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Polynuclear Aromatic Hydrocarbons (PAHs) in fish from the Red Sea Coast of Yemen

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Abstract

A detailed analytical study using combined normal phase high pressure liquid chromatography (HPLC), gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) of Polynuclear Aromatic Hydrocarbons (PAHs) in fish from the Red Sea was undertaken. This investigation involves a preliminary assessment of the sixteen parent compounds issued by the U.S. Environmental Protection Agency (EPA).

The study revealed measurable levels of Σ PAHs (the sum of three to five or six ring parent compounds) (49.2 ng g^{-1} dry weight) and total PAHs (all PAH detected) (422.1 ng g^{-1} dry weight) in edible muscle of fishes collected from the Red Sea. These concentrations are within the range of values reported for other comparable regions of the world. Mean concentrations for individual parent PAH in fish muscles were; naphthalene 19.5, biphenyl 4.6, acenaphthylene 1.0, acenaphthene 1.2, fluorene 5.5, phenanthrene 14.0, anthracene 0.8, fluoranthene 1.5, pyrene 1.8, benz(a)anthracene 0.4, chrysene 1.9, benzo(b)fluoranthene 0.5, benzo(k)fluoranthene 0.5, benzo(e)pyrene 0.9, Benzo(a)pyrene 0.5, perylene 0.2, and indeno(1,2,3-*cd*)pyrene 0.1 ng g^{-1} dry weight respectively. The Red Sea fish extracts exhibit the low molecular weight aromatics as well as the discernible alkyl-substituted species of naphthalene, fluorene, phenanthrene and dibenzothiophene. Thus, it was suggested that the most probable source of PAHs is oil contamination originating from spillage's and/or heavy ship traffic.

It was concluded that the presence of PAHs in the fish muscles are not responsible for the reported fish kill phenomenon. However, the high concentrations of carcinogenic chrysene encountered in these fishes should be considered seriously as it is hazardous to human health. Based on fish consumption by Yemeni's population it was calculated that the daily intake of total carcinogens were 0.15 μg/person/day.

Introduction

The need to identify organic and inorganic pollutants in the Red Sea has become a major concern for all countries in the region within the past few years. There are good reasons for this concern, among which is the need for baseline data (or background levels), the chronic pollution from industrial and other anthropogenic sources, and the acute oil pollution of the area which is a fishing ground. Because of the potential impact on marine life and fisheries, it is therefore important to know the extent of the pollution, how much it has affected marine life, and how long that effect will last, particularly from oil pollution. Due to the variety of organic and inorganic compounds that can be present in the marine environment and the complexity involved in analyzing all of them, the present study was limited to investigating the presence of certain Polynuclear Aromatic Hydrocarbons (PAHs).

PAHs are among the most ubiquitous organic pollutants in the marine environment. The study of PAHs in coastal marine environment is of great importance since these areas are biologically active and receive considerable pollutant inputs from land-based sources via coastal discharge. The carcinogenic properties of some compounds coupled with the stability of PAHs during their atmospheric and aquatic transport and their widespread occurrence have, in recent years, gener-

ated interest in studying their sources, distribution, transport mechanisms, environmental impact, and fate (Bouloubassi & Saliot, 1993). The proposed primary sources of the environmental complex assemblages of PAHs include petroleum-related sources (ship traffic, oil seepage, or spillage) as well as combustion of various fossil fuels, natural fires, and road runoff/street dust. Other sources of more localized significance but worthy of note include domestic and industrial waste waters and sewage (Bouloubassi & Saliot, 1991).

Carcinogenic PAHs are suspected toxicants to marine organisms (Malins et al., 1984) and can be transported over long distances adsorbed onto airborne and waterborne particles. Being hydrophobic, they tend to accumulate in sediment and biota (Govers, 1990). Adsorption onto suspended particles and accumulation on bottom sediments may remove PAHs from the water column and reduce the chance of their photo-decomposition (Literathy et al., 1991).

There is evidence linking haemosiderosis, and also internal and external lesions, in bottom dwelling/feeding fish with PAHs in sediments (Malins et al., 1984; Gibbs et al., 1986). It is, therefore, essential to assess temporal influences on the accumulation of PAHs in fish.

In Yemen the marine and coastal areas are of a major economic significance. Marine resources are exploited for local consumption as well as export. The report of Haskoning (1991) suggested that the main impacts presently affecting Yemen marine environment are pollution and over-exploitation of certain natural resources. Qualitative and quantitative data are still lacking on the expected source/s of pollution, and their impacts on the coastal environment. Thus, we present here, for the first time to the best of our knowledge, a detailed analytical study using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) of anthropogenic PAH in fish samples from the Southern Red Sea along the coast of Yemen. This investigation involves screening of PAHs in several fish species from the Red Sea to determine if these animals show evidence of oil contamination. Furthermore, coastal sediments as well as mussels were also collected to assess damage to there resources.

Description of the region

The Red Sea is a long, narrow body of water, separating north-east Africa from Arabian Peninsula. Its nearly 2000 km of navigable waters connects at the south with the Indian Ocean via Bab el-Mandeb. The average width of the Red Sea is 280 km, however, the width is only 28 km at the strait of Bab el-Mandeb. The maximum depth is 2246 m with an average of 700 m. The mean surface temperature increase southward, maximum surface water temperature is observed from June to September and attain 30 to 32 °C in the south (Edwards & Head, 1987). The shallow coastal waters may reach a temperature of 38 °C. The average salinity is about 35‰, but it is higher in shallow coastal areas as a result of evaporation.

