Indonesian Journal on Health Science and Medicine

Vol. 3 No. 1 (2026): July
DOI: 10.21070/ijhsm.v3i1.370

ISSN 3063-8186 (online), https://ijhsm.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Indonesian Journal on Health Science and Medicine

Vol. 3 No. 1 (2026): July
DOI: 10.21070/ijhsm.v3i1.370

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ISSN 3063-8186 (online), https://ijhsm.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

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Indonesian Journal on Health Science and Medicine

Vol. 3 No. 1 (2026): July
DOI: 10.21070/ijhsm.v3i1.370

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Indonesian Journal on Health Science and Medicine

Vol. 3 No. 1 (2026): July
DOI: 10.21070/ijhsm.v3i1.370

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Vol. 3 No. 1 (2026): July
DOI: 10.21070/ijhsm.v3i1.370

Seasonal Distribution of Petroleum Hydrocarbons in Imported Rainbow Trout Organs

Ali M. Galo, AliM.Galo_8888mlynam@gmail.com (*)
Basrah Education Directory, Basrah, Iraq

Rajaa N. Alyassein, AliM.Galo_8888mlynam@gmail.com
University of Basrah/ College of Science/ Department of Pathological analyses, Iraq

Ghassan A. Al-Najare, AliM.Galo_8888mlynam@gmail.com
University of Basrah/ Marine Sciences Center/ Department of Marine Vertebrates, Iraq

Ali T. Yaseen , AliM.Galo_8888mlynam@gmail.com
University of Basrah/ Marine Sciences Center/ Department of Marine Vertebrates, Iraq

(*) Corresponding author

Abstract

General background: Petroleum hydrocarbons are persistent aquatic contaminants capable of bioaccumulation in fish tissues, posing ecological and public health concerns. **Specific background:** Rainbow trout (*Oncorhynchus mykiss*) are widely consumed in Basra Governorate and are sensitive bioindicators of hydrocarbon pollution. **Knowledge gap:** Limited information exists on seasonal and organ-specific distribution of total petroleum hydrocarbons (TPHs) in imported trout sold in local markets. **Aims:** This study quantified seasonal TPH concentrations in selected organs of spotted and pink *O. mykiss* obtained from Basra markets. **Results:** TPH levels varied significantly among organs and seasons ($P \leq 0.05$), with the highest concentrations consistently detected in the liver, particularly during spring (up to 2.88 µg/g dry weight), while muscles and gonads showed the lowest levels (as low as 0.01 µg/g dry weight). **Novelty:** The study provides market-based evidence of differential TPH accumulation patterns in imported trout, rather than wild-caught populations, highlighting seasonal trends linked to biological and environmental processes. **Implications:** Although muscle tissues showed lower contamination, detectable TPHs indicate potential long-term bioaccumulation risks, underscoring the need for routine monitoring of imported fish to safeguard consumer health.

Highlights:

- Liver consistently exhibited the highest TPH accumulation across seasons.
- Spring recorded peak TPH concentrations in both trout varieties.
- Edible muscle tissues contained lower but detectable TPH levels.

Keywords:

Total Petroleum Hydrocarbons; Rainbow Trout; Fish Organs; Seasonal Variation; Bioaccumulation

ISSN 3063-8186 (online), https://ijhsm.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Published date: 2026-01-12

Introduction

Environmental pollution and lack of access to clean water are significant and widespread problems affecting organisms in their habitats [1]. Assessing and predicting the effects of pollution on the aquatic environment is a highly urgent and important matter due to urban development and industrial and agricultural activities [2]. There are major sources of hydrocarbons in the aquatic environment, such as crude oil and its derivatives, in addition to natural sources and the burning of organic fuels; therefore, they are considered among the most dangerous pollutants in water bodies [3]. Petroleum hydrocarbons are among the most significant and serious problems facing the ecosystem, whether their source is benzene, pesticides, or other toxic organic materials [4]. Living organisms are affected by petroleum hydrocarbons as pollutants in their environment and also by their toxic properties, such as bioaccumulation [5]. Because they are harmful to most forms of life, they have raised significant environmental and health concerns for environmental organizations [6]. Approximately one million tons of oil enter seawater annually as a result of accidents during production or transportation, in addition to petroleum products entering with sewage [7]. All these activities have potential environmental impacts and require assessment. In the last two decades, efforts have been made to address this issue. There is an increasing effort underway to develop multi-impact methods that can integrate multiple environmental pressures and thus provide a comprehensive assessment [8].

