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## Determination of Hydrocarbons in Bivalves from the Egyptian Mediterranean Coast

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### Abstract

*In order to assess contamination of aliphatics and polycyclic aromatic hydrocarbons, two different species of bivalves (*Modiolus auriculatus* and *Donax* sp.) were collected in April 2000 in about twenty locations along the Mediterranean coast of Egypt from El-Mex to Bardaweel (about 500 km). The results showed that the concentration of total aliphatics (average 180 ng g<sup>-1</sup> wet weight) and PAHs (average 8180 ng g<sup>-1</sup> wet weight) was generally lower than that reported from some of the published surveillance and monitoring studies of coastal areas from various regions of the world. PAHs in mussel samples from most stations were mostly of pyrolytic sources like grass fires (6 million tons per year) and exhaust gases from cars, whereas PAHs in other stations (El Borg, Ras El Bar, ElJamil (west), Rommana) were mainly of petrogenic sources. However, other pollution sources are involved.*

**Keywords:** Mediterranean Coast, Egypt, PAHs, Aliphatic hydrocarbons, Organic pollution, *Modiolus auriculatus*, *Donax* sp.

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### Introduction

Polycyclic aromatic hydrocarbons (PAHs), a group of hydrophobic organic compounds with two or more fused aromatic rings, are introduced into the environment via natural and anthropogenic processes (LA FLAME & HITES, 1988, NRC, 1985). However, some PAHs such as benzo(a)pyrene and benz(a)anthracene, have mutagenic and carcinogenic properties (MCCANN *et al.* 1975; IARC 1983). PAHs accumulate in aquatic organisms, particularly in invertebrate species that have

a low metabolizing capability (PAYNE, 1977; VARANASI *et al.*, 1985; FORSTER & WRIGHT, 1988) and have been detected in marine mammal tissue (HELLON *et al.*, 1990; HELLON *et al.*, 1991). Moreover, PAHs are suspected of inducing cancer in marine and fresh water fish (VARANASI *et al.*, 1987; BLACK & BAUMANN, 1991; MYERS *et al.*, 1991).

Several possible sources for PAHs in the environment exist (FOUCHECOURT, *et al.*, 1999, NEFF JM. 1979, Mc ELROY A.E., *et al.*, 1989)). Polycyclic aromatic hydrocarbons

can result from natural processes, but anthropogenic activity is generally considered to be the major source of PAH input into the environment. Concerning natural sources pyrolytic PAHs can be generated by forest or grass fires. Marine seeps can release hydrocarbon compounds into seas and oceans and natural compounds can derive from biogenic precursors. As for pollution due to anthropogenic activity, the most important generation pathway of PAHs is the combustion at high temperature of organic matter due to industrial activity (pyrolytic source). Some petroleum hydrocarbons can also be released into the environment, mainly due to offshore oil production or petroleum transportation. Each PAH source (pyrolytic, diagenetic, and petroleum hydrocarbons) gives rise to a characteristic PAH pattern and it is therefore possible to determine the processes that generated these compounds.

Bivalves and particularly mussels are used world-wide as sentinel organisms to rapidly assess the status of the contamination of the marine environment for large number of pollutants. Where, PAHs are lipophilic and coplanar; they can accumulate in adipose tissues or secretions. Their metabolism in vertebrates is essentially realized by cytochrome P450-dependent monooxygenases (LEVIN *et al.*, 1982), while in invertebrates an

oxidative process is the major route of biotransformation (LIVINGSTONE & PIPE, 1992). They offer the advantage of a wide geographic distribution, facilitating comparison of data, and of integrating chemical pollutants over long periods at the same site (FARRINGTON *et al.*, 1987). The aim of this work was to investigate the present status of the contamination by selected organic hydrocarbons in two species of bivalves (*Modiolus auriculatus* and *Donax sp.*) collected from Egyptian Mediterranean Sea Coast

### Materials and Methods

The sampling cruise took place in April 2000, from an area extending for about 500 Km from the beginning of the El Mex in the west to the Bardaweel in the east (Fig. 1). Twenty sampling sites were chosen to provide an adequate as possible geographical coverage of the study area. Two different bivalves were collected from these areas. *Modiolus auriculatus* from 15 sites and *Donax sp.* from 5 sites. The specimens were collected and wrapped in three sheets of clean, heavy duty aluminum foil, where the dull side of the foil was in contact with the sample and the samples were then kept in a deep-freezer at  $-20^{\circ}\text{C}$ .

