene and indeno(1,2,3-cd)pyrene by dilution to create a series of calibration standards of PAHs at 0.1, 0.25, 0.5, 0.75, 1.0, 2.0, 5.0, and 10 μg/ml. The detection limit was approximately 0.01 μg/ml for each PAH.

To control the analytical reliability and assure recovery efficiency and accuracy of the results, 8 analyses were conducted on PAH compound reference materials, HS-5 and SRM 1491 (provided by International Atomic Energy Agency (IAEA)). The laboratory results showed recovery efficiency ranged from 90-114% with coefficient of variation (CV) from 6-15 %.

RESULTS AND DISCUSSION

Concentrations of PAHs and aliphatics in coral reef skeleton presented (Table 1) are the means of three replicated extractions. Petroleum derived PAHs (containing three or less aromatic rings with a high proportion of alkylated homologues) and pyrogenic PAHs (parental compounds with four or more aromatic rings) were present. The results represent average concentrations of sixteen detectable n-alkanes (n-C$_{12}$ to n-C$_{40}$) in coral reef skeleton ranged from 0.5 ng g^{-1} at Hibeiq Ras Nabar (St. No. 16) to 78 ng g^{-1} at Ras Mamlah (St. No. 15) with an average 22 ng g^{-1}. On the other hand, the range of sixteen detected PAHs in the coral reef skeleton ranged between 269 ng g^{-1} at El Hamarawien ref (St. No. 4) and 3985 ng g^{-1} at Nakhlat El-Tal (St. No. 13) with an average 1719 ng g^{-1}.

The range of total hydrocarbons (F1 + F2) in coral reef skeleton was 291 to 3944 ng g^{-1} with an average of 1740 ng g^{-1}. The total aliphatic concentration in fraction (F1) was lower than the corresponding aromatic in fraction (F2) for all collected coral samples.

Table 1. Concentration (ng g^{-1}) of PAHs in coral reef samples.

Sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
NAPH	179	288	537	nd	153	34	175	542	81	nd	nd	nd	140	80	99	nd	200	266	nd	nd
ACTHY	246	972	2278	nd	1140	113	1076	1743	140	nd	nd	nd	189	161	155	13	249	241	nd	nd
ACE	273	268	408	nd	261	40	565	532	84	nd	nd	nd	97	62	66	nd	162	500	17	nd
FL	180	116	51	nd	134	123	229	169	397	nd	nd	nd	121	64	23	nd	51	279	71	202
PHE	53	31	38	nd	55	106	37	40	112	16	20	nd	91	16	20	7	37	130	150	16
ANT	3	10	19	nd	5	34	9	4	243	nd	68	44	12	7	51	nd	4	143	nd	nd
FLTH	93	56	154	nd	148	150	131	55	329	40	49	44	58	58	74	96	50	90	46	23
PYR	31	22	147	nd	48	41	49	20	116	14	22	15	13	19	21	34	24	19	15	10
BaA	103	22	161	nd	86	187	57	70	243	22	39	26	140	48	119	103	122	78	nd	72
CHR	87	33	46	18	60	118	51	48	248	12	71	25	14	26	106	18	87	415	408	52
BbF	5	13	7	nd	8	41	21	9	75	16	129	6	14	8	35	nd	13	231	112	nd
BkF	9	8	13	13	8	72	12	7	79	nd	129	182	22	7	62	4	21	209	232	13
BaP	93	32	34	20	77	171	57	51	283	111	215	22	1255	51	73	16	87	336	409	44
DBA	42	10	15	54	27	48	39	29	86	31	17	30	842	57	48	33	37	25	9	39
BghiP	16	15	8	88	12	46	56	50	75	41	45	38	63	32	28	20	145	36	23	58
InP	46	10	15	78	29	67	71	28	110	53	49	141	915	27	23	27	103	90	32	85
Σ PAH	1461	1904	3931	269	2250	1390	2635	3396	2701	356	853	531	3985	723	1002	373	1393	3089	1524	613
Σ PAH$_{CARC}$	299	94	244	164	234	585	257	193	876	233	577	408	3188	199	359	184	384	970	794	252
n-C$_{16}$	0.3	0.3	1.2	1.8	0.7	0.3	0.4	0.4	0.2	0.8	0.2	0.1	0.1	nd	8.6	nd	0.1	9.1	1.7	0.1
n-C$_{26}$	3.1	0.2	0.4	0.9	1.5	0.4	0.6	0.5	0.2	0.4	0.3	0.2	0.1	nd	2.1	nd	0.1	3.2	1.3	0.2
Σ n-C$_{12}$- n-C$_{40}$	37.7	7.7	13.0	20.9	27.0	14.9	5.6	6.8	7.9	34.9	4.3	6.6	3.8	1.1	78.8	0.5	2.2	87.7	26.2	5.8
pristane	nd	nd	nd	nd	nd	0.09	nd	nd	nd	0.12	nd	nd	nd	nd	nd	nd	nd	nd	0.1	0.1
UCM-ALP	0.02	0.05	0.03	0.04	0.02	0.02	0.03	0.05	0.03	0.03	0.02	0.03	0.03	0.03	0.02	0.04	0.03	0.02	0.03	0.03
Σ ALP	37.7	7.7	13.0	21.0	27.0	15.0	5.7	6.9	7.9	35.0	4.4	6.6	3.8	1.1	78.8	0.6	2.2	87.7	26.4	5.9

