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Study of N-Alkanes and Polycyclic Aromatic Hydrocarbons in the Mussel *Unio tigris* at Tigris River in Iraq

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ABSTRACT

N-alkanes and 18 polycyclic aromatic hydrocarbons (PAHs) with fat content in the tissue of the mussels *Unio Tigris* were collected from seven locations along the Tigris River in Maysan Province, Iraq. These sites were distributed along Al-Uzair, Qal'at Salih, Al-Majar, Al-Amara, Al-Kumait, Ali Al-Sharqi, and Ali Al-Garbi. Sampling locations along the Tigris River in Amara city showed high levels of pollution. The values of the n-alkanes in the samples ranged from 0.240 at station 7 to 3.724 at station 4 ug/ g dry weight, respectively, while the values of PAH compounds ranged from 5.070 at station 7 to 9.230 at station 4 ug/ g dry weight. In the study area, carbon numbers C19 through C31 were predominant. Sources of petroleum and microbes are responsible for the even n-alkanes in the C14–C24 range. In contrast, the domination of the odd n-alkanes in the range C15-C33 is pointed to biogenic sources, particularly the C23, C25, C27, C29 and C31, otherwise the mean of fat ranged from 0.75 at station 7 to 0.97 at station 4. The HMW-PAHs are prevalent at elevated quantities. Nevertheless, LMW-PAHs are absent in the majority of locations. The prevalence of HMW-PAHs in numerous locations was ascribed to oil refineries, oil fields, various power plants, road traffic, vehicle emissions, electrical generators, waste incineration, and other activities reliant on high-temperature fuel combustion, which generates substantial quantities of PAHs, leading to the accumulation of these pervasive pollutants in the samples. High molecular weight PAH concentrations predominated in the samples, and the LMW/HMW ratio was below one, signifying petrogenic pollution origins. The overall findings revealed that the alkanes consistently exhibited moderate pollution levels. The PAH pollution was quantified by the total concentration of PAHs, which showed the highest contamination among all the PAHs throughout the year-long study. This study is among the most extensive investigations into the presence and distribution of n-alkanes and polycyclic aromatic hydrocarbons in mussels from the Tigris River. In addition, it offers important data on other sources of n-alkane and PAH inputs through the use of such mussels as bioindicators of oil pollution in this area.

INTRODUCTION

Water pollution has a significant environmental issue affecting humans and ecosystems in advanced and emerging countries. There are many materials that can be classified as pollutants such as the heavy metals, polynucleic hydrocarbons (PAHs), n-alkanes, polychlorinated biphenyls (PCBs), and pesticides. These organic pollutants are highly resistant in the environment and can cause prolonged effect on living organisms and ecosystems **(Behera & Prasad, 2020)**.

Polynucleic aromatic hydrocarbons (PAHs) are toxic in their nature, which can be found in the environment by two sources, natural process and human activities. Animals and aquatic organisms may be exposed to these pollutants through long-time accumulation or genetic tendency to their surroundings. Polycyclic aromatic hydrocarbons have been found inflow and outflow of wastewater treatment plants. Moreover, they are determined in sediments, fish, and both aquatic systems, nature and man-made. Due to their persistence and potentiality to accumulation in sediments and tissues of living organisms, the PAHs had represented a threat by bioaccumulation **(Saleh *et al*., 2021)**.

Consequently, implementing a monitoring program to assess environmental exposure to these hazardous materials has become crucial. Therefore, many species can be regarded as a bioindicator, specially *Unio Tigris* mussels, for their ability to quickly accumulate lipophilic organic pollutants. Additionally, their stationary in nature and filter-feeding patterns made them perfect indicators of the environment. This is done since they incompletely absorb the contaminations.

This study focuses on determining the concentrations and sources of n-alkanes and PAHs in *Unio Tigris* mussels on the Tigris River in the Amara Province. The Tigris River is an essential source of many activities in the governorate such as drinking water, irrigation, and fishing resources. Unfortunately, the water quality has become deteriorating as a result of industrial, agricultural, and human activities. Because of their high protein and low-fat content, the *Unio Tigris* mussels serve as an important economic and dietary resource in the densely populated northern governorates of Iraq. Consequently, the exposure of these compounds in a long-time due to serious health risk whether direct or indirect exposure through the consumption of the hazardous pollutants by mussels and other species in river.

The goal of this study was to grasp comprehensive data on the hydrocarbon composition of *Unio Tigris* mussels in Iraq. In addition, determining the sources of PAHs and n-alkanes. This research highlights the critical need for continuous monitoring to reduce the health and environmental risks associated with pollution in the Tigris River.

