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Sources of Total Petroleum Hydrocarbons in Mussel of *Unio tigris* at Tigris River at Maysan Governorate in Iraq

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ABSTRACT

Total petroleum hydrocarbons pose vital environmental problems in the Tigris River. *Unio tigris* samples were collected from seven different sites of the Tigris River in Meysan Governorate during 2024. Total petroleum hydrocarbons concentrations were determined by using spectroflurometer technique. The highest level (12.116µg/ g) was recorded at Al-Kumait region (St. 5), and this station had the highest concentration of total petroleum hydrocarbons, which is mainly due to the increased population density beside the station, leading to increased human activities and the introduction of various wastes, while the lowest level (3.050µg/ g) was recorded at Ali Al-Garbi region (St. 7). Additionally, total fat content was determined, revealing a correlation between fat content and concentration of total petroleum hydrocarbons detected.

INTRODUCTION

This research aimed to examine and track oil residues present in down section of the Tigris River at Meysan Governorate. To facilitate this investigation, seven specific sampling sites were designated along the river. These samples were subsequently analyzed using spectroflurometer techniques. The main goal of the study was to measure the concentration of total petroleum hydrocarbons in mussels, with mussels being employed as bioindicators for evaluating the pollution levels in the Tigris River. The findings of the study highlight potential risk factors related to petroleum products and include a biological hazard risk assessment concerning petroleum pollution in the river. Furthermore, the research provides recommendations aimed at managing and reducing the sources of this pollution **(Arjoon & Speight, 2020; Ahmad, 2022; Almutairi, 2022; Shi *et al.*, 2024)**.

Total petroleum hydrocarbons are significant contributors to the accumulation of pollutants in mussel tissues and the overall pollution levels of riverbeds. Various studies indicate that the concentration of these hydrocarbons in mussels is closely tied to the performance of concentration within water-sediment systems.

In this study, researchers were successful by figuring out how to pick out petroleum hydrocarbons from freshwater critters in a polluted water source. They used mussels as bioindicators to track the environment. Mussels are good indicators at showing how healthy their surroundings are. This is because they can move around and they filter feed, which lets them soak up all those tiny metallic elements. The petroleum hydrocarbons in mussels from seven different river locations were checked out, covering the whole river stretch from the north to the south of Meysan Province. We found species of river mussels from the Unioninae family-*Unio tigris*, that were ideal for measuring these hydrocarbons. Many studies conducted quick checks on the PHC levels in *U. tigris* at many sites, finding higher PHC concentrations than in *U. indocus*. These studies have found that *U. tigris* accumulate PHC in their tissues and are considered as a bioindicator for pollution **(Abdulla *et al.*, 2023; Borea, 2024; Hemati *et al.*, 2024; Hura, 2024)**.

This study is the first to illustrate that concentrations of these hydrocarbons are widely dispersed in mussels throughout the down region of the Tigris River at Meysan Governorate. Therefore, the purpose of this research was to measure the levels of total petroleum hydrocarbons (TPH) found in the tissues of freshwater mussels belonging to the Unio species in the Tigris River.

The concentration trends observed in the mussel tissues closely linked to the overall disturbances within the ecosystem. TPH contamination represents one of the prevalent types of environmental pollution found in various waters along the Tigris River. This pollution comprises a blend of different substances primarily originating from natural gas and fossil-derived crude minerals. Over the past twenty years, instances of such pollution have escalated, particularly in the world's major rivers, as a result of diverse fishing and industrial operations. The impact of this pollution on the ecosystem is multifaceted, leading to a temporary decline in environmental quality, which results in diminished nutritional value for both aquatic organisms and terrestrial animals. In certain regions of Iraq, especially in the northern Tigris, the untreated wastewater, is released into the river. Consequently, it is vital to both human and animal consumption. Furthermore, the sources of oil pollution are crucial in exacerbating the detrimental effects of contaminants on marine ecosystems **(Ibrahim & Adnan, 2021; Garabedian, 2023; Kareem *et al.*, 2023; Resen *et al.*, 2024)**.

