

**SOURCES OF POLYCYCLIC AROMATIC HYDROCARBONS
(PAHS) IN FISH SAMPLES FROM THE NORTH-WEST ARABIAN
GULF AND THE RED SEA COAST OF YEMEN**

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ABSTRACT

Fish samples were taken from both Red Sea-coast of Yemen and the North-West Region of the Arabian Gulf for determination of Polycyclic Aromatic Hydrocarbons (PAHs) using capillary gas chromatography. The study revealed measurable levels of PAHs in muscles of edible fishes collected from both areas. The source of PAHs is mainly petrogenic originating from oil spillage, refinery effluents, and/or heavy ship traffic. This study indicates that the accumulation of PAHs in the collected fish samples was very low, and it may not represent a serious hazard on human health at present, however, long term exposure via diet may impose certain undesirable health consequences. These concentrations are within the range of PAHs values reported for other comparable regional samples.

INTRODUCTION

Recently there has been a global growing concern on the release of harmful organics into the marine environment. So, the need for identification of organic and inorganic pollutants in the Red sea and the Arabian Gulf has become an important issue, too. That is because both areas are considered among the oil producing regions, and among the busiest in ship traffic.

Among the reasons for the concern is the need for baseline data to reveal the background levels, and the chronic oil pollution in such vital areas from industrial and other anthropogenic sources, which is described in some literature as "acute oil pollution" (DouAbul *et al.*, 1997).

Polycyclic aromatic hydrocarbons (PAHs), which are also known as polynuclear aromatic hydrocarbons are organic molecules belonging to the family of compounds known as "Aromatic Hydrocarbons". They are formed by the combustion of carbon-containing fuels during processes such as forest fires, coal combustion, and from the operations of gasoline and diesel engines. Many of the PAHs are known carcinogens. PAHs are generally released into the atmosphere but eventually reach surface waters. Low molecular weight PAHs, such as naphthalene, phenanthrene, and anthracene, tend to exist as vapors in the atmosphere. Higher molecular weight compounds, such as pyrene, exist as solids and are usually associated with soot particles. In general, the high molecular weight PAHs tend to be more carcinogenic than the smaller molecules. Due to their nonpolar behavior, PAHs are hydrophobic and have very low water solubility.

On the other hand, carcinogenic Polycyclic Aromatic Hydrocarbons are a class of compounds of special interest due to their possible harmful effects to man as well as organisms. There are over 100 different PAH compounds, which are usually found as a mixture containing two or more of these compounds, such as soot, coal tar, crude oil, creosote, and tar, (NEHC, 2006). Some are used in medicines or to make dyes, plastics, and pesticides. Man made sources such as automobile exhausts and coal burning can contribute far more PAHs to the environment than natural sources. PAHs enter water through discharges from industrial and wastewater treatment plants. Most PAHs do not dissolve easily in water. They stick to solid particles and settle to the bottoms of lakes or rivers, (NEHC, 2006). Most of the PAHs introduced to the marine environment tend to accumulate in bottom sediment and biota (Govers, 1990).

Animal studies have shown PAHs to cause cancer, problems with reproduction, and harmful effects on the skin, body fluids, and ability to fight disease after both short-and long-term exposures. Other than cancer, these effects have not been well documented in people, (NEHC, 2006).

The anticipated primary sources of environmental complex assemblages of PAHs include petroleum-related sources, such as ship traffic, oil seepage, and drilling and refinery activities (petrogenic sources). The other sources are combustion of various fossil fuels and natural fires (pyrogenic sources). Among sources road runoff, street dust, domestic and industrial waste waters, and sewage discharges (Al-Saad and Al-Timari 1989; DouAbul *et al.*, 1997).

The study of PAHs in costal areas of Yemen and the Northern Arabian Gulf is of great importance, since these areas are ecologically, biologically,

and economically of great importance. These locations are known to face considerable pollutant input from variety of land-based sources via coastal discharges (Al-Saad *et al.*, 1998).

Fishes have the ability to avoid oil impacted areas if the area is not restricted in size. Also, fishes are more resistant than other organism to toxic effects of oil, since their external surfaces are coated with mucous. Furthermore, certain fish species can smell oil polluted areas, thus, they avoid them. On the other hand, however, extensive fish mortality has been reported in connection with spills of toxic light oils such as gasoline, diesel oil, and light fuel oil (Al-Saad *et al.*, 1997).