UCM: unresolved complex mixture; ALP: aliphatics; Σ PAH$_{CARC}$: B[a]An + B[b]Fl + B[b]F1 + B[a]Py + dB[a,h]An + I[1,2,3-cd]Py (IARC 1991, probable and possible human carcinogens); nd: Non detectable; NAPH: Naphthalene; ACTHY: Acenaphthylene; ACE: Acenaphthane; FL: Fluorene; PHE: Phenanthrene; ANT: Anthracene; FLTH: Fluoranthene; PYR: Pyrene; BaA: Benzo(a)anthracene; CHR: Chrysene; BbF: Benzo(b)fluoranthene; BkF: Benzo(k)fluoranthene; BaP: Benzo(a)pyrene; DBA: Dibenzo(a,h)anthracene; BghiP: Benzo(g,h,i)perylene; InP: Indeno(1,2,3-cd)pyrene.
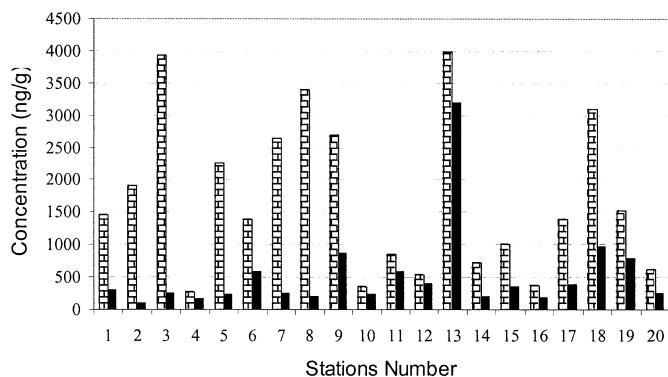
Figure 2. Concentration of Σ PAHs (block) and Σ PAH$_{CARC}$ (black) in coral reef.

The low proportion of unresolved complex mixture (UCM, appeared as a broad unimodal hump in the range C_{12} to C_{36}) of aliphatics to resolved aliphatics (Table 1) suggests that most n-alkane contaminants were recently discharged into the marine environment. This distribution suggests that some of these contaminants may originate from urban runoff, municipal wastes, petroleum industries, oil refineries and oil distribution.

The n-alkane C_{16} (n-hexadecane) is rarely found in biolipids (Thompson and Eglinton 1978) but appeared in all chromatograms. The presence of n-alkane C_{26} (n-hexacosane) in all studied coral reef samples suggests possible bacterial contamination (Shaw and Wiggs 1980). Pristane (C_{19}) and phytane (C_{20}) are common isoprenoids deteced in coastal marine sediments. They are present in most petroleums, usually as the major constituents within a much wider range of isoprenoid alkanes and usually considered as good indicators of petroleum contamination (Readman et al. 2002). Phytane and pristane were detected at four stations (St. No. 6, 10, 19 and 20) at very low concentrations ($\sim$0.1 ng g^{-1}) of pristane (pristane may be obtained from oxidation of the phytol side-chain of chlorophyll).