Crude oil and its derivatives are recognized as significant contributors to organic pollutants in aquatic environments. When water becomes polluted with petroleum products, the solubility of specific organic compounds in oils results in the introduction of petroleum hydrocarbons into the water. These hydrocarbons are found in the environment as either aliphatic or aromatic components, which can be quantified together

as total petroleum hydrocarbons. Aquatic organisms can absorb total petroleum hydrocarbons as their surroundings become contaminated. As a result, organisms that accumulate these hydrocarbons may suffer direct physical damage due to the ingestion of hydrocarbons and the infiltration of total petroleum hydrocarbons into the cells of vulnerable tissues **(Ossai *et al.*, 2020; Asejeje *et al.*, 2021; Hoang *et al.*, 2021; Marvin *et al.*, 2021; Uddin *et al.*, 2021)**.

The uptake of total petroleum hydrocarbons and their metabolites is affected by the physiological and ecological tolerances of the organism. Consequently, the composition of polycyclic aromatic hydrocarbons, isoprenoid derivatives, and carbon ranges are essential factors that contribute to the high toxicity of total petroleum hydrocarbons in aquatic ecosystems, especially in areas impacted by weathered and resistant petroleum products. Generally, organisms do not significantly accumulate total petroleum hydrocarbons. Notably, the enhancement of granules and the stable set length of phase 4 seem to restrict the buildup of total petroleum hydrocarbons in aquatic proteins. Although the process that governs the interaction between pollutants and their integration into the water matrix during the accumulation of total petroleum hydrocarbons in living organisms is not well understood, information regarding the uptake of total petroleum hydrocarbons and the corresponding cellular responses to contaminant dispersion can clarify the cause-and-effect relationship linked to the toxicity of total petroleum hydrocarbons or affected habitats. This understanding can support the formulation of regulations concerning total petroleum hydrocarbons **(Haider *et al.*, 2021; Sun *et al.*, 2021; Jesus *et al.*, 2022; Mallah *et al.*, 2022; Yilmaz & Donaldson, 2022)**.

Certain aquatic species exhibit varying levels of tolerance to different types of oil pollution present in their habitats. The prevalence of hydrocarbon contamination in marine environments has notably risen due to the discharge of waste from petroleum refinement processes. The primary aim of this research was to assess the levels of total petroleum hydrocarbons present in bivalve species from the Tigris River, particularly those situated near oil processing facilities, and to examine any variations throughout different study periods. Various bivalve indicator species were selected for this analysis, revealing that mussels displayed a notable sensitivity to total petroleum hydrocarbons while remaining within their tolerance range. Findings indicated that these mussels can differentiate between total petroleum hydrocarbons originating from various sources, particularly by distinguishing natural tarballs from offshore sources and oils from onshore origins. This implies that mussels are concurrently exposed to two distinct sources of total petroleum hydrocarbons. Furthermore, the results provided evidence of an additional source of total petroleum hydrocarbons contributing to the sampled materials **(Karam & Al-Wazzan, 2021; Dellali *et al.*, 2022; Marigómez *et al.*, 2024)**.

In relation to the ecological risks posed by total petroleum hydrocarbons, mussel tissues have shown the most significant inhibition when compared to non-edible tissues. Within the entire homogenate, there appears to be an average decrease in the ability to

detoxify these hydrocarbons. Extended future research is necessary to evaluate the levels of total petroleum hydrocarbons in inland waters and to assess the damage inflicted on fish and mussels communities. Such studies are crucial for informing pollution control strategies and for safeguarding a vital food source. Fishing plays a critical economic and social role for the nations adjacent to the Tigris River, thus chronic exposure to polluted river water poses serious threats to human health and food security **(Hadidi *et al.*, 2022; Takahashi *et al.*, 2022; Salem *et al.*, 2023; Ng *et al.*, 2024)**.

This research was conducted along the Tigris River within Meysan Governorate in Iraq, focusing on combustion as a primary contributor to Total Petroleum Hydrocarbons (TPH) and trace element pollution in the river. The study highlighted practices such as burning oil in desert conditions, which produces significant smoke, and the operations of oil transport trucks. These trucks are often under police protection, facilitating negotiations with business operators to unload and transport oil at reduced costs. The study analyzed TPH concentrations in the soft tissue of mussels. The native population of *Unio tigris* (Fig. 1) is confined to Maysan Governorate and the mudflats along the banks of the Tigris River. Seven locations along the river were chosen for placing mussels at a depth of 20cm beneath surface water, with subsequent measurements of TPH concentrations conducted over designated time periods. This is important to monitor timeframes and the unaccounted discharges from illicit oil traders.

