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Spatial and Temporal Variation of Total Petroleum Hydrocarbons and Aliphatic Compounds in Muscles of *Planiliza subviridis* in Shatt Al-Basrah Canal, Southern Iraq

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Abstract: The levels of petroleum hydrocarbons and the concentrations of aliphatic compounds were measured in the muscles of the mugilid fish *Planiliza subviridis* population at Abu Sakhir and Zubair Bridge sites in Shatt Al-Basrah Canal, Southern Iraq. The total hydrocarbons showed significant differences in fish muscles ($P \leq 0.05$) in summer and winter with no noticeable differences during spring and autumn. The highest concentrations of aliphatic compounds were during spring in the first station (24370.35 µg/g), while the lowest (1419.15 µg/g) in the second station during summer. Carbon Preference Index (CPI) values were calculated in the studied fishes and exhibited a highest percentage of 7.123 in the first station and a lowest percentage of 0.174 in the second station during the spring. The CPI values showed evidence that these compounds are of biological and anthropogenic origins. The results of the statistical analysis indicated that there were highly significant differences ($P \leq 0.05$) in the muscle fat at the two stations and during the seasons.

Keywords: Aliphatic compounds, Muscles, *Paniliza subviridi*s, Total petroleum hydrocarbons, Shatt Al-Basrah Canal

Introduction

Oil pollution is one of the most important contemporary issues and pollution of the aquatic ecosystem with natural and anthropogenic hydrocarbon inputs is attributed to industrial and marine activities and these increased activities have led to widespread environmental pollution (Maurya et al., 2018). In addition to the input of pollutants and nutrients from sewage discharge and the use of fertilizers in agriculture (Saadali et al., 2020), the presence of these compounds in the aquatic environment will cause a lot of disturbances at the level of water bodies. Thus, it affects the water quality (Mohiddin et al., 2020), as well as burning biofuels and other sources (Salata et al., 2018). The release of petroleum hydrocarbons into rivers and coastal areas during the process of oil production, transportation or processing on land and at sea has negative impacts on the environment and human

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health (Ihunwo et al., 2021). Also, the entry of pollutants into the aquatic environment through oil spill accidents, navigation, oil transportation, washing loading docks, water balancing and export ports, which amount to about six million tons annually (Cai et al., 2021), causes harm to living organisms. Its effects vary depending on the metabolism and photo-oxidation process, petroleum hydrocarbons, which are highly sensitive to fats, enter the fish body through the respiratory and digestive systems (Rodrigues et al., 2010) as it is distinguished by its ability to dissolve in fats, fishes can concentrate it in their bodies (McConville et al., 2018). Fishes are of great importance in the food chain due to the presence of high-quality protein which provides a mixture of easily digestible amino acids. On the other hand, they act as an important indicator of a number of environmental pollution issues in the aquatic system (Wang et al., 2021). The reason for the increase in the levels of pollutants, as a result of their direct absorption from the aquatic environment, is due to the bio concentration (Muijs & Jonker, 2012).

The variation in the level of fish contamination depends on type and food habits, biological availability of chemicals in food and water, influencing environmental conditions, nature of nutrition, type and abundance of food and breeding season (Nasir, 2007). Its effect appears in the form of physiological or biochemical signs, and it may appear in the form of a tissue defect, a genetic disease, or a behavior disorder (Leonzio & Fossi, 2020). Aliphatic compounds (AHs) are one of the most abundant types of hydrocarbons in the environment worldwide, derived from several sources of anthropogenic and biological origin (Duan et al., 2010). The main components of oil (organic fuels), whether crude or refined (Huguet et al., 2019). Iraq is among the oil-producing countries in the Organization of Petroleum Exporting Countries (OPEC) and the city of Basrah is located in the southern part of Iraq, so its crude oil production is estimated to be approximately 90% in the onshore oil fields in the southern part of the country (USEIA, 2019).