The tides are semi-diurnal and spring tide ranges vary from 0.6 m in the north to 0.9 m in the south. The sea level is strongly influenced by the rate of evaporation and the balance between the inflow and outflow of the water from and to the Gulf of Aden. Surface water transport in summer is directed south by the prevailing northerly winds for about 4 months, at a velocity of 12–50 cm s^{-1}, while in winter the flow is reversed, pushing water into the Red Sea from the Gulf of Aden, the net value of the latter movement is greater than the summer outflow.

The Red sea is unique amongst deep bodies of water for having an extremely stable warm temperature throughout its deeper water. Below about 250–300 m, the water maintains a constant temperature of about 21.5 °C, which extends down to the sea floor in all areas except where heated brine pools exist (Edwards & Head, 1987).

Materials & methods

Materials

All solvents were redistilled in an all-glass distillation apparatus equipped with a 150 cm vacuum jacketed fractionation column filled with 3 mm diameter glass helices. Blanks of 1000-fold concentrates were determined by gas chromatography with flame ionization detection. Water used for cleaning the adsorption resin and sample work-up was purified with a Millipore Milli-Q system. Sodium chloride and sodium sulfate were kiln fired at 450 °C overnight and cooled in a greaseless desiccator. Silicagel used for column chromatography was solvent extracted with n-hexane in a glass cartridge inserted into an extraction apparatus, as described by Ehrhardt (1987). After extraction, the silica gel was first dried in the same cartridge by passing ultrapure nitrogen through it and was then activated by heating the cartridge in an electric tube oven to 200 °C

for 6 h with the stream of nitrogen reduced to a few ml per minute.

The high performance liquid chromatograph (HPLC) was a Perkin-Elmer series 4 equipped with a microprocessor controlled solvent delivery system and fitted with a Reodyne 7125 injection valve. The detection system was composed of a Perkin-Elmer 560S scanning fluorescence spectrophotometer and an LC 75 variable wave length spectrophotometric detector with auto-control and equipped with a Perkin-Elmer analytical LC-PAH 0258 column (250 mm $\times$ 5 mm i.d.) with acetonitrile-distilled water gradient elution at a flow rate of 1 ml min^{-1}. Quantification of peaks and identification of PAHs in chromatograms was achieved by an LCI-100 laboratory computing integrator (Perkin-Elmer). The gas chromatograph was a Hewlet Packard HP5890-GC with split/splitless injector furnished with a 25 m $\times$ 0.32 mm fused silica capillary with a chemically bonded gum phase SE54 (J & W Scientific, Inc.).

Sampling

The study was carried out along the Yemen coast in the Red Sea during January–March 1995. The site were chosen according to their importance as a hot spots and the suitability of obtaining samples (Figure 1). Fish samples were also taken from fishermen fishing off the Yemen coast. After collection, the fish samples were wrapped in aluminum foil, stored in a cool box and frozen upon return to the city center (2 hours on an average). A composite samples of fish, having similar size (length and weight) were chosen for each species. Sub-sample of each of the following species *Solea solea, Scombermorus malculatus, Rhochycentron canadum, Chorinemus lysan* and *Variola louti.*

Surface sediments from the sub-tidal coastal areas were collected by scuba divers, wrapped in aluminum foil, stored in a cool box and then frozen. The mussel *Thais sarignyi* (Reshayes, 1844) was found and collected from three location only. The whole samples were used for analysis after removing off the shells, and the body was washed with distilled water to remove any traces of sand, and then taken a known weight of the composite samples for chemical analysis.

Methods

The bulk sediment and tissue extraction procedure used was adapted from a method developed by Mcleod et al. (1985). Approximately 15 g of wet tissue were used for PAH analysis. After the addition of internal standards (surrogates) and 50 g of anhydrous Na_2SO_4, the tissue was extracted three times with dichloromethane using a tissuemizer. A 20 ml sample was removed from the total solvent volume and concentrated to one ml for lipid percentage determination. The 380 ml of remaining solvent was concentrated to approximately 20 ml in a flat-bottom flask equipped with three-ball Synder column condenser. The tissue extract was then transferred to a Kuderna-Danish tube heated in a water bath (60 °C) to concentrate the extract to a final volume of 2 ml. During concentration, the dichloromethane was exchanged for hexane.

The tissue extracts were fractionated by alumina:silica (80–100 mesh) open column chromatography. The silica gel was activated at 170 °C for 12 h and partially deactivated with 3% distilled water (v/w). Twenty grams of silica gel were slurry-packed in dichloromethane over 10 g of alumina. Alumina was activated at 400 °C for 4 h and partially deactivated with 1% distilled water (v/w). The dichloromethane was replaced with pentane by elution. The extract was then applied to the top of the column. The extract was sequentially eluted from the column with 50 ml of pentane (aliphatic fraction) and 200 ml of 1:1 pentane:dichloromethane (aromatic fraction). The aromatic fraction was further purified by HPLC to remove lipids. The lipids were removed by size exclusion using dichloromethane as isocratic phase (7 ml min^{-1}) and two 22.5 $\times$ 250 mm Phenogel 100 columns (Krahn et al., 1988). The purified aromatic fraction was collected from 1.5 min prior to the elution of 4,4′-dibromofluoro-biphenyl to 2 min after the elution of perylene. The retention times of the two marker peaks were checked prior to the beginning and at the end of a set of 10 samples. The purified aromatic fraction was concentrated to 1 ml using Kuderna-Danish tube heated in a water bath at 60 °C.