The Arabian Gulf is considered an extremely demanding shipping route for oil, with accidental spillage being almost unavoidable. Also, oil refinery effluents and lose, during operations have been identified as the major source of oil contamination in the waters of the Arabian Gulf. In combination, these sources provide long term input of petroleum hydrocarbons into the Gulf ecosystem, whereas major oil spills, either being unintentional or as an outcome of military activities, have added occasional dramatic pulse of oil contamination to the long term background of oil pollution of this area (Al-Saad *et al.*, 1998).

In Yemen the marine and coastal areas are of major economic significance, too. In this region, the natural marine resources are well exploited, both for local consumption as well as export. Haskoning (1991) suggested that the impacts presently affecting the Yemeni marine environment are pollution and over-exploitation of the natural resources.

Qualitative and quantitative data concerning sources and impacts of pollution in these geographical regions are still scarce. Thus we aimed to present in this research project the following targets; First; to perform a detailed analytical study using capillary gas chromatography for screening PAHs in fish species from the Red Sea coast, and from the North-West region of the Arabian Gulf. This is to see if these animals show any evidence of oil contamination. Second; to provide a logic base for the comparison of PAHs in the fish samples collected from the Red Sea coast of Yemen, and the North-West of Arabian Gulf. Third; to compare the obtained results with those obtained from nearby regional countries.

MATERIALS AND METHODS

This study was carried out along the Red Sea coast of Yemen. The sites were chosen according to their importance, as active fishing spots, and for the suitability of sampling (Fig.1). Fish sample were also taken from fishermen fishing off the Yemeni Coast. After collection, the fish samples

were wrapped in aluminum foil, stored in cool boxes, and frozen upon return to laboratory. Combined samples of fish, having similar size (length and weight) were chosen for the fish species; *Solea solea*, *Scomberomus malculatus*, and *Rhocycentron canadum*. On the other hand, composed samples of five fish species; *Jobnieops sina*, *Acanthopagrus latus*, *Otoliths argenteus*, *Lize subviridis*, and *Hilsa illisha* were collected from the North-West Arabian Gulf (Fig.1) at various locations during the study period.

The extraction of fish sample were done according to the method described by DouAbul *et al.* (1997) ,and based upon that of Grimalt and Oliver (1993). In a brief description, exactly 10 gm of dried fish muscles were placed in a pre extracted cellulose thimbles and soxhelt extracted with 150 ml methanol/ benzene (1:1 ratio) for 24 hours. At the end of this period the extracted samples were transferred to storage flasks, and each sample was further extracted with fresh solvent. The combined extractions for each sample were reduced in volume to about 10 ml by means of a rotary vacuum evaporator, and was then saponified for 2 hours with a solution of 4N KOH in 1:1 methanol / benzene. After extraction of the unsaponified matter with hexane, the extract was dried over anhydrous Na₂SO₄, and concentrated by applying a flow of purified Nitrogen. The concentrated extract was cleaned up by column chromatography. The column filled with 8gm; of 5% water deactivated alumina (100-200 mesh) on the top, and silica (100-200 mesh) in the bottom. The extract was then applied to the head of the column, and eluted (washed) with 50 ml of n-hexane to isolate the aliphatic fraction, and 50 ml of benzene to isolate the aromatic fraction. The aromatic fractions were then reduced to a suitable volume prior to analysis for PAHs by means of capillary gas chromatography. The samples were injected in the "splitless mode" on to a "50 mx 0.25 mm" i.d. SE-30 (Methyl Silicone) fused silica capillary column, at an initial temp of 50°C and following temperature regimen programmed at 4°C min, to 280 °C max, then held at the final temperature for 30 min.

The tissue fat content determination of the fish samples, were done in the following methodology: 3gm of each dried sample were soxhlet extracted within 2:1 mixture of petroleum ether and acetone for 24 hours. The extracts were reduced in volume by means of a rotary vacuum evaporator, and subsequently reduced to exactly 1 ml by applying a flow of purified Nitrogen. Accurately 10 µl of concentrated extracts were taken by means of a Hamilton syringe, and weighed after evaporation of solvent on CAHN 29 automatic electro balance.