This resulted in the discharge of high concentrations of petroleum hydrocarbons into the aquatic environment, as in electric power plants, gas production plants and other industrial plants (Al-Saad et al., 2015). *P. subviridis* is a species of the family Mugilidae that lives in Iraqi waters, enters through coastal marine waters and reaches into the rivers and swamps of southern Iraq for feeding (Mohamed et al., 2018). It is abundant in Shatt Al-Arab River and the Arabian Gulf, and the studied stocks in Shatt Al-Arab River have not been overexploited (Mohamed & Abood, 2020). Two types of fishes mentioned in the study (Resen et al., 2014) were recorded in Shatt Al-Basrah Canal. They are *P. klunzingeri* and *P. subviridis*. The aim of this research is to assess the total levels of petroleum hydrocarbons, types and concentrations of aliphatic compounds and percentage of fat in muscles of *P. subviridis* in Shatt Al-Basrah Canal.

Materials and Methods

Study Area

Shatt Al-Basrah Canal is an industrial drainage channel that starts from the north of Basrah and extends towards the southeast. It covers about 37,157 km and is situated within the lands of the alluvial plain of Basrah, located between latitudes 3027-3028 in the north and 4750-4749 in the east. It was used to transfer flood waters from the Al-Hammar Marsh to Khor Al-Zubair and then to the Arabian Gulf. It is also used to withdraw saline water returning from irrigated agricultural lands that later turned into marsh reclamation. Moreover, this channel is a waterway connecting the southern marshes with the Arabian Gulf from its northern part (Hassan et al., 2018). The sampling was collected from two stations. First station called Abu Sakhir, located northwest of Basrah (N: 47.702856 and E: 30.561467). Second station called Al-Zubair Bridge, located in the southwestern part of the city of Basrah (E: 30.442322 N: 47.760947). It is about 6,423 km from the center of Basrah and about 15,272 km from the second station (Figure 1).

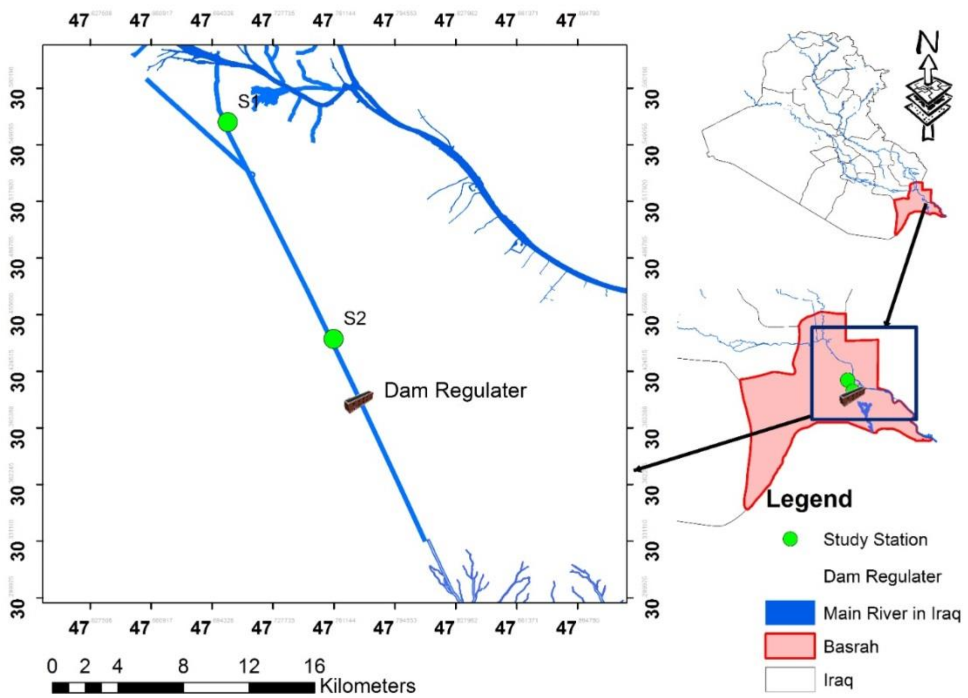

Figure 1: Map of collection sites from Shatt Al-Basrah Canal.

Fish Sampling and Preparation

Fish samples were collected seasonally to measure total petroleum hydrocarbons and aliphatic compounds in their muscles. Sampling was carried out on 21 October and 23 December 2021 (autumn and winter), 21 March and 21 June, 2022 (spring and summer). Water temperature on those sampling dates was 19, 28, 22 and 15 ^{O}C, respectively. Fishes were collected by gill nets. The samples were frozen and taken to the laboratory, where they were individually weighed, morphometrically measured, and stored at 4 ^{O}C. To obtain the muscles, fishes were dissected by using aseptic equipment and dishes, then labeled for further analysis.

Total Petroleum Hydrocarbons Extraction

Extractions were carried out according to Goutx & Saliot (1980). A 3 g dry muscle tissue was crushed in a ceramic mortar with pestle. The grinded samples were taken separately and placed in a thimble into 100 ml of a mixture of methanol: benzene (1:1 v/v) for 24 hours which was used as the extraction solvent. The extract was saponified for two hours at 40 °C by adding 15 ml of an aqueous solution of 4N MeOH (KOH). The contents were poured into separating funnel and 50 ml of normal n-hexane was added to it. The sample was shaken well and then left to settle. As it was observed, two layers were formed, the layer containing hydrocarbons was taken, and passed on a separation column containing at the bottom of the glass wool, topped with a layer of silica gel, alumina, and then anhydrous sodium sulfate. Finally, the total petroleum hydrocarbons were measured after dissolving them with pure hexane using a spectrofluorometer. The types and concentrations of aliphatic compounds in fishes were measured by using a capillary Gas Chromatography (GC) device, type Agilent 7890, equipped with a Flame Ionization Detector (FID). The flow rate of helium gas was 3 ml/min.

Calibration

Basrah crude oil was used for calibration by dissolving a known weight of oil with a specific volume of n-hexane to prepare standard solutions by using a fluoridation device to determine the concentrations of total petroleum hydrocarbons in fishes. The emission intensity was measured at a wavelength of 310 nm and at an excitation of 360 nm.

Lipid Extraction

Concentration of fat in the fish muscles was determined according to the method described in Egan et al. (1981). Two grams of dried and ground fish muscles were taken, placed on filter paper, weighed (filter paper + sample weight before extraction), then it is placed in a thimble inside the extraction part, and the organic solvent is added to it. The device is gradually operated for nine hours at a temperature of 70 °C. The process is repeated 6-10 times every hour. After the period ends, the thimble was extracted and left to dry in the air, the filter paper + the weight of the sample after extraction was weighed and the percentage of fat was calculated according to the equation (Egan et al., 1981):

Fat percentage = (leaf weight + sample weight before extraction) - (leaf weight + sample weight after extraction)/ sample weight * 100.

Statistical Analysis

The statistical analysis of the data was performed by using SPSS 20 software (Statistical Package for Social Sciences). Also, L.S.D. test was used to determine those differences under the significance level of $P<0.05$ through the ANOVA test in the current study sites.

Results

The results of the current study showed a discrepancy in the hydrocarbon concentrations for the two studied areas on the Shatt Al-Basrah Canal, as the highest rate of total petroleum hydrocarbons concentrations in *P. subviridis* was 57.5 and 42.71 µg/g dry weight for the first and second stations, respectively, during the summer.

The lowest rate was at 9.08 and 6.2 µg/g dry weight for the first and second stations, respectively, during the autumn (Figure 2). The results of the concentrations of aliphatic compounds showed that the highest value of 24370.35 µg/g was in the first station during the spring and the lowest value was 1419.15 µg/g in the second station during the summer (Table 1). There was also a clear discrepancy in the values of CPI in that it is a biological or human source during the seasons of the year.

For example, CPI recorded the highest percentage of 4.085 in the first station and the lowest percentage was 1.764 in the second station during the autumn. The CPI values recorded in the study stations gave evidence that the aliphatic compounds in the studied fishes are of biogenic source. The highest CPI value was 7.123 in the first station and the lowest percentage was 4.079 in the second station during the spring. CPI values recorded in the studied stations gave evidence that these compounds are of a biological source. Biogenically, the highest value of CPI during the summer was 6.535 in the second station and the lowest value was 3.604 in the first station. CPI values recorded in the studied stations gave evidence that these compounds are of biogenic source, while the highest percentage was 0.483 in the first station and the lowest was 0.174 in the second station during the winter CPI values recorded in the studied stations gave evidence that these compounds are anthropogenic (Table 1). The highest value of fat in fish muscles was recorded during the spring (18.69% and 21.77%), while the lowest value was in the winter (11.68% and 5.68%) in the first and second stations, respectively (Figure 3).

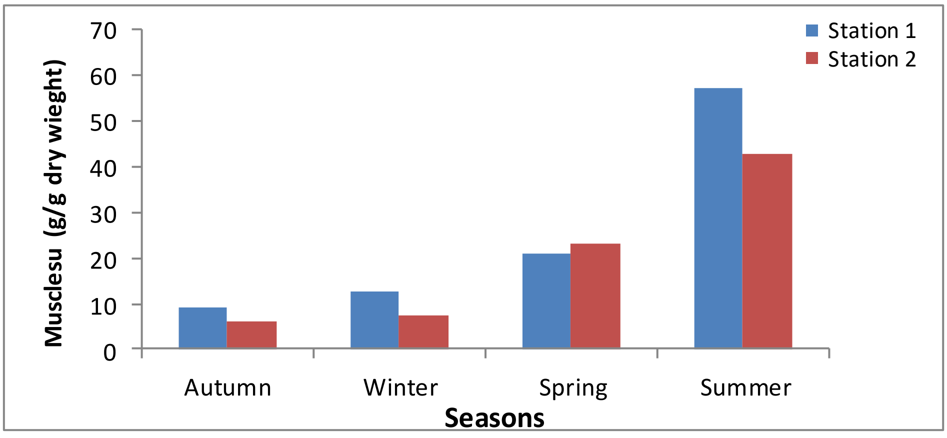

Figure 2: Levels of TPHs in *P. subviridis* muscles from Shatt Al-Basrah Canal during the study period.

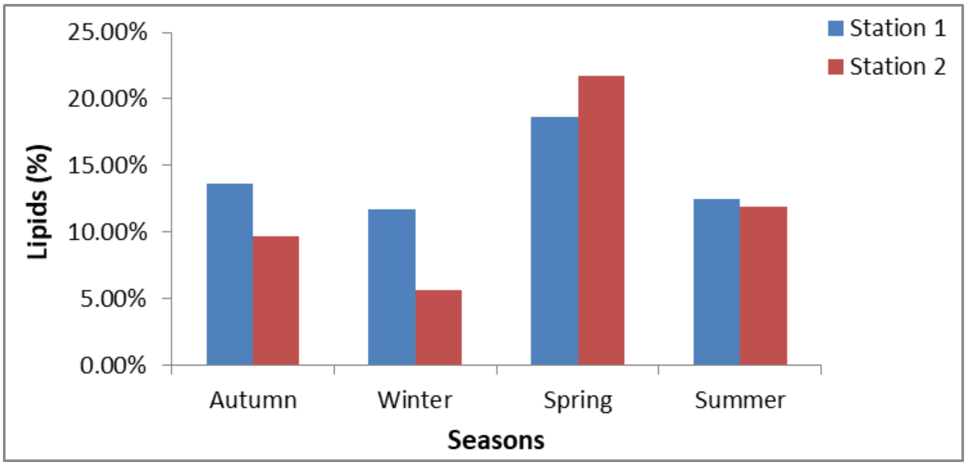

Figure 3: Percent distribution of lipids in muscles of *P. subviridis* from Shatt Al-Basrah Canal during the study period.