The Iraqi major rivers are the Tigris and the Euphrates rivers, both of which are derived from Turkey. The Tigris River has contributed to many important civilizations, including ancient Mesopotamia. Iraq is located at the head of the Arab Gulf; it contains a large network receiving different pollutants from various sources. The Turkish part of the Tigris River contains significant dams. The water of the river is used to irrigate agricultural land and the construction business.

Iraq is greatly dependent on it for drinking purposes, agricultural lands, and several industries. The river is suffering from many natural disasters, including drought, salinity, and pollution from both the Turkish and Iraqi sides. The population has increased, leading to a large amount of waste being disposed. Therefore, the government in Iraq published environmental laws and rules to control this matter. It is required to test different parameters to assess the environmental effectiveness of those laws.

Fig. 1. *Unio tigris*

MATERIALS AND METHODS

Mollusca samples were collected from seven different sites of the Tigris River in Meysan Governorate (Fig. 2) during 2024. The external surface of the mussels was cleaned with a sponge and tap water to remove traces of silt, and then tap water was rinsed again. The mussels were then removed from the water; the gills and internal soft tissue were dissected from each mussel. The tissues isolated from each individual mussel were then air-dried at room temperature. Dried tissue samples were homogenized in a stainless steel mill using a tungsten carbide ball for 15 seconds. The homogenized dried tissue samples were retained in a labeled container before being analyzed. Each sample was used for determining the total petroleum hydrocarbons concentrations. Data from site triplicates were averaged for each sampling site **(Al-Saad, 1995)**.