In general, living organisms are used as biological indicators to determine pollutant concentrations, allowing the study to provide a comprehensive picture of the environmental situation [7]. Fish, especially adult fish, are important for assessing ecosystem pollution, this is due to their continuous exposure to pollutants through skin, gills, or by consuming contaminated food. The large size of fish, their ease of handling, and their nutritional importance all contribute to their selection for this task [9]. Because fish have a high capacity to absorb pollutants from water, as particles transported from rivers enter directly through their skin or gills, and other pollutants such as tar balls found in sediment enter through the fish's intestines when they swallow water [10].There is a direct relationship between the rate of bioaccumulation of pollutants and the rate of blood flow within tissues, and the accumulation of high concentrations in the liver and kidneys of fish is attributed to the high rate of blood flow within these organs [11].

Rainbow trout (*Oncorhynchus mykiss*) are widely farmed around the world as a food supplement in natural ecosystems and for commercial aquaculture [12].They are partially migratory fish, they complete their life cycle and reach the large size achievable in freshwater, maturing at 2 to 7 years of age and measuring approximately 500 to 1100 mm in length [13].Fish that migrate spend one to five years in the open ocean and one to four years in freshwater [14]. There are many factors that affect survival, reproduction, and fertility, such as body size, growth, and energy content [15]. Due to the sensitivity of the fish (*O. mykiss*) to chemicals, it is a useful model for toxicology studies and for assessing the health of aquatic ecosystems [16]. Therefore, the object of this study is to evaluate total of petroleum hydrocarbons levels and determine the suitability of various biomarkers in selected parts of *O. mykiss* fish obtained from local markets for detecting oil pollution, comparing these findings with other studies.

Material and Methods

Study area and sampling

Over a twelve-month period, from winter 2022 to autumn 2023, approximately 196 fish were purchased, including 98 of each species (spotted and pink), from the Basra Central Wholesale Market in Basra Governorate. Their average weight was 2650 grams, and their average length was 41 cm.

Total petroleum hydrocarbons extraction

The [17] guidelines were followed during the fish extraction procedure. Three grams of dried fish organs (gill, liver, gonads, internal organs, and muscle) were mashed in a ceramic mortar and pestle. After that, the samples were separated and kept in a thimble for a whole day in 100 milliliters of a 1:1 v/v methanol: benzene combination, which served as the extraction solvent. After adding 15 ml of an aqueous solution of 4N MeOH (KOH), the extract was saponified for two hours at 40°C. After pouring the contents into the separating funnel, 50 milliliters of regular n-hexane were added. The sample was shaken well and then left to settle. Two layers were created: the hydrocarbon-containing layer was transferred to a separation column with a glass wool base, followed by a layer of silica gel, alumina, and finally anhydrous sodium sulfate. At room temperature, the extracted samples were allowed to dry. Ultimately, a spectrometer was used to measure the total petroleum hydrocarbons after they had been dissolved in pure hexane.

Calibration

Basra crude oil was calibrated by dissolving a known weight of oil with a predetermined amount of n-hexane to form standard solutions, which were then used to calculate the total petroleum hydrocarbon concentrations in fish. A fluorescence device was used to measure the emission intensity at a wavelength of 310 nm with an excitation of 360 nm.

Statistical analysis

SPSS 2024 (Statistical Package for Social Sciences) was used to do the statistical analysis. Using the ANOVA test, the LSD test was utilized to identify those differences below the significance level of $P<0.05$ [18].