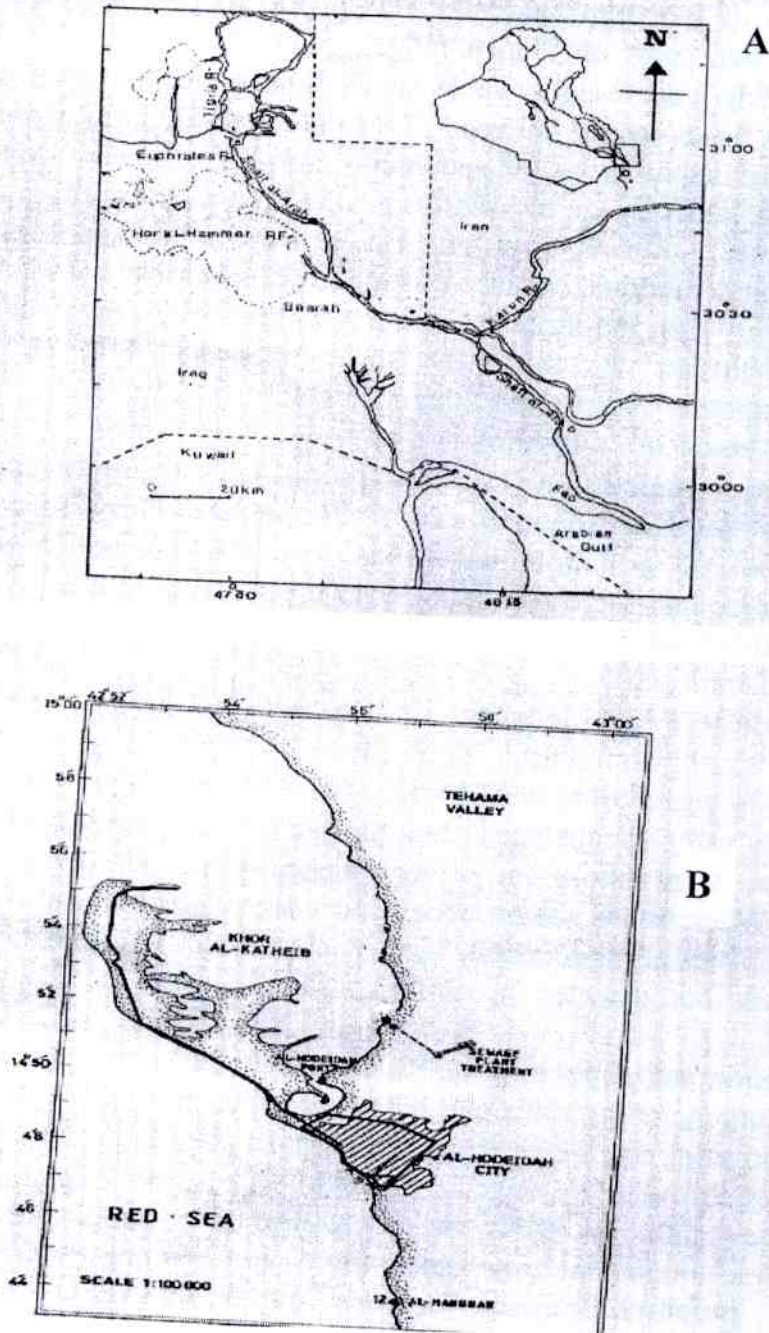


Fig. (1): Sample locations in: A) N.W. Arabian Gulf. B) Red Sea-Coast of Yemen

RESULTS AND DISCUSSION

Concentrations of individual parent PAHs in the fish muscles collected from the Red Sea coast of Yemen ranged from less than one ng/g dry weight to 21.15ng/g dry weight (Table 1). The total concentration of PAHs in the Red Sea fishes ranged between 23.90 ng/g dry weight to 57.90 ng/g dry weight. The naphthalene, phenanthrene and flourene were dominant PAHs, which more likely are derived from petroleum inputs (petrogenic oil pollution sources), (DouAbul *et al.*, 1997). Napthalene was the most prevalent parent compound, because it is more water soluble and has lower particular affinity than the large molecular weight aromatic hydrocarbons, this same conclusion was found by DouAbul *et al.*(1987). The other abundant compound was phenanthrene, which is a standard PAHs component in crude oil. Chrysene is one such PAHs, which is normally produced through combustion (Readman *et al.*,1986) was present in fish samples of the Red Sea. This particular PAH compound may indicate the other source of oil pollution, known as pyrogenic oil pollution. The mentioned compounds were known to cover a high proportion of "Marib" crude oil product from Yemen, which is exported via a terminal in the Red Sea (DouAbul *et al.*, 1997).

The results of PAHs detection in fish samples of the North-West Arabian Gulf are shown in (Table 2). The total PAHs varied from 6.78 ng/g dry weight in *Johnieops sina* to 23.83 ng/g dry weight in *Hilsa ilisha*.

In all fish samples analyzed the highest average individual PAHs concentrations were those of Fluoranthene, Fluorene, Pyrene, and Anthracene. Whereas the lowest recovered concentrations were those of Acenaphthene, Benzo(ghi)perylene, and Phenanthrene. Naphthalene and Chrysene were not detected in any fish sample from the North-West Arabian Gulf.