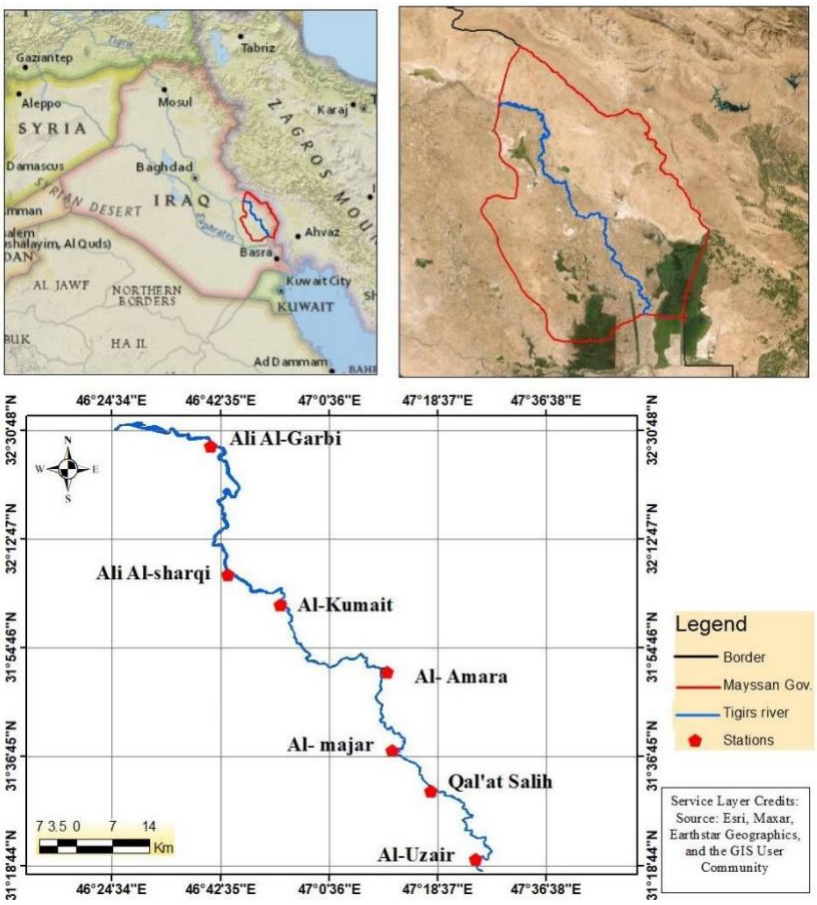

Fig. 2. Study area and sample locations

RESULTS

This study examined the levels of total petroleum hydrocarbons found in the soft tissues of *U. tigris* mussels sampled from the Tigris River across four different stations, with the results quantified in parts per million (ppm), as shown in Table (1), and Fig. (3), The findings indicate that the mussels exhibit measurable impacts from petroleum product activity, with the highest levels detected at the 5th station and the lowest at the 7th station.

Additionally, there was a minor increase in concentration at the stations. The average concentration of hydrocarbons identified in this investigation surpasses the levels reported in neighboring nations affected by similar environmental issues. However, the present concentrations in *U. tigris* mussels are lower than those documented in previous studies conducted in Meysan and surrounding areas of the lower Tigris River.

Table 1. Concentration of total petroleum hydrocarbon (µg/g) in stations

Station	Conc.	Range	Mean	±SD	±SE
1	6.31	5.89-6.36	6.186	0.258	0.149
	6.36				
	5.89				
2	7.54	7.21-7.63	7.460	0.221	0.127
	7.63				
	7.21				
3	9.52	8.89-9.52	9.156	0.325	0.188
	8.89				
	9.06				
4	11.21	10.64-11.21	10.913	0.285	0.164
	10.64				
	10.89				
5	12.53	11.79-12.53	12.116	0.377	0.217
	11.79				
	12.03				
6	5.32	5.1-5.43	5.283	0.168	0.097
	5.43				
	5.1				
7	3.3	2.88-3.3	3.050	0.221	0.127
	2.88				
	2.97				

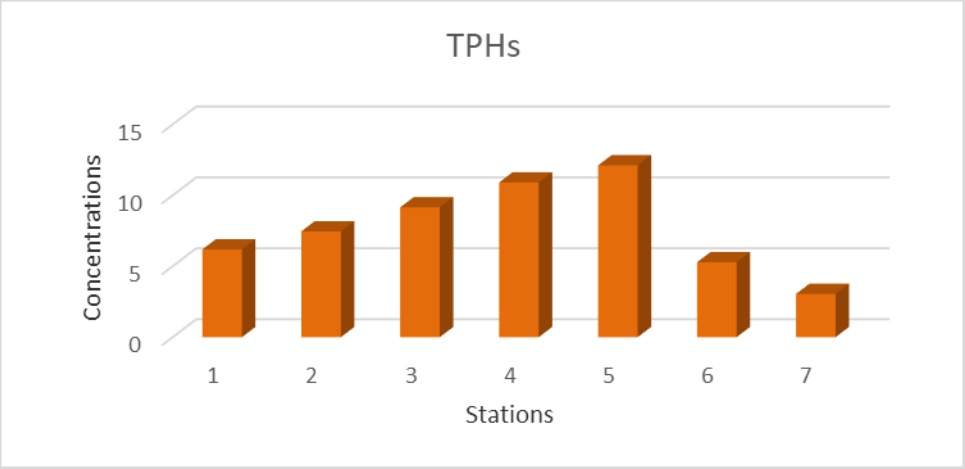

Fig. 3. Means concentration of total petroleum hydrocarbon (µg/g) at stations

Table 2. Means concentration of fat content (%) at stations

Station	Conc.	Range	Mean	SD	SE
1	0.85	0.84-0.86	0.85	0.01	0.006
	0.84				
	0.86				
2	0.86	0.85-0.88	0.863	0.015	0.009
	0.88				
	0.85				
3	0.88	0.88-0.92	0.897	0.021	0.012
	0.89				
	0.92				
4	0.98	0.96-0.98	0.97	0.01	0.006
	0.97				
	0.96				
5	0.92	0.9-0.92	0.91	0.01	0.006
	0.9				
	0.91				
6	0.85	0.81-0.85	0.827	0.021	0.012
	0.82				
	0.81				
7	0.75	0.74-076	0.75	0.01	0.006
	0.76				
	0.74				