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Results and Discussion

A. Result

According to the findings, the TPHs in the gills of the fish study ranged between 2.03 and 0.74 µg/g (dry weight) in autumn and spring, respectively (Figure 1). As for the liver, its concentrations ranged between 2.88 and 0.11 µg/g (dry weight) in autumn and spring, respectively (Figure 2). The spotted fish showed substantial variations ($P < 0.05$) between the seasons, its contents in the gonads varied from 0.07 to 0.01 µg/g (dry weight) (Figure 3). Seasonal differences were significant ($P \leq 0.05$), internal organs showed amounts of 0.18 and 0.02 µg/g dry weight, respectively (Figure 4). Significant differences $P \leq 0.05$ were found between seasons, meanwhile, concentrations in muscles ranged between 0.05 and 0.01 µg/g dry weight in winter and spring, respectively (Figure 5). Seasonal changes were found to be statistically significant ($P < 0.05$), Table (1) shows the results of the research on rainbow trout parts and its two varieties (spotted and pink) throughout the year. In the spring, the trout's liver had the highest average concentration of total petroleum hydrocarbons (2.88 µg/g dry weight), whereas in the winter, the gonads and muscles had the lowest average (0.01 µg/g dry weight). Nevertheless, the greatest mean concentration of all petroleum hydrocarbons or the pink trout was 2.74 µg/g (dry weight) in the liver during the spring, while the lowest average was 0.03 µg/g dry weight in the internal organs during the summer.

This does not affect the scientific meaning but slightly impacts the linguistic precision.