MATERIALS AND METHODS

In this work, the mussels (*Unio Tigris*) depicted in Fig. (1), were gathered from seven sites along the Tigris River in Amara Province, Iraq. These sites were distributed along Al-Uzair, Qal'at Salih, Al-Majar, Al-Amara, Al-Kumait, Ali Al-Sharqi, and Ali Al-Garbi, as shown in Fig. (2).

The dried mussel samples were grounded into a fine powder using mechanical mortar and were then sifted through a 63µm filter. The protocol was conducted for analysis of hydrocarbon according to the method of **Goutx and Saliot (1980)** and **Al-Hejuje (2014)**.

Fig. 1. *Unio tigris*

A Soxhlet extraction was performed intermittent on five grams of mussel in a thimble, using 100 milliliters of a methanol-benzene solution (1:1 v/v). Consequently, the resulting of extraction was separated into two fractions, the aromatic and aliphatic hydrocarbons, via column chromatography by cleanup process. The column was prepared packing it with ten grams of silica gel (100-200 mesh). This layer is positioned after the glass wool, supporting the material and is followed by an alumina with a mesh of silica gel. The extracted chemicals were subsequently separated into aromatic and aliphatic hydrocarbons. Ten grams of silica (100-200 mesh) and ten grams of alumina (100-200 mesh) were activated at 200°C for four hours, followed by partial deactivation with five percent of water. One gram of anhydrous sodium sulfate was sprinkled on the surface. The extract was poured at the top of the column, then 25ml of hexane was used to elute the aliphatic. Similarly, 25ml of benzene was used for eluting the aromatic hydrocarbons to keep the top layer from being disturbed when the solvent was added. The two fractions, aromatic and aliphatic hydrocarbons, were concentrated utilizing a rotary evaporator until the volume became 1ml by nitrogen (N2), then they were transferred into a vial. The chromatography-mass spectrometry analyses was performed on both fractions.