Naphthalene, acenaphthylene and acenaphthane were the prominent PAHs in most studied samples. On the other hand, benzo[a]pyrene and the sum of six carcinogenic PAHs (Σ PAH$_{CARC}$)(IARC 1991) were highest at Stations No. 8, 13, 18 and 19, showing a concentrations of 876, 3188, 970 and 794 ng g^{-1}, respectively (Fig. 2).

The simultaneous occurrence of isomer ratios phenanthrene/anthracene < 10 and fluoranthene/pyrene >1 in most stations indicates that most PAHs are of pyrolytic (combustion) origin (Garrigues et al. 1995; Bentahcen et al. 1997). The values of PHE/ANT can be plotted against the values of FLTH/PYR (Fig. 3) showing that PAHs are from pyrolytic resources (Baumard et al. 1998; Readman et al. 2002).
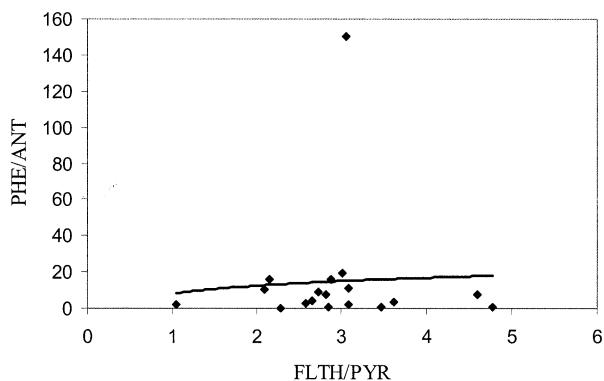
Figure 3. Plot of isomeric ratios PHE/ANT (phenanthrene vs anthracene) vs FLTH/PYR (fluoranthene vs pyrene) for coral reef from the Egyptian Red Sea coast.

Table 2. Sources of pollution in the Gulf of Aqaba and Red Sea.

Sources of Pollution and others impacts	Main sites and/or other impacts
Sewage from cities and tourist resorts	Hurghada (land-filling, tourism)
Shipment of mineral products (mainly phosphate)	Safaga, Quseir and El Hamarawein (tourism and shipment of phosphate)
The northern part of the Red Sea (offshore and onshore oil production and transport facilities)	Aqaba in Jordan and Eilat in Israel (tourism, oil terminals and phosphate loading operations)
Land-filling, dredging and siltation	Sharm El Sheikh, Na`ama Bay, Ras Nasrani, Dahab, Nuweiba and Taba

The major inputs of hydrocarbon pollutants in the Egyptian Red Sea coast are summarized in Table 2. The PAHs can be divided into three main classes according to the characteristics of their fingerprints. First, the most important input of PAHs into the environment is from combustion of organic matter, anthropogenic industrial activity, and/or natural fires. These sources give rise to complex mixtures of PAHs characterized by a high abundance of parent PAHs and a low abundance of alkylated PAHs. The second class is constituted of petroleum hydrocarbons due to petroleum transportation, off-shore exploitation, and/or natural seeps. The composition of petroleum hydrocarbons is very complex and is characterized by a high abundance of alkylated PAHs. Finally, some compounds may have a diagenetic origin. For instance, perylene can be derived from biogenic precursors via short-term diagenetic processes. The origin of perylene is quite controversial. Perylene could also derive from aquatic material or diatoms (Budzinski et al. 1997; Laflamme and Hites 1988; Venkatesan 1988). In the case of diagenetic origin only a few compounds are generated in

comparison to the complex mixtures of PAHs generated by the other sources. The ranges for total aliphatic (F1) and aromatic (F2) content in the present monitoring were shorter than the corresponding ranges reported in some of the published surveillance and monitoring studies of coastal areas from various regions (Hughes 1994; Loya and Rinkevch 1980). Accordingly, the coastal area in the Red Sea, Egypt, might in this sense be considered as less polluted especially for total aliphatics.

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