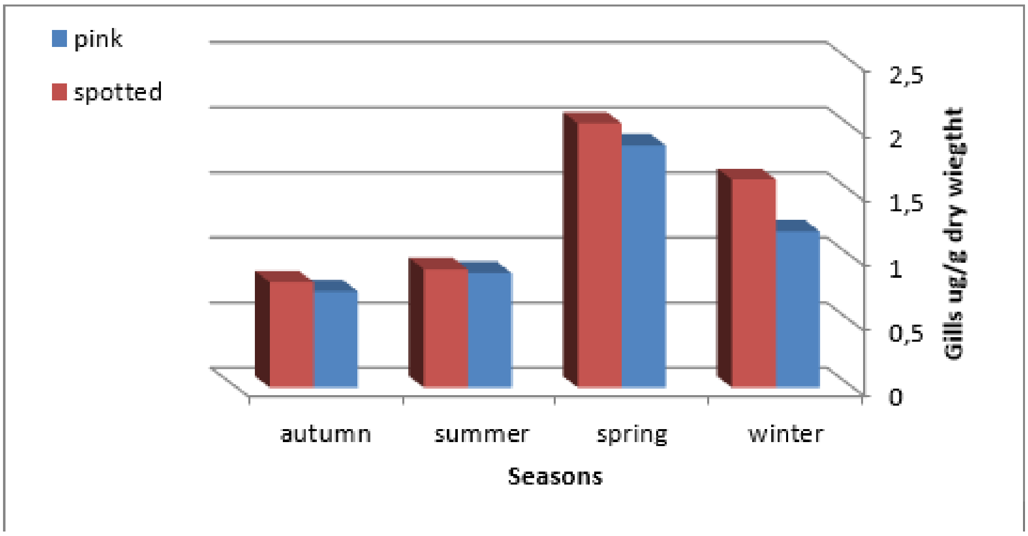

Figure 1. TPHs concentration in the gills of *O. mykiss*

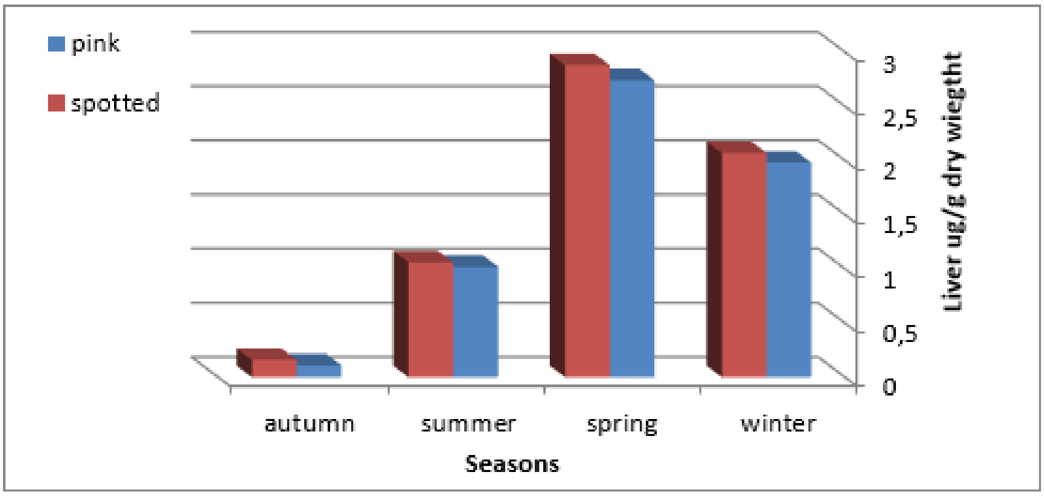

Figure 2. TPHs concentration in the liver of *O. mykiss*

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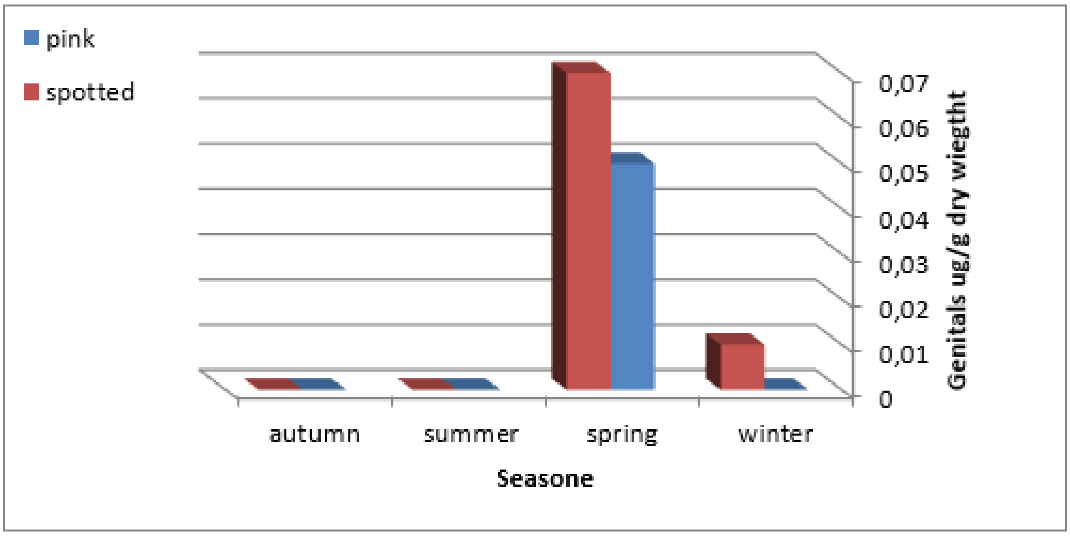

