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Sources and apportionment of aliphatic hydrocarbons in freshwater fish species from Shatt Al-Arab River, Iraq

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ABSTRACT

Aliphatic hydrocarbon n-alkanes, pristane (Pr), and phytane (Ph) were determined in the tissues of six economic freshwater fish species (*Luciobarbus xanthopterus*, *Ctenophyngodon idella, Cyprinus carpio, Tilapia zillii, Palaniza abu, and Leuciscus vorax*) collected from the Shatt Al-Arab river. They were analyzed by capillary gas chromatography - flame ionization detector (GC-FID). The carbon chain lengths of n-alkanes in the fish tissue samples ranged from nC_{13} to nC_{36}. The highest concentration of the total n-alkanes was detected in the filter feeder *P. abu* tissue (23.14µg/g dry weight) and the lowest concentration was in the omnivorous *C. carpio* tissue (3.76 µg/g dry weight). The major sources of the n-alkanes, pristanes, and phytanes were from petroleum hydrocarbons based on the results of the molecular biomarker indices and the ratios of CPI (carbon preference index), Pr/Ph, LMW/HMW (total nC_{13}-C_{24}/total nC_{25}-nC_{36}), nC_{17}/Pr, and nC_{18}/Ph. The percentage of n-alkanes from petroleum related-products ranged from 83.2% to 94.8%, whereas the natural biogenic sources were in the order of bacterial (3.7-11.7%) > higher plant wax (0.0-9.1%) > algal (0.1-1.2%) sources. The occasion of high fractions of n-alkanes and isoprenoids from petroleum hydrocarbons in these fish tissues indicated that the foster habitats were highly contaminated by crude oil. Further studies are required to investigate the bioaccumulation of other anthropogenic contaminants such as plasticizers, pesticides, and polycyclic aromatic hydrocarbons fresh-water organisms.

Key word: *n-Alkane; Fish; Shatt Al-Arab; Gas-Chromatography.*

Introduction

Aliphatic hydrocarbons in the environment are derived from natural biogenic origins (Eglinton and Hamilton, 1967; Simoneit, 1978; Klenkin *et al.*, 2010) and/or from anthropogenic hydrocarbons (Zakaria *et al.*, 2002; Peters *et al.*, 2005). Oil spills or wastes in the aquatic environment introduce hydrocarbons into the water ecosystem and become parts of the food supplies for various organisms. Organisms in general and fish in particular consume hydrocarbons from the surrounding resources either directly by absorption through gills and skin from water or with food.

Crude oil, which is the most common source of hydrocarbon contaminants in the environment (Harrison *et al*., 1975), consists of n-alkanes (paraffin), cycloalkanes (naphthenes), aromatic hydrocarbons (asphaltenes), and inorganic compounds containing sulfur, nitrogen, and heavy metals like nickel, iron, vanadium, and copper (NRC, 1985; Kathi and Khan, 2011). n-Alkanes are saturated unbranched straight-chain hydrocarbons. Branched alkanes are also saturated hydrocarbons with an alkyl substituent or a side branch from the main chain (Arora, 2006; Abed Ali, 2013).

n-Alkanes consists of odd and even carbon atom numbers, where the odd carbon numbers are dominant in organic matter from plants and the even carbon numbers are derived from bacterial sources (Bray and Evan, 1961; Mackie *et al.,* 1974; Dembicki *et al.,* 1976; Nishimura and Baker, 1986; Grimalt and Albaiges, 1987; Simoneit, 1989; Guerra-García *et al.,* 2003; Ekpo *et al.,* 2005; Rushdi *et al.,* 2006a, 2019; Wang et al. 2010; Fagbote and Olanipekun 2013; Li et al. 2018; Stout 2020). The long-chain n-alkanes (nC_{25}-nC_{35}) are molecular markers for most of the higher plant inputs and the short-chain n-alkanes (nC_{15}-nC_{20}) are indicators of marine phytoplankton sources (Blumer *et al.,* 1971; Duan, 2000; Commendatore and Esteves, 2004). The n-alkanes from crude oil are usually known by a normal (unimodal) distribution pattern with no significant odd-to-even carbon preferences (Mazurek and Simoneit, 1984; Roushdy *et al.,* 2011; Commendatore *et al.,* 2012). The isoprenoids hydrocarbon pristane (Pr) and phytane (Ph) are often used to determine hydrocarbon crude oil and also as good indicators for terrestrial and marine organic origin (Tissot and Welte, 1978; Peters *et al.,* 2005). Pr is produced by zooplankton or other marine organisms; whilst Ph is found in crude oil components and can be produced by methanogenic and photosynthetic bacteria (Peters *et al.,* 2005). These diverse characteristics of aliphatic hydrocarbon distribution patterns and concentrations are useful means to examine their potential sources in the background. Therefore, carbon preference index (CPI), pristine/phytane (Pr/Ph) and low molecular weight-to-high molecular weight (LMW/HMW) ratios have used to define the sources of hydrocarbons in the different environmental settings (Bray and Evan, 1961; Lijmbach, 1975; Didyk *et al.,* 1978; Simoneit, 1989; Al-Saad, 1991; Al-Saad, 1995; Al-Khatib 2008; Talal *et al.,* 2010; Abed Ali, 2013; Farid *et al.,* 2010; Al-Hejuje *et al.,* 2015; Kadhim *et al.,* 2019). These indices, ratios, and molecular parameters are also useful and can be applied to identify the main sources of hydrocarbons bioaccumulated by aquatic organisms.