An initial screening of the 16 EPA-listed PAHs [naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenz(*ah*)anthracene, indeno(1,2,3-*cd*)pyrene, and benzo(*ghi*)perylene] were carried out (see Table 1). Results of HPLC analysis were complemented by a detail GC and GC/MS analysis at Texas A&M University, for the above EPA-listed PAHs as well as alkyl-substituted PAHs. PAHs were separated and quantified by GC-MS (HP5890-GC interfaced to a HP5970-MSD). The samples were injected in the splitless mode on to a 30 $\times$ 0.32 mm (0.32 μm film thick-

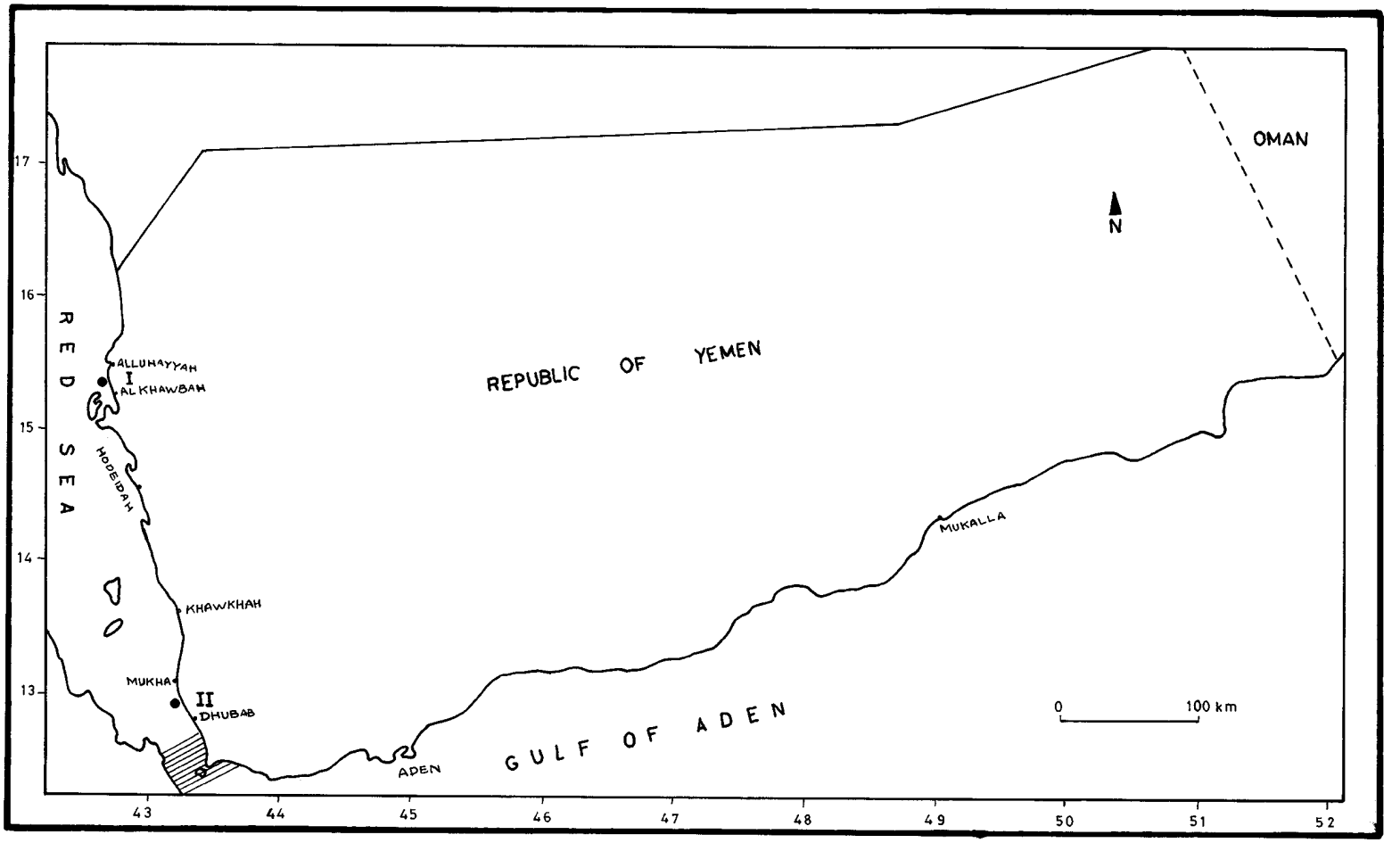
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Figure 1.

Table 1. Mean ∗ concentrations (μg g^{-1} wet weight) of 16 EPA-PAH in fish, mussels and coastal sediments from the Red Sea as determined by HPLC.