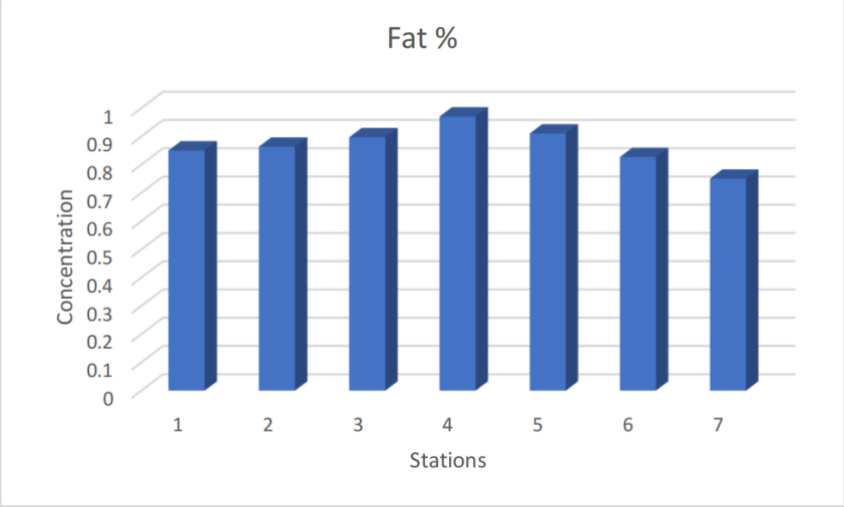

Fig. 4. Means concentration of fat content (%) at stations

DISCUSSION

The analysis of total petroleum hydrocarbons (TPHs) in adult *Unio tigris* in this study indicated relatively elevated concentrations, with an average of 14.3 ± 2.1mg/ kg wet weight. These levels are comparable to those found in certain European freshwater mussels. The increased concentrations can be attributed to the fact that Iraqi rivers often receive bodies for substantial amounts of crude oil, as well as various forms of unrefined, untreated, or inadequately treated wastewater and industrial discharges. This situation is exacerbated by the inadequate design, siting, and operation of facilities in the rapidly growing petrochemical sector near Basrah, which is situated close to the southern rivers. The proximity of these facilities to the rivers raises the risk of both accidental and intentional oil discharges, some of which may persist in the environment without biodegradation, eventually accumulating in the sediments of riverbeds inhabited by the mussels. Additionally, the construction of dams has altered the natural water flow essential for maintaining river water quality and promoting self-purification processes, thereby allowing for the accumulation of various chemicals, including TPHs, in the river.

Several factors such as nutritional status may also influence the uptake of total petroleum hydrocarbons by mussels, in addition to concentration and bioaccumulation effects. This area warrants further investigation in future research efforts. This phenomenon may be linked to the differing physicochemical properties of total petroleum hydrocarbons when compared to polycyclic aromatic hydrocarbons and hexachlorobenzene. Notably, most prior studies have not addressed pollution levels in both mussels and sediments within the Tigris River ecosystem.

Our study revealed that the highest concentrations of hydrocarbons in the *Unio* species were found in the Tigris River, in contrast to the lowest concentrations, which were recorded at the Euphrates River. While several previous studies have investigated hydrocarbon levels in Iraq's environment, we are not aware of any prior research specifically focusing on Unio as a biological specimen sampled from water bodies in Iraq. The hydrocarbon levels identified in our research were typically greater than those reported in earlier studies concerning Unio in Iran, as well as findings related to freshwater mussels in various other nations **(Ollard *et al.*, 2023)**.

As a general rule, field data were not available for TPH levels in *U. tigris* at the Tigris River in Iraq for comparison. However, our results are almost similar to those obtained for other taxa from the surface of the Tigris River.

Several potential sources for the TPH in the Tigris River include municipal wastewater, where the degradation of lubricating oil and high amounts of vehicular oil contribute to environmental contamination. Additionally, at large distances from the city, the sources are likely to be recreational; this is especially important as it pertains to the presence of a high number of motorboats, which contribute to the TPH concentrations in

surface water primarily as a result of the use of internal combustion engines **(Al-Saad, 1995)**. The TPHs measured in the study in mussels were derived mainly from highway traffic and any residues from spills collected at the study sites abutting the alignment of the roads, gas stations, or weirs.