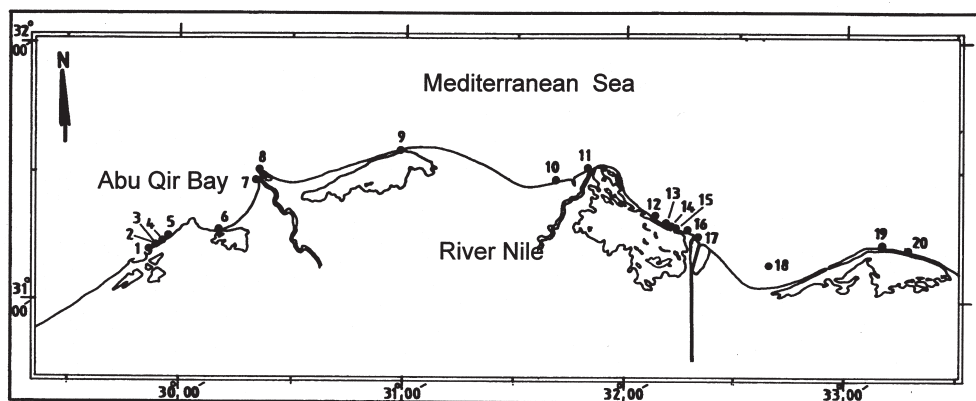


Fig 1: Location of collected samples.

10 g bivalve tissue (wet weight) was treated with 30 g of sodium anhydrous sulfate and the mixture was blended at high speed for 5 minutes. Then the mixture was extracted with a Soxhlet extractor with 200 ml of methanol for 8 hours (UNEP/IOC/IAEA, 1981). Then, 0.7 M KOH (20 ml) and distilled water (30 ml) were added to the flask and the reflux was continued for 2 hours to saponify the lipids. The content of the extraction flask was extracted in a separatory funnel with 80 ml / 3 hexane. Then the extracts were combined, dried with anhydrous sodium sulfate and filtered through glass wool. The hexane fraction was concentrated with a rotary evaporator down to about 15 ml at 30 °C followed by concentration with nitrogen gas stream down to a volume of 1 ml. A chromatography column was prepared using a 50 ml burette; 10 g of silica gel was transferred into the column, followed by 10 g of alumina and finally 1 g of sodium sulfate.

The extract (1 ml) was sequentially eluted from the column with 20 ml of hexane for the saturated aliphatic fraction (F1). Then 30 ml of hexane and dichloromethane (90:10) for the unsaturated and aromatic hydrocarbons fraction (F2). F1 and F2 were concentrated using stream of nitrogen for instrumental analysis.

To control the analytical reliability and assure recovery efficiency and accuracy of the results, 7 analyses were conducted on PAH compound reference materials, HS-5 (sediment) provided by NRC-IMB of Canada and SRM-2974 (Freeze-dried mussel tissue) (*Mytilus edulis*) provided by NIST of USA. The laboratory results showed recovery efficiency ranged from 89-110% with coefficient of variation (CV) of 10-14% and standard deviation (SD) of  $\pm$  7-15.

All solvents were pesticide grade purchased from Merck. Blanks of 1000 fold concentration were analyzed by Gas Chromatography with a flame ionization detector (FID). The Gas Chromatographer was a Hewlett Packard HP-5890 series II equipped with split/splitless

injector and a fused silica capillary HP-1 (30 m, 0.32 mm, 0.17 mm) 100% dimethylpolysiloxane. The temperature was programmed from 50-290 °C with rate of 5 °C min<sup>-1</sup> and was, then, maintained at 290 °C for 25 min. Nitrogen was used as a carrier gas at a flow of 1.3 ml min<sup>-1</sup>.