The major sources aliphatic hydrocarbons in the aquatic environment of the Shatt Al-Arab river and Iraqi wetlands are from oil refinery effluents during loading operations (Bedair and Al-Saad, 1992); where about 48 tones of oil are shipped annually across the river for domestic usage (DouAbul and Al-Saad, 1985). Significant levels of petroleum hydrocarbon have been recently reported in the water column and sediments of aquatic systems of Iraq including Shatt Al-Arab and coastal zone (Rushdi *et al.,* 2006a, 2017, 2018; Al-Saad *et al.,* 2011; Douabul *et al.* 2012; Kadhim *et al.,* 2019). Other potential sources include coastal higher plants, freshwater phytoplankton, and biogeochemical alteration of sedimentary organic matter (Rushdi *et al.* 2006a,b,2018).

Few studies have investigated the bioaccumulation and the sources of aliphatic hydrocarbons in aquatic biota, especially from locales with high concentrations of petroleum hydrocarbons, such as Iraqi waters (De Mora *et al.,* 2005, 2010; Tolosa *et al.,* 2005; Ashraf and Mian, 2010; Al-Khion *et al.,* 2016). Therefore, the present study aims to determine the occurrence, levels, and the main sources of the n-alkanes, pristanes, and phytanes in the tissues samples of freshwater fish species from Shatt Al-Arab River using aliphatic hydrocarbon biomarker indices and ratios.

Materials and Methods

Fish species and tissue sample collection:

The fish species were collected from the Shatt al-Arab river, which consists of the confluence of the Tigris and Euphrates rivers in the city of Al-Qarnah, about 70 kilometers north of Basra. The river runs south-east and extends for 204 kilometers, inflowing the waters of the Arabian Gulf for a distance of 5 kilometers with loads of sediments and silt. Shatt Al-Arab River is the most important rivers in Iraq where many types of organisms such as fishes grow and flourish. The current study was conducted on six types of freshwater fishes, (*Luciobarbus xanthopterus; Ctenophyngodon idella; Cyprinus carpio; Tilapia zillii; Palaniza abu; and Leuciscus vorax*) caught from Shatt Al-Arab River to determined the levels of aliphatic hydrocarbons (i.e. n-alkanes, and the isoprenoid pristane and phytane) by using capillary gas chromatography. The description of the fish species is shown in Table SM1. The tissues of the fish samples were freeze-dried, ground using a food grinder and at least three replicates of 15g each sample were freeze-dried and sieved through a 63 µ metal sieve for chemical analyses.

Extraction of aliphatic hydrocarbons

The procedure described by Goutex and Saliot (1980) was used to extract the aliphatic hydrocarbons from fish tissue samples. Twenty five grams of each fish tissue were Soxhlet-extracted for 24 hours with 250 ml Methanol: Benzen (1:1; v:v). The extract was then concentrated to about 2 ml by a rotary evaporator. The concentrated extract was fractionated by column chromatography that contained anhydrous sodium sulphate (1 g) above alumina (10 g) and silica gel (10 g). Saturated hydrocarbons were ob-tained by successively eluting with hexane (25 ml). The aliphatic hydrocarbon fractions were concentrated again on a rotary evaporator, transferred to a vial, and the volume was adjusted to exactly 1 ml using a stream of nitrogen gas.

Determination of aliphatic hydrocarbons and fats

The determination of aliphatic hydrocarbons was performed on an Agilent capillary gas chromatography (GC) with a flame ionization detector (FID) (Agilent, USA). Fused silica capillary column (10 m × 250 µm × 0.5 µm) was used; it was wall-coated open tubular (methyl silicone) (Agilent US2463233H DB-petro, Agilent, USA), with helium as a gas carrier at a flow rate of 1.5 ml/min. The operating temperatures for the detector and injector were 320°C and 300 °C, re-spectively. The temperature of the column was held at 50 °C for 8 min, then 8°C/min to 350°C. Before GC analysis, a standard of n-alkane and isoprenoid pristane and phytane mixture (Supelco company) was injected for calibration and the quantification of the sample aliphatic hydrocarbons. Individual n-alkanes and isoprenoids were quantified based on the retention time of the authentic mixed standard, and the concentrations of each were calibrated based on the standard calibration curve. The concentrations based on indi-vidually resolved peak were summed to obtain the total n-alkanes and isoprenoid concentrations, where the concentrations were reported in mg/g DW.

Determination of the fat content of the fish samples was done by taken three grams of each freeze-dried sample and soxhlet extracted with a 2:1 mixture of petroleum ether: acetone for 24-hours. The extracts were reduced in volume in a rotary vacuum evaporator and subsequently reduced to exactly 1 ml. Ten ìl of the concentrated extracts were taken by a Hamilton syringe and weighted after solvent evaporation.