In addition, three pollutant source groups producing TPH can be designated: natural sources, including oil, sea oil, and oil spills. These sources are responsible for the natural inflow of TPH into waters, creating a consistent background pollution level. Production sources, which influence the detected TPH concentration by surrounding activities, include municipal and industrial wastewaters, fast water current sites, bank erosion and trash.

Many industrial activities use petroleum products for various purposes, particularly in large quantities, directly or indirectly reducing TPH in aquatic systems. Crude oil and petrochemicals are transported to industries or through pipelines to and from port areas located along rivers. The movement of transfer, shipping, loading, unloading, and storage of crude oil and petrochemicals into process units are critical points where spillage during handling and abnormal or unexpected accidental releases into the environment are known to occur. Examples of industries and activities with potentially high exposure or load of total petroleum hydrocarbons include pipelines, disposal sites, suppliers, refiners, operators of pulp and paper mills, operators of metalworking companies, producers of food and feed, and companies that deal with the preservation of leather, as well as companies in the chemical business, insulation, and so on. The TPH signature from each industry is different; therefore, a clear profile and near-source will be requested to resolve from the local TPH background.

Oil pollution of aquatic ecosystems can be controlled in the following way. Both regulations and policies must be established. A national regulation for the discharge of oil pollutants from rivers, canals, and similar sources must be set. The maximum permissible annual average and maximum annual average amounts that are allowable for oil pollution content in river water, sediments, and biota for each of the natural ecosystems must be established. Such regulations can serve as an incentive for the prevention of oil contamination from both municipal and industrial sewage. In order to control and reduce oil pollution in aquatic ecosystems, the amount of oil in municipal wastewater must be monitored accordingly. Industrial enterprises operating in areas of high oil pollution potential need to install special treatment plants to eliminate oil pollution from their wastewater. In addition, it is necessary to investigate the actual sources of pollution to assess their compliance with national regulations.

Industrial waste and industrial wastewater can be especially dangerous. The mere dumping of any oily industrial waste or chemical additives into the waterway will change the condition of the water. Oil pollution of aquatic ecosystems can also be controlled by policy. To avoid further pollution from industrial sewage, preventive action must be taken. Both the community and industry must develop cooperative methods for the

reduction of oil production and pollution. Industries involved in exploration, production, refining, and distribution of oil all have a role to play in relation to continuous pollution estimation. Preventive measures, such as proper maintenance and installation of equipment to control the discharge of water, and national standards for wastewater discharge prevention, could serve as a basis for development. Since the oil and gas industry, refineries, users, and owners of the facilities all show an interest in being active participants with a common goal, the imperative for developing such a national plan is evident.

When studying the uptake of pollutants in mussels to assess the environmental health complications, it is important to take into consideration 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 mussel tissue from the Al-Amara site (Table 2 & Fig. 4). In this study, a significant correlation (R=0.79) between values was found, which shows a that there is a correlation between fat content and TPHs levels in *Unio tigris*.

CONCLUSION

The use of *Unio tigris* as indicators of ecological quality and the health of these invertebrates can be utilized for environmental management and conservation policy development. Furthermore, in future studies, the tissue-specific dose-response of these compounds can be explored in different aquatic organisms in this region. Future studies on total petroleum hydrocarbons should be carried out that involve atypical histological examination with emphasis on the safety assessment of these large complexes of aquatic ecosystems. Scientists also need to propose any possible solutions to the range of pollutants found in the water. The TPHs measured in the study in mussels were derived mainly from highway traffic and any residues from spills collected at the study sites abutting the alignment of the roads, gas stations, or weirs.

In addition, three pollutant source groups producing TPH can be designated: natural sources, including oil, sea oil, and oil spills. These sources are responsible for the natural inflow of TPH into waters, creating a consistent background pollution level. Production sources, which influence the detected TPH concentration by surrounding activities, include municipal and industrial wastewaters, fast water current sites, bank erosion and trash.

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