PAH Analyte	Fish		Mussel (*T. sarignyi*)				Sediment			
Site	*Solea solea*		*S. malculatus*		*R. canadum*		*C. lysan*		*V. louti*	
	I	II	I	II	I	II	I	II	I	II
Napthalene	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Acenaphthylene	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Acenaphthene	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Fluorene	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Phenathrene	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.03	<0.01	<0.01
Anthracene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fluoranthene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyrene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Benz(*a*) anthracene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chrysene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Benzo(*b*) fluoranthene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	<0.02
Benzo(*k*) fluoranthene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Benzo(*a*) pyrene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Dibenz(*ah*) anthracene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Benzo(*ghi*) perylene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	<0.02
Indeno(1,2,3-*cd*) pyrene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	<0.02
% Fat	3.4	2.7	2.7	3.04	0.78	14.91	0.41	0.50		

∗ = Mean of at least 3 determinations.

ness) DB-5 fused silica capillary column at an initial temperature of 60 °C and temperature programmed at 12 °C min^{-1} to 300 °C and held at the final temperature for 6 min. The mass spectral data were acquired using selected ions for each of the PAH analytes.

The GC/MS was calibrated and its linearity determined by injection of a standard containing 11 analytes at five concentrations ranging from 0.01 ng μl^{-1}. Sample component concentrations were calculated from the average response factor for each analyte. Analytical identifications were based on correct retention time of the quantitation ion (molecular ion) for the specific analyte and confirmed by the ratio of quantitation ion to confirmation ion. Calibration check samples were run with each set of samples (beginning, middle, and end), with no more than 6 h between calibration checks. The calibration check must maintain an average response factor within 10% for all analytes, with no one analyte greater than +25% of the known concentration. A laboratory reference sample (oil spiked solution) was also analyzed with each set of samples to confirm GC/MS system performance and calibration.

Quality assurance for each set of ten samples included a procedural blank, matrix spike, and tissue standard reference material (NIST-SRM 1974) which were carried through the entire analytical scheme. Internal standards (surrogates) were added to the sample prior to extraction and were used for quantitation. The surrogates were d-naphthalene, d-acenaphthene, d-phenethrene, d-chrysene, and d-pyrylene. Surrogates were added at a concentration similar to that expected for the analytes of interest. To monitor the recovery of the surrogates, chromatography internal standards d-fluorene and d-benzo(*a*)pyrene were added just prior to GC/MS analysis.

Recovery studies with fortified samples indicated that the recovery efficiency for naphthalene, acenaphene, phenantherene, chrysene, and perylene were 76.8%, 87.9%, 72.4%, 85.0% and 72.1% respectively. Results were not adjusted for percent recovery.

Table 2. Mean ∗ concentrations (ng g^{-1} dry weight) of 16 EPA-PAH in fish, mussels and coastal sediments from the Red Sea as determined by GC.

PAH Analyte	Fish (*Solea solea*)		Mussel (*T. sarignyi)*			
Site	I	II	I	II	I	II
Napthalene	19.6	20.4	21.0	21.3	12.2	19.0
Acenaphthylene	1.0	1.0	0.8	0.9	0.6	0.9
Acenaphthene	1.2	1.1	1.8	1.4	0.2	1.5
Fluorene	5.5	5.0	2.7	2.1	1.2	1.4
Phenathrene	14.0	13.0	8.4	7.6	2.0	1.6
Anthracene	0.8	0.7	0.7	0.7	0.3	0.3
Fluoranthene	1.5	1.5	2.2	2.0	0.7	1.0
Pyrene	1.8	1.7	2.4	2.0	0.5	0.5
Benz(*a*) anthracene	0.4	0.3	0.3	0.3	0.2	0.3
Chrysene	1.9	1.6	0.8	0.7	0.7	0.2
Benzo(*b*) fluoranthene	0.5	0.3	0.3	0.3	0.2	0.2
Benzo(*k*) fluoranthene	0.5	0.3	0.3	0.3	0.2	0.2
Benzo(*a*) pyrene	0.9	0.7	0.3	0.3	0.3	0.2
Dibenz(*ah*) anthracene	0.1	0.1	0.2	0.1	0.2	0.1
Benzo(*ghi*) perylene	0.5	0.2	0.2	0.2	0.3	0.1
Indeno(1,2,3-*cd*) pyrene	0.1	0.1	0.1	0.1	0.2	0.3
ΣPAH	50.3	48.0	42.5	40.3	20.0	27.8
% Fat	2.92	2.89	0.42	0.47		

∗ = Mean of at least 3 determinations.