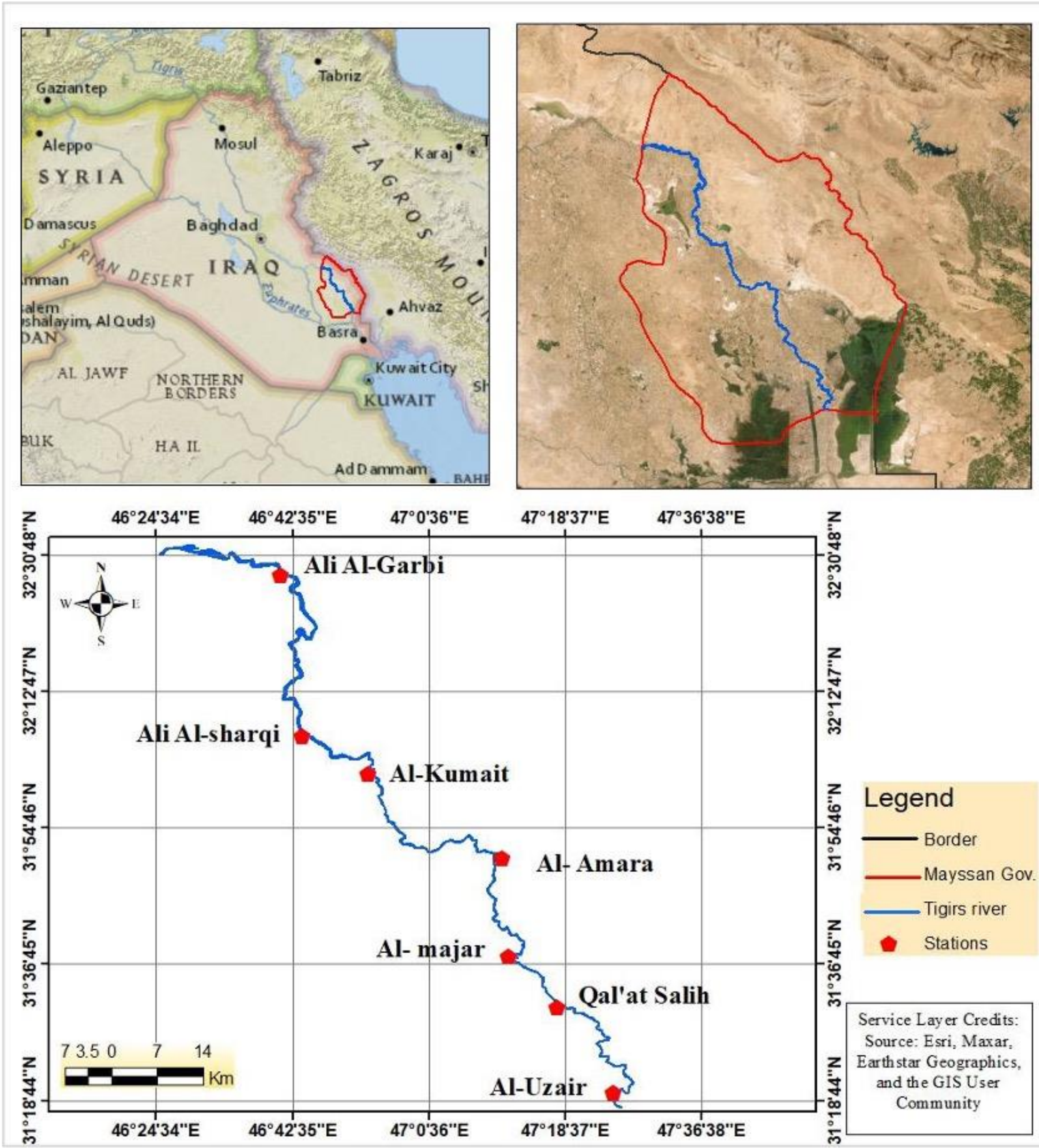

Fig. 2. The study area and stations

RESULTS

A sample of *Unio Tigris* was elected from the Amara region along the Tigris River in Iraq. The study aimed to identify n-alkanes and 18 polycyclic aromatic hydrocarbons (PAHs) in the tissue of the mussels.

The n-alkanes in the samples varied from 0.240ug/ g dry weight at station 7 to 3.724ug/ g dry weight at station 4 (Table 1 & Fig. 3). However, the values of PAH compounds ranged from 5.070ng/ g dry weight at station 7 to 9.230ng/ g dry weight at station 4 (Table 3 & Fig. 5). On the other hand, the mean of fat ranged from 0.75 at station 7 to 0.97 at station 4 (Table 5 & Fig. 7).

The recovery rates for n-alkanes and PAHs were found to be satisfactory. The results indicated the presence of both n-alkanes and PAHs at most sites, with a predominance of multi-methylated PAHs and low-methylated PAHs. Monitoring the Tigris River is crucial for identifying additional pollution sources and for supporting the development of policies focused on improving the river conservation **(Al-Humaidan *et al.*, 2021; Pešić & Glöer, 2021)**.

The overall findings revealed that the alkanes consistently displayed moderate levels pollution. The PAH pollution was quantified by the total concentration of PAHs, which revealed the highest contamination among all the PAHs throughout the year-long study. This study is one of the most comprehensive investigations into the presence and distribution of n-alkanes and polycyclic aromatic hydrocarbons in mussels from the Tigris River. In addition, it provides important data on other sources of n-alkane and PAH inputs through monitoring the Tigris River. The study established a framework for evaluating the types and presence of hydrocarbons in the sediment, enabling the identification of key source areas responsible for introducing hydrocarbons into the river. These data are essential for developing policies focused on improving the river's conservation **(Kang *et al.*, 2020; Li *et al.*, 2021; Chen *et al.*, 2022)**.

Table 1. Concentrations of n-alkanes (μg/g) dry weigh in samples of stations

N-alkanes	Stations						
	1	**2**	**3**	**4**	**5**	**6**	**7**
C14	0.00484	0.02	0.030	0.017	0.015	0.014	0.010
C15	0.012	0.0494	0.032	0.042	0.032	0.023	0.021
C16	0.012	0.0534	0.028	0.046	0.036	0.021	0.001
C17	0.015	0.012	0.031	0.055	0.053	0.041	0.003
Pri	0.014	0.01	0.064	0.078	0.071	0.060	0.005
C18	0.01584	0.01	0.059	0.052	0.042	0.210	0.007
Phy	0.016	0.008	0.028	0.018	0.092	0.008	0.004
C19	0.018	0.0174	0.099	0.083	0.074	0.063	0.006
C20	0.017	0.016	0.085	0.094	0.085	0.074	0.009
C21	0.014	0.01	0.089	0.093	0.064	0.042	0.004
C22	0.016	0.014	0.110	0.102	0.063	0.054	0.003
C23	0.017	0.014	0.110	0.123	0.093	0.073	0.002
C24	0.016	0.016	0.121	0.125	0.098	0.084	0.005
C25	0.02084	0.0274	0.12	0.123	0.024	0.012	0.010
C26	0.023	0.028	0.208	0.207	0.026	0.018	0.012
C27	0.02984	0.0534	0.31	0.315	0.045	0.035	0.014
C28	0.033	0.062	0.452	0.462	0.062	0.041	0.02
C29	0.031	0.064	0.43	0.531	0.037	0.028	0.01