## Results

In this study, the results of the analysis represent average concentrations from at least two determinations. It is well established that aquatic bivalves are the final accumulation site of water-borne constituents derived from natural sources (living organisms and their detritus) in situ, surroundings, and artificial (domestic, urban-industrial and agricultural wastes) sources. Molluscs have been used for monitoring contaminants in the environment (FARRINGTON *et al.*, 1983)

*n*-Alkanes in the range of C<sub>12</sub> to C<sub>40</sub> are present in most samples, the concentration of twenty detected alkanes in bivalve tissue (Table 1) lays in the range 4.5 ng g<sup>-1</sup> at station No. 16 (Port Said West) to 850 ng g<sup>-1</sup> at station No. 11 (Ras El-Bar) wet weight with an average 180 ng g<sup>-1</sup>. A distinctively high tissue content of C<sub>12</sub>, C<sub>14</sub> at stations No. 2, 4, and 13 and of C<sub>12</sub>, C<sub>22</sub>, C<sub>24</sub>, C<sub>28</sub>, C<sub>30</sub> at stations No. 1, 6 - 8, 10, 11 and 13 were recorded. On the other hand, the range of sixteen detected aromatic hydrocarbons in the tissues lay between 1219 ng g<sup>-1</sup> at station No. 11 (Ras El-Bar) and 46741 ng g<sup>-1</sup> at station No. 1 (El Mex Bay) with an average 8180 ng g<sup>-1</sup> of wet weight (Table 2). Pristane (C<sub>19</sub>) and phytane (C<sub>20</sub>) are present in most petroleum oils, so the detection of these two components is often used as a good indicators of petroleum contamination. However, in this study, phytane was not detected in all twenty locations and pristane was detected in low concentrations (0.1 to 2.0 ng g<sup>-1</sup>) at only nine locations, which reflected low petroleum contamination. The detection of pristane in nine locations with no detection of phytane may reflect biogenic origins [biogenic sources of the compounds