Results

The use of HPLC for the initial screening of the 16 EPA-listed PAHs *viz.* naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenz(*ah*)anthracene, indeno(1,2,3-*cd*)pyrene, and benzo(*ghi*)perylene in fish from the Red Sea has revealed that these pollutants were below the detection limits of 0.05, 0.05, 0.05, 0.05, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, and 0.01 respectively (Table 1). However, the combined use of normal phase HPLC, GC and GC/MS analysis in the present study provided a powerful tool to isolate and further resolve the complex fish muscle PAH assemblages into simpler fractions. Thus, more than 40 compounds were identified and quantified. They comprise unsubstituted two- to six-ring PAHs (i.e. parent PAH), several alkyl-substituted homologs as well as sulfur-heterocyclics (benzothiophenes, benzonaphthiophenes and their alkylated homologs). The concentrations of individual parent PAH in fish muscles collected from the Red Sea ranged from less than 1 to tens of nanograms per gram dry weight (Table 2). In many environmental studies dealing with PAH, the concentrations have been reported as the sum of three- to five- (or six-) ring parent compounds, i.e. PAH with molecular weight 178 (phenanthrene, anthracene), 202 (fluoranthene, pyrene), 228 (chrysene + triphenylene, benzo(*a*)anthracene), 252 (benzofluoranthenes, benzo(*a*)pyrene, benzo(*e*)pyrene) and 276 (indeno [1,2,3-*cd*]pyrene, benzo[*ghi*]perylene). Their sum is referred to here as ΣPAH (mean concentration of ΣPAH in the Red Sea fish was 49.2 ng g^{-1} dry weight), whereas the sum of all PAHs detected is noted as totPAH (mean concentration of totPAH in the Red Sea fish was 422.1 ng g^{-1} dry weight). While the widespread use of ΣPAH for assessing pollution levels can facilitate comparison between various studies, this parameter does not however, represent the bulk amount of PAH occurring in environmental samples especially in a region like the Red Sea and Gulf of Aden. Our data showed that the concentration of total PAH (tot PAH) were higher by a factor of more than 8. This underline the quantitative importance of the compounds not-included in the

ΣPAH parameter. Although this can facilitate comparisons, it may also underestimate, sometimes severely, the bulk amount of PAH occurring in investigated samples. Moreover, from qualitative point of view, this parameter does not take into account PAH derived mainly from fossil sources, since the latter are characterized by high abundances of alkylated homologes and sulfur-heterocyclics (Readman et al., 1991) as it will be discussed bellow. The latter source is particularly important in a region like the Red Sea and Gulf of Aden where crude oil contribution of PAH exceed that of pyrolytic/urban source. In terms of toxic effects, some of alkylated homologes have mutagenic or carcinogenic properties (e.g. 1-methyl-phenethrene whose mean concentration in fish muscle was 5.0 ng g^{-1} dry weight). For this reason we preferred to use in this study the sum of all PAH compounds identified and quantified as summarized in Table 3. These constituents derived from anthropogenic sources with the exception of two compounds, tetrahydrochrysenes (αTHC and βTHC) which have natural terrestrial precursors.

Discussion

Unsubstituted (parent) PAH

Unsubstituted compounds were the minor fraction of PAH components in all fish samples. Among them, two-ring (naphthalene MW 128, biphenyl MW 154) and three-ring PAH (phenanthrene MW 178 and fluorene MW 166) dominated the distribution. Such patterns are characteristic of PAH mixtures generated by petrogenic pollution (Sauer et al., 1993). Naphthalene was the most prevalent parent compound (mean concentration was 19.5 ng g^{-1} dry weight) because it is more water soluble and has lower particulate affinity than the larger molecular weight aromatic hydrocarbons. In common with our findings, studies of PAHs indicated that naphthalenes are the compounds accumulated in highest concentrations by marine organisms (DouAbul et al., 1987). The second more abundant parent compound was phenathrene (mean concentration was 14.0 ng g^{-1} dry weight) which is a principal PAH component of crude oil. PAHs generated during high temperature combustion are mainly higher molecular weight (>4 aromatic ring) non-alkylated compounds, many of which are carcinogenic. One such PAH, chrysene, is normally produced through combustion (Readman et al., 1986) and was present in fish

Table 3. Mean ∗concentrations (ηg/g dry weight) of PAHs(parent + alkyl substituted) in fish, mussel and coastal sediment from the Red Sea

PAH Analyte	Fish (*Solea solea*)	Mussel (*T. sarignyi*)	Sediment
Naphthalene	19.5	21.0	12.2
C1-Naphthalenes	20.8	27.6	10.3
C2-Naphthalenes	28.6	90.6	8.9
C3-Naphthalenes	50.4	92.3	10.9
C4-Naphthalenes	25.4	32.5	8.0
BiPhenyl	4.6	3.7	1.2
Acenaphthylene	1.0	0.8	0.6
Acenaphthene	1.2	1.8	0.2
Fluorene	5.5	2.7	1.2
C1-Fluorenes	9.8	6.6	ND
C2-Fluorenes	20.2	9.8	ND
C3-Fluorenes	21.7	ND	ND
Phenanthrene	14.0	8.4	2.0
Anthracene	0.8	0.7	0.3
C1-Phen-Anthr	19.7	9.8	ND
C2-Phen-Anthr	18.3	8.1	ND
C3-Phen-Anthr	14.6	13.3	ND
C4-Phen-Anthetr	ND	ND	ND
DiBenzoThio	10.3	2.5	0.8
C1-DiBen	22.1	8.5	1.8
C2-DiBen	24.2	20.6	3.4
C3-DiBen	12.1	16.8	3.6
Fluoranthene	1.5	2.2	0.7
Pyrene	1.8	2.4	0.5
C1-Fluoran-Pyr	3.7	3.1	ND
Ben(a)Anthracene	0.4	0.3	0.2
Chrysene	1.9	0.8	0.7
C1-Chrysene	4.6	3.0	ND
C2-Chrysene	7.7	2.6	ND
C3-Chrysene	ND	1.4	ND
C4-Chrysene	ND	ND	ND
Ben(b)Fluoran	0.5	0.3	0.2
bEN(k)Fluoran	0.5	0.3	0.2
Ben(e)Pyrene	0.9	0.3	0.3
Ben(a)Pyrene	0.5	0.3	0.3
Perylene	0.2	0.2	0.4
I123cdPyrene	0.1	0.1	0.2
DBahAnthra	0.1	0.2	0.2
BghiPerylene	0.5	0.2	0.3
2-MethylNaph	10.5	14.9	5.6
1-MethylNaph	10.2	12.7	4.7
2,6-DiMethNaph	11.3	32.9	2.6
1,6,7-TriMethNaph	15.4	22.0	1.7
1-Methyl Phen	5.0	2.3	1.5
Total PAHs	421.5	480.3	85.1

