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# Regional and seasonal variation of Total Petroleum Hydrocarbons in Water and Mollusca at Quarna North Shatt AL-Arab River

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**Abstract:**

Water samples and four species of molluscs*(Theodoxus Jordani*, *Melanoides tuberculate Melanopsis nodosa, Bellamya bengalensis* ) were collected seasonally from three stations at Al-Quarna in Shatt Al Arab river during the low tide period from September 2018 to March 2019 to determine the regional and seasonal variations of total Petroleum hydrocarbons using a spectrofluorometer. Physical and chemical parameters were measured, such as water temperature range from (13°C to 39°C), Dissolved oxygen range from (6.5 mg/l to 3.84 mg/l),pH range from (8.15-7.17), and Electrical conductivity (2.59 ms/cm- 4.75 ms/cm). The concentration of total petroleum hydrocarbons ( TPHs) in water range from (0.33µg/l)in summer at Station 3 to (2.89 µg/l ) in winter at Station 2, while TPHs in molluscs range from 0.55µg/g dry weight in the *T. jordani* in station 3 during summer to 8.93µg/g dry weight in the *B. bengalensis* during winter .The highest TPHs in the four species were arranged as fellow:*Bellamya bengalensis* > *Melanopsis nodosa* > *Melanoides tuberculate* > *Theodoxus Jordani*. When we compare the concentration of TPHs in water and molluscs with other studies, it allies within these concentrations.

Keywords: Pollution, oil, mollusca, Shatt AlArab River

**Introduction**:

The Iraqi freshwater environment suffered from various types of pollution especially that related to pesticides, hydrocarbons, or heavy metal toxicants (Al-Saad *et al*., 2017). One of the most dangerous pollutants for the water environment is petroleum hydrocarbons and its derivatives (Al-Hejuje, 2015). Therefore, their biological effects need to be established at each level of biological organization (El-Nemr et al., 2004). Long-term exposure to hydrocarbon compounds can cause various disturbances in human life in comfort and health (Azhari et al., 2011). A hydrocarbon, by definition, is one of a group of chemical compounds composed only of hydrogen and carbon. Typically, hydrocarbons are broken down into three main classes; aliphatic, alicyclic, and aromatics. Further sub-classes can also be defined. Simply stated, though, hydrocarbons are organic compounds made up of hydrogen and carbon (Reeves, 2000). Hydrocarbons enter the aquatic environment either anthropogenic, which occurred by the discharge of petroleum products such as spillages, tanker operations, industrial effluents, refineries, transport of petroleum products, uncontrolled releases from power plants, accidental discharges , municipal waste discharges, or biogenic which occurred by decomposition of organisms containing biogenic hydrocarbons or by natural seepage (Al-Imarah et al ,2010; Farid et al, 2014). Molluscs are one of the species used in monitoring programs as sentinels of marine pollution, and their tissues are an important hydrocarbon storage site (Farid et al., 2010). It is reported that the molluscs are ideal monitoring species as they are dominant members of coastal and estuarine communities, accumulate different toxicants in their tissues, are responsive to many environmental pollutants, and have a wide geographical distribution (Farid et al., 2008).

UNEP (1993), Cajaraville et al. (1995) and NRC (2003) reported that the indigenous species of molluscs represent ideal subjects for evaluating contaminants in the aquatic environment because they are sedentary and, excepting the larval sojourn, spend their entire lives in the same location; molluscs populations inhabit water which are polluted by domestic sewage, petroleum by–products and industrial waste products; they tend to concentrate almost all noxious substances in the environment in their body; they are relatively easy to locate and sample; it is possible to obtain or locate spat (young molluscs that have recently metamorphosed from the free-swimming larval stage to the permanently attached form) and thus, it is possible to monitor the rate of uptake and incorporation of various toxic chemical in molluscs populations starting with essentially zero-aged animals; their ubiquitous distribution permits comparisons to be made with other workers; and large number of molluscs can be analyzed and examined

The water of Shatt Al-Arab River receives oil contaminants from varying origins, including; accidental spills, normal operations of boating activities, runoff from land and introduction via sewage outfalls and disposal of oil waste material. In addition, some oil originates from atmospheric transport, which includes hydrocarbons from combustion engines (Al-Saad, 1995)

The present study aims to determine the distribution and seasonal variation of Total Petroleum Hydrocarbons (TPHs) in the water and 4 species of molluscs collected from three stations in Quarna at the northern of Shatt al-Arab River.