Accumulation and depuration of PAHs in fish can be influenced by a range of factors including route and length of exposure time, lipid content of tissues, and some marine environmental factors such as the salinity, and temperature of sea water (Al-Saad *et al.*, 1998).

The concentration of PAHs in the studied fish samples could be attributed to accumulation from water, food, and environmental sources. Hence, the proposed primary sources of environmental complex assemblages of PAHs may include petroleum related sources from ship traffic, oil seepage and refinery effluents, which we collectively described as petrogenic sources. The other sources are from combustion of various fossil fuel and natural fires, which was described as pyrogenic oil pollution. Also, other sources may include road runoff and street dust, domestic and

industrial waste waters and sewage discharges (DouAbul *et al.*, 1997; Al-Saad and Al-Timari, 1989).

Table (1) Mean concentration (ng/g dry weigh) of PAHs in fish samples from the Red Sea Coast of Yemen*.

<i>PAHs analyte</i>	<i>Sola sola</i>	<i>S.malculatus</i>	<i>R.canadum</i>
Naphalene	20.00	21.15	15.60
Acenaphthylene	1.00	0.85	0.75
Acenaphthene	1.15	1.60	0.85
Fluorene	5.25	2.40	1.30
Phenetherene	13.50	8.00	1.80
Anthracene	7.50	0.70	0.30
Fluoranthene	1.50	2.10	0.85
Pyrene	1.75	2.20	0.50
Benzo(a)anthrecene	0.35	0.30	0.25
Chysene	1.75	0.75	0.45
Benzo(b)fluoranthene	0.40	0.30	0.20
Benzo(k)fluoranthene	0.40	0.30	0.20
Benzo(a)pyrene	0.80	0.30	0.25
Diben(ah)anthracene	0.10	0.15	0.15
Benzo(ghi)perylene	0.35	0.20	0.20
Indeno(1,3,4-cd)pyrene	0.10	0.10	0.25
Total PAHs	57.90	49.50	23.90
% Fat	2.90	0.44	0.40

* Adopted from DouAbul *et al.*(1997).

Presence of anthracene, phenanthrene, pyrene, and benzeo (ghi) perylene may indicate that discharges of refinery processing into coastal waters and fishing boats engine effluents were the main contributors of PAHs in the studied samples. As we have mentioned elsewhere, another source of PAHs in these fish samples may be of a biogenic source, which is obtained through the food web (Al-Saad *et al.*, 1998).

The analysis of fish muscles revealed the fish ability to accumulate PAHs in their lipid pool. On the basis of different PAHs concentrations observed in the analyzed fish samples, an obvious, direct relationship has been existed between PAHs and fat content of the muscle tissues of the fish species.

Table (2) Concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) in fish samples (ng/g) from North-
Arabian Gulf West

Species	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	B(ghi) p	Total	%Fat
<i>Johnieops sina</i>	-	2.11	0.32	1.04	3.31	-	-	6.78	1.9
<i>Acanthopagrus latus</i>	-	3.98	-	4.13	2.09	2.52	0.09	12.81	5.6
<i>Otoliths argenteus</i>	-	3.67	1.66	2.98	5.74	2.20	-	16.25	5.9
<i>Liza subviridis</i>	-	2.65	-	1.48	4.18	4.82	-	13.13	4.8
<i>Hilsa ilisha</i>	2.18	3.74	2.13	5.56	6.27	3.95	-	23.83	6.3

- = Not detected

The significance of this study data is best appreciated upon comparing them with the values of total PAHs found in fish samples collected from other areas of the same region (Table 3). The levels of PAHs in the fish samples were generally low. Hence, an agreement between the spatial tendency of fish contamination by PAHs is observed between the present study and that performed by Readman *et al.*, (1992), and by Fowler *et al.*, (1993). These studies were carried out following the 1991 massive oil spill happened in the Arabian Gulf due to war activities. About 6 to 8 million barrels of crude oil were estimated to intentionally released to the Arabian Gulf during the conflict.

Table (3) Comparison of values of PAHs in Fish samples of Red Sea and North-West Arabian Gulf with other comparable values obtained in the nearby Countries.