∗ Mean of at least 3 determinations. ND = Below the detection limit of 0.1 ηg/g dry weight.

muscle extracts at a mean concentration of 1.9 ng g^{-1} dry weight.

The rather low concentrations of individual PAH observed in the Red Sea fishes may be attributed to rapid metabolism of PAHs by fish coupled with the rather limited source of pyrolytic/urban PAHs in the Red Sea thus led to steady state tissue levels of these compounds, and accounts for the failure in the present study to demonstrate appreciable levels of most parent PAHs in the examined samples. Accumulation and depuration of PAHs in fish can be influenced by various factors including route and length of exposure, lipid content of tissues, environmental factors (e.g. salinity, temperature... etc.), differences in species, age and sex and exposure to other xenobiotics (Varanasi et al., 1989). It is also indicated in the literature that fish efficiently metabolize PAH (Ahokas & Pelkonen, 1984). However, the metabolism of benzo(*a*)pyrene can lead to the formation of products which are more toxic than B(*a*)P itself (Gmur & Varanasi, 1982). During the metabolism of petroleum-derived aromatic hydrocarbons, the ability of an organism to process PAHs may be altered by the presence of polar components (Varanasi & Stein, 1991), including the concentrations of produced PAH metabolites (Schmeltz et al., 1978). Furthermore, the pattern of distribution of carcinogenic PAHs (including the levels in edible flesh) could be potentially affected by exposure to other xenobiotics which induce or inhibit xenobiotic-metabolizing enzyme systems (Gooch et al., 1989) or alter excretory pathways (Pritchard & Renfro, 1984).

The significance of our data (mean concentration of ΣPAH was 49.2 ng g^{-1} dry weight) is best appreciated, however, by comparing them with values of ΣPAH found in fish collected from other part of the world. For example it is lower than the maximum PAHs concentration in fish from Puget Sound, Washington USA (160 ng g^{-1} dry weight) reported by Landlot et al. (1987). It is comparable to the maximum PAHs concentration in sand flathead fillet collected from Port Phillip Bay, Victoria (55.7 ng g^{-1} dry weight) (Nicholson et al., 1994). DouAbul et al. (1987) have reported a maximum PAHs value in fish from the Arabian Gulf of 118 ng g^{-1} dry weight.

For the purpose of comparison between our results and those reported in the literature in areas of variable PAH contamination, it is necessary to convert the PAH concentration from ng g^{-1} dry weight to ng g^{-1} wet weight. In this investigation, an average reduction of weight of 75% during drying was used in the estimation. The mean ΣPAH concentration 49.2 ng g^{-1} dry weight × 0.25 dry weight/(wet weight) = 12.3 ng g^{-1} wet weight indicates that the level of PAH encountered in edible muscles of fish from the Red Sea lie within the range of values reported for zones defined as unpolluted (<0.5–148.0 ng g^{-1} wet weight) (Pancirov & Brown, 1977; Losifidou et al., 1982; Takatsuki et al., 1985). Rainio et al. (1986) found few PAH components and at low levels in fish muscle collected from the Finnish archipelago sea. Neff & Anderson (1981) state that the work conducted by a number of researchers indicates that the majority of marine fin fish contain very low or undetectable levels of PAHs. Strikingly enough, we found that the mean concentrations of ΣPAHs in our samples were only 50% of the average concentration of ΣPAH (105.3 ng g^{-1} dry weight) in edible tissue of fish collected from the Arabian Gulf after the 1991 oil spill (Al-Yakoob et al., 1993). Despite the fact that between 6 to 10 million barrels of crude oil were released to the Gulf during the conflict (Thorhaug, 1992).

Alkyl-substituted PAH

The average concentration of totPAHs in the edible parts of fish from the Red Sea was 422.1 ng g^{-1} dry weight. However, the alkyl-substituted species comprise the bulk of this total (mean concentration was 372.9 ng g^{-1} dry weight), which indicates that the major source of PAHs in the Red Sea and the Gulf of Aden is petrogenic. It is unfortunate that to the best of our knowledge, there is no available relevant data to compare our results with. Most of the published work dealt with the parent PAH compounds. This is reasonable in the light of the fact that the bulk of these studies were carried out in industrialist nations whose major source of PAH originate from pyrolytic/urban source rather than from crude oil spillage's. Mean concentrations of individual alkyl-substituted PAH in the fish muscles from the Red Sea ranged from ND (below the detection limit of 0.1 ng g^{-1}) to tens of nanograms per gram dry weight (Table 3). Prominent among these are; methyl-naphthalenes, alkyl-fluorenes, Dibenzothiophenes, and methyl-phenantheren.