Figure 3. TPHs concentration in the gonads of *O. mykiss*

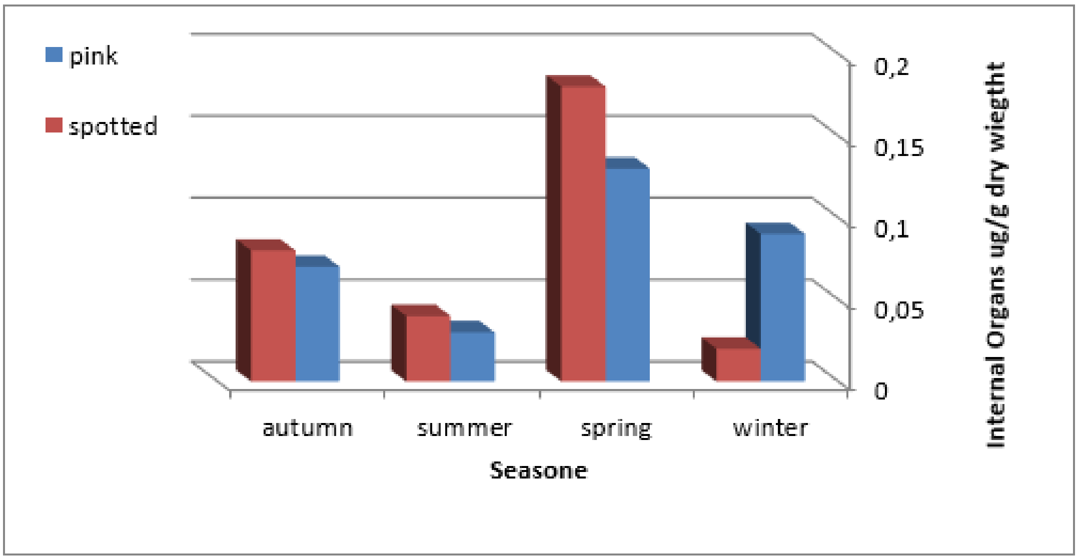

Figure 4. TPHs concentration in the internal organs of *O. mykiss*

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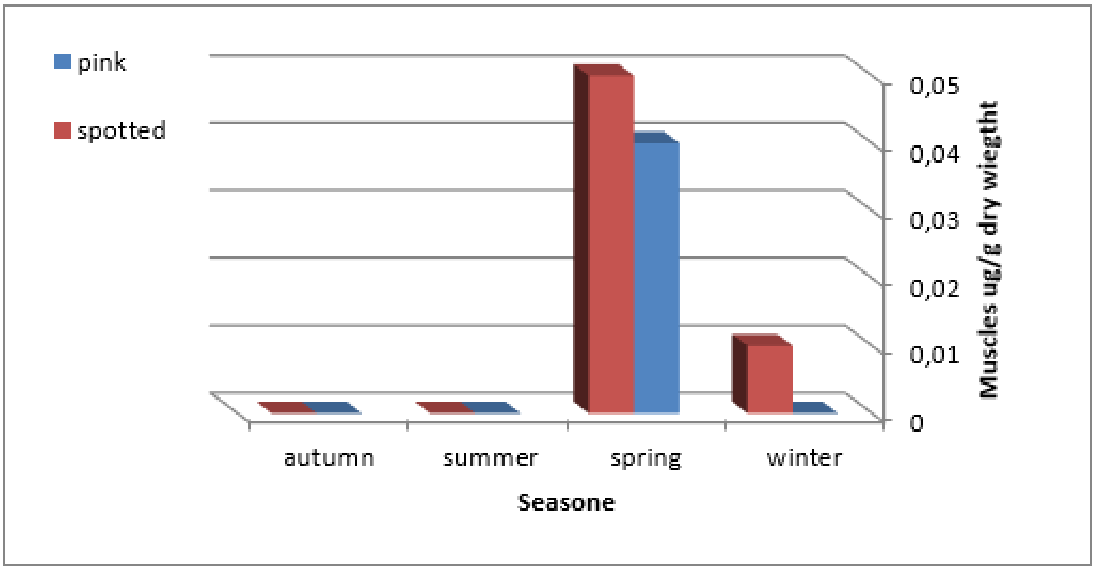

Figure 5. TPHs concentration in the muscles of *O. mykiss*

Fish organs	Gills (µg/g)		Liver (µg/g)		Muscles (µg/g)		Genitals (µg/g)		Internal Organs (µg/g)	
Seasons	**pink**	**spotted**	**pink**	**spotted**	**pink**	**spotted**	**pink**	**spotted**	**pink**	**spotted**
Winter	1.2	1.6	1.98	2.07	ND	0.01	ND	0.01	0.09	0.02
Spring	1.86	2.03	2.74	2.88	0.04	0.05	0.05	0.07	0.13	0.18
Summer	0.88	0.91	1.02	1.06	ND	ND	ND	ND	0.03	0.04
Autumn	0.74	0.81	0.11	0.16	ND	ND	ND	ND	0.07	0.08
TPHs	4.68	5.35	5.85	6.17	0.03	0.05	0.05	0.08	0.32	0.32