Quality control

The technical accuracy and precision of the research have been emphasized throughout the different phases of the research activities. These included the sample collection and preparation, chemical analytical method, bimolecular marker approach, and index and ratio application. All solvents were of analytical grade and were dis-tilled before use. Sodium sulphate ashed at 400 °C and silica gel and alumina (100–200 mesh) were washed using methanol and chloroform and dried before use. The organic and inorganic reagents and solvents used in the analytical procedure and the extractions of the samples were tested for potential contaminants. The background contamination as a result of laboratory procedures has been qualitatively and quantitatively assessed by examining procedural blanks. Plank extracts were also carried out after three sample batches through the entire procedures.

Hydrocarbon n-alkanes indices and ratios

The following indices and ratios were used to determine the origin of aliphatic hydrocarbons in the fish tissue samples:

1. Carbon Preference Index (CPI): It is the concentration ratio of the odd to even carbon number of n-alkanes. If the CPI value is ~ 1, it indicates that the n-alkanes are of a petrogenic origin; CPI < 1 indicates biogenic bacteria with bitumen and oils (pyrogenic), a CPI > 1 indicates biogenic sources of n-alkanes from higher plants (Bray and Evan, 1961; Simoneit, 1989; Rushdi *et al.,* 2006a, 2019; Fagbote and Olanipekun, 2013; Diefendorf *et al.,* 2014).
2. LMW/HMW Ratio: LMW (Low Molecular Weight) is the total concentrations of aliphatic hydrocarbons from nC_{13} to nC_{24}, and the HMW (High Molecular Weight) is the sum concentrations of aliphatic hydrocarbons from nC_{25} to nC_{36} (Bouloubassi *et al.,* 2001; Rushdi *et al.,* 2006a; 2018; Fagbote and Olanipekun, 2013). The source is from natural terrestrial biogenic inputs if the ratio < 1, natural input from marine biogenic source if the ratio > 1, and from petroleum source if the ratio = 1 (Gelpi *et al.,* 1970; Meyers 1997; Bouloubassi *et al.,* 2001; Rushdi *et al.,* 2006a, 2018; Fagbote and Olanipekun, 2013).

3- Pristane/Phytane (Pr/Ph) ratio: a petrogenic source is suggested if the ratio of Pr/Ph = 1, the biogenic source if the Pr/Ph > 1, and pyrogenic source if the Pr/Ph < 1 (Lijmbach 1975; Didyk *et al.*, 1978; Broman *et al.*, 1987; Al-Saad 1995; Peters *et al.*, 2005).

4. Heptadecane-to-pristane (nC_{17}/Pr) and octadecane-to-phytane (nC_{18}/Ph) ratios: if the nC_{17}/Pr and nC_{18}/Ph ratios are more than 1 they indicate that the sources of hydrocarbons are from fresh petroleum input and if the ratios are less than 1 then the sources are degraded oil (Lijmbach, 1975; Didyk *et al.*, 1978; Colombo *et al.*, 1989; UNEP, 1995; Peters *et al.*, 2005; Harji *et al.*, 2008; Gao and Chen, 2008)

Statistical analysis

The normal distribution of the data was used for all statistical analyses. Varimax rotation with Kaiser Normalization was applied for principal component analysis. The relationships between different variables were defined by Pearson's correlation. The software SPSS 16.0 (IBM-Statistical Package for Social Sciences, version 16.0) was used for the data analyses.

Results and Discussion

Aliphatic hydrocarbon distribution, levels, and similarity

The distribution and characteristics of aliphatic hydrocarbons measured in the various freshwater fish species tissues are shown in Figure 1 and their concentrations are listed in Table 1. The carbon chain lengths of n-alkanes in the fish sample tissues ranged from nC_{13} to nC_{36} and their distributions varied between samples as shown in Figure 1. The total n-alkane concentrations in the fish tissues ranged from 7.27 µg/g Dwt in *C. idella* (herbivorous) to 23.14 µg/g Dwt in the *P. abu* (filter feeder). They were similar to the concentration ranges reported in fish tissues and muscles from different rivers and lakes (Colombo *et al.*, 2007; Olaji *et al.*, 2014; Al-Imarah *et al.*, 2016; Wang *et al.*, 2019). The n-alkane maximum carbon number (C_{max}) was at nC_{24} for all fish species except for *C. carpio*, which was at nC_{29} (Table 1; Fig. 1.), which suggested different sources of n-alkanes. The concentrations of pristanes and phytanes varied from 0.068 µg/g Dwt in *C. carpio* (omnivorous) to 0.232 µg/g Dwt in *L. vorax* (carnivorous) and from 0.076 µg/g Dwt in *C. carpio* (omnivorous) to 0.391µg/g Dwt in *P. abu* (filter feeder), respectively (Table 1).

The predominance of nC_{24} in the fish tissues may be due to the fish feeding on algae and detritus because the major source of nC_{24} is likely from the algae, phytoplankton, and bacteria (Gomes and Azevedo, 2003; Shahbazi, 2009). The distribution of n-alkanes and the high level of nC_{29} (0.393 µg/g) in the fish tissue of *C. carpio* may be attributed to their feeding on a freshwater higher, mainly emergent, plants because usually nC_{27} nC_{29} and nC_{31} are the major n-alkanes in plant waxes of these plants (Eglinton and Hamilton, 1967; Simoneit, 1978; Ficken *et al.*, 2000; Duan and Xu, 2012).