**Table 1**  
**Concentration (ng/g wet weight) of total aliphatic Hydrocarbons in mussel**  
**from Mediterranean Sea**

<b>Chemical Name</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>
n-Dodecane	10.0	110.2	ND	39.6	10.2	16.4	3.9	1.6	5.2	5.5
n-Tetradecane	13.4	66.5	0.4	20.8	3.6	26.6	0.1	17.9	0.7	10.6
n-Hexadecane	13.6	14.3	1.0	4.4	0.7	8.8	2.7	23.7	0.8	23.2
n-Heptadecane	12.8	6.8	1.5	2.3	0.3	10.9	8.6	19.2	0.6	32.9
n-Octadecane	14.0	2.6	5.0	0.7	0.2	9.3	3.4	20.7	1.0	25.4
n-Eicosane	26.2	5.0	1.9	1.4	0.3	25.5	10.4	42.5	0.5	25.8
n-Docosane	15.6	1.4	4.0	0.4	0.5	29.6	8.0	26.1	0.6	20.5
n-tetracosane	15.7	1.3	12.0	0.4	ND	12.1	8.4	27.9	0.4	23.2
n-Hexacosane	10.6	4.3	3.2	1.1	0.1	6.5	5.1	19.0	0.5	30.1
n-Octacosane	19.1	7.4	5.9	1.9	0.3	8.0	11.5	45.8	1.0	49.0
n-Triacontane	19.4	3.9	3.9	1.1	0.3	16.8	10.8	46.6	1.8	39.5
n-Dotriacontane	12.2	2.5	17.4	1.1	0.4	12.8	5.2	29.5	1.5	21.9
n-Tetratriacontane	10.1	3.1	11.5	0.6	0.7	7.2	5.2	34.2	2.8	26.7
n-Hexatriacontane	4.1	2.6	6.0	2.2	0.2	2.0	2.4	17.4	1.1	14.1
n-Octatriacontane	1.1	1.5	1.6	1.1	0.1	0.4	0.4	4.7	0.4	3.7
tetracontane	7.1	2.3	5.2	2.8	ND	0.6	2.2	14.7	0.8	12.6
pristane	ND	ND	2.0	ND	0.1	ND	ND	0.2	ND	0.3
<b>Total aliphatics</b>	<b>205.1</b>	<b>235.7</b>	<b>82.3</b>	<b>81.9</b>	<b>18.0</b>	<b>193.4</b>	<b>88.4</b>	<b>391.9</b>	<b>19.7</b>	<b>365.2</b>
<b>UCM-ALI</b>	<b>0.03</b>	<b>0.01</b>	<b>0.03</b>	<b>0.02</b>	<b>0.02</b>	<b>0.03</b>	<b>0.05</b>	<b>0.04</b>	<b>0.03</b>	<b>0.04</b>
1 = El-Mex; 2 = Eastern Harbour; 3 = El-Shatby; 4 = Sidi Gaber; 5 = Gleem; 6 = Maadiya; 7 = Rashid; 8 = Rashid; 9 = El-Borg; 10 = New Damietta, UCM-ALI = unresolved Aliphatics, ND: not detected.										
<b>Chemical Name</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>	<b>18</b>	<b>19</b>	<b>20</b>
n-Dodecane	2.3	4.0	19.1	0.5	0.2	2.9	2.8	2.0	0.2	1.1
n-Tetradecane	21.8	3.8	32.0	2.4	1.2	0.1	0.4	2.5	0.3	0.1
n-Hexadecane	40.6	1.8	36.9	2.6	2.2	ND	0.5	1.0	0.5	0.7
n-Heptadecane	62.1	2.4	59.2	1.4	5.4	ND	0.6	1.7	0.6	1.9
n-Octadecane	41.4	1.0	37.7	1.6	1.9	ND	ND	0.5	0.4	0.3
n-Eicosane	60.0	1.1	47.7	2.8	3.5	ND	0.3	1.8	0.9	1.3
n-Docosane	42.8	0.8	56.3	1.3	1.7	ND	0.3	0.7	0.6	1.2
n-tetracosane	45.5	1.3	28.3	2.1	2.0	ND	0.1	1.3	1.3	0.9
n-Hexacosane	60.8	1.6	30.6	2.0	2.0	ND	0.2	1.4	0.9	1.5
n-Octacosane	121.1	0.8	52.4	1.7	3.4	0.7	0.4	1.6	0.8	2.1
n-Triacontane	110.6	0.9	59.7	2.2	2.4	ND	0.5	1.4	0.5	2.4
n-Dotriacontane	69.6	0.8	31.2	0.7	1.6	ND	0.3	1.1	0.3	1.6
n-Tetratriacontane	96.2	1.5	38.9	0.6	1.5	0.1	0.6	0.9	0.3	1.5
n-Hexatriacontane	35.3	1.1	17.8	0.5	0.8	0.2	0.2	1.0	0.3	0.7
n-Octatriacontane	7.7	0.2	4.2	0.2	0.6	0.1	0.0	0.2	0.1	0.2
tetracontane	31.2	0.4	18.7	8.9	1.8	0.5	0.6	2.1	1.8	0.7
pristane	0.9	ND	0.3	4.0	ND	ND	ND	0.2	ND	0.1
<b>Total aliphatics</b>	<b>849.8</b>	<b>23.5</b>	<b>571.1</b>	<b>35.6</b>	<b>32.2</b>	<b>4.5</b>	<b>7.9</b>	<b>21.3</b>	<b>9.7</b>	<b>18.3</b>
<b>UCM-ALI</b>	<b>0.03</b>	<b>0.03</b>	<b>0.03</b>	<b>0.03</b>	<b>0.02</b>	<b>0.01</b>	<b>0.01</b>	<b>0.02</b>	<b>0.02</b>	<b>0.04</b>
11 = Ras El-Bar; 12 = El-Jamil west; 13 = El-Manzala; 14 = El-Manzala; 15 = El-Jamil east; 16 = Port Said west; 17 = Port Said; 18 = Rommana; 19 = Bardaweel; 20 = Bardaweel. UCM-ALI = unresolved Aliphatics, ND: not detected										