C30	0.036	0.058	0.532	0.546	0.052	0.043	0.02
C31	0.021	0.04	0.063	0.330	0.021	0.01	0.027
C32	0.025	0.026	0.062	0.076	0.032	0.012	0.01
C33	0.02384	0.024	0.093	0.086	0.022	0.010	0.013
C34	0.015	0.012	0.044	0.066	0.054	0.063	0.014
C35	0.015	0.008	0.042	0.054	0.046	0.042	0.010
SUM	**0.4612**	**0.645**	**3.242**	**3.724**	**1.239**	**1.081**	**0.240**
C17/Pri	1.250	1.200	0.484	0.705	0.746	0.683	0.600
C18/Phy	2.000	1.250	2.107	2.888	0.456	26.250	1.75
Pri/Phy	0.666	1.250	2.285	4.333	0.771	7.500	1.25
Odd	0.126	0.181	1.419	1.835	0.511	0.379	0.12
Even	0.125	0.163	1.731	1.793	0.565	0.634	0.111
CPI	1.008	1.110	0.819	1.023	0.904	0.597	1.081

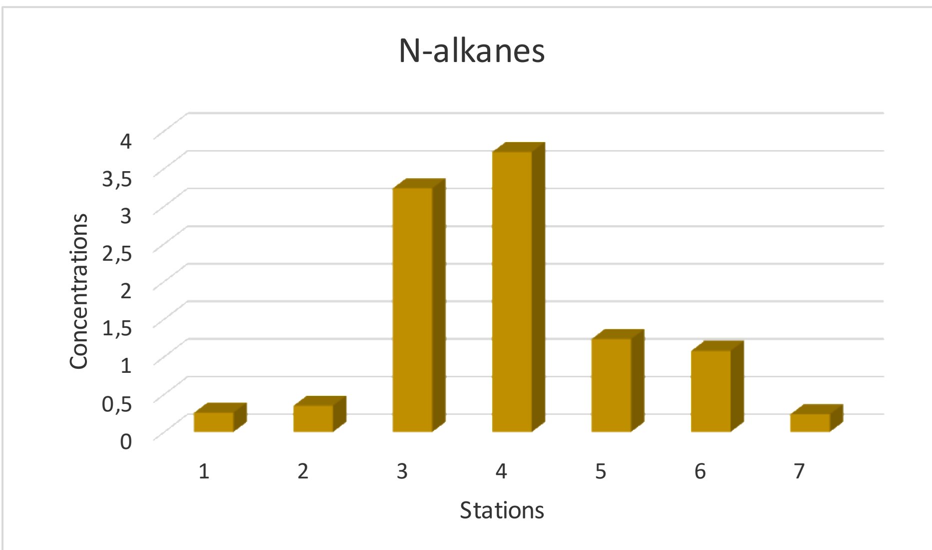

Fig. 3. Total concentrations of n-alkane (µg/g) at stations

Table 2. Diagnosis ratio of n-alkane in study stations

N-alkanes	Stations						
	1	2	3	4	5	6	7
C17/Pri	Presence of oil components	Presence of oil components	Oil and hydrocarbons are weathering	Oil and hydrocarbons are weathering	Oil and hydrocarbons are weathering	Oil and hydrocarbons are weathering	Oil and hydrocarbons are weathering
C18/Phy	Presence of oil components	Presence of oil components	Presence of oil components	Presence of oil components	Oil and hydrocarbons are weathering	Presence of oil components	Presence of oil components
Pri/Phy	Anthropogenic	Biogenic	Biogenic	Biogenic	Anthropogenic	Biogenic	Biogenic
CPI	Biogenic	Biogenic	Anthropogenic	Biogenic	Anthropogenic	Anthropogenic	Biogenic

The current study revealed that high molecular weight (HMW) n-alkane compounds surpassed low molecular weight (LMW) n-alkane compounds. The low molecular weight (LMW) n-alkane compounds, such as C9-C16, can be easily evaporated or decomposed by microbes, but the high molecular weight (HMW) n-alkane compounds, such as C28-C31, exhibit greater resistance to biodegradation **(Al-Hejueje *et al.*, 2016)**. The sample in the current investigation encompassed n-alkane carbon chain lengths ranging from C14 to C35. In the studied area, carbon numbers C19 to C31 were prominent. The even n-alkanes within the C14-C24 range are derived from microbial and petroleum sources, while the prevalence of odd n-alkanes in the C15-C33 range indicates biogenic origins, namely the presence of C23, C25, C27, C29, and C31 as indicators of terrestrial plants **(Wang *et al.*, 2011; Farid, 2017; Dong *et al.*, 2024)** (Fig. 3).

The Tigris River, an essential water source in Iraq, has been the focus of numerous research investigating the levels of polycyclic aromatic hydrocarbons (PAHs) and n-alkanes. These chemicals are notable environmental contaminants that can exert profound ecological and health effects.

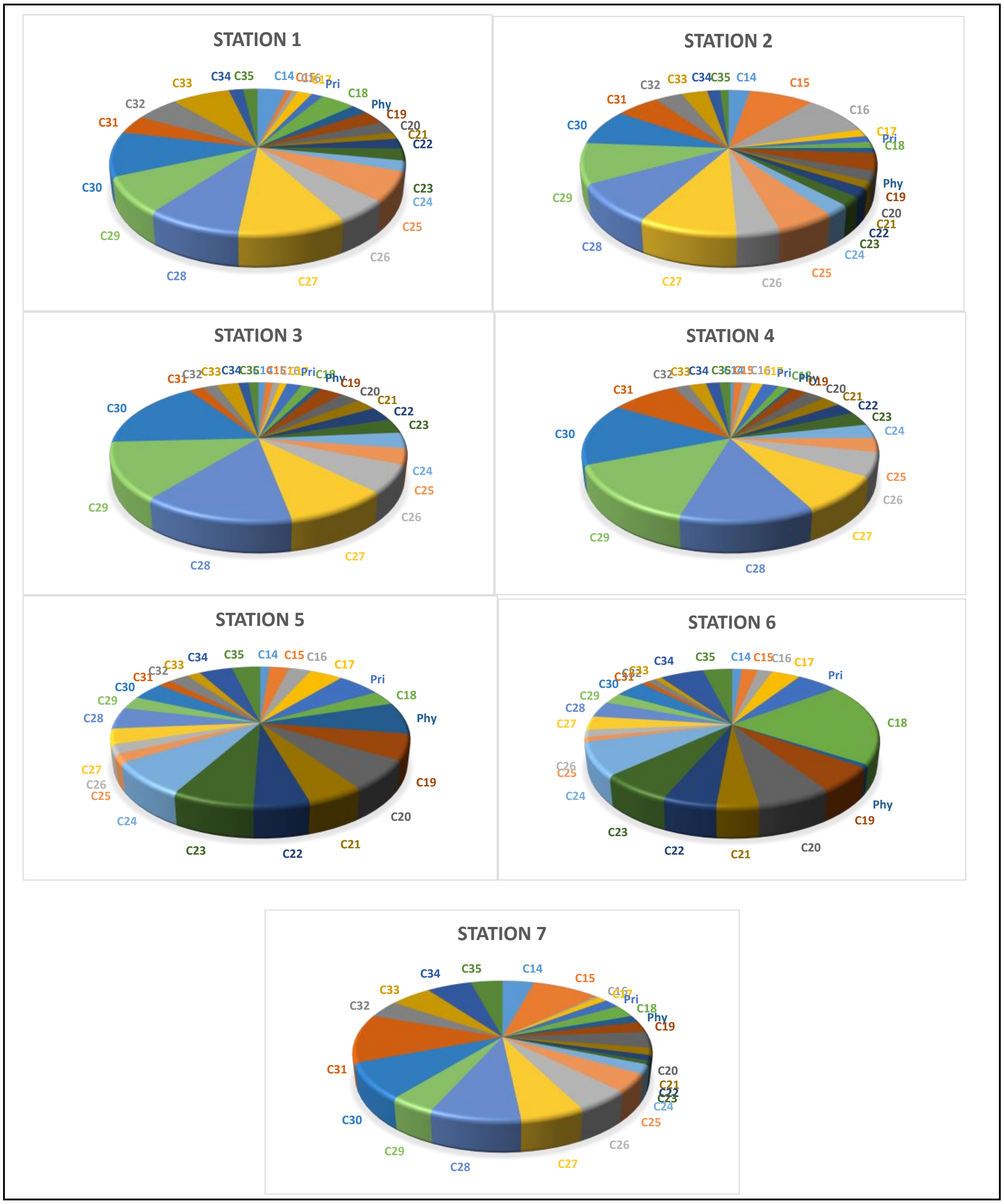

Fig. 4. Concentrations of n-alkanes (μg/g) at stations

Table 3. Concentration of polycyclic aromatic hydrocarbons (ng/g) dry weight in samples of stations