Location	Concentration ($\mu\text{g/g}$)	Reference
NW Arabian Gulf	14-106	DouAbul <i>et al.</i> ,(1987)
Kuwait	21	Fowler <i>et al.</i> ,(1993)
Saudi Arabia	7.6-239	Fowler <i>et al.</i> ,(1993)
Bahrain	1.1-6.4	Fowler <i>et al.</i> ,(1993)
UAE	3.8-7.7	Fowler <i>et al.</i> ,(1993)
Oman	2.2-110	Fowler <i>et al.</i> ,(1993)
Qatar	55.8-165.9	Fowler <i>et al.</i> ,(1993)
NW Arabian Gulf	6.78-23.83	Present study
Red Sea-Coastal, Yemen	23.9-57.9	Present study

PAHs are an important class of environmental contaminants to study because some of these compounds are carcinogenic and/or mutagenic to mammals; in addition, they have both acute toxicity and sub-lethal effects on some aquatic organisms. PAHs may also bioaccumulate in edible fish and shellfish, which gives them a pathway to humans, (Hartmann and Quinn, 2006). In this study, the accumulation of PAHs in fish was very low, and the concentration of carcinogenic chrysen and benzo(a) pyrene, which were found in the tissues of fishes collected from the Red Sea and North-west region of the Arabian Gulf, respectively, may be considered seriously hazardous to humans, who include and consume fish caught from these areas in their diet on regular bases, which may represent a long term exposure to such potentially toxic chemicals.

As a conclusion for this study:

- 1- The concentrations of PAHs in the edible parts of the fish samples from the Red Sea Coast of Yemen and the North-West Arabian Gulf showed that the major source of PAHs in the two areas was mostly a petrogenic source.
- 2- The rather low concentrations of individual PAHs observed in the Red Sea and North-West Arabian Gulf fishes may be attributed to the rapid metabolism of PAHs by the fish species of this region.
- 3- The most probable source of PAHs is oil contamination originating from spillage, refinery effluents and lose, and /or heavy ship traffic prevalent in both areas.
- 4- Fish and other marine organisms in both areas are subject to the same source of oil pollution, which is a natural consequence of oil operations and ship traffic, which are characteristics to these areas.
- 5- The presences of carcinogenic PAHs in fish muscles may be considered hazardous to humans, who include and consume fish caught from these areas on regular bases. This fish consumption by populations may represent a real health threat based on the long term exposure to such potentially toxic hydrocarbons.
- 6- Last, PAHs are an important class of environmental contaminants to study, because some of these compounds are carcinogenic and/or mutagenic to mammals. In addition, they have both acute toxicity and sub-lethal effects on some aquatic organisms. PAHs may also be bioaccumulated in edible fish and shellfish, which gives them a pathway to humans.

Accordingly, we recommend conduction of a thorough investigation to be carried out on regional scale to include all aspects of oil pollution associated with air, water, and soil of these areas to help formulation of a unified strategy to combat oil pollution in the region.

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مصادر الهيدروكربونات الاروماتية متعددة الأنوية في اسماك

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الخلاصة

اخذت عينات اسماك من كل من ساحل البحر الأحمر في الجمهورية اليمنية، وشمال غرب الخليج العربي لغرض دراسة مصادر المركبات الهيدروكربونية متعددة الأنوية، وذلك باستخدام تقنية التحليل بالغاز كروماتوغرافي. وقد أظهرت الدراسة وجود تراكيز مختلفة ولكنها قليلة في عضلات الاسماك في عينات كل من المنطقتين. ويعتقد على أساس النتائج والفرضيات بان مصادر هذه المركبات تتأتى من مصادر لها علاقة بالنفط كالمصافي والتسربات النفطية وحركة النقل البحري، ومصادر أخرى كمياء الصرف الصحي في هاتين المنطقتين. كما وبينت الدراسة إن قابلية الأسماك لتتركيز هذه المركبات قليلة ومحدودة، وان وجود هذه المركبات لا يمثل خطراً في الحالات الإعتيادية على صحة الإنسان لكونها تقع ضمن الحدود المسموح بها، ولكن وجود بعض المركبات التي تعتبر من المسرطنات ولو بتراكيز نزره قد تشكل خطراً على المدى البعيد من التعرض لهذه المركبات المتجمعة في الأسماك والأغذية البحرية المصطادة أو المستزرعة في هذه المناطق. وعند مقارنة التراكيز المستحصلة من هذه الدراسة مع التراكيز المستحصلة من دراسات أخرى في بلدان قريبة، نجد بأنها جميعاً تقع ضمن حدودها الدنيا، مما قد لا يمثل خطراً حقيقياً على الثروات البحرية الطبيعية في الوقت الحاضر، ولكن هذا لا يمنع من قيام دراسات بحثية متخصصة وشاملة للمنطقة لغرض توفير المعلومات الأساسية التي ستساعد على الحد من التلوث النفطي ومعرفة أسبابه ومصادره في مثل هذه المناطق الحيوية من العالم.