The principal component analysis (PCA) method has been applied to identify the similarity of hydrocarbon n-alkanes and isoprenoids in the various tissues of the fish species. The PCA analysis using Eigen value > 1.0 has recognized three major components (Table 2). The interpretation was based on factor loadings of > 0.85 and the results showed that 95.29% of the total variance extracted three principal components (PC1, PC2, and PC3). PC1 revealed that 67.00% of the variance, with tissue samples of the fish species *C. idella* (herbivorous), *T. zillii* (herbivorous), and *P. abu* (filter feeder) as shown in Figure 2. It may signify that certain major food sources were controlling the aliphatic hydrocarbons in the fish samples. PC2 clarified 21.07% of the total variance showing a significant factor loading for *L. vorax* (carnivores), and PC3 explained 5.22% of the total variance for *C. carpio* (Omnivorous) confirming that different food sources controlled the aliphatic compounds in fish tissues. The PCA result may perhaps indicate that feeding habits and food sources are key factors that have controls on the levels and content of aliphatic hydrocarbons in the tissues and muscles of the fish species.

Aliphatic hydrocarbon sources

The presence of aliphatic hydrocarbons in an aquatic environment is generally from biogenic and anthropogenic sources (Eglinton and Hamilton 1967; Rieley *et al.*, 1991; Volkman *et al.*, 1992; Rushdi *et al.*, 2018). Also, biogeochemical processes such as alteration and diagenesis of organic matter in the water column and bottom sediments are additional sources of aliphatic hydrocarbons in an aquatic ecosystem (Brassell and Eglinton 1980; Prahl *et al.*, 1980; Colombo *et al.*, 1997; Li *et al.*, 2017; Jafarabadi *et al.*, 2019). Therefore, the levels and contents of hydrocarbons vary between the different environmental compartments. The occurrence of high levels of hy-

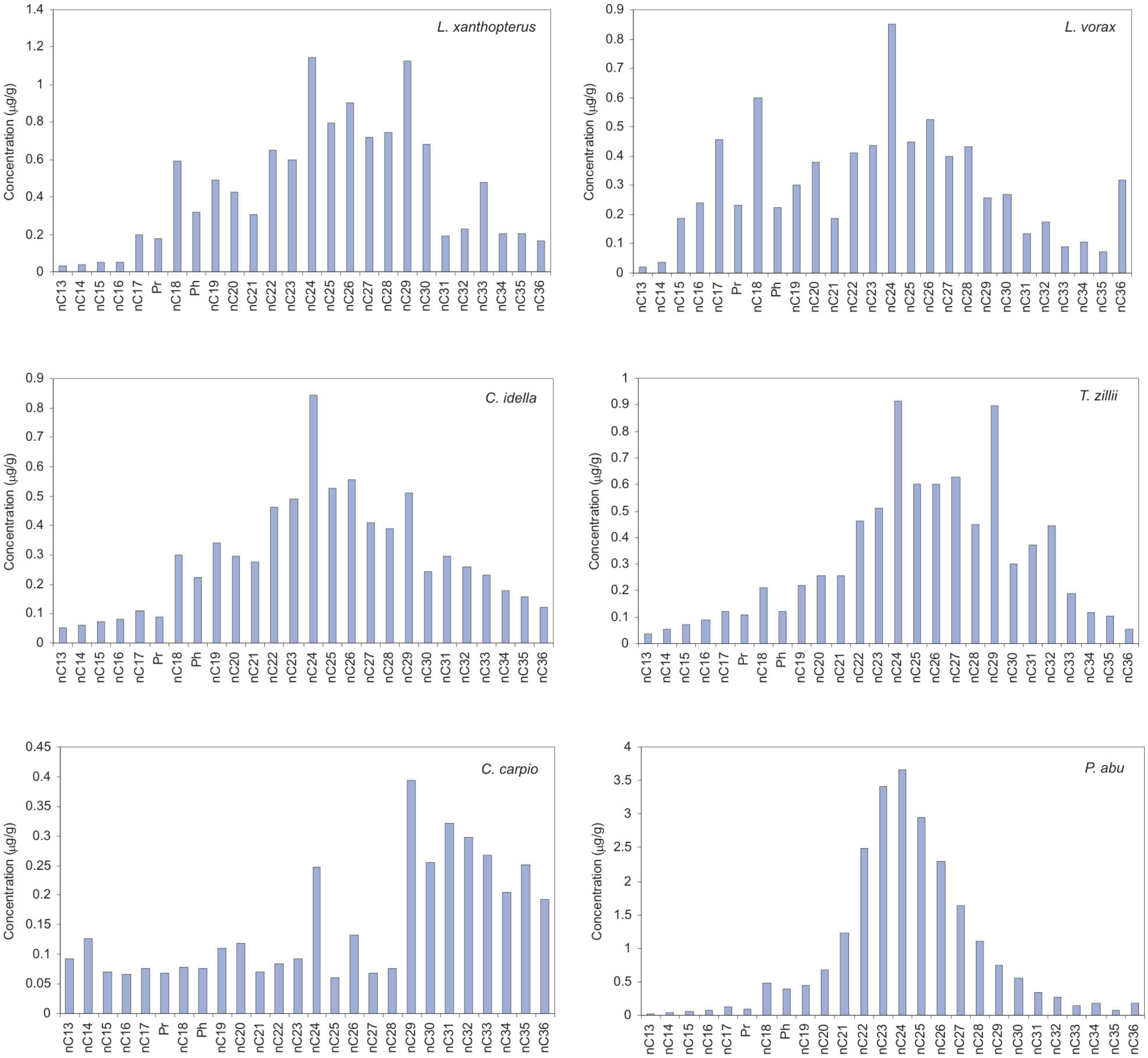

Fig. 1. The distributions and concentrations of n-alkanes in the tissues of the different fish species from the Shatt Al-Arab river- Iraq.

drocarbons will directly become food supply for marine organisms including fish species.