Table 1: Quality and concentrations of aliphatic compounds in *P. subviridis* samples (µg/g dry weight) during the study period.

Study stations								
	Station 1				Station 2			
Conc.	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
C12	ND	11.626	ND	ND	ND	ND	41.372	ND
C13	ND	0	ND	ND	ND	ND	14.075	ND
C14	66.851	58.381	9.612	5.454	7.059	449.069	674.369	5.454
C15	36.941	42.782	11.853	47.769	7.513	44.148	28.891	47.769
C16	411.047	1180.834	472.891	510.582	68.168	1184.811	721.813	510.582
C17	79.833	106.296	28.037	180.170	13.522	89.970	120.155	180.170
Pr	39.898	52.405	27.065	106.143	9.038	54.893	12.067	106.143
C18	464.124	1076.91	487.090	555.235	57.644	808.735	458.133	555.235
Ph	48.627	100.308	17.320	65.828	5.632	75.990	17.737	65.828
C19	11.687	45.444	26.967	147.509	1.488	29.628	23.284	147.509
C20	37.169	636.312	279.511	390.867	2.377	547.016	274.456	390.867
C21	69.887	100.123	24.097	108.732	7.425	42.110	26.541	108.732
C22	152.370	239.407	155.680	229.691	17.291	299.281	154.590	229.691
C23	47.804	270.834	52.841	100.076	5.465	143.983	49.623	100.076
C24	87.469	397.310	108.1	135.379	9.000	248.587	104.686	135.379
C25	40.579	288.359	59.623	80.802	4.517	187.385	54.455	80.802
C26	52.524	322.374	88.813	98.077	5.171	238.946	75.118	98.077
C27	28.154	253.487	57.919	69.825	2.712	200.840	50.610	69.825
C28	23.361	108.379	54.215	48.453	2.353	169.167	47.995	48.453
C29	20.410	47.928	33.833	39.651	1.978	30.315	33.879	39.651
C30	14.167	120.213	27.303	31.816	1.888	124.443	28.236	31.816
C31	4680.981	18015.82	529.861	8196.988	1171.09	6614.989	27.931	8196.988
C32	30.574	332.546	19.553	96.695	9.780	84.658	74.209	96.695
C33	10.844	146.628	8.497	67.509	1.272	51.209	25.749	67.509

C34	8.911	44.378	9.182	40.611	1.864	56.128	27.379	40.611
C35	11.755	30.109	7.402	12.851	1.107	39.777	21.438	12.851
C36	49.249	15.213	27.821	76.167	4.790	23.946	49.593	76.167
Total	6525.229 µg/g	24044.41 µg/g	2625.099 µg/g	11442.89 µg/g	1419.158 µg/g	11840.04 µg/g	3238.393 µg/g	11442.89 µg/g
CPI	3.604	4.085	0.483	7.123	6.535	1.764	0.174	4.079

* ND= Not Detected.

Discussion

There are several sources through which petroleum hydrocarbons reach the aquatic environment, such as entry from adjacent lands, atmosphere and industrial sources (Salata et al., 2018) as well as biosynthesis by living organisms (Liu & Li, 2020). The concentrations of petroleum hydrocarbons are indicators of the nature of the aquatic environment and its pollution, which requires special emphasis in order to control (Jazza, 2015). The study showed a seasonal variation in the rate of accumulation of total petroleum hydrocarbons concentrations in *P. subviridis* in Al-Akhdar during the summer of 57.5 µg/g dry weight in the first station, while the lowest rate was 6.2 µg/g dry weight in the second station during the autumn.