PAHs	Stations						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Naphthalene	0.28	0.172	0.209	0.300	0.4	0.1934	0.01
Acenaphthene	0.21	0.192	0.219	0.520	0.12	0.6434	0.02
Fluorene	0.43	0.512	0.549	0.460	0.46	0.4534	0.31
Phenanthrene	0	0	0.109	0	0	0	0
Anthracene	0.18	0.082	0.119	0.120	0.1	0.0534	0.22
Fluoranthene	0.14	0.112	0.159	0.060	0	0.01	0.04
Pyrene	0.59	0.692	0.819	0.840	0.63	0.8434	0.62
Benzo[a] anthracene	0.22	0.34	0.529	0.730	0.2	0.8834	0.74
Chrysene	0.43	0.55	0.58	0.680	0.7	0.75	0.5
Benzo[b] flo	0.63	0.78	0.83	0.930	0.1	0.63	0.63
Benzo[k]fluoranthene	0.98	1.28	1.29	1.540	1.37	1.18	0.76
Benzo[a] pyrene	0.03	0.01	0.01	0.050	0	0.03	0.01
Indeno [1,2….]	0.32	0.43	0.48	0.650	0.4	0.473	0.23
Dibenzo [a.h….]	0.89	0.95	0.97	1.660	1.5	1.333	0.31
Benzo[ghi] perylene	0.64	0.86	0.89	0.690	3	0.243	0.67
Total	5.97	6.962	7.762	9.230	8.98	7.72	5.07
LMW/HMW	0.175	0.120	0.120	0.178	0.136	0.240	0.121
Phen/Ant	0	0	0.666	0	0	0	0
Fl/Py	0.692	0.714	0.630	0.547	0.730	0.580	0.500
BaA/ (BaA+ Chry)	0.338	0.382	0.431	0.517	0.222	0.535	0.596
InP/ (InP+ BghiP)	0.326	0.333	0.350	0.485	0.117	0.635	0.255
Ant/ (Ant+ Phen)	1	1	0.600	1	1	1	1

PAH were mostly categorized into two groups based on their molecular weight. Anthracene, naphthalene, acenaphthylene, acenaphthene, fluorene, and phenanthrene were all part of the first group of compounds. These small compounds boast two or three fused aromatic rings and a low molecular weight. The second group consists of nine compounds including indeno (1, 2, 3, c, d), benzo (g, h, i) perylene, benzo(k), benzo(a), benzo(b), chrysene, benzo(a)anthracene, benzo(b), fluoranthene, and pyrene. These large molecules had a high molecular weight and contained four fused aromatic rings.

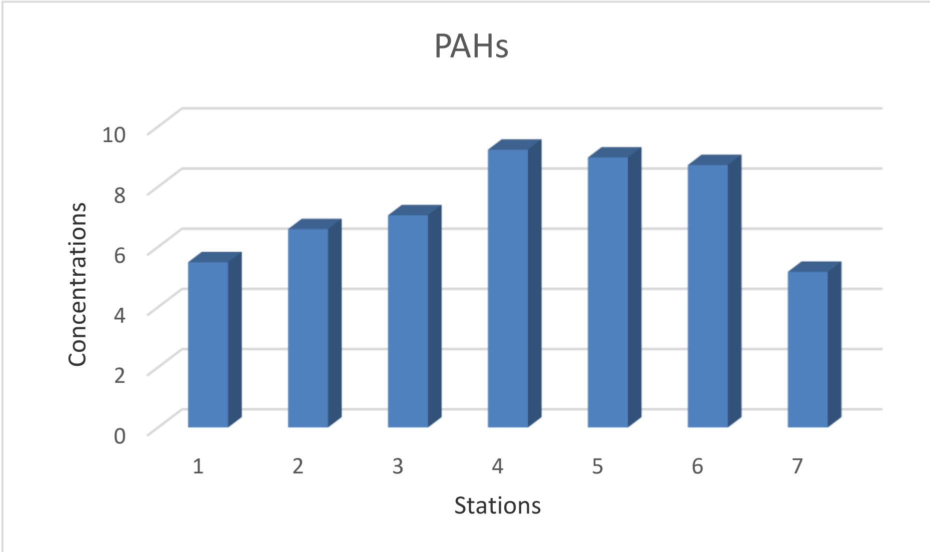

Fig. 5. Total concentration of polycyclic aromatic hydrocarbons at stations

Table 4. Diagnosis ratio of PAHs compounds in study stations

PAHs	**Stations**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
LMW/HMW	pyrogenic	Pyrogenic	Pyrogenic	pyrogenic	Pyrogenic	pyrogenic	pyrogenic
Phen/Ant	pyrogenic	Pyrogenic	Pyrogenic	pyrogenic	Pyrogenic	pyrogenic	pyrogenic
Fl/Py	petrogenic	Petrogenic	Petrogenic	petrogenic	Petrogenic	petrogenic	petrogenic
BaA/(BaA+Chry)	petrogenic or pyrogenic	Pyrogenic	Pyrogenic	pyrogenic	petrogenic or pyrogenic	pyrogenic	pyrogenic
InP/(InP+ BghiP)	petrogenic or pyrogenic	petrogenic or pyrogenic	petrogenic or pyrogenic	petrogenic or pyrogenic	Petrogenic	pyrogenic	petrogenic or pyrogenic
Ant/(Ant+ Phen)	pyrogenic	Pyrogenic	Pyrogenic	pyrogenic	Pyrogenic	pyrogenic	pyrogenic