**Table 2**  
**Concentration (ng/g of wet wt.) of PAHs in mussel samples from Mediterranean Sea**

<b>Chemical Name</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>
Naphthalene	6648	61	308	395	123	485	178	2049	123	ND
Acenaphthylene	9867	178	1997	716	417	2198	1416	1349	685	434
Acenaphthene	2902	105	540	192	6	238	159	ND	511	ND
Fluorene	4906	42	ND	ND	20	270	618	766	259	633
Phenanthrene	15766	21	167	141	25	327	261	70	438	74
Anthracene	1948	28	ND	83	28	66	67	ND	535	ND
Fluoranthene	1932	133	147	84	71	87	511	302	104	35
Pyrene	ND	70	85	70	74	43	228	132	149	ND
Benz(a)anthracene	ND	227	230	497	ND	137	1066	263	119	155
Chrysene	500	259	24	139	272	62	109	62	137	ND
Benzo(b)fluoranthene	ND	976	283	247	350	356	479	138	228	44
Benzo(k)fluoranthene	667	18	ND	53	14	61	88	ND	250	ND
Benzo(a)pyrene	839	507	159	140	226	268	178	55	346	47
Dibenz(a,h)anthracene	319	331	143	237	132	85	88	152	111	59
Benzo(ghi)perylene	ND	112	ND	ND	33	32	476	ND	92	ND
Indeno(1,2,3-cd)pyrene	447	567	88	ND	131	213	236	86	130	82
<b>Total PAHs</b>	<b>46741</b>	<b>3636</b>	<b>4172</b>	<b>2993</b>	<b>1920</b>	<b>4929</b>	<b>6159</b>	<b>5424</b>	<b>4220</b>	<b>1562</b>
1 = El-Mex; 2 = East. Harbour; 3 = El-Shatby; 4 = Sidi Gaber; 5 = Gleem; 6 = Maadiya; 7 = Rashid; 8 = Rashid; 9 = El-Borg; 10 = New Damietta; ND: not detected.										
<b>Chemical Name</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>	<b>18</b>	<b>19</b>	<b>20</b>
Naphthalene	193	297	422	270	377	189	ND	ND	33	447
Acenaphthylene	498	1299	1363	1285	1509	1000	ND	521	443	1222
Acenaphthene	130	162	223	253	160	229	ND	ND	ND	195
Fluorene	13	844	767	555	881	664	682	1430	530	304
Phenanthrene	20	137	300	936	804	85	112	872	437	170
Anthracene	12	ND	64	1492	24	ND	ND	339	177	23
Fluoranthene	12	82	105	552	267	61	52	181	829	68
Pyrene	53	192	54	133	87	ND	ND	495	476	19
Benz(a)anthracene	134	1727	921	273	1133	ND	702	7045	2484	644
Chrysene	16	ND	31	230	16	ND	ND	1315	275	19
Benzo(b)fluoranthene	8	217	203	2622	177	ND	86	1610	1393	116
Benzo(k)fluoranthene	19	232	334	1488	342	112	111	738	401	63
Benzo(a)pyrene	17	209	294	2251	505	142	122	1889	1013	186
Dibenz(a,h)anthracene	20	172	237	616	172	135	213	1745	433	124
Benzo(ghi)perylene	9	1560	540	2058	170	681	899	698	223	162
Indeno(1,2,3-cd)pyrene	64	117	2730	795	1835	ND	569	2431	905	204
<b>Total PAHs</b>	<b>1219</b>	<b>7247</b>	<b>8589</b>	<b>15810</b>	<b>8459</b>	<b>3297</b>	<b>3547</b>	<b>21306</b>	<b>10052</b>	<b>3964</b>
11 = Ras El-Bar; 12 = El-Jamil west; 13 = El-Manzala; 14 = El-Manzala; 15 = El-Jamil east; 16 = Port Said west; 17 = Port Said; 18 = Rommana; 19 = Bardaweel; 20 = Bardaweel; ND: not detected.										

are important, for example, reduction of the chlorophyll phytol side led to phytane, where its oxidation gave pristane and can also originate from lipids of zooplankton and bacteria, however, the high ratio of pristane to phytane indicates a biogenic source (VILLENEUVE *et al.*, 1999)].

Data represented in Table 1 and 2 indicate that the range for total hydrocarbon (F1 + F2) tissue content lay between 1930 ng g<sup>-1</sup> at station No. 11 (Ras El-Bar) and 46950 ng g<sup>-1</sup> wet weight at station No. 1 (El Mex Bay) with an average 8360 ng g<sup>-1</sup>. The total aliphatic fraction (F1) was lower than the corresponding aromatic fraction (F2) in all bivalves collected. The ranges for total aliphatic (F1) in the present study (5 ng g<sup>-1</sup> to 850 ng g<sup>-1</sup>) were lower than the corresponding ranges reported in some of the published survey and monitoring studies of coastal areas from various regions, for example. The ranges for total aliphatics in mussels (*Mytilus galloprovincialis*) from Venice, Italy (Lagoon) were from 8000 ng g<sup>-1</sup> to 87000 ng g<sup>-1</sup> wet weight (FOSSATO & SIVIERO, 1974) and Spanish Western Mediterranean Coast were between 1800 and 58400 ng g<sup>-1</sup> wet weight (ALBAIGES *et al.*, 1982).

On the other hand, the ranges for total aromatic (PAHs) (F2) content (12200 ng g<sup>-1</sup> to 46740 ng g<sup>-1</sup>) in the present study were higher than the corresponding 200-30600 ng g<sup>-1</sup> wet weight on the Spanish western Mediterranean coast and from 1000 to 20500 ng g<sup>-1</sup> in the eastern Mediterranean sea (French Riviera, Corsica, Sardinia) (BAUMARD *et al.*, 1998a) but were lower than the ranges reported in other published surveillance and monitoring studies of coastal areas from various regions, such as the ranges of total PAH in San Francisco Bay, California, USA (5800-75000 ng g<sup>-1</sup>) (DISALVA *et al.*, 1975) and Monterey Bay, Central California, USA (14000-208000 ng g<sup>-1</sup>) (MARTIN & CASTLE, 1984) and on the Spanish Atlantic coast (Galicia) 10400 to 218000 ng g<sup>-1</sup> (SOLER *et al.* 1989).

In this study all the stations showed that the total aliphatics is lower than the total aromatics by about 1660 times at station 1 (El-Mex, which receives a large input of industrial waste) to 1.5 times at station 11 (Ras El-Bar). Saturated hydrocarbons analyzed by gas chromatography can be represented by two general features: resolved compounds and an unresolved complex mixture (UCM; the GC capillary columns cannot resolve the mixture of many structurally complex isomers and homologues of branched and cyclic hydrocarbons which appeared as a broad hump in the GC chart) (Table 1). All the samples in this study had a low UCM (0.01 to 0.05 ng g<sup>-1</sup>) as a broad unimodal hump in the range C<sub>12</sub> to C<sub>40</sub>. The low proportion of unresolved (0.01 to 0.05 ng g<sup>-1</sup> of wet wt.) compared to resolved aliphatics (4.5 to 850 ng g<sup>-1</sup>) suggested that the source of alkanes is not petroleum. This spatial distribution for aliphatics and PAHs suggested that most of the contaminants may originate from urban runoff, municipal wastes and petroleum industries.

The isomer ratios phenanthrene / anthracene < 10 in most stations and fluoranthene / pyrene > 1 in most stations, except stations 9, 11, 12 and 18, indicate that the polycyclic aromatic hydrocarbons (PAHs) are of pyrolytic (high temperature combustion) origin (GARRIGUES *et al.*, 1995; BENLAHCEN *et al.*, 1997). The most likely source for these pyrolytic PAHs are the grass fires (6 million tons per year come from rice grass) and exhaust gases from cars.

In the present study, a very good correlation was found between the total hydrocarbons and PAHs (r=0.999) as shown in Figure 2, which indicates that the two classes of compounds may come from the same primary sources. On the other hand there was no correlation between the total hydrocarbons and the total aliphatic compounds (r=0.06). The values of PHE/ANT can be plotted against the values of FLTH/PYR (Fig. 3), showing that PAHs are from pyrolytic sources (BAUMARD *et al.*, 1998b; READMAN *et al.*, 2002).

## Conclusions

Bivalves were collected from Mediterranean Sea off the coast of Egypt for coastal environment contamination in April 2000. This study showed that the concentrations of aliphatics are relatively low in comparison with other coastal seas. On the other hand, the concentration of PAHs was relatively high in comparison with other coastal areas in the Mediterranean Sea, but may be much lower than those reported for some of the published

survey and monitoring studies of coastal areas from other regions in the world. Accordingly, the coastal area in the north of Egypt might, in this sense, be considered as relatively less polluted. However PAHs in the Mediterranean Sea are generally of pyrolytic origin(s), and in the region, this indicates the frequent grass fire as a common source of these compounds together with the car exhaust fumes. Other sources such as petrogenic, non-petroleum industries, oil refineries, oil distribution and heavy ship traffic may be involved.

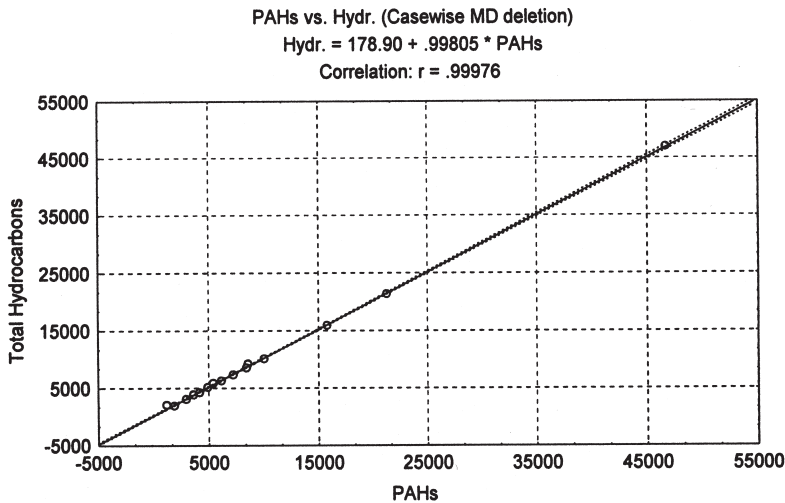


Fig 2: Correlation between total hydrocarbons and PAHs.

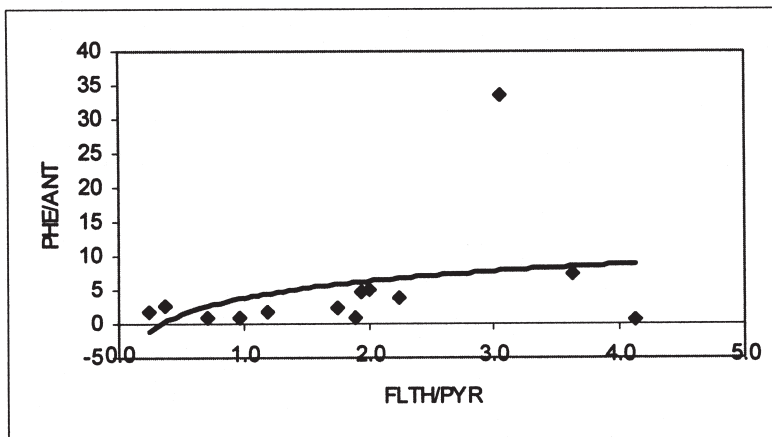


Fig 3: Plot of isomeric ratios PHE/ANT (phenanthrene / anthracene) vs FLTH/PYR (fluoranthene / pyrene) for mussels from Mediterranean Sea.



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