In comparison with what was recorded in Iraqi waters (Table 2), 8.3-40.6 (Al-Saad, 1990), 310-9.6 (Fowler et al., 1993), 12.55-26 (Hantoush et al., 2001) and 11.44-48.16 (Nasir, 2007), the concentrations recorded in the current study were found to be higher than 2 g/kg for the fish standard set by the European Union (Enuneku et al., 2015).

The study showed a seasonal variation in the rate of accumulation of total petroleum hydrocarbons concentrations in *P. subviridis* during the summer of 57.5 µg/g dry weight in the first station, while the lowest rate was 6.2 µg/g dry weight in the second station during the autumn (Figure 2). It is also higher than the rest of the studies, but it is lower than the concentrations recorded in the coasts of the Arabian Gulf (Saudi Arabia) due to what is issued by oil refineries, as well as the release of quantities of exhaust oils and ballast water from giant tankers and washing docks and loading platforms as they are large areas for the export of oil (Al-Saad, 1995). The high levels of concentrations recorded in summer may be attributed to biotic and anthropogenic processes, including the alteration and composition of organic matter in the water column and bottom sediments in aquatic environments (Rushdi et al., 2018), as well as the movement of boats used in fishing and high inputs of hydrocarbons as a result of water pollution with oil product discharges (Huguet et al., 2019). The high inputs of hydrocarbons affect the feeding habitats of fishes, leading to eventual accumulation in fish tissues, as long as it is present in polluted habitats (Al-Saad et al., 2011). The first station had the highest concentrations for all seasons except for the spring due to oil leakage resulting from industrial processes, municipal and sewage water and surface runoff (Al-Saad et al., 2017).

The reason for the high levels of concentrations during the spring in the second station may be due to the nature of nutrition and the type and abundance of food, as plants and phytoplankton flourish, which have the ability to accumulate hydrocarbons (Al-Bidhani, 2014). Fish contamination with petroleum hydrocarbons is a serious health problem, largely caused by oil-related activities (Al-Khion et al., 2021). The statistical analysis showed that there were highly significant differences ($P \leq 0.05$) in muscle fat in the two studied stations and during the seasons of the year.

However, no significant differences ($p > 0.05$) were recorded in the summer between the two stations, as the highest value of fat in fish muscles was recorded during the spring (18.69% and 21.77%), and its lowest value was in the winter (11.68% and 5.68%) in the first and second stations, respectively. The current results showed a high concentration of hydrocarbons compared to other studies as a result of the multiple sources of oil pollutants in the Shatt Al-Basrah Canal, which leads to its accumulation in the studied fishes with higher concentrations than other areas. There is a direct relationship between the percentage of fat and the concentration of petroleum hydrocarbons (Ackman et al., 1996). The total fat content of fishes is also mainly related to fish species, nutritional status, fishing seasons, fish life cycle, age and sex (Das & Biswas, 2019). The main components of oil are aliphatic compounds (Huguet et al., 2019), as are biological, synthetic and biogeochemical processes, such as the alteration of organic matter in the water column and bottom sediments (Rushdi et al., 2018). Aliphatic compounds reach fish tissues via the digestive system either directly from water or suspended particles or digest and eventually accumulate contaminated phytoplankton, zooplankton and crustaceans (Wang et al., 2021). The concentrations of aliphatic compounds in fishes of the studied stations varied from the lowest concentrations (1419.15 µg/g) in the summer to the highest concentrations (24370.35 µg/g) in the spring (Table 1). In general, the cause of the increase in concentrations may be due to water pollution with the exhaust of petroleum products and their derivatives, as these high inputs of hydrocarbons affect the feeding habitats of fishes. This leads to the accumulation of these compounds, eventually in fish tissues, as long as they are present in their polluted habitats (Al-Saad et al., 2011). The reason for the decrease in concentrations is due to the effects of extensive weathering (Chapman, 2021), as the evaporation process increases with increasing temperature for alpha compounds of low molecular weights (C10-C22), as well as increasing the processes of biodegradation of microorganisms by heat (Talal et al., 2010). As the length of the lighting period during the day causes an increase in the decomposition of aliphatic compounds by light oxidation, and leads to the cracking of oil compounds in the water column, which coincides with high temperatures,

One of the reasons is the low concentration of hydrocarbons in summer (Talal, 2008). The mode and duration of exposure to pollution, tissue lipid content, differences in species, age and sex affect the accumulation of aliphatic compounds in fish tissues (Al-Ali et al., 2016). The high levels of aliphatic compounds in the tissues of these fishes indicated that their aquatic environment was heavily polluted by petroleum hydrocarbons (Al-Khion et al., 2021). CPI, which is defined as the sum of odd-to-even carbon alkanes, used to indicate the relative relationship between organisms of biological or synthetic origin for hydrocarbons (Saleh et al., 2020).

The CPI data in this study (Table 1) was less than 1 µg/g in winter which means that the origin of hydrocarbons was human either autumn, spring and summer is greater than 1 µg/g which means that the origin of hydrocarbons was biological. There are unnatural sources of hydrocarbons produced from various human activities, where fishing boats and air are added in addition to the oil compounds carried by rivers. The current study showed that the length of the carbon chains of aliphatic compounds in fishes ranged between C36 and C12, the individual carbon compounds with lower molecular weights less than C23 such as C21, C19, C17, C15 and C13 were sourced from zooplankton and algae (Al-Saad, 1995). The carbon compounds with the range C31and C23 come from higher plants (Frena et al., 2017). The source of aliphatic compounds with even carbon numbers (C24,26C and 28C) in fish tissues is mainly from a mixture of marine organisms including algae, bacteria, higher plant waxes and oil inputs (Rushdi et al., 2018). When comparing the results of the current study with the results of other studies in the region and the world (Table 3), one can conclude that the results of the current study were higher compared to the results of other local studies. The concentrations were also very high compared to the concentrations recorded in the Arabian Gulf. The differences in the concentration of AH and its sources in fish tissues may be attributed to the feeding habitats, locations (surface vs. bottom waters), the main sources of food and conditions of contamination (Johnson-Restrep et al., 2008). Pollution of feeding habitats resulting from oil-related activities causes fishes to pollute those habitats with petroleum hydrocarbons (Al-Khion et al., 2021). As for the reason for the high concentrations of the current study, it may be due to the fact that the sources of pollution of Shatt Al-Basrah Canal are large and diverse due to the presence of industrial facilities (Aziz & Sabbar, 2013) and the movement of fishing boats, in addition to large quantities of petroleum compounds as untreated domestic and sewage products (Al-Saad et al., 2017).

Table 2: Comparison of TPHs concentrations in fish muscles from different Iraqi waterbodies and other marine areas.

Regions	TPHs (µg/g)	References
Khor Al-Zubair	8.3-40.6	Al-Saad (1990)
The Arabian Gulf	9.6-310	Fowler et al. (1993)
Northwest of the Arab Gulf	12.55-26	Hantoush et al. (2001)
Northwest Arabian Gulf	11.44-48.16	Nasir (2007)
Shatt Al-Basrah Canal	6.2-57.5	The current study

Table 3: Comparison of aliphatic compounds (AH) concentrations in fishes from different Iraqi water bodies and other marine areas.

Regions	AH (μg/g)	References
Kuwait	48	Fowler et al. (1993)
Saudi Arabia	10.3-2290	Fowler et al. (1993)
Bahrain	5.2-30	Fowler et al. (1993)
The United Arab Emirates	23-174	Fowler et al. (1993)
Oman	8.3-190	Fowler et al. (1993)
Northwest of the Arab Gulf	6.4-32.6	Al-Saad (1990)
Shatt Al-Arab River	3.45-13.11	Al-Saad (1995)
Northwest of the Arab Gulf	0.82-8.85	Al-Saad et al. (2008)
Shatt Al-Basrah Canal	1419.15-24370.3	The current study

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