The HMW-PAHs are prevalent at elevated concentrations, but LMW-PAHs are largely unrecorded in most areas. The prevalence of high molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs) in various locations can be attributed to oil refineries, oil fields, numerous power plants, vehicular traffic, emissions from vehicles, electrical generators, waste incineration, and other activities reliant on high-temperature fuel combustion, which generate substantial quantities of PAHs. These pervasive pollutants have accumulated in the samples **(Wu *et al.*, 2017; Aoeed *et al.*, 2021)**. The biodegradation rates of low molecular weight polycyclic aromatic hydrocarbons (LMW-PAHs), like naphthalene and acenaphthene, are more accelerated than those of high molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs) such as pyrene, fluoranthene, and benzo(a)pyrene **(Obayori & Salam, 2010; Karem *et al*., 2016)**. In addition to biodegradation, low molecular weight polycyclic aromatic hydrocarbons (LMW-PAHs) evaporated more rapidly than high molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs) due to the elevated vapor pressure of LMW-PAHs. This constituted communication with more reports **(Al- Saad *et al*., 2017; Al-Hijaj *et al*., 2019; Al- Saad *et al*., 2019)**.

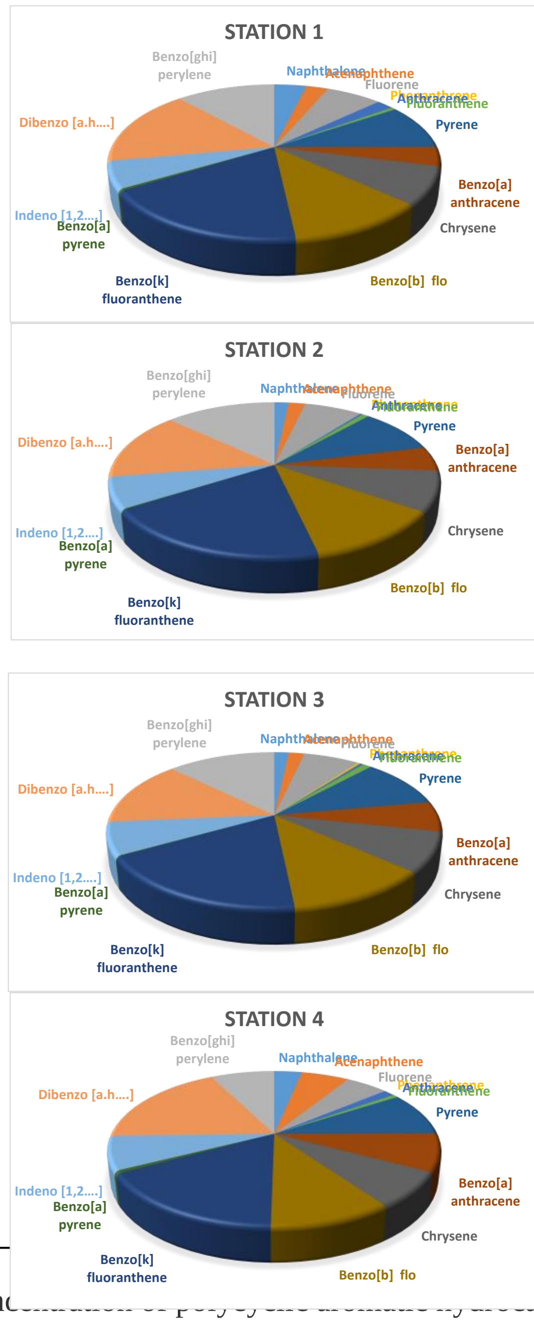

Fig. 6. Concentration of polycyclic aromatic hydrocarbons at stations

Table 5. Concentration of fat % in samples of stations

Station	Con.	range	mean	±SD	±SE
1	0.85	0.84-0.86	0.85	0.01	0.005
	0.84				
	0.86				
2	0.86	0.85-0.88	0.863	0.015	0.008
	0.88				
	0.85				
3	0.88	0.88-0.92	0.896	0.020	0.012
	0.89				
	0.92				
4	0.98	0.96-0.98	0.97	0.01	0.005
	0.97				
	0.96				
5	0.92	0.9-0.92	0.91	0.01	0.005
	0.9				
	0.91				
6	0.85	0.81-0.85	0.826	0.020	0.012
	0.82				
	0.81				
7	0.75	0.74-0.76	0.75	0.01	0.005
	0.76				
	0.74				