The low molecular weight compounds (<3 aromatic ring) and their alkylated homologues are principal constituents of crude oil (Gundlach et al., 1983; Readman et al., 1986), and our analyses demonstrated that the total resolved aromatic components generally covaried with the total petroleum hydrocarbons in fish samples. The concentration of alkyl-substituted PAHs exceed the concentrations of parent PAHs in all classes,

as is typical for petroleum residue (Ehardht & Burns, 1993).

High concentrations of petroleum-related PAHs e.g. alkylated phenantherens and dibenzothiophenes were evident in all samples of fish muscle by GC/MS. In particular, the alkylated dibenzothiophenes were present in higher proportions than were other PAHs (Table 3). These compounds are known to comprise a high proportion of Marib light oil a crude that is produced from Yemen and exported via a terminal in the Red Sea (Geochem Group Limited, 1990). However, pyrogenic PAHs were present in low or non-detectable concentrations. Analogous results i.e. high concentrations of petroleum-related PAHs characteristic of Kuwait crude oil were found by HPLC and GC/MS analyses of fish following the Gulf War (Krahn et al., 1993a). Similarly high concentrations of petroleum-related PAHs characteristic of Prudhoe Bay crude oil were found by HPLC and GC/MS analyses of sediments following the *Exxon Valdez* oil spill in Alaska (Krahn et al., 1993b).

Among the sulfur-heterocyclics detected in the edible tissues of fish from the Red Sea, dibenzothiophene (DBT) and its alkylated homologs (C_1-, C_2-, and C_3-DBT) were the most abundant. The sum of their concentration (ΣDBT) was 68.7 ng g^{-1} dry weight (Table 3). The compounds dominance of the dibenzothiophenes in Marib light oil samples which is a distinct chemical feature of crude oil in this area of the world. In the Red Sea fishes this distribution was similar in almost all samples, characterized by the predominance of the mono- and di-methyl DBT.

Origin of PAH

Assessment of the origin of PAHs in environmental samples requires detailed analysis of individual components in order to compare composition with those of known pollutant emissions. This approach is also necessary for evaluating the fate and impact of PAHs since their environmental behavior and hazardous properties depend on various components (Bouloubassi & Saliot, 1991). In many cases fossil imprints are less readily recognizable in environmental PAH mixture than pyrolytic ones, unless important petroleum inputs have occurred. Low molecular weight, mono-, bi- and tri-cyclic aromatics are generally the most abundant constituents of unburned fossil fuels with only minor relative amounts of tetracyclic and larger PAH (Neff, 1979). In contrast, combustion PAH mixtures are dominated by compounds of three or more condensed rings. The low molecular weight PAH of primarily fossil origin are known to degrade more severely than the larger PAH through physical-chemical and microbial processes. Jones et al. (1986) reported preferential biodegradation of oil-derived PAH with respect to pyrolytic ones. They suggested that the latter show specific association with particles (sequestration, occlusion) which render them relatively inaccessible to bacterial action, while oil-derived PAH are mainly introduced in the aquatic environment as emulsions presenting a large surface area to the degrading organisms. Hence, the commonly observed apparent predominance of pyrolytic PAH in environmental samples may results from both high contributions of related sources and better preservation of these imprints.

In order to gain some information on the probable source of PAHs (Hites et al., 1980), fish extracts were subjected to capillary gas chromatography/mass spectrometry. To investigate oil contamination (Neff, 1979), the following compound specifications were selected; m/z 128, 142, 156, 170-naphthalene and alkyl-naphthalenes: 166, 180, 194-fluorene and alkyl-fluorenes: 178, 192, 206, 220-phennathrene and alkyl-phenanthrenes: 184, 198, 212, 226-dibenzothiophene and alkyl dibenzothiophenes. In the Red Sea fishes the discernible parent and alkyl-substituted species are for and in particular dibenzothiophenes which represent evidence for petrogenic contamination (Hites et al., 1977; DouAbul et al., 1987; Bouloubassi & Saliot, 1993).

To investigate combustion/urban runoff the molecular ions for the typical 'parent' (unsubstituted) PAHs (Blumer & Youngblood, 1975; Herrmann, 1981) were selected: m/z 178-phenanthrene/anthracene; 202-fluoranthene/pyrene; 228 benzoanthracene/chrysene; 252-benz-fluoranthenes/benz-pyrenes; 276-benz(ghi) perylene/indeno (1,2,3,-cd)pyrene. It is apparent that these compounds are present in the Red Sea fishes thus indicating combustion/urban runoff.

