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Distribution and seasonal variations of n-alkanes in some species of molluscs from Shatt Al-Arab river

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Abstract - The seasonal variations of n-alkanes compounds in molluscs of Shatt Al-Arab river have been studied. The species of molluscs were the snails, *Lymnaea auricularia*, *Theodoxus jordani*, *Physa acuta*, *Melanopsis nodosa*, *Melