

Original Article

Total petroleum hydrocarbons in water, sediment, and Redbelly tilapia, *Coptodon zillii* in Shatt Al-Basrah Canal, Iraq

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Abstract: Water pollution is one of the most common global problems resulting from increased industrial and agricultural activities. Petroleum hydrocarbons have extremely dangerous to the aquatic environment. The total petroleum hydrocarbon (TPHs) was investigated in water, sediment, and muscles of *Coptodon zillii* at Abu Sakhir and Al-Zubair Bridge stations seasonally in the Shatt al-Basra Canal. The results showed a variation in the TPHs levels in the studied stations. In addition, a significant difference in the TPHs was recorded during the seasons in the water, and sediments between stations. The results showed significant differences in the TPHs in the muscles in the spring but no significant in other seasons between the two stations. The results of the lipid contents of fish revealed significant differences between the two studied stations in the fall, spring, and summer seasons but not significant in winter.

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Introduction

Water contamination is a crucial global problem resulting from expanding industrial and agricultural activities (Wang et al., 2019). Many pollutants enter the aquatic environments directly or indirectly due to human activities, affecting aquatic organisms (Li et al., 2019). One of the main environmental pollutants worldwide is crude oil-based hydrocarbons (Ławniczak et al., 2020). Petroleum hydrocarbon pollution is a significant environmental issue that threatens the aquatic environment, whether from benzene or other toxic organic materials (Abha and Singh, 2012). Increased urbanization and intensive industrial activities have led to an increase in the consumption of oil and its products worldwide (Liu et al., 2019).

There are many ways to enter oil contaminants in the aquatic environment e.g. discharged through oil spill accidents or by-products for individual or commercial uses (Cai, 2021). Furthermore, navigation, transporting oil, washing loading docks,

balance water, and export ports are the main sources of pollution of aquatic ecosystems that have been reported as approximately 6 million tons annually (Chougule et al., 2009). In addition, there are other sources of hydrocarbon pollution, such as domestic, agricultural, and power plant releases (Frena et al., 2017). Therefore, when petroleum hydrocarbons are discharged into aquatic ecosystems, they cause harm to living organisms, with toxic effects ranging from acute to chronic depending on the metabolism and photo-oxidation process (Kuppusamy et al., 2020). This damage may appear direct or indirect after a long period (Rodrigues et al., 2010).

Releasing large quantities of petroleum hydrocarbons into rivers and coastal areas during oil production and transportation negatively impacts the environment and human health (Ihunwo et al., 2021). It would be affected water in its dissolved particles, organisms, and sediments phase (Raja et al., 2022). Because of their hydrophobic nature, hydrocarbons tend to bind to organic particles in aquatic

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ecosystems where they are deposited in sediments (Qiu et al., 2009). Fish can rapidly absorb soluble petroleum hydrocarbons that are highly sensitive to lipids, which enhances living organisms' ability to absorb petroleum hydrocarbons through the respiratory and gastrointestinal tracts (Rodrigues et al., 2010).

Fish plays a significant role in the food chain and is an important indicator for many environmental pollutions in the aquatic system (Wang et al., 2021). On the other hand, fish is an important part of the human diet due to having high-quality protein (Holub and Holub, 2004). In addition, it contains a wide variety of vitamins and minerals, containing vitamins A and D, magnesium, selenium, and phosphorus (Fischer and Gleis, 2015). Fishes can absorb petroleum hydrocarbons from contaminated water directly or indirectly through food and sediments (Scheuhammer et al., 2016). The uptake of contaminants directly from the aquatic environment is known as bioconcentration (Muijs and Jonker, 2012). Fish generally dissolve petroleum hydrocarbons due to their high lipid solubility (McConville et al., 2018).

Tilapia can be found in freshwater or brackish waters such as rivers, lakes, estuaries, shallow streams, and ponds (Iyabo, 2015). It is the second most important fish in the world aquaculture after common carp, with production reaching 6.3 million tons in 2018 (FAO, 2019). Redbelly tilapia, *Coptodon zillii* has a widespread distribution within the Iraqi inland waters. It might come from Iraq's bordering countries, such as Turkey, Syria, and Iran (Mutlak and Al-Faisal, 2009). Based on above-mentioned background, this study aims to evaluate the total petroleum hydrocarbon levels in the water, sediment, and muscles of *C. zillii* in Shatt al-Basra Canal, Iraq.

Material and Methods

Study area and sampling: The Shatt al-Basra Canal is a drainage channel that starts north of Basra and extends southeast. It covers about 37,157 km and is situated within the alluvial plain of Basrah, located

between latitudes 3027-3028 in the north and 4750-4749 in the east. It transfers flood waters from the Al-Hammar Marsh to Khor Al-Zubayr and then to the Persian Gulf. It is also used to withdraw saline water from irrigated agricultural lands, which later turned into marsh reclamation. Moreover, this channel is a waterway connecting the southern marshes with the Persian Gulf from its northern part (Hassan et al., 2018).

The sampling was done in two stations, including (1) Abu Sakhir, in the northwest of Basrah (47.702856N, 30.561467E), and (2) Al-Zubair Bridge, in the southwestern part of the city of Basrah in (47.760947N, 30.442322E). It is about 6,423 m from the center of Basrah and about 15,272 m from the second station (Fig.1). Fish, water, and sediment samples were collected seasonally to measure total petroleum hydrocarbons from the selected stations from fall 2021 to summer 2022. Fish were collected by gill net, and the samples were frozen and taken to the lab, where they were weighed, measured, and stored at -4°C. They were dissected using aseptic equipment to obtain the muscles, and the samples were labeled for analysis. Water samples were collected from the surface in 2-liter amber glass bottles by adding 10 mm of CCL₄, carbon tetrachloride. Before water sampling, all bottles were washed with warm water and liquid detergent, then properly rinsed with tap water and dried for 24 hours, afterward the bottles were cleaned with acetone, dried for about 30 seconds, and finally rinsed with n-hexane. Sediments were collected during the low tide period from the slightly submerged riverbank at approximately 2-3 m using a small shovel and placed in plastic bags.

Total petroleum hydrocarbons extraction: Fish and sediments extractions were performed according to Goutx and Saliot (1980). Three gram of fish muscles was dried, weighted, and crushed in a ceramic mortar with a pestle. For sediment, 3 g of the dried sediment was passed through a sieve of 63 μm mesh and put into an extraction thimble. The fish and sediments' ground samples were placed separately in thimbles with 100 ml of methanol: benzene (1:1 v/v)

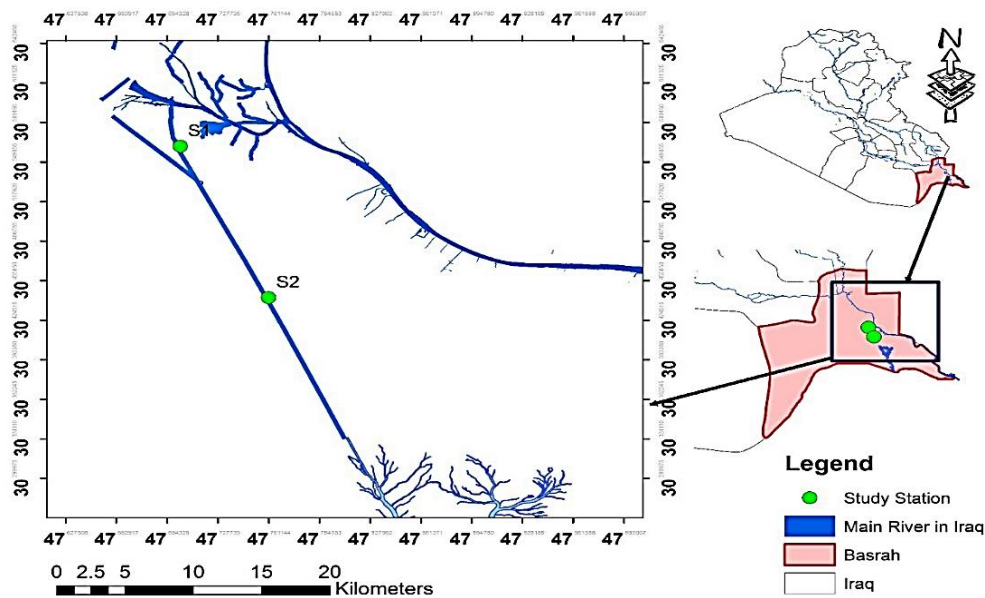


Figure 1. Map of sampling sites in the Shatt al-Basrah Canal.

for 24 h, as the extraction solvent. The extract was saponified for two hours at 40°C by adding 15 ml of an aqueous 4N MeOH (KOH) solution. The contents were poured into a separating funnel, and then 50 ml of normal n-hexane was added; afterward, the sample was well-shaken and left to settle. The two layers were formed, and the layer containing hydrocarbons was taken and passed on a separation column from the bottom of the glass wool, topped with a layer of silica gel, alumina, then anhydrous sodium sulfate.

The extraction of total hydrocarbons from the water was based on UNEP (1989), that in this procedure, 10 ml of CCL₄ was added to 1 liter of sampled water. Using an electric mixer, the sample was shaken for 30 min; then, the contents were poured into a separating funnel and left to settle. The organic layer (the lower layer containing carbon tetrachloride and hydrocarbons) was collected and passed over a separation column containing glass wool at the bottom, topped with a layer of aqueous sodium sulfate Na₂SO₄ to remove water. The extracted muscles, water, and sediment samples were dried at laboratory temperature. Finally, the total petroleum hydrocarbons were measured after dissolving them with pure hexane using a spectrofluorometer.

For calibration, Basrah crude oil was used by

dissolving a known weight of oil with a specific volume of n-hexane to prepare standard solutions using a fluoridation device to determine the concentrations of total petroleum hydrocarbons in fish, water, and sediments. The emission intensity was measured at a wavelength of 310 nm and at an excitation of 360 nm. To determine the lipid concentrations in muscles, aliquots of the lipid extracts were dried and dissolved with a mixture of n-hexane (Egan et al., 1981).

Statistical analysis: The statistical analysis was performed using SPSS 20 (Statistical Package for Social Sciences). LSD test was used to determine those differences under the significance level of $P < 0.05$ through the ANOVA test (Snedecor and Cochran, 1989).

Results

The results showed differences in the total petroleum hydrocarbons in the water, sediment, and muscles for both stations in the Shatt Al-Basrah Canal study areas. The highest TPHs in the water were 21.58 and 30.53 $\mu\text{g.l}^{-1}$ in winter in the first and second stations, respectively. The lowest values were 7.45 and 8.61 $\mu\text{g.l}^{-1}$ in summer and fall in the first and second stations, respectively (Fig. 2). The sediments results showed the highest level of TPHs as 39.59 and 41.37 $\mu\text{g/g}$ dry weight in spring in the first and second

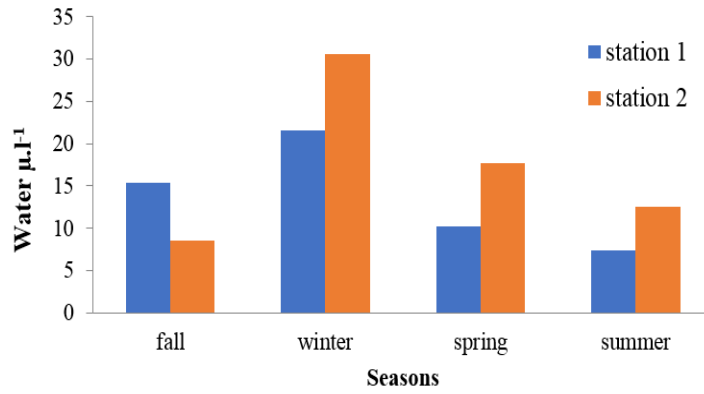


Figure 2. TPHs in the water of Shatt Al-Basrah Canal.

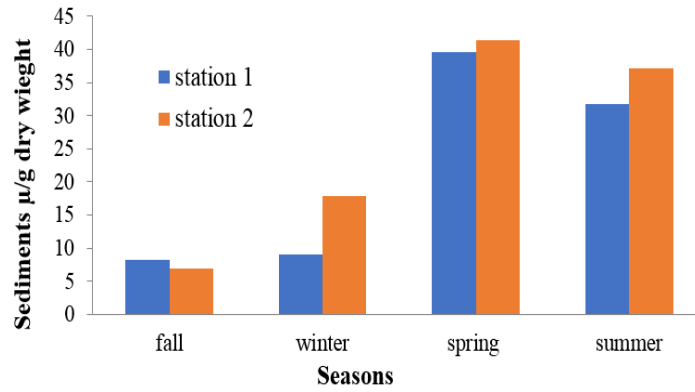


Figure 3. TPHs in the sediments of Shatt Al-Basrah Canal.

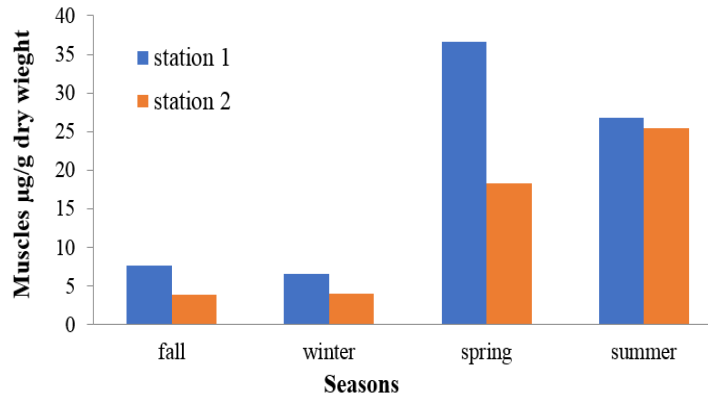


Figure 4. TPHs in the *Coptodon zillii* muscles of Shatt Al-Basrah Canal.

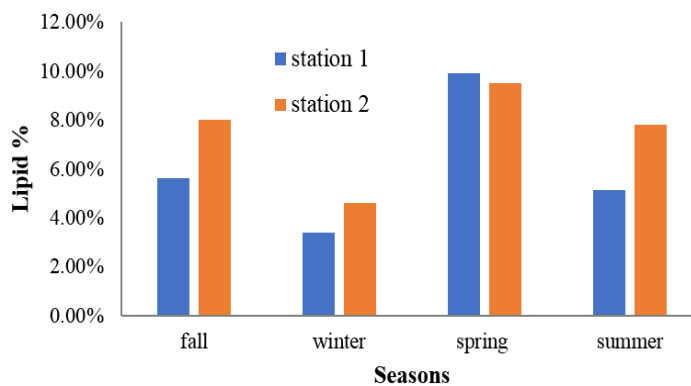


Figure 5. Percent distribution of lipids in the muscles of *Coptodon zillii* in Shatt Al-Basrah Canal.

stations, respectively. The lowest values were 8.17 and 6.98 $\mu\text{g/g}$ dry weight in fall at the first and second stations, respectively (Fig. 3).

A highly significant difference ($P \leq 0.05$) at both stations in the TPHs level in the muscles of *C. zillii* was recorded. The highest value was in spring at the first station as 36.56 $\mu\text{g/g}$ dry weight, while the lowest value was 3.87 $\mu\text{g/g}$ dry weight in fall at the second station (Fig. 4). The highest lipids of *C. zillii* were 9.90 and 9.51% in spring at the first and second stations, but the lowest values were 3.41 and 4.61%, respectively in winter at the first and second stations (Fig. 5).

Discussion

Petroleum hydrocarbons enter the aquatic environment through various sources, including the atmosphere, industrial sources, household waste, and untreated civil waste (Eganhouse et al., 1981). The current study showed a seasonal variation in the TPHs in water in all seasons in both stations. A highly significant difference in TPHs was observed in the water of both stations. The least TPHs in water was 7.45 $\mu\text{g.l}^{-1}$ in summer in the first station (Fig. 6). However, the highest TPHs was 30.53 $\mu\text{g.l}^{-1}$ in winter at the second station (Fig. 7). However, a higher level of TPHs recorded in the surface waters was observed in summer in the study of Adeniji et al. (2017). Depending on the regions and seasons, the amount of petroleum hydrocarbons in water varies e.g. the highest TPHs were 4.92-46.4 $\mu\text{g.l}^{-1}$ in Khor Abdullah water (Nasir, 2005), but the lowest TPHs at Shatt al- Basrah was 0.05-26.44 $\mu\text{g.l}^{-1}$ (Atti, 2014), whereas in the current study, it was 7.45-30.53 $\mu\text{g.l}^{-1}$. Conversely, the TPH was 45.07 to 307 $\mu\text{g/L}$ in the water of Algoa Bay in South Africa, and the ranges of TPH in a seasonal investigation were 45.07 to 273 g/L in surface water and 55.72 to 307 g/L in bottom water (Adeniji et al., 2017).

Based on the results, the second station had the highest concentrations of TPHs, which may be due to the accumulation of oil residues from the Basrah oil refinery (Aziz and Sabbar, 2013). Furthermore, the small fishing boats, and the presence of main

drainage pipes that receive untreated domestic and sewage waste to the east of the canal, are the most important sources of petroleum hydrocarbon pollution (Eganhouse et al., 1981). The ratio of TPH was higher in the winter because of the low temperatures that prevented evaporating compounds (Al-Saad and Al-Timari, 1993). In addition, the effectiveness of microorganisms in breaking down hydrocarbons decreases with decreasing temperature (Khillare et al., 2014; Marić et al., 2020). Due to the high temperatures, the oil loses 20-50% of its components through evaporation (Harnstrom et al., 2009). Light oxidation also leads to the breaking of oil compounds in the water column, which coincides with the high temperatures in the summer, as this factor depends on the length of the illumination period during the day and is one of the reasons for the low concentrations of TPHs in the summer (Talal, 2010).

There were also highly significant differences in TPHs in the sediments of both stations. The highest concentration of TPHs in sediment was 41.37 $\mu\text{g/g}$ dry weight in spring in the second station. A consistent rise in TPH in sediments was noted from summer to autumn (Al-Saad et al., 2017b). There was a seasonal variation, with the dry season having the most variability because of the higher level of increased oil activity during that time (Clinton et al., 2009). Massoud et al. (1996) reported TPH in sediments between 10-15 mg/kg should be regarded as unpolluted and 15-50 mg/kg as slightly polluted. The levels of PHCs in the sediments were high in our study, however 279 to 17 900 mg.kg^{-1} was reported by Lindén and Pålsson (2013), and the high level of PHC in the surficial sediment of the inshore area of Ratnagiri has been reported as 107.7 ppm, dry weight (Chouksey et al., 2004).

Table 2 shows the petroleum hydrocarbon (PHC) levels in the sediments of different water bodies in Iraq. The values of PHCs in Iraqi waterbodies were 2.3-50.2, 4.76-45.24, and 19.43-49.09 $\mu\text{g/g}$ dry weight (Hantoush, 2006; Al-Hejuje, 2014; Al-Ali et al., 2016) and in our work, it was 6.98-57.5 $\mu\text{g/g}$ dry weight. Pollutants can enter the aquatic environment

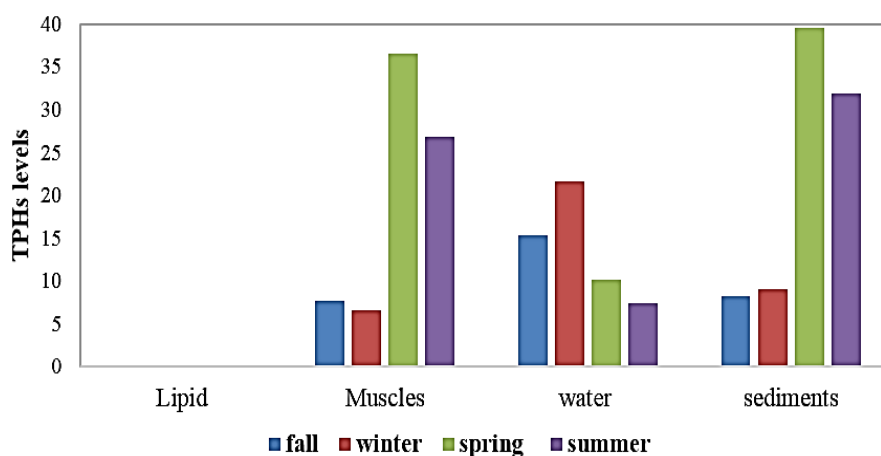


Figure 6. TPHs levels in different sample types at station 1.