Molluscs have been used for monitoring contaminants in the environment (Farrington et al., 1983). These are sentinel organisms which concentrate pollutants from the marine environment, yet do not readily metabolize contaminants such as petroleum hydrocarbons and heavy metals (Farrington & Quinn, 1973). The concentration of a contaminant in a mussel is the difference between uptake and excretion of that contaminant. Thus, the contaminants found in mussels reflect the current contaminant burden of an ecosystem (Jackson et al., 1994). Furthermore, mussels were also collected to assess damage to these resources. It is well

established that aquatic sediments are the final accumulation site of water-borne constitutes derived from natural sources (living organisms and their detritus) *in situ* and surroundings, and artificial (domestic, urban-industrial and agricultural wastes) sources (DouAbul et al., 1984). The aquatic sediments can thus provide not only a historic record of sedimentary environment, but also reserve the features of average sedimentary environmental constituents. Besides they are *vice versa*, also a possible source of chemicals in water. Based upon the foregoing facts mussels and sediments were collected from the Red Sea and analyzed for their PAHs contents in order to correlate the distribution patterns of the pollutants found in them with that of the fish, consequently it will be possible to pin-point the origin of contamination/s i.e. whether it is local or transported. Furthermore, mussels were also collected to assess damage to these resources.

Mussel and sediment extracts were also subjected to capillary gas chromatography/mass spectrometry in order to investigate the probable source of PAHs (Table 3). Similarly, in both mussels and sediments naphthalene was the most prevalent compound which gives some evidence that fish, mussels and sediments were subjected to the same source of PAHs contamination in the Red Sea namely crude oil. Again, in the Red Sea mussels and sediments the discernible parent and alkyl-substituted species are for and in particular dibenzothiophenes which represent further evidence for petrogenic contamination (Hites et al., 1977; DouAbul et al., 1987).

In the light of the above reasoning we may thus conclude that the dead fishes were subjected to the same source of oil contamination as the life sentinel mussels and sediments. This pollution is a consequence of localized oil operations (Rushdi et al., 1991) and/or heavy ship traffic crossing Bab el-Mendeb, currently 100 million tons of oil transit the Red Sea annually (PERSGA,1995).

Toxicity

Based on qualitative classification of PAH carcinogencity (IARC, 1983) detected PAHs classified as having sufficient or limited evidence for carcinogenicty [benzo(a)pyrene, benzo(a)anthracene and chrysene] were lower in concentration and frequency of detection than those classified as having insufficient or no evidence for carcinogenicity [naphthalene, biphenyl, acenaphthylene, acenaphthylene, Fluorene, anthracene, phenanthrene, fluoranthene, and pyrene].

Among PAH, benzo(a)pyrene, a well known PAH because of its carcinogenic properties, shows a detectable level (0.5 ng g^{-1} dry weight). Chrysene (1.9 ng g^{-1} dry weight) is also classified as having sufficient evidence for carcinogenicty (IARC, 1983), thus the presence of this PAH in the edible muscles of the Red Sea fishes should be treated with absolute care (Futoma et al., 1981).

To assess health implications associated with PAHs, benzo(a)pyrene is often used as a toxicologic surrogate for all carcinogenic PAHs (Collins et al., 1991). Based on fish consumption by Yemeni's population (in 1994 the total fish consumption was 84 150 tones divided over 15 400 000 persons = 0.546 kg wet wt/person/month) estimated by Ministry of Fish Wealth, and considering the likely preference of consumption of highly contaminated fish, the daily intake of carcinogenic PAHs concentration as a potential extreme in exposure:

$$[50\ \mu g/kg\text{dry wt} \times 0.5\text{Kg wet wt /mo} \times$$

$$\text{mo (30 d)}^{-1} \times 0.2\text{dry wt (wet wt)} = 0.15\ \mu g\text{/person/day}]$$

This estimate accounts for 6%, 18.8%, (5.17–12.5%) and 15% of the total intake of benzo(a)pyrene from food reported by Dennis et al. (1983), Vaessen et al., (1988), DeVos et al. (1990) and Menzie et al. (1992) respectively. Evidently that the total intake of chrysene is a much higher 0.0063 μg/person/day.

The contribution of fish to food [the major source of exposure of man to PAHs (Santodonato et al., 1981)] is influenced mainly by the way fish is prepared (Fazio & Howard, 1983). Charboiled or smoked fish are major additional sources of carcinogenic PAHs (Menzie et al., 1992).

Evaluation of possible risks imposed on different population segments in Yemen and/or the rest of the Red Sea region due to prolonged seafood consumption requires further investigations involving seasonal analysis of edible tissues of different species/size/age categories of locally consumed fish in addition to conducting seafood consumption surveys.

Conclusions

In the light of the above reasoning we may thus conclude:

(1) the presence of carcinogenic PAHs in fish muscles are hazardous to human health and should be considered seriously. Based on fish consumption by

Yemeni's population it was calculated that the daily intake of carcinogenic PAHs was 0.15 μg/person/day.

(2) The fish extracts exhibit the discernible parent and alkyl-substituted species of naphthalene, fluorene, phenanthrene and dibenzothiophene. Hence the most probable source of PAHs is oil contamination originating from spillage's and/or heavy ship traffic.

(3) The Red Sea fishes are subjected to the same source of oil contamination as the life sentinel mussels. This pollution is a consequence of oil operations and heavy ship traffic crossing Bab el-Mendeb.

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