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Bioaccumlation Of Total Petrolum Hydrocarbons In *Pseudodontopsis euphraticus* And *Bellamya bengalensis* , In Euphrates River , Al-Nassiriyah City / Iraq.

QASIM MOHAMMED AL-TAHER[1] , MANAL MOHAMMED AKBAR[2] AND IMAD HADI Al-QAROONI[3]

[1]MSc. General Directorate of Education In Thiqar, Iraq.

[2]Prof. [3]Ass.Prof. Department Of Biology-College Of Education For Pure Sciences -University Of Basrah, Iraq.

E-mail: [1]qasim20020@gmail.com

ABSTRACT

The current study was conducted for a full year (from December 2018 to November 2019) to study and determine the concentration of total petroleum hydrocarbons in the soft tissues of two species of Molluscs Snails *Bellamya bengalensis* and Clams *Pseudodontopsis euphraticus* whose samples were collected from four stations located on the Euphrates River in the city Nasiriyah, southern Iraq .The total petroleum hydrocarbon concentration was calculated in *B. bengalensis*, *P. euphraticus,* water and sediments using an ultra violet fluorescence apparatus, as it was found through the study that the highest concentration was recorded during the winter and the lowest during the summer, and the range from the average concentration of total hydrocarbons in *B. bengalensis* (1.52-12.96) µg /g dry weight , *P. euphraticus* (16.69 -1.46) µg/g dry weight , water (8.43-31.28) µg/l and for sediments(205-118) µg/g dry weight.

The second and third stations also recorded the highest concentration .The lowest concentration levels in the first and fourth stations. It was noticed through the results that there is a seasonal and local variation in the concentration of total oil hydrocarbons in water, sediments and living organisms in the Euphrates river, as it was found that the highest concentration rate in the sediments followed by water, then *P. euphraticus* and *B. bengalensis*, was a percentage the TPHs in the study area are within the global and Iraqi determinant.

Key words: Total petroleum hydrocarbons, water, sediments, *B. bengalensis* , *P. euphraticus.*

INTRODUCTION

Oil pollutants occupied an important rank among those polluting sources of the environment, and this importance comes from the continuous increase in global oil production, as the water environment has suffered from increased concentrations of pollutants in recent years, as is the case with other environmental systems, especially the increase in the concentrations of oil hydrocarbons [1]. Oil pollution is the phenomenon of entering complex compounds consisting of several elements, the most important of which are hydrogen and carbon, adding damage to the aquatic environment, as these complex compounds work to change those physical, chemical and biological properties and damage to living organisms. As a result, which leads to direct harm to humans, directly or indirectly [2, 3] as the increase in the level of these

pollutants has become a source of concern due to their significant impact not only on the quality of water and aquatic organisms, but they also pose a threat to human health [4] The distribution of petroleum hydrocarbons and their accumulation in the aquatic environment has been studied by many researchers [5 ,6, 7 ,8].The reason behind choosing *B. bengalensis* and *P. euphraticus* its ability to accumulate pollutants in their tissues in a manner that reflects the levels of pollutants in the environment, mollusca consider a good example of bioindicaters [9]. In addition, the large size, slow movement, and availability of mollusc helped researchers in study these organisms as a bioindicaters [10].The present study aimed to estimate Total Petrolum Hydrocarbons in water, sediments, and fresh tissues of *B. bengalensis* and the *P. euphraticus* from the Euphrates river / south of Iraq.

MATERIALS AND METHODS

Four stations along the stretch of the Euphrates river in / Al-Nassiriyah - south of Iraq, were selected (Figure 1). The upstream station is located in the northwestern part of the city of Nasiriyah, near the Sharif station1, and it is characterized by a vegetation cover and a small population density(N=31^{0}3' 36.46" , E=46^{0}7' 45.42").Three other downstream station at power plant station 2,(N=31^0 2' 74.35, E=46^0 11' 63.69"), city center (station 3, N=31^0 2' 5.88", E=46^0 16' 84.24"), and Ur district (station 4, N=30^0 58' 59.69", E=46^0 19 '76.35") were designated as polluted/experimental sites. The station 2 is characterized by its nearly to thermal pollution source. Station 3 is receiving sewage inputs from city sanitation networks. Station 4 is receiving agricultural inputs from rural areas along the river.

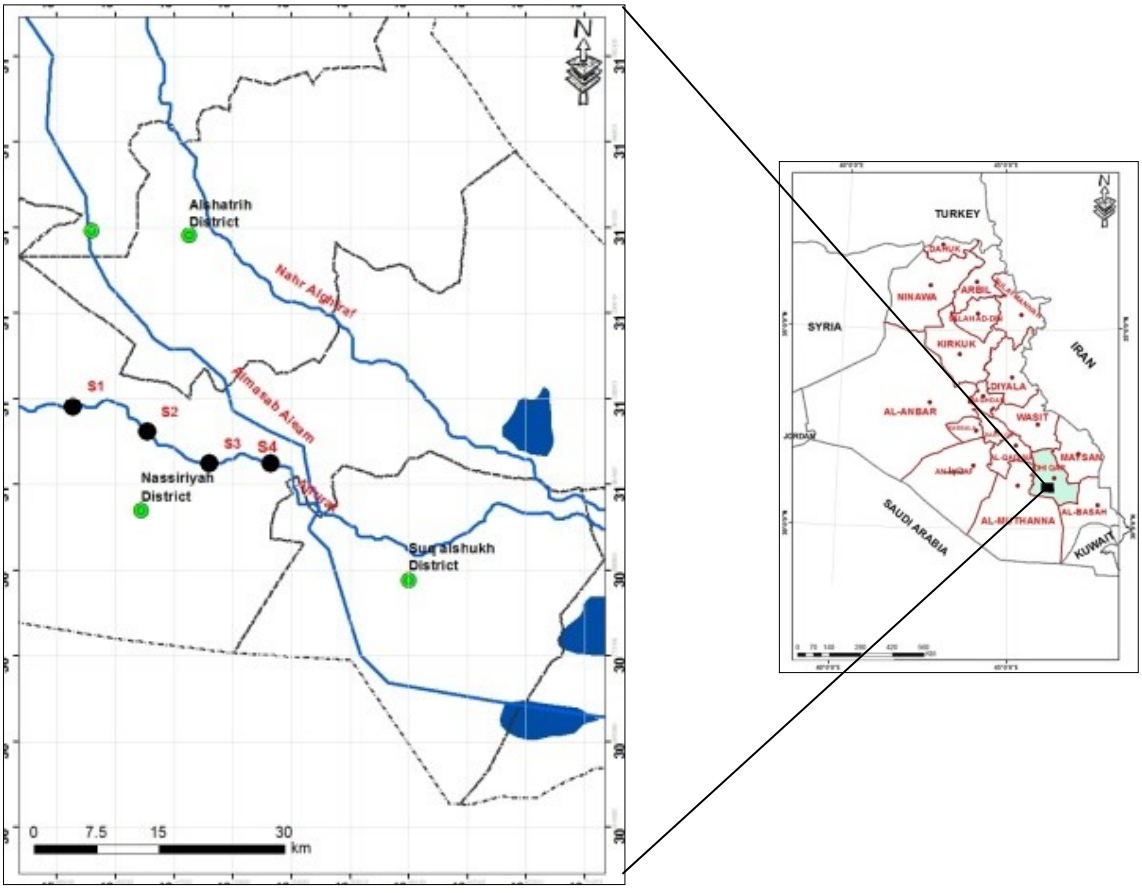

Figure 1: Stations Location Of Euphrates River.(Arc Gis program,10.2)

Water samples were collected from the sampling station were collected by 5-liter dark brown glass bottles, and collected at a depth of one meter away from the river banks in such a way that no bubbles were allowed. These water samples were filtered and preserved by adding 25 mm of CCl4 carbon chloride added to it, per liter of water to prevent TPHs adsorption on the inner surface of the container and stored at 4°C before their analysis.

In each station, the samples of sediment were collected from a depth of 10 cm from the surface of water, using a collector of sediment with an acid-washed stanlis steel scoop then taken by glass bottles marked to the laboratory. They were dried and ground with a ceramic mill. After that, they were sifted with a 2 mm diameter sieve to get rid of stones and impurities then kept in labeled glass bottles until the TPHs were extracted, [11] .

Mollusca were seasonally collected from freshwater from their natural inhabitants at the four stations involved. Species that were sampled for this study are *P.euphraticus* and *B. bengalensis* . Mollusca were collected by hand and washed with distilled water several times, then placed in containers carrying water. They were taken to the laboratory. The biomass was isolated by a stanliss forceps on the filter papers to dry in the laboratory temperature, and put in the Freeze Dryer until it reached drought, then transferred to the dryer at room temperature. The samples were thoroughly ground using a ceramic mortar, and the powder was kept in clean, labeled glass boxes tightly closed until the heavy elements were extracted.

The method [12] was used to extract the total petroleum hydrocarbons from the Molluscas samples. Samples to be ready for measurement in the Shimadzu type Ultra Violet fluorescence (U.V.F).

Total Petrolum Hydrocarbons detection in water Using the method adopted by the United Nations Environmental Protection Program [13] to extract the total petroleum hydrocarbons from the water.

The method [14]by [15] was used to extract total petroleum hydrocarbons from sediments to make the sample ready for measurement with a UV (Ultra Violet fluorescences) device.

Mechanical analysis of sediment samples was performed using the Hdrometer method to determine the percentage of sediment components (Grain Size Analysis), and the percentage of sediment minutes (sand, silt and clay) was calculated according to method [16] and a method [17] was adopted to estimate the total organic carbon content in the sediments. (TOC%) using potassium dichromate and sulfuric acid and by correction method against Fe $(SO4)_2$.6H2O (NH4) 2 and expressed results as a percentage.

An extraction similar to the extraction process was performed except for the presence of samples for the purpose of ascertaining the solvent purity and not contaminating the samples and glass bottles that were used during the work..

One-way ANOVA test was used to compare the various sets of data collected from the stations using SPSS v.17.0 software for Windows.

RESULTS :

The results given in Figer 2 indicate Total Petrolum Hydrocarbons concentrations in water samples collected from stations during all study seasons. The highest concentration of TPHs was in Winter at st.3 (31.28±0.81), while the lowest concentration was in summer at st.1 (8.43±1.00) dry weight.

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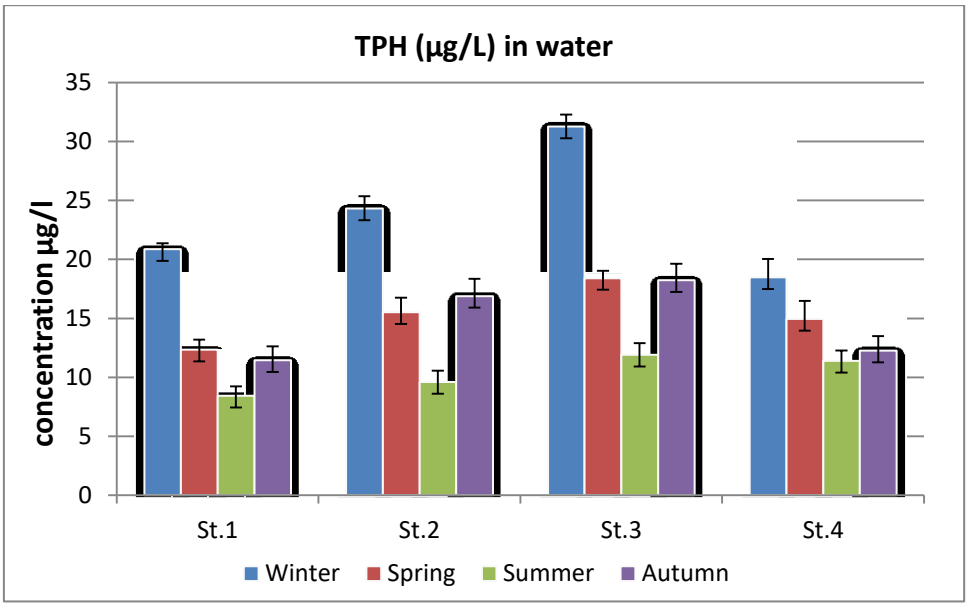
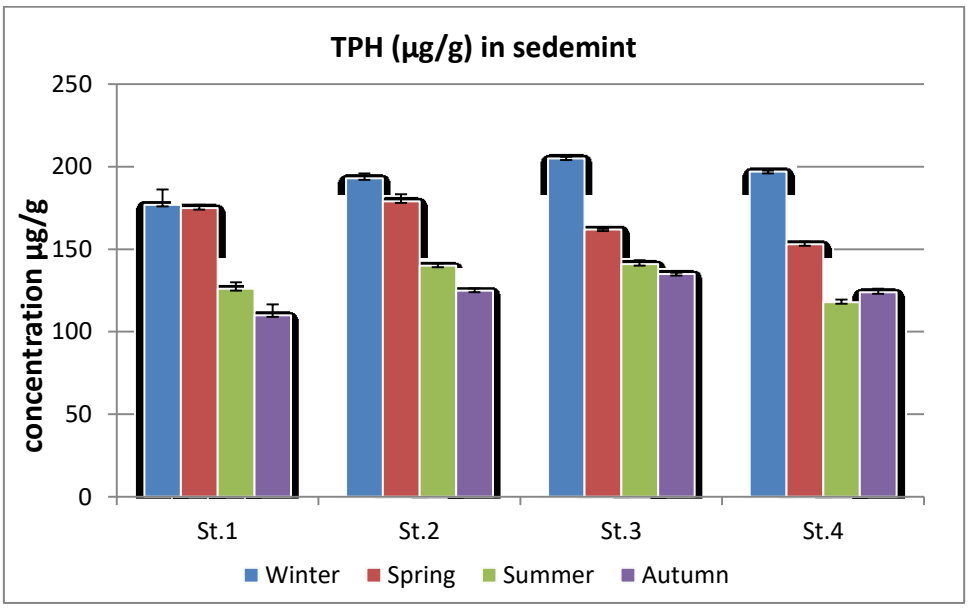


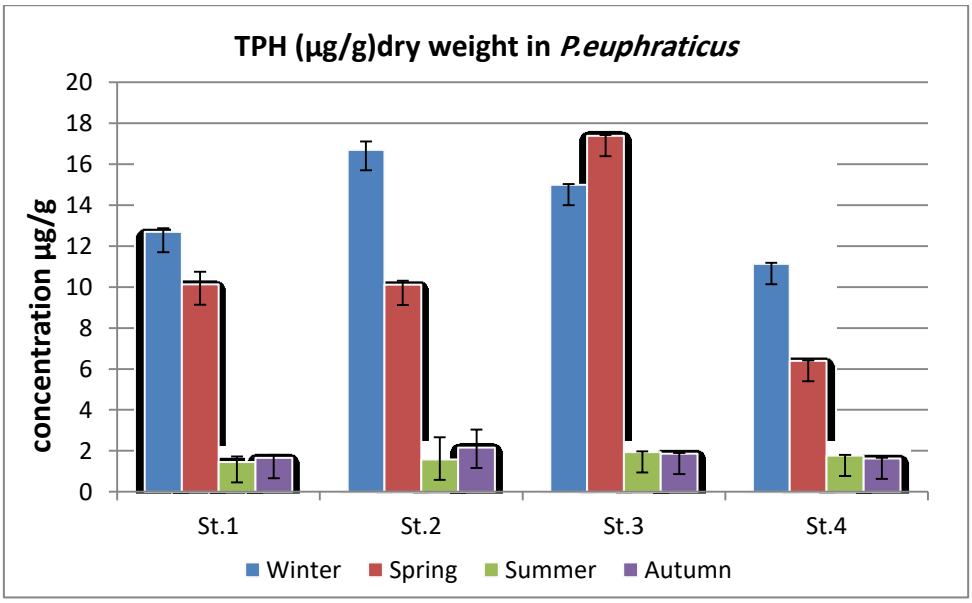
Figer 2: Mean Concentration Of TPHs (µg/l) In Water

The results given in Figer 3 indicate TPHs concentrations in sediments samples collected from stations during all study seasons. The current study showed that highest value of TPHs concentration was (205±3.94) dry weight measured in Winter at st.3, whereas the lowest value (110±0.61) dry weight was measured in Autumn at st.1.



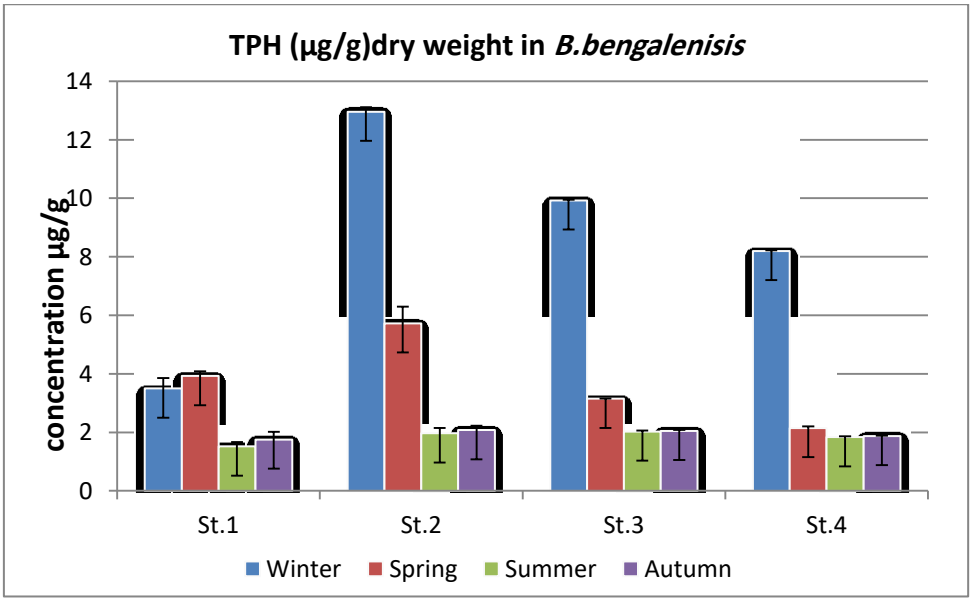
Figer 3: Mean Concentration Of Total Petrolum Hydrocarbons (µg/g) Dry Weight In Sediments

The results given in Figer 4 indicate Total Petrolum Hydrocarbons concentrations in *P.euphraticus* samples. The present study showed that maximum concentration of TPHs (15.00±0.26) dry weight was found in fresh tissues of *P.euphraticus* collected from st.3 in Winter, while the minimum concentration (1.46.14±0.02) dry weight was collected from st.1 in Summer.



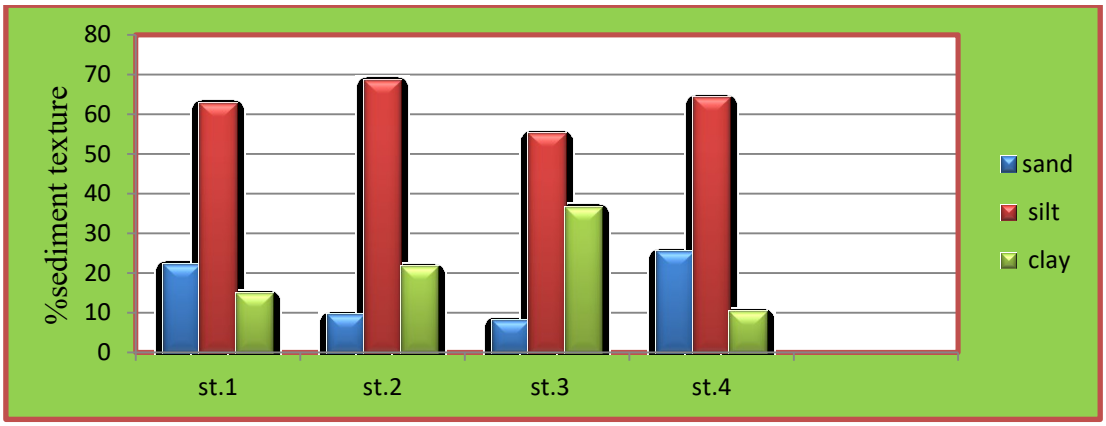
Figer 4: Mean Concentration Of Total Petrolum Hydrocarbons (µg/g)Dry Weightt In *P.euphraticus*

The results given in Figer 5 indicate Total Petrolum Hydrocarbons concentrations in *B.bengalensis.* The highest concentration of TPHs (12.96±0.15) dry weight was found in *B.bengalensis* collected from st.2 in Winter, while the lowest concentration (1.52 ±0.02) was collected from st.1 in Summer.



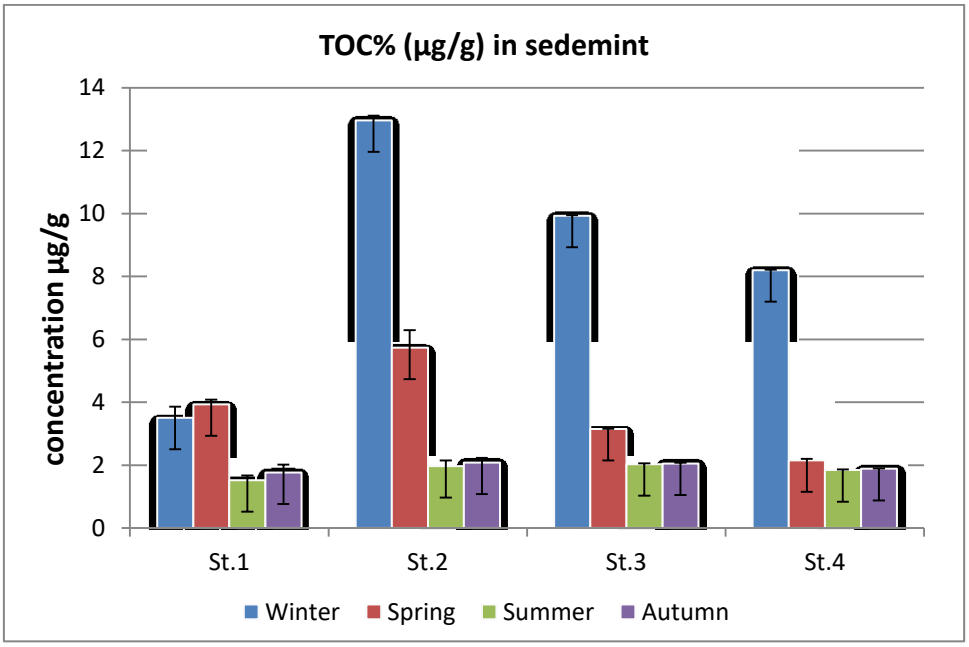
Figer 5: Mean Concentration Of Total Petrolum Hydrocarbons (µg/g) Dry Weight In *B.bengalensis*

The presence of hydrocarbons is also associated with the type of sediments, as the percentage of these compounds increases in Clay and Silt alluvial deposits more than sandy deposits due to the increase in the capacity of the clay deposits in the containment of hydrocarbon compounds [18] as shown in Figur (6).



Figer (6) Percentage Of Sediment Texture.

As the study recorded the highest rate concentration of TPHs in st.3 in winter (205 µg / g dry weight), which is the same station that recorded the highest concentration of organic carbon, this means that a higher amount of TPHs indicates a higher amount of organic matter [(19)] as shown in figer (6, 7) and the lowest mean concentration of TPHs was recorded in the St.4 in summer (118 µg / gm dry weight. The decrease of pollutants in the St.4 may be due to the self-purification carried out by the river as it is a large and running river that is rich in biological diversity [20] .



Figer (7) Mean Concentration TOC% In Sediment

The study proved, as it shows in table (2) the presence of the direct relationship at the level of significant difference $P \leq 0.01$. Between the concentration of lipids in Molluscas with the concentration of petroleum hydrocarbons in water and the correlation coefficient r = 0.878, and the presence of a direct correlation relationship between the concentration of lipid rate in the fresh tissues with the hydrocarbons in the water and sediments, and the correlation coefficient for them was r = 0.760, r = 0.958, respectively.

Table (2) Seasonal Significant Differences In Lipid Concentrations Between Both Species Of Study

lipid rate / Season	*P.euphraticus*		*B.bengalensis*	
	M	SD±	M	SD±
Winter	%4.12a	0.80	%3.43a	0.20
Spring	%3.24b	0.57	%2.90b	0.15
Summer	%1.76c	0.30	%1.53c	0.16
Autumn	%2.18c	0.48	%1.42c	0.20
LSD	0.68		0.21	

DISCUSSIONS

There are many paths through which hydrocarbons reach the aquatic environment, such as natural exudation and life synthesis within the bodies of living organisms and industrial sources.The environment of the Euphrates river water is directly affected by the pollutants resulting from the dumping of untreated wastewater directly to the river and what is offered by the factories , factories located on the Euphrates river , the electric power station that reach the river by shoveling this soil with water by rains also include random urbanization on the bank of the river, the spread of large diesel generators, oil change operations thereof, and other sources [9] .

The results of the current study showed that the lowest rates of total petroleum hydrocarbon concentrations in water reached (8.43) µg / l in the summer in the first station, And the highest concentration rate (31.28) µg / liter in st. 3 at the winter, as shown in table No. (1), and the reason may be due to the rise in temperature, which plays an important role in evaporation Petroleum hydrocarbons during the summer, in particular those on the surface layer of water that are exposed to direct sunlight and this is consistent with [21] .

Indicated [22] that biodegradation processes occur due to the presence of analyzed bacteria of petroleum hydrocarbons due to the rise in temperature. It was found that there were significant differences between the seasons and between the stations, this confirms the role of temperature in the disposal of petroleum hydrocarbons and their removal from water [23] .

As for the local differences, the high concentration in the st.3 may be due to the proximity of the sanitry sewage station and the high population density at the center of Nasiriyah city and the disposal of oil from electric power generators in addition to using boats that usually replace their oil on the edge of the river.

When comparing some previous studies (Table 1), the study recorded higher concentration than some previous studies conducted on the Euphrates river, but it remained within the limits of Iraqi and international standards for pollution.

Table (1) Comparing The Average Concentration Of TPHs In The Water Of The Current Study With Some Studies Of The Region.

Study Area	Concentrations µg/ l	Source
Euphrates River -Nassiriyah	12.35-2.50	Abed Ali (2013)[5]
Shaat Al-Arab- Basrah	38.88-23.36	Al-Taee (2017)[7]
Euphrates River - Nassiriyah	12-5	Kadhum (2017) [24]
Marsh Abo- Zarag /South of Iraq	5.1-2.07	Farhood (2017) [25]
Al- Garaaf River Thiqar Province	8.29-4.12	Ali (2019)[26]
Shaat Al-Arab North West Gulf	27.119-3.132	Haider (2019) [27]
Euphrates River – Nassiriyah	31.28-8.43	Present study

[28] Indicated the possibility of pollutants in the water environment reaching sediments either by depositing them directly when they are introduced into the area because of their weight or with water currents as they carry them to further distances and then settle after a period when the speed of these currents decreases and may enter into the bodies of living organisms and transfer through the food chain. After the death of these organisms, they reach the sediments. Adsorption of pollutants takes place on suspended matter and particles in the water column and settles to the bottom. This study agreed with the results[29].

The summer recorded the lowest total petroleum hydrocarbon concentration in sediments, while winter was the opposite. It was found that there was an inverse correlation between the concentrations of TPHs in the sediments with water temperature ($r = -0.566$, $P < 0.05$) and showed significant correlation with the concentration of TPHs in the water ($r = 0.496$), $P < 0.05$.

Increased death rates of aquatic plants and phytoplankton during the winter leads to an increase in the organic matter in the sediments and thus an increase in the percentage of adsorbed hydrocarbons on the surface of these sediments, The results of this study were agreed with [19] . As for the site, the third station with the highest concentration and the reason behind that rise may be due to the fact that this station is close to an important source of pollution, which is the presence of a saintary sewage station whose waste is dumped directly into the river, as the presence of hydrocarbons in the sediments is linked to the presence of organic materials [30] .

It was evident that the highest concentration of total petroleum hydrocarbons (TPHs) was recorded in both species during the winter and spring seasons, while the lowest concentration was during the summer.

It is possible that the source of these hydrocarbons is either biogenic or anthropogenic that accumulates in the tissues of the snails by feeding on phytoplankton or through water or the adsorbent suspended particles, as they are nourished and preserved as part of their fatty stock that is used to release energy during the winter season [31] as well as the biological constraction that organisms perform within their bodies and this is what was confirmed by [23] which will subsequently accumulate in the tissues of different organisms, including Mollusca, as it was found that temberature has an adverse effect on the concentration of hydrocarbons and this is confirmed in the current study, as the higher of temperature will be lower the hydrocarbons concentration due to the evaporation factor [29],

and the entry of these compounds into the bodies of living organisms helps them to spread within the food chain and thus damage the aquatic environment of various species [32] there is a direct relationship between the concentration of petroleum hydrocarbons and the high lipid content in tissues [33], as the current study recorded an increase in the concentration of lipids for both species in the winter and a lower concentration in the summer table (5).

So the greater the lipid content in the body of the organism, the more it is proportional to the concentration of the total hydrocarbons.This was consistent with [23, 28, 34] indicating that hydrocarbons are lipophilic and therefore accumulate in the lipid tissue of these organisms.

The variation in the hydrocarbon content of both species may be due to the feeding pattern, type of food, and fat content [35,33] , as demonstrated by the current study to the existence of a difference in the ability of these two species to accumulate total hydrocarbons from the surrounding environment, and the ability of *P.euphraticus* On the accumulation of total hydrocarbons higher than *B.bengalensis*, [36] explained when studying different species of Molluscs that they differed in the absorption rates of hydrocarbons, and may be due to the difference in the filtering rates and the amount of fats.