Algal, phytoplankton, and bacteria have been identified as the major sources of low molecular weight n-alkanes, mainly nC_{17}, nC_{18}, and nC_{19} for algae and phytoplankton and even-to-odd predominance (nC_{16}-nC_{24}) for bacteria (Youngblood and Blumer 1973; Grimalt *et al.*, 1985; Nishimura and Baker, 1986; Grimalt and Albaiges, 1987; Stephanou 1992; NRC, 2003; Ji-xiang and Jia, 2012; Punyu *et al.* 2013). The high value of nC_{17} has been suggested due to the presence of sulfuric reducer bacteria (*Desulfovibrio desulforicans*) in the sediments, and the high level of nC_{19} to the algal sources (Talal, 2008). The presence of low molecular weight n-alkanes, mainly nC_{23} and nC_{25}, as major components is generally related to non-emergent aquatic plant sources (Fickens *et al.*, 2000; Duan and Xu, 2012). The n-alkanes of high molecular weight such as nC_{27}, nC_{29}, and nC_{31} in the environment are attributed to higher plant inputs including emergent aquatic plants and their tissue decomposition (Eglinton and Hamilton, 1967; Simoneit, 1978). The dominance of the n-alkanes with no carbon number preference suggests that petroleum input is the main source (Wang *et al.* 2011). Pristane is derived from zooplankton and other aquatic animals, while phytane is a normal component of oil and can be contributed to methanogenic and photosynthetic bacteria or deposition algae (Guerra-García *et al.*, 2003; Abed Ali 2013; Punyu *et al.*, 2013; Al-Hejuje *et al.*, 2015).

The CPI, which has been used to assess the contribution of biogenic versus anthropogenic inputs (Scalan and Smith, 1970; Simoneit, 1989), has been grouped into nC_{13}-nC_{24} ($CPI_{(o/e, \leq 24)}$), C_{25}-C_{36} ($CPI_{(o/e, 25)}$) and for the entire range ($CPI_{(o/e, E)}$) (Rushdi *et al.* submitted). The $CPI_{(o/e, £24)}$ values varied from 0.57 to 0.71 (Table 1) indicating that the major sources of n-alkanes were bacterial and fossil fuel residues. The $CPI_{(o/e, ^{3}25)}$ ranged from 0.77 to 1.42 suggesting that the n-alkanes were derived mainly from petroleum sources with a minor contribution from higher plant waxes. The $CPI_{(o/e, E)}$ values varied from 0.69 to 1.42 confirming a mixture of major anthropogenic and

Table 1. The concentrations (µg/g dry weight) and the biomarker indices and ratios (CPI, Ph/Pr, LMW/HMW parameters of n-alkanes and isoprenoid pristane and phytane in the tissues of freshwater fish species from Iraq.

Compound	Composition	M.W.	*L. xanthopterus*	*L. vorax*	*C. idella*	*T. zillii*	*C. carpio*	*P. abu*
			Carnivorous		Herbivorous		Omnivorous	Filter feeder
n-Alkanes								
Tridecane	$C_{13}H_{28}$	184	0.029	0.022	0.053	0.037	0.093	0.024
Tetradecane	$C_{14}H_{30}$	198	0.04	0.037	0.062	0.052	0.126	0.037
Pentadecane	$C_{15}H_{32}$	212	0.048	0.189	0.071	0.071	0.071	0.062
Hexadecane	$C_{16}H_{34}$	226	0.054	0.242	0.083	0.088	0.066	0.068
Heptadecane	$C_{17}H_{36}$	240	0.195	0.455	0.11	0.119	0.076	0.12
Octadecane	$C_{18}H_{38}$	256	0.595	0.597	0.298	0.21	0.079	0.482
Nonadecane	$C_{19}H_{40}$	268	0.489	0.302	0.339	0.218	0.111	0.449
Eicosane	$C_{20}H_{42}$	282	0.426	0.377	0.296	0.255	0.118	0.672
Heneicosane	$C_{21}H_{44}$	296	0.303	0.187	0.277	0.254	0.071	1.221
Docosane	$C_{22}H_{46}$	310	0.647	0.411	0.462	0.462	0.084	2.489
Tricosane	$C_{23}H_{48}$	324	0.597	0.436	0.489	0.511	0.093	3.422
Tetracosane	$C_{24}H_{50}$	338	1.146	0.851	0.844	0.914	0.247	3.667
Pentacosane	$C_{25}H_{52}$	352	0.793	0.448	0.528	0.6	0.061	2.946
Hexacosane	$C_{26}H_{54}$	366	0.903	0.527	0.554	0.599	0.133	2.288
Heptacosane	$C_{27}H_{56}$	380	0.72	0.401	0.41	0.626	0.069	1.627
Octacosane	$C_{28}H_{58}$	394	0.746	0.43	0.389	0.449	0.076	1.104
Nonacosane	$C_{29}H_{60}$	408	1.126	0.255	0.511	0.898	0.393	0.754
Triacontane	$C_{30}H_{62}$	422	0.678	0.268	0.243	0.3	0.255	0.554
Hentriacontane	$C_{31}H_{64}$	436	0.191	0.134	0.296	0.372	0.322	0.339
Dotriacontane	$C_{32}H_{66}$	450	0.228	0.174	0.261	0.446	0.298	0.266
Tritriacontane	$C_{33}H_{68}$	464	0.479	0.088	0.23	0.189	0.267	0.135
Tetratriacontane	$C_{34}H_{70}$	478	0.205	0.106	0.179	0.115	0.204	0.172
Pentatriacontane	$C_{35}H_{72}$	492	0.203	0.073	0.158	0.104	0.251	0.069
Hexatriacontane	$C_{36}H_{74}$	506	0.168	0.316	0.122	0.055	0.192	0.169
Total			11.01	7.33	7.27	7.94	3.76	23.14
Pristane	$C_{19}H_{40}$	268	0.177	0.232	0.09	0.109	0.068	0.097
Phytane	$C_{20}H_{42}$	282	0.321	0.226	0.224	0.122	0.076	0.391
$CPI_{(o/e, E)}^{a}$			0.89	0.69	0.92	1.01	1.00	0.93
$CPI_{(o/e, \leq 25)}$			1.20	0.77	1.22	1.42	1.18	1.29
$CPI_{(o/e \leq 24)}$			0.57	0.63	0.65	0.61	0.72	0.71
LMW/HMW			0.71	1.28	0.87	0.67	0.49	1.22
Pr/Ph			0.55	1.03	0.40	0.89	0.89	0.25