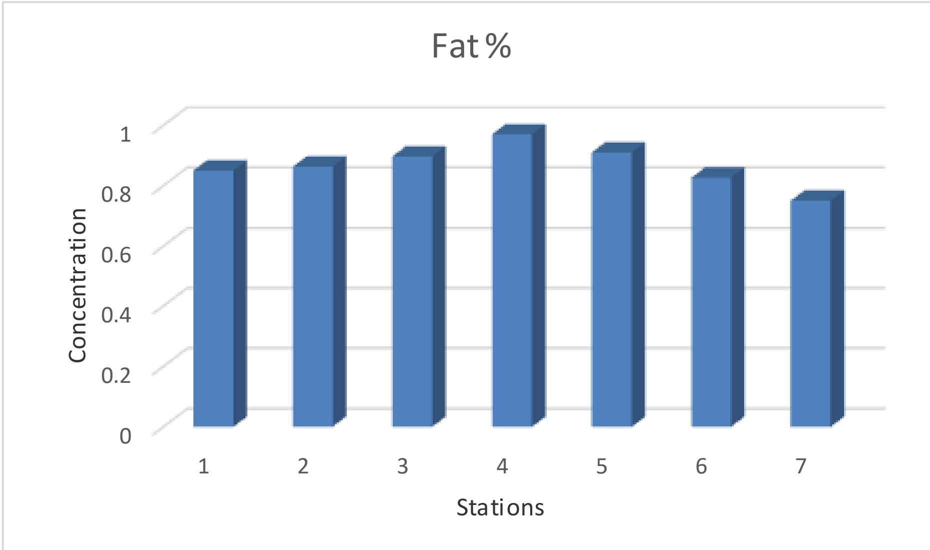

Fig. 7. Total concentration of fat% at stations

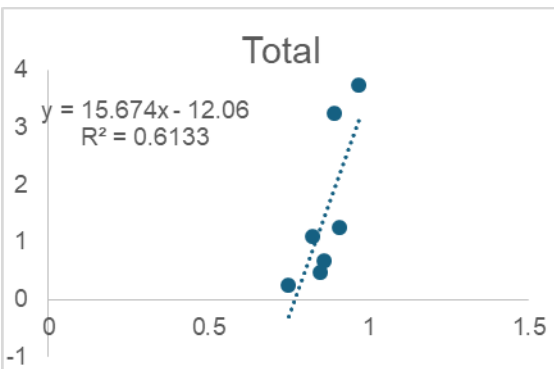

Fig. 8. Relationship between percentage of fat with aliphatic

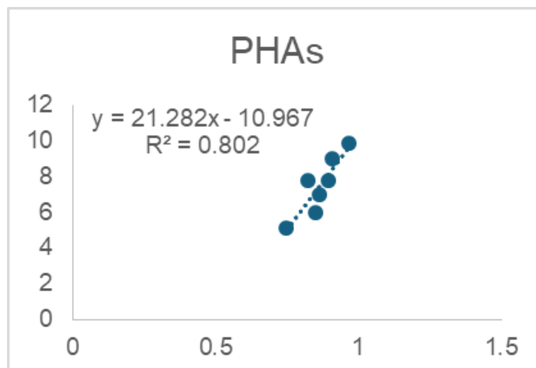

Fig. 9. Relationship between percentage of fat with polycyclic aromatic hydrocarbons

Direct correlation was established between the mussels and the biota in the vicinity. They stored the fat content in mussels, which varies over the year, and suggest that it is necessary to have individual correlations to many parameters of the environment rather than global relations between pollutant concentrations in biota. Moreover, it was shown that hydrocarbon depuration in the mussels can influence the relationship between hydrocarbon biodynamic model parameters and hydrocarbon levels in mussels. Thus, site-to-site variations in contaminant and mussel artificial feeding regimes can bring variability in the hydrocarbon relationship with the muscle tissue triacylglycerol content. The uptake of pollutants in mussels for addressing environmental health issues is important to consider the soft tissue fat content. Medium (or low) fat content may serve as an internal reservoir of hydrophobic contaminants and also clarify their bioavailability in this spatiotemporal species of the hydrosphere **(Ollard *et al.*, 2023; Cordeiro *et al.*,**

2024). The fat content was determined to be 0.75-0.97% depending on the site. The highest amount of fat was observed in muscle tissue from the Al-Amara site. It was found that there is a significant correlation between values, which means a significant correlation (R=0.61)>between fat content and n-alkane and PAH levels in *Unio Tigris* in this study, as shown in Fig. (8). However, the fat has a significant correlation (R=0.80) with the PAHs compounds, as displayed in Fig. (9).

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