**Materials and Methods:**

Samples of water and four species of molluscs, *(Theodoxus Jordani*, *Melanoides tuberculate Melanopsis nodosa*, *Bellamya bengalensis )* were collected from the three stations at Quarna in the northern of Shatt al-Arab River during the period Spt. 2018 to Mar.,2019. (Figure 1). Water samples were collected at least 20 -30 cm under the water surface and whenever it was possible at the middle of the river using dark glass bottles and preserved in situ with 25 ml. $CCl_4$. Samples were never taken when it was raining, molluscs Samples were collected at least 350 adult individuals of uniform size of each species

The tissues of the animals were pooled and macerated in a food liquidizer from which at least 3 replicates of 15g each were freeze-dried, grounded and sieved through a 63 μ metal sieve.


Figure 1: Map of Shatt al-Arab River showing the three sampling stations

**Water measurements:**
Water physical and chemical parameters, including Dissolved oxygen (DO) and Water Temperature (WT), Electrical Conductivity (EC), and pH were measured in situ using the Multimeter type (Multi 350 i SET 5 ).

**Extraction of hydrocarbons from water:**
Hydrocarbons in the water sample (about 5L) were extracted according to (UNEP,1989) by mixing with another (25 ml ) CCl4 for 20 min. using Water Mixer, the liquid fraction was drained, and the residual ( about 1L) was transferred into a separator funnel. The organic (lower) phase was carefully poured into a glass column containing (5g) of anhydrous sodium sulfate ($Na_2SO_4$), collected, and dried. The residual was dissolved with n-hexane (25 ml ) and passed through a 20 cm glass column ( packed with glass wool at the bottom , about 10 g deactivated silica gel (100-200 mesh), 10 g deactivated alumina ( 100-200 mesh), and 5g anhydrous sodium sulfate ($Na_2SO_4$) at the top ). The aliphatic fractions were eluted from the column with n-hexane (25 ml), while the aromatics were eluted with benzene (25 ml). The samples dried and stored until detection with a spectrofluorometer (for total petroleum hydrocarbons(TPHs)

**Extraction of hydrocarbons from molluscs tissues:**
The procedure of Grimalt and Oliver (1993) was used in the extraction of hydrocarbons from molluscs tissues. Ten grams of dried molluscs tissues were placed in a pre-extracted cellulose thimble and soxhlet extracted with 150 ml methanol: benzene (1 : 1 ratio) for 24 - hours. The extract was then transferred into a storage flask. The sample was further extracted with a fresh solvent. The combined extracts were reduced in volume to ca 10 ml in a rotary vacuum evaporator. They were then saponified for 2 - hours with a solution of 4 N KOH in 1: 1 methanol: benzene. After extracting the unsaponified matter with hexane, the extract was dried over anhydrous $Na_2 SO_4$ and concentrated by a stream of $N_2$ for UVF analysis. A Shimadzu RF–540 spectrofluorometer equipped with a DR–3 data recorder was used to determine total petroleum hydrocarbons. The quantitative basis measurements were made by measuring emission intensity at 360 nm with excitation set at 310 nm and monochrometer slits of 10 nm.

The procedure used by Al-Saad (1995) was employed to determine the fat content of molluscs samples. Three grams of each freeze- dried sample was soxhlet extracted with a 2 : 1 mixture of petroleum ether and acetone for 24-hours. The extracts were reduced in volume in a rotary vacuum evaporator and subsequently reduced to exactly 1 ml. Ten µl of the concentrated extracts were taken by a Hamilton syringe and weighted after evaporation of the solvent.

The reference oil was used in the spectroflurometer obtained from Iraqi South Oil Company (Basrah regular crude oil)

**Results and Discussion:**

The basic statistical seasonal variations for the water quality parameters are summarized in (Table 1) and illustrated in (Figures 2, 3,4 and 5). Temperature is an important water quality parameter due to its influence on other parameters. Temperature affects the solubility and, consequently, the availability of gases such as oxygen in water (Rubio-Arias *et al*, 2013). It also affects the toxicity of some chemicals in water systems and the sensitivity of living organisms to toxic substances ( Dojlido and Best 1993). It was found that the concentrations of hydrocarbons in Shatt Al-Arab river water varied inversely with water temperature (Al-Saad, 1995). In this study, the variability in temperature values at the study locations may have resulted from the weather condition at the time of study (13°C to 39°C)

The dissolved oxygen is essential for aquatic life, as it is needed to keep organisms alive. The sources, raw water temperature and chemical or biological processes taking place in the aquatic system (Al-Hejuje, 2014) influence the DO content of water. Our results showed that the DO concentrations range from (3.84 mg/l to 6.5 mg/l)

The pH results show seasonal differences but for all. Stations fall within the acceptable range of (7.17- 8.15); the average values tend to be slightly alkaline during the study period, consistent with previous studies made on aquatic ecosystems in southern Iraq (Al-Hejuje, 2014; Moyel and Hussain 2015). The pH is an important parameter that determines the suitability of water for different purposes.

EC estimates the amount of total solids or amount of total dissolved ions in water. The EC of water generally increases as the levels of dissolved pollutants and salinity increase ( Prathumratana *et al*.,2008). EC showed clear seasonal differences (2.59 ms/cm- 4.75 ms/cm).

**Table (1) Environmental measurements of the three stations during different seasons.**

| | Station 1 | | Station 2 | | Station 3 | | Season |
|---|---|---|---|---|---|---|---|
| **Parameters** | Range | Mean ± SD | Range | Mean ± SD | Range | Mean ± SD | |
| **pH** | (7.15-7.16) | 7- 0.005 | (7.21--7.32) | 7.25-0.06 | (7.55--7.71) | 7.62-0.08 | Winter |
| **EC** | (3.55-3.69) | 3.61 -0.07 | (3.55-3.66) | 3.59-0.06 | (3.28-3.46) | 3.35-0.094 | |
| **DO** | (6.3-6.5) | 6.4 -0.1 | (6.3-6.4) | 6.3-0.057 | (5.1-5.3) | 5.2-0.1 | |
| **Water Temp.** | (14-15) | 14 -0.57 | (15-16) | 16-0.57 | (13-15) | 14-1 | |
| **pH** | (7.17-7.18) | 7.17-0.005 | (7.10--7.28) | 7.21-0.101 | (7.47--7.56) | 7.53-0.058 | Spring |
| **EC** | (3.32-3.44) | 3.39-0.062 | (3.21-3.24) | 3.22-0.015 | (2.61-2.73) | 2.67-0.061 | |
| **DO** | (5.4-5.6) | 5.5-0.1 | (5.4-5.5) | 5.4-0.057 | (5.2-5.4) | 5.3-0.1 | |
| **Water Temp.** | (17-19) | 18-1 | (18-20) | 19-1 | (17-18) | 17-0.577 | |
| **pH** | (8.14--8.16) | 8.15 -0.01 | (8.15--8.16) | 8.15-0.005 | (7.89--7.98) | 7.88-0.095 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **EC** | (4.75-4.88) | 4.82 -0.066 | (3.64-3.76) | 3.68-0.066 | (4.67-4.76) | 4.72-0.049 | Summer |
| **DO** | (4.6-4.7) | 4.6 -0.057 | (4.5-4.7) | 4.6-0.1 | (3.84-3.98) | 3.9-0.07 | |
| **Water Temp.** | (38-39) | 39 -0.577 | (38-39) | 38 -0.577 | (37-38) | 38-0.577 | |
| **pH** | (7.83--7.84) | 7.83 -0.005 | (7.53--7.63) | 7.56-0.055 | (7.43--7.63) | 7.53-0.1 | |
| **EC** | (2.69-2.85) | 2.75 -0.085 | (2.89-2.93) | 2.91-0.020 | (2.59-2.69) | 2.63-0.051 | |
| **DO** | (5.1-5.3) | 5.2 -0.1 | (5.0-5.2) | 5.1-0.115 | (5.1-5.4) | 5.2-0.152 | Autumn |
| **Water Temp.** | (21-22) | 21 -0.577 | (20-22) | 21-1 | (21-22) | 21-0.577 | |

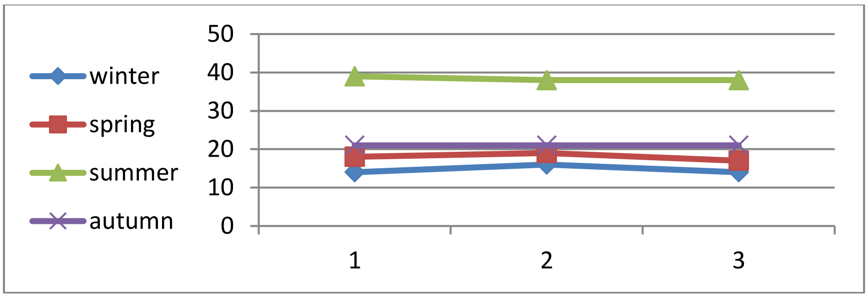


Fig.2: Water temperatures (°C) at the studied station

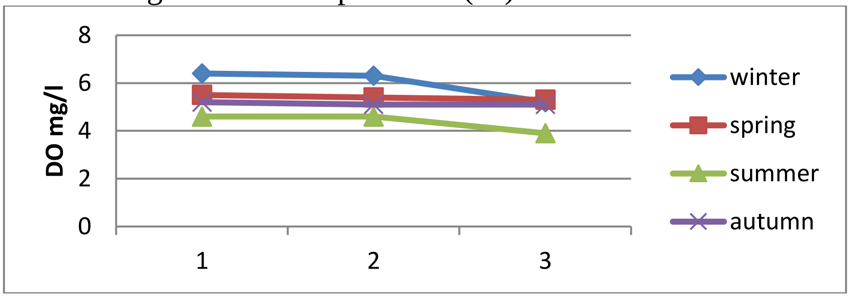


Fig.3: Dissolved Oxygen (DO) concentrations (mg/l) at the studied stations

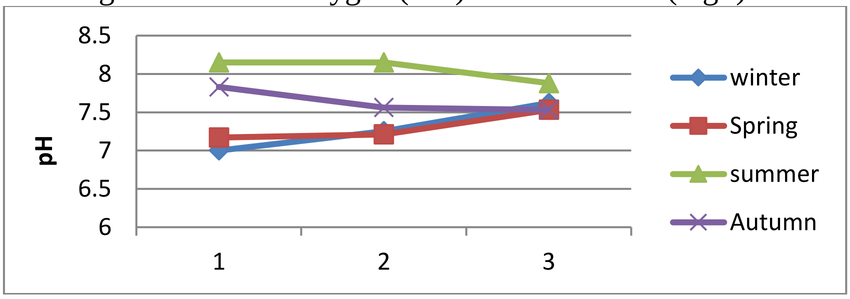


**Fig.4: Seasonal variation of (pH) at the studied stations**

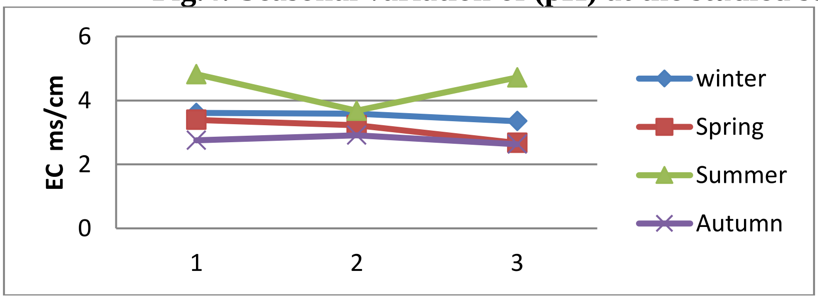


**Fig.5: Electrical Conductivity (EC ms/cm) at the studied stations**

The TPHs are a complex mixture of hundreds of hydrocarbons, categorized into a wide range of structures from light compounds characterized by a volatile fraction with a short chain of carbon atoms to a heavy fraction composed of long chain ( Al-Khatlan *et al*.,2019).

In the present study, the concentrations of TPHs compounds were measured seasonally in water and species of molluscs from three stations at Quarna in the northern of Shatt al-Arab river, the concentration of TPHs in water samples ranged between (0.33 µg/l - 0.89 µg/l )in the third station during Summer to (2.44 µg/l - 2.89 µg/l) in the second station during winter. In contrast, the highest mean concentrations of total TPHs in water (2.69µg/l) were recorded at station 2 during the Winter season, while the lowest mean concentrations (0.68 µg/l) were recorded at station 3 during Summer (table 2 and Figure 6). Based on elicitation of numerous expert opinions, the variations in the total hydrocarbons in the Shatt Al-Arab river were due to many factors mainly: the discharging of hydrocarbons compounds to the environment, uncontrolled releases from power plants, petroleum tanker traffic, temperature changes, evaporation, photo-oxidation, biosynthesis, biodegradation, adsorption and absorption by suspended matters and organisms, and sedimentation. The intense solar radiation coupled with relatively high water temperature is the characteristic features of the climate of the subtropical region of Basrah city. These two factors could account for a rather low level of hydrocarbons in water (Al-Hejuje,2015). While the concentrations of total Petroleum Hydrocarbons varied from 0.81µg/g dry weight in the *T. jordani* to 3.81µg/g dry weight in the *B. bengalensis* during summer and from 1.02µg/g dry weight to 4.08µg/g dry weight during autumn. In contrast, they range from 2.99µg/g dry weight to 8.66µg/g dry weight and from 1.05µg/g dry weight to 5.14µg/g dry weight during winter and spring in station 1. While the concentrations of (TPHs) varied, from 0.72µg/g dry weight in the *T. jordani* to 3.39µg/g dry weight in the *B. bengalensis* during summer and from 1.06µg/g dry weight to 4.21µg/g dry weight during autumn. In contrast, they range from 2.89µg/g dry weight to 8.78µg/g dry weight and from 1.13µg/g dry weight to 5.56µg/g dry weight during winter and spring in station 2. and the concentrations of (TPHs) in station 3 varied from 0.55µg/g dry weight in the *T. jordani* to 3.59µg/g dry weight in the *B. bengalensis* during summer and from 1.12µg/g dry weight to 4.45µg/g dry weight during autumn, whereas they range from 2.36µg/g dry weight to 8.93µg/g dry weight and from 1.43µg/g dry weight to 6.09µg/g dry weight during winter and spring in station 2 (Figure 7,8 and 9). a direct relationship is found between concentrations of TPHs and fat contents of the species of molluscs (r = 0.8 - 0.9) Other investigators reported similar results, Gold-Bouchot et al. (1995) found that oyster with high lipid content took up more fuel oil (314 µg/g) from the water than low lipid oysters (161 µg/g) Menon and Menon (1999) reported that the clams, oysters and mussels differed in their rates of hydrocarbons uptake, possibly due to difference in filtering rates and mounts of lipids.

The higher concentrations of TPHs were recorded in all species of molluscs and water samples during winter and autumn. The lowest levels were recorded during summer and spring. (Al-Saad, 1995) showed that the possible explanation of higher concentrations during winter is that total hydrocarbons discharge was greater than in summer due to the wider occurrence of combustion processes and the higher association of these hydrocarbon compounds with atmospheric particles at lower ambient temperature. Furthermore, the TPHs compounds deposited on land during summer would not reach the aquatic environment to the same extent as during winter when runoff from land is much more extensive due to rainstorms in the region.

The hydrocarbons were probably biogenic or anthropogenic accumulated into species of molluscs tissues from phytoplankton or through water, either from solution or adsorbed to suspended particles while feeding and retained as part of the lipid energy store and utilized during the winter(Al-Saad *et al.*,2011). Abdul-Retha (1997) reported that the temperature of water controls the activity of oil-degrading bacteria of Shatt Al-Arab river water. Shamshoom et al. (1990) found that oil-degrading bacteria were more active in summer than in winter in the Shatt AlArab River. The photo-oxidation may also degrade the components of oil in a water environment (Ehrhardt and Burns, 1993). The intense solar radiation coupled with relatively high water temperature is the characteristic feature of the climate of the subtropical regions of Iraq. These two factors could account for rather low levels of hydrocarbons compounds encountered in Shatt Al-Arab river water (Bedair and Al-Saad, 1992; Al-Saad and AlTimari, 1993; Al-Saad, 1995). This can conclude that THC concentrations in mollusca during different seasons are due to factors such as temperature, photo oxidation, and bacterial activity (AL-Saad1995). If we compare our data with other studies (Table 3), it seems to lie within these studies.

Table (2) Total PAHs (µg / L) in water in the study area during the seasons.

| Season | Station | Range | Mean µg/l)( | SD |
|---|---|---|---|---|
| **Winter** | Station 1 | (2.13-2.64) | 2.41 | 0.258 |
| | Station 2 | (2.44-2.89) | 2.69 | 0.230 |
| | Station 3 | (2.22-2.57) | 2.40 | 0.175 |
| **Autumn** | Station 1 | (1.02-1.06) | 1.03 | 0.020 |
| | Station 2 | (1.03-1.07) | 1.05 | 0.02 |
| | Station 3 | (1.04-1.09) | 1.06 | 0.025 |
| **Summer** | Station 1 | (0.48-0.98) | 0.72 | 0.250 |
| | Station 2 | (0.51-0.83) | 0.70 | 0.168 |
| | Station 3 | (0.33-0..89) | 0.68 | 0.309 |
| **Spring** | Station 1 | (1.24-1.53) | 1.37 | 0.145 |
| | Station 2 | (1.34-1.78) | 1.59 | 0.227 |
| | Station 3 | (1.46-1.55) | 1.50 | 0.045 |

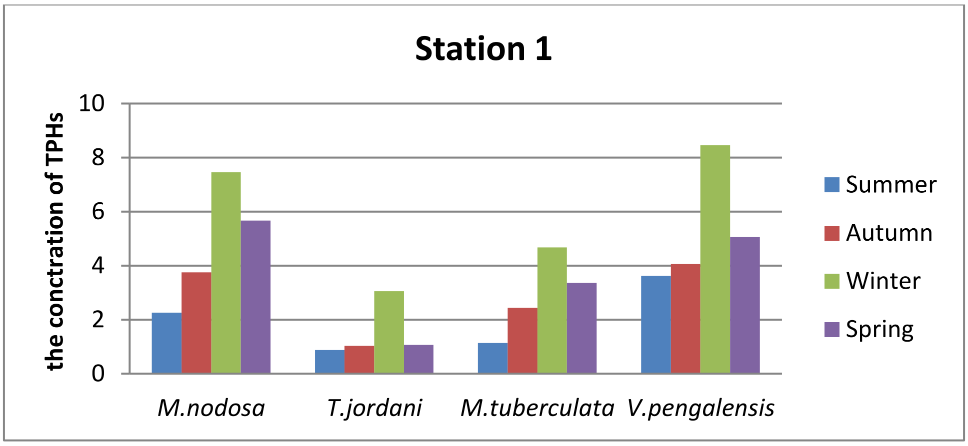


**Fig.6: TPHs in mollusca during the seasons in station 1**

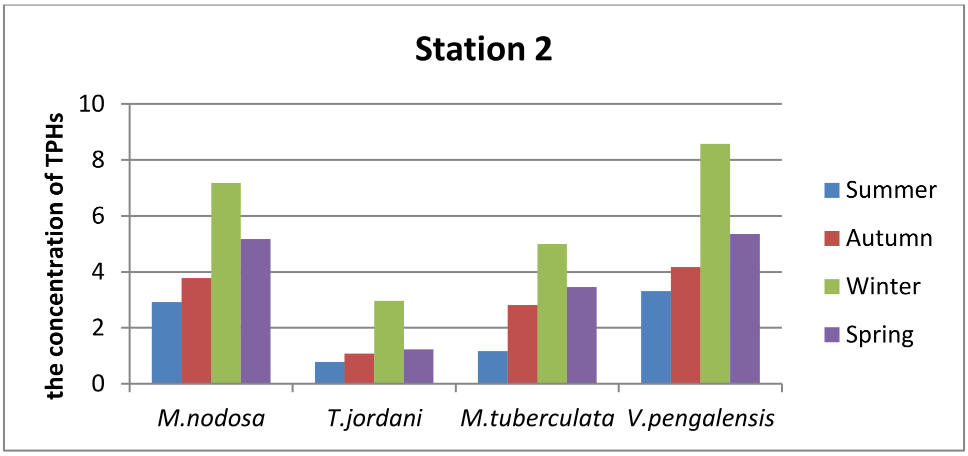


**Fig.7: TPHs in molluscs during the seasons in station 2**

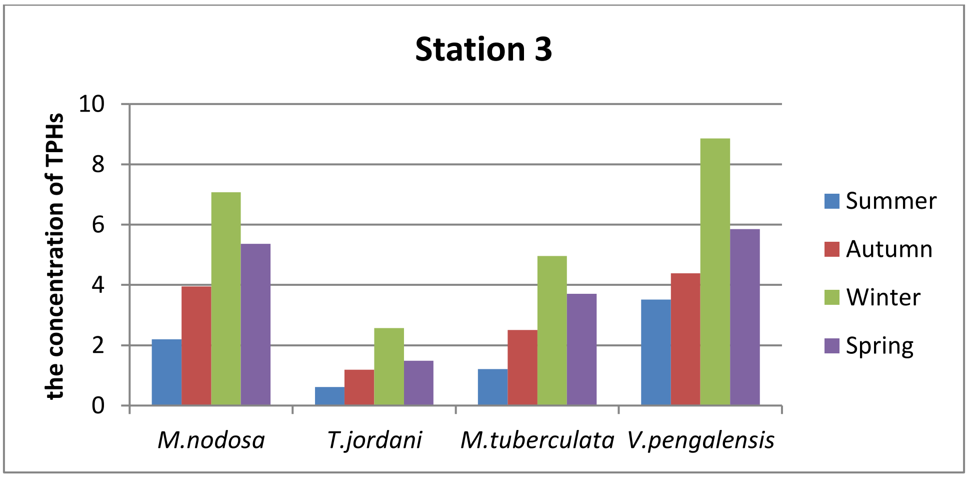


**Fig.8: TPHs in molluscs during the seasons in station 3**

**Table (3): Comparison between the concentrations of total hydrocarbons in water for the present study with the other previously studied ones**

| Studied Areas | Total Hydrocarbons (μg/l) | References |
|---|---|---|
| Shatt Al-Arab river &NW Arabian Gulf | 3.25-25.33 | Al-Saad (1995) |
| Al-Howaiza Marsh | 1.005-11.965 | (Al-Khatib,2008) |
| Al-Hammar Marsh | 0.411-0.824 | (Talal, 2008) |
| Shatt Al Arab Northern | 5.67-9.48 | (Al-Imarah et al.,2010) |
| Iraqi Coast Region | 1.51-18.14 | (Al-Khion,2012) |
| Shatt Al-Arab river | 16.77-42.6 | (Farid et al., 2014) |
| Shatt Al-Arab river | 0.22 - 17.78 | (Al-Bidhani 2014) |
| Shatt Al-Arab river | 5.18 – 37.58 | (Al-Hejuje,2015) |
| Shatt Al-Arab river | 5.294-21.738 | (AL-Imarah *et al*.,2017) |
| Shatt Al Arab Northern | 0.33-2.89 | Present study |

**Table (4) Comparison between the concentrations of total hydrocarbons in molluscs for the present study with the other previously studied ones**

| Species | Concentrations of Total Petroleum Hydrocarbons | | | | References |
|---|---|---|---|---|---|
| | Summer | Autumn | Winter | Spring | |
| *T . jordani* | 1.93 ± 0.06 | 2.90 ± 0.04 | 3.58 ± 0.08 | 2.60 ± 0.02 | (Al-Adhub *et al.*,2009) |
| *M . nodosa* | 4.28 ± 0.04 | 6.27 ± 0.04 | 8.16 ± 0.05 | 5.55 ± 0.03 | (Al-Adhub *et al.*,2009) |
| *M . tuberculata* | 3.21 ± 0.03 | 5.15 ± 0.05 | 6.85 ± 0.04 | 4.23 ± 0.06 | (Al-Adhub *et al.*,2009) |
| *T . jordani* | 0.87-0.055 | 1.03-0.015 | 3.05-0.056 | 1.07-0.02 | Present study |
| *M . nodosa* | 2.26-0.045 | 3.75-0.026 | 7.45-0.1 | 5.67-0.399 | Present study |
| *M . tuberculata* | 1.14-0.096 | 2.44-0.120 | 4.68-0.145 | 3.36-0.205 | Present study |
| *B.bengalensis* | 3.62-0.162 | 4.06-0.026 | 8.46-0.176 | 5.07-0.065 | Present study |

**Conclusion:**

Water and four species of Mollusca have some concentrations of total petroleum hydrocarbons. The highest TPHs in the four species were arranged as fellow: *Bellamya bengalensis* > *Melanopsis nodosa* > *Melanoides taberculata* > *Theodoxus Jordani*. The sources of TPHs came from many sources, and there is seasonal variations of TPHs in the water due to many factors such as temperature, photooxidation and bacterial degradation.

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**التباين الموقعي والفصلي للهيدروكربونات الكلية فى مياه وأحياء القرنة فى القرنة شمال شط العرب**

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المستخلص:
تم جمع عينات المياه وأربعة أنواع من النواعم (*Melanopsis Melanoides tuberculate,Theodoxus Jordani*, *Bellamya bengalensis nodosa*) فصليا من ثلاث محطات من القرنة في شط العرب خلال فترة المد المنخفض من سبتمبر 2018 إلى مارس 2019 لتحديد الاختلافات الموقعية والفصلية للهيدروكربونات النفطية باستخدام مقياس الطيف. تم قياس المعلمات الفيزيائية والكيميائية، فقد تراوحت درجة حرارة الماء من (13 درجة مئوية إلى 39 درجة مئوية)، و الأكسجين المذاب من (6.5 ملغم/لتر إلى 3.84 ملغم/لتر)، و الاس الهيدروجيني من (7.17-8.15)، والتوصيلية الكهربائية (2.59 مللي ثانية/سم- 4.75 مللي ثانية/سم). وكان تركيز الهيدروكربونات النفطية الكلية (TPHs) ضمن نطاق الدراسات الاخرى...