Figure 6. Table (1): Total petroleum hydrocarbons concentrations in Organs of *Rainbow trout (O. mykiss)* micrograms/g dry weight during the seasons of the year. * ND= Not Detected

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Region	TPHs (μg/g)	References
Shatt Al-Arab river	2.47- 21.52	[19]
Shatt Al-Arab River and Southern Iraqi Marshes	9.97- 38.56	[20]
Shatt Al-Basrah Canal	6.2-57.5	[21]
Shatt Al-Basrah Canal	5.9-48.9	[22]
Local markets in Basra	0.01-2.88	The current study

Figure 7. Table (2): Comparison of petroleum hydrocarbon concentrations in Organs of the rainbow trout (O. mykiss) with fish from various Iraqi water bodies and other marine areas.

B. Discussion

The present study showed a seasonal variation in the accumulation rate of total petroleum hydrocarbons in the studied fish parts throughout the year. When contrasting the results of this investigation with those found in Iraqi seas (Table 2), it was discovered that the study's concentrations were lower than those obtained in other regional investigations. The creation and modification of organic matter in bottom sediments and the water column in aquatic environments are among the biological, artificial, and biogeochemical processes that could be responsible for this decline [23]. Untreated sewage and domestic wastewater are added to this, and when comparing the concentrations (Table 1) with EU standards (2 micrograms/kg), it was found that fish have the ability to accumulate hydrocarbons in their bodies. [22]. The observed increase in TPH concentrations throughout the body during the spring may be attributed to the proliferation of phytoplankton and aquatic plants capable of accumulating hydrocarbons, which form the base of the food chain. These types of fish also contain fats in their tissues that have a positive correlation with hydrocarbon concentrations in the water [10]. Which can integrate into the fish's biological system through the ability to accumulate in its fatty tissues [24].

The decrease in TPHs concentrations observed during the summer (Table 1) is likely due to increased evaporation and microbial biodegradation processes resulting from higher temperatures [23]. In addition, there is increased photo-oxidation of petroleum hydrocarbons in the water column, which reduces their concentrations in feeding habitats. [25].The high concentrations of TPHs in the gills (Table 1) may be attributed to their continuous exposure to pollutants in the water as a result of respiration [26]. The higher concentrations of hydrocarbons in the liver compared to the gills (Table 1) can be explained by the physiological function of each organ [28]. The liver is the organ responsible for the accumulation, metabolism, conversion, and excretion of toxic substances from the bloodstream [27]. Since TPHs enter the digestive system either directly from water or suspended particles or are digested and eventually accumulate, their presence in the intestines throughout the year is indicative of environmental conditions, dietary patterns, food type, and availability [10]. The concentrations of TPHs in the reproductive glands that increase fat content during winter and spring can be attributed to the fish reaching sexual maturity in preparation for the spawning season, as there is a direct relationship between total hydrocarbon (TPH) levels and fat content [13]. The lower TPHs content in muscles may indicate lower bioaccumulation compared to the absorption shown by the gills TPH concentrations in muscles (the portion consumed by humans) were lower than in other analyzed organs, they were still higher than the EU recommended standard of 2 μg/kg. This may be attributed to bioaccumulation occurring in muscles [29]. The study also showed that the liver was the most affected organ, followed by the gills, internal organs, and gonads, while muscles were the least affected.

Conclusion

The study showed the basic distribution of TPHs in parts of imported and consumed *O. mykiss* fish in Basra. All fish samples analyzed contained TPH concentrations in their organs. The value of fish tissues study exposed to TPHs in the following order: liver > gills > internal organs > gonads > muscles. Although the concentration in the muscles (the source of fish protein) was low compared to the other organs analyzed, the potential consequences of bioaccumulation should not be ignored, especially in communities that consume fish in large quantities. In order to protect public health, it is recommended that TPHs levels of imported fish be periodically monitored.

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