In the current study, it was found that the high concentrations of petroleum hydrocarbons in Molluscas were in the second and third stations, while their concentration decreased in the first and fourth stations. Perhaps the reason is the presence of pollution sources near the second station, which is represented by the thermal power plant and the proximity of the third station to the sewage station that throwing wastewater directly to the river, as for the first station, the decrease in pollution may be due to its distance from the source of pollution.

As for the fourth station, the concentration in the Molluscas members of this station decreased, and the reason may be due to the occurrence of the self-purification process in the river and the presence of dense vegetation that helps to get rid of high rates for hydrocarbons and leads to lower their concentrations. It is known that plants, plankton and algae have the advantage of accumulating higher concentrations of petroleum hydrocarbons than their outer surroundings.

It became clear from the study that the second and third stations had the highest rate of concentration of petroleum hydrocarbons in the winter in water, sediments and organisms compared to other stations , the lowest in the summer, as it became clear that two stations were affected by human activities being the largest population gathering between the rest of the studied stations. The stations 2 and 3 are exposed to direct excreta from the sanitary sewage plant and from the thermal power in Nasiriyah, as well as the presence of motor boats that are used for fishing and wandering in the river water that add the amount of excreta of oils in the water that cause Increasing the amount of petroleum hydrocarbons, and that the seasonal variation is a result of the effects that occur in the disposal and removal of compounds from the water, as there are many processes that affect simultaneously or individually on the events of variation in the concentrations of petroleum hydrocarbons, including the temperature and variation that occurs between the winter and summer, and this is consistent with [23, 28, 25, 34] .

CONCLUSION

In conclusion, the results indicate high concentrations of TPHs in Euphrates River, but stay in the range of Iraqi limitation Increasing concentration of TPHs in water, sediments and molluscs, refers to the importance of pollution in the aquatic environment which requires taking the possible actions to save the Euphrates river from pollution risks.

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التراكم الحيوي للمركبات النفطية الكلية في المحار ***Pseudodontopsis euphraticus*** والقوقع ***Bellamya bengalensis*** في نهر الفرات ، مدينة الناصرية/ العراق .

قاسم محمد الطاهر1 ،منال محمد أكبر 2 وعماد هادي القاروني3

1م.م المديرية العامة لتربية ذي قار،2أستاذ. ،3 أستاذ.مساعد /قسم علوم الحياة، كلية التربية للعلوم الصرفة

E-mail: [1]qasim20020@gmail.com

الخلاصة :-

أجريت الدراسة الحالية على مدار عام كامل (بداية شهر كانون الأول للعام 2018 وحتى نهاية تشرين الثاني للعام 2019) لدراسة وتقدير تركيز الهيدروكربونات النفطية الكلية في الأنسجة الرخوة لنوعين من النواعم هما القوقع *Bellamya bengalensis* وللمحار *Pseudodontopsis euphraticus* التي تم جمع عيناتها من أربع محطات تقع على نهر الفرات في مدينة الناصرية جنوب العراق. تم حساب تركيز الهيدروكربونات النفطية الكلية في القوقع *B. bengalensis* و المحار *P. euphruticus* والماء والرواسب بأستخدم جهاز التفلور بالأشعة فوق البنفسجية Ultra violet flouresence أذ تبين من خلال الدراسة بأن أعلى التراكيز سجلت خلال فصل الشتاء وأقلها خلال فصل الصيف ، وتراوح المدى لمعدل تركيزالهيدروكاربونات الكلية في القوقع *B . bengalenisis* (12.96-1.52) مايكروغرام/غم وزن جاف وللمحار(1.46 – 16.69) مايكروغرام/غم وزن جاف وللماء(31.28-8.43) مايكروغرام / لتر وللرواسب (118-205) مايكروغرام/غم وزن جاف.

كما سجلت المحطة الثانية والثالثة أعلى معدلات للتركيز وأقل تركيز سجلته الدراسة في المحطتين الأولى والرابعة ولوحظ من خلال النتائج وجود تباين فصلي وموقعي في تركيز الهيدروكربونات النفطية الكلية في مياه ورواسب والكائنات الحية في نهر الفرات ، كما تبين أن معدلات التركيز هي الأعلى في الرواسب يليها الماء ثم المحار والقوقع وكانت نسبة الهيدروكربونات النفطية الكلية في منطقة الدراسة ضمن المحددات العالمية والعراقية .

الكلمات المفتاحية : الهيدروكربونات النفطية الكلية، الماء ، الرواسب، المحار،القوقع .