$CPI(o/e) = \Sigma(nC_{odd})/\Sigma(nC_{even})$,
a = entire raange,
$LMW/HMW = \Sigma(nC_{13}+....+nC_{24})/\Sigma(nC_{25}+....+nC_{36})$,
Pr/Ph = Pristane/Phytane

minor natural and sources. The Pr/Ph ratio is another useful molecular marker for distinguishing the origin of hydrocarbons in the environment (Lijmbach, 1975; Didyk *et al.,* 1978; Broman *et al.* 1987; Commendatore and Esteves 2004; Peters *et al.* 2005). The ratios of Pr/Ph in the fish tissue samples varied from 0.25 to 1.03 (Table 1), indicating that they were from both alteration and transformation processes under redox conditions and petroleum inputs. The nC_{17}/Pr ratios ranged from 1.09 to 1.28 and for nC_{18}/Ph ratios were 1.04 - 2.64 (Table 1), indicating that the sources of hydrocarbons were from fresh petroleum input (UNEP, 1995; Gao and Chen 2008; Harji *et al.,* 2008). The major anthropogenic sources of fresh hydrocarbons to the Iraqi rivers and wetlands are factory wastes, effluents during refinery loadings and natural seepage (Law, 1981; Bedair and Al-Saad, 1992). The ratios of LMW/HMW in the fish tissue samples were all < 0.87 except for *L. vorax* (1.28) and *P. abu* (1.22) (Table 1). Assuming no or minor sources from petroleum hydrocarbons, these values suggested that the contribution of algae, bacteria and non-emergent aquatic plants is slightly lower than the contribution from emergent plant inputs.

Table 2. The results of principal component analysis (PCA) explaining the relative loadings of aliphatic hydrocarbons in the fish tissue samples.

	PC1	PC2	PC3
L. xanthopterus	0.751	0.537	0.231
L. vorax	0.468	**0.837**	-0.232
C. idella	**0.882**	0.429	0.118
T. zillii	**0.863**	0.328	0.299
C. carpio	0.068	-0.084	**0.977**
P. abu	**0.909**	0.233	-0.272
% Variance	68.996	21.068	5.223

Rotation Method: Varimax with Kaiser Normalization.

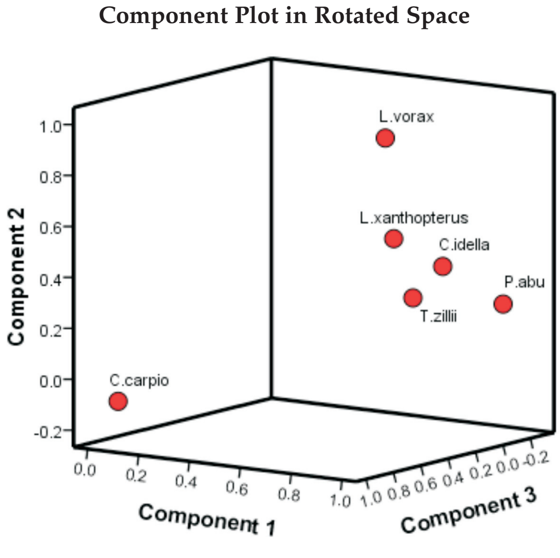

Fig. 2. A plot showing the principal component analysis (PCA) statistical outputs for the aliphatic hydrocarbon n-alkanes and isoprenoids in the fish species tissues from Shatt Al-Arab River-Iraq.