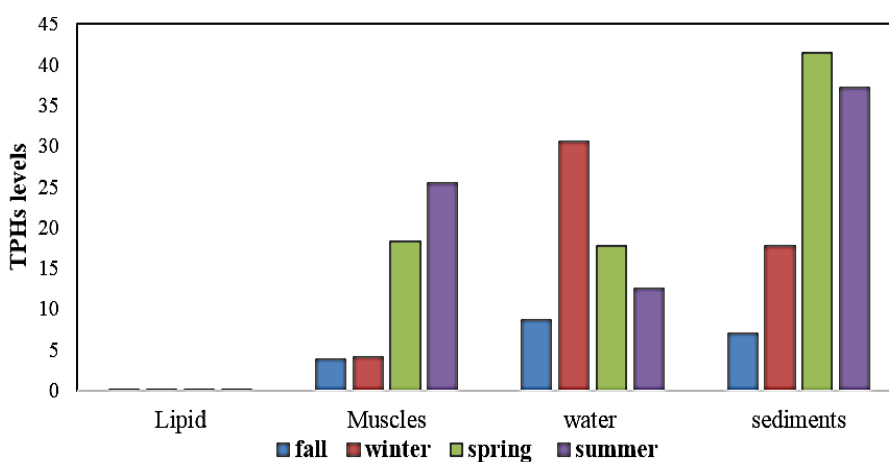


Figure 7. TPHs levels in different sample types at station 2.

Table 1. PHCs concentrations of water in different Iraqi aquatic ecosystems.

Region	TPHs $\mu\text{g.l}^{-1}$	References
Khor Abdullah	4.6-22.6	Al-Timar et al. (2002)
Khor Abdullah	0.9-23	Al-Timar et al. (2003)
Khor Abdullah	4.92-46.4	Nasir (2005)
Khor Abdullah	11.23-26.57	Nasir (2007)
Northwest Persian Gulf	6.38-13.36	Al-Imarah et al. (2007)
Shatt al- Basrah	0.05-26.44	Atti (2014)
Shatt al- Basrah	7.45-30.53	Current study

and settle in the sediment in various ways, including direct deposit because of their weight or long-distance water current transport because of their stability, which causes them to deposit when the speed of the currents decreases (Liu et al., 2019). These compounds enter the living organisms and reach the sediments after death (Al-Saad, 1995). Therefore, THP levels in the sediment were consistently higher than in surface water, showing that it accurately indicates pollution in the river

systems (Osuji et al., 2004). The reason for increasing PHCs levels in sediment after many years is the establishment of shore refineries (Aziz and Sabbar, 2013)). Similarly, variation in different regions could be related to the transfer of PHCs loads from the inshore areas to the open-shore sediments and probably to an important role of benthic organisms in the biological transfer of PHC into the sediment due to the remnants of an oil spill (Chouksey et al., 2004). Due to the particle size, the

Table 2. PHCs concentrations in sediments of different Iraqi aquatic ecosystems.

Region	TPHs $\mu\text{g.l}^{-1}$	References
Khor Abdullah	34-192	Nasir (2005)
Shat Al-Arab	2.3-50.2	Hantoush (2006)
North-West Persian Gulf	34.37-66.02	Nasir (2005)
Iraqi coast regions	2.39-30.88	Al-Khion (2012)
Shat Al-Arab	4.76-45.24	Al-Hejuje (2014)
North-West Persian Gulf	19.43-49.09	Al-Saad et al. (2017a)
Shatt Al-Basrah	6.98-57.5	Current study

Table 3. PHCs concentrations in fishes of different Iraqi aquatic ecosystems.

Region	TPHs $\mu\text{g/g}$ dry weight	References
Shat Al-Arab	29.6-45.9	Al-Saad (1989)
Khor Alzubair	8.3-40.6	Al-Saad (1990)
Shat Al-Arab estuary and northwest Persian Gulf	1.7-10.91	Al-Saad et al. (1997)
Shat Al-Arab estuary and northwest Persian Gulf	2.55-26	Hantoush et al. (2001)
Iraqi marine waters	11.44-48.16	Nasir (2007)
North-West Persian Gulf	2.545-7.25	Al-Ali et al. (2016)
Shatt Al-Basrah	3.87-57.5	Current study

sediments' bottom structure with silt and clay has a greater potential to preserve TPH. Therefore, sediment analysis is a good indicator of the distribution of petroleum hydrocarbons (Raja et al., 2022).

An increase in the PHC during the summer at both stations (31.82 and 37.21 $\mu\text{g/g}$ dry weight, respectively) was recorded, maybe because of irregular oil leaks from industrial areas, the accumulation of oil residues from fishing boats that are active in the summer, municipal sewage water, and surface runoff from the land (Al-Saad et al., 2017a). Water temperature also impacts the speed of organic matter sedimentation and the solubility of hydrocarbons (Al-Dossari, 2008). The lowest concentration of petroleum hydrocarbons in the sediments was in the fall (6.98 $\mu\text{g/g}$ dry weight), and with the decrease in temperature. TPHs gradually increased during the winter at station two due to the reduction in temperature. Lower temperatures reduce the evaporation process, photo-oxidation, and biodegradation by microorganisms in the sediments, which can oxidize more than 70% of the crude oil in roughly 18 days (Atlas and Bartha, 1973). Moreover, the higher phytoplankton mortality rates during the winter will result in higher organic matter levels in sediments, increasing the proportion of hydrocarbons that are adsorbed to the surface of

these sediments (Al-Khafaji, 2007).

Petroleum hydrocarbons were found in all fish samples. There was a correlation between THC in the organisms and their surrounding water and sediments (Clinton et al., 2009). The PHCs in *C. zillii* muscles range from 3.87-57.5 $\mu\text{g/g}$ dry weight; however, it was 2.545 to 48.16 $\mu\text{g/g}$ dry weight in other Iraqi waterbodies (Table 3). Differences in PHC are found based on the seasons and fish species, e.g. in summer and winter seasons, *Tenuulosa ilisha* had the highest PHC (6.85 and 7.65 g/g dry weight, respectively), whereas in summer and the winter, *Euryglossa orientalis* had the lowest THC concentration (2.45 and 2.64 g/g dry weight, respectively) (Al-Ali et al., 2016).

There is a significant fluctuation in the TPHs of fish between the seasons (Ansari et al., 2012). Al-Baidani (2014) indicated that the abundance of phytoplankton, the primary food for fish, causes high TPHs during the spring season. Additionally, physical and chemical factors of water bodies affect the composition and spread of petroleum compounds (Akaahan et al., 2014). Due to their feeding habits, some fish have higher PHCs in their muscle (Veerasingam et al., 2011). Nasir (2007) explained that the variations in THC concentrations in fish muscles in different seasons are caused by the influence of environmental factors, nutrition, type

and amount of available food, and the breeding season. Chronic exposure to PHCs causes physiological and histopathological alternations at all life stages of fish (Rodrigues et al., 2010). The primary threat posed by these pollutants is accumulating in the tissues of aquatic species and then transferring to humans (Wang et al., 2021). Thus, PHCs in fish tissues more than the normal level significantly negatively impact human health (Lindén and Pålsson, 2013).

Lipids had significant differences between the two studied stations at most seasons. The values in the first station were 3.41, 5.12, 5.61 and 9.90%, respectively (Fig. 6) and 4.61, 7.80, 7.99 and 9.51% in the second station (Fig. 7). The present results were higher than previous studies because the fish of the Shatt al-Basra Canal are exposed to oil pollutants continuously. Increasing the hydrocarbon levels in fish could be related to the high concentration of lipids in the fish muscle tissue (Shriadah, 2001). The amount and nature of fat vary in different fish species (Suzuki, 1981). There is a direct correlation between THCs ratio and fat percentage (Jamoussi et al., 2022) i.e. fatty parts of fish possess higher concentrations of organic pollutants (Shriadah, 2001). Ansari et al. (2012) observed a decrease in the level of TPH in the tissues of lean fish. Feed type is also responsible for fish muscles' high and low-fat content (Tolosa et al., 1996). In addition, many factors, such as the exposure time, the lipid content of tissues, environmental conditions, age, and sex, might affect the accumulation of PHs in fish (Froehner et al., 2011). In conclusion, there were significant differences in the TPHs levels of fish and their surrounding environment. TPHs concentrations in fish samples are based on the expected levels of the TPH; therefore, *Tilapia* can be a good indicator to assess TPH pollution in aquatic systems.

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