The input concentrations from different sources were estimated following the method described by Simoneit *et al.* (1991) for terrestrial plant wax n-alkanes and have been applied to compute the n-alkane contribution of marine algae and bacteria (Rushdi *et al.,* submitted). The n-alkane concentrations of the higher plant (mainly aquatic emergent plants), microbial, and algal (including non-emergent plants) sources were found to range from 0.00 µg/g Dwt (*L. vorax* and *P. abu),* to 0.693 µg/g Dwt (*L. xanthopterus*), 0.243 µg/g Dwt (*C. carpio*) to 0.933 µg/g Dwt (*L. xanthopterus*), and 0.010 µg/g Dwt (*L. xanthopterus*) to 0.359 µg/g Dwt (*P. abu*), respectively (Table 3). The n-alkane concentrations from fossil fuel residues were prevailing and estimated to range from 3.125 µg/g Dwt (*C. carpio*) to 21.929 µg/g Dwt (*P. abu*). Accordingly, the major source of n-alkanes in the tissues of these fish species was from petroleum waste. This was also designated by the significant Pearson's correlation ($r < 0.05$) between the total n-alkanes of the samples and petroleum n-alkanes ($r = 0.999$) (Table 4). The correlation was also significant ($r < 0.05$) between total and algal/non-emergent plant n-alkanes ($r = 0.882$), suggesting that petroleum and algal sources were the main contributors to the n-alkanes in the tissues of the fish species. The significant correlation of the latter might be due to the feeding of fishes on contaminated algae and non-emergent plants. The insignificant correlations between total n-alkanes and fat contents, total lengths, and total weights (Table 4) suggested that the fat contents, lengths, and weights of the fishes had no influence on the accumulation of hydrocarbons in the fish tissues. Internal physiological routes of fat metabolism and transformation as well as the occurrence of different contaminants may affect the uptake and bioaccumulation of organic compounds by the aquatic organism (Pruell et al. 1986; Barton 1990; Varanasi *et al.,* 1993).

The percentage ranges of n-alkane sources in the fish species tissues were 0.0% - 9.1% from higher plant and emergent plants, 3.5% - 11.7% from bacteria, 0.1% - 1.6% from algae, and 83.2% - 94.8% from petroleum sources (Fig. 3). The petroleum by-products were the major sources of n-alkanes in the tissues of these fishes. As shown in Figure 3, the petroleum n-alkanes in the tissues of the filter feeder *P. abu* (94.8%) was slightly higher than in the tissues of the carnivore *L. xanthopterus* and *L. vorax*, the herbivorous *C. idella* and *T. Zillii*, and the omnivorous *C. carpio* (83.2-88.9%). The higher plant and aquatic emergent plant n-alkanes were slightly higher in herbivorous (0.45%) than in carnivorous (0.35%), omnivorous (0.34%) filter feeder (0.00%); the marine bacterial n-alkanes were relatively higher in carnivorous and filter feeder (0.90% and 0.85%, respectively) than the herbivorous (0.49%) and the omnivorous (0.24%); and the algal n-alkanes were higher in filter feeder (0.36%) than the carnivorous (0.05%), the omnivorous 0.05%) and the herbivorous (0.04%). These results indicated that the accumulation of hydrocarbon n-alkanes and isoprenoids might be influenced by the feeding habits of the fishes. Other factors that affected the levels of n-alkanes in fish tissues could be the differences in species, age, sex, route and duration of contact, and exposure to other xenobiotics (Johnson-Restrep *et al.*, 2008; Al-Ali *et al.*, 2016). The aliphatic hydrocarbons were most likely added to the fish species tissues directly from solution or suspended particles or via the digestion processes of contaminated phytoplankton, zooplankton, crustacean, and higher plant and eventually accumulated in the tissues of the species (Al-Saad *et al.*, 2011; Wang *et al.*, 2019).

Conclusion

The analyses of aliphatic hydrocarbons in the tissues of six fish species from the Shatt Al-Arab River of Iraq showed that oil hydrocarbons are major components. The molecular biomarker indices and ratios of n-alkanes and the isoprenoid pristane and phytane indicate that the major sources of the aliphatic hy-

Table 3. The concentrations (µg/g Dwt) of different aliphatic hydrocarbon sources measured in different freshwater fish species from Iraq.

	L. xanthopterus	*L. vorax*	*C. idella*	*T. zillii*	*C. carpio*	*P. abu*
Higher plants and aquatic emergent plants	0.693	0.000	0.257	0.645	0.342	0.000
Bacteria	0.933	0.860	0.488	0.499	0.243	0.848
Algae and non-emergent plants	0.010	0.089	0.064	0.012	0.046	0.359
Total Natural	0.703	0.089	0.321	0.657	0.388	0.359
Petroleum	9.374	6.378	6.457	6.789	3.125	21.929

Table 4. Pearson correlation between the different sources of n-alkanes in the tissues of the fish species and their physicochemical characteristics.

	Total n-alkanes	Higher/ emergent plant n-alkanes	Bacterial n-alkanes	Algal/non emergent plant n-alkanes	Natural n-alkanes	Petroleum n-alkanes	Fat	Total length	Total weight
Total n-alkanes	1	-.352	.602	**.882***	.046	**.999****	.361	-.389	-.579
Higher/emergent plant n-alkanes		1	-.176	-.705	**.912***	-.385	.101	-.321	-.091
Bacterial n-alkanes			1	.355	-.026	.576	-.085	.307	.061
Algal/non emergent plnat n-alkanes				1	-.351	**.901***	.273	-.265	-.490
Natural n-alkanes					1	.014	.292	-.577	-.403
Petroleum n-alkanes						1	.364	-.392	-.583
Fat							1	-.809	**-.871***
Total length								1	**.897***
Total weight									1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

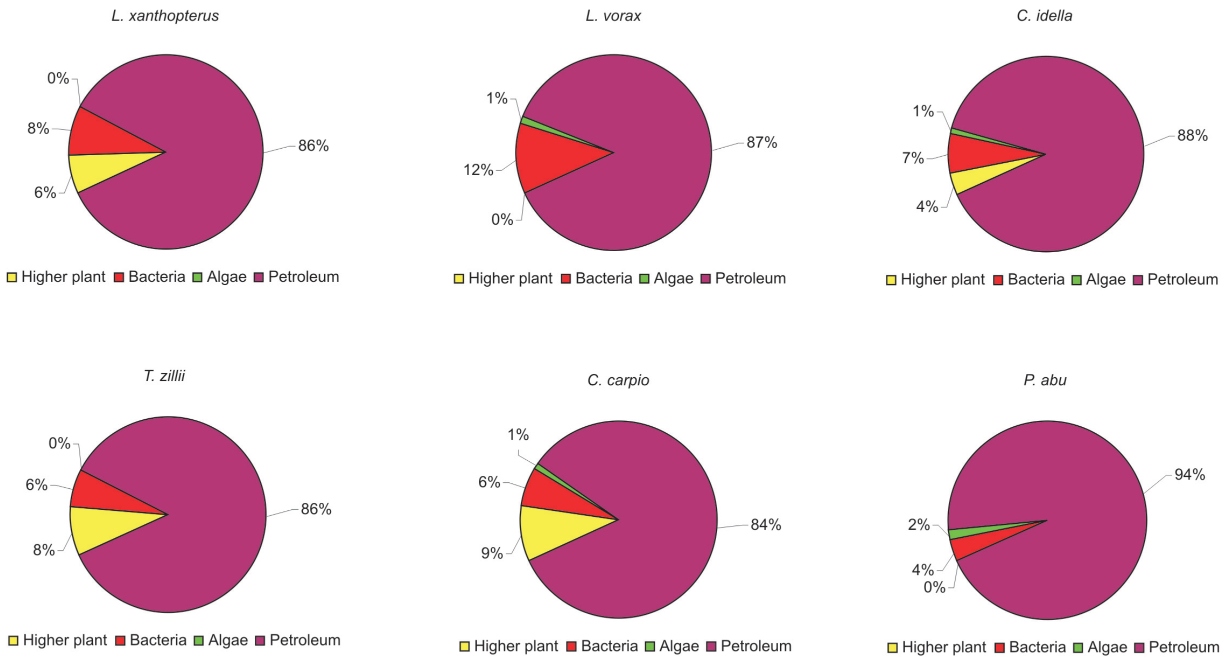

Fig. 3. The fractions of different sources of aliphatic hydrocarbons in the tissues of fish species from the Shatt Al-Arab river of Iraq.

Table (SM-1). Description and information of the collected fish samples from Shatt Al-Arab River, Iraq.

Fish species	Common name	Feeding habit	No. of fish	Total weight (g)	Total length (cm)	fat %
Luciobarbus xanthopterus	Cattan	Carnivores	10	916	40	2.03
Ctenophyngodon idella	Gareeba	Herbivorous	18	1117	57	3.22
Cyprinus carpio	Samti (commen c.)	Omnivorous	30	1019	35	4.1
Tilapia zillii	Bultti	Herbivorous	60	75	15	11.1
Leuciscus vorax	Shilling	Carnivores	30	750	35	2.51
Detritus and phytoplankton feeding	Khishni	*Palaniza abu*	46	43	21	7.8

drocarbons in the fish tissues are from petroleum sources with minor sources from aquatic plants. The n-alkanes fractions of petroleum-related products in the fish tissues are relatively high (83.2-94.8%). The natural biogenic sources are comparatively low (0-9.1% from higher plant waxes, 0.1-1.2% from algae, and 3.7-11.7% from bacteria). Contamination of the river fishes by oil-related hydrocarbons as shown by the results could be a very serious health problem. This is mainly attributed to oil-related activities in the area such as loading operations, discharges from refineries and tanker traffic, and the possibility natural oil seeps in the area. The accumulation of the aliphatic hydrocarbons in the fish tissues is not directly related to the fat contents of the fish, where other possible factors such as metabolism and biotransformation of lipids may have some control on the bioaccumulation of contaminants in aquatic organisms.

More researches are required to study the occurrence of other anthropogenic organic compounds such as pesticides, plasticizers, and polycyclic aromatic hydrocarbons in aquatic fauna and flora of the river and wetlands of Iraq.

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Consent to publish

The authors gave the consent to the publisher to publish this work.

Authors' Contributions

Balqees S. AlAli: sampling, chemical analysis and data preparation; Dhafar D. Alkhion: field work, sampling and sample preparation; Hamid T. Al-Saad: laboratory work and first draft writing; Ahmed I. Rushdi: data and result manipulation, statistical analyses, and final draft editing submission.

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Competing of Interests

Availability of data and Materials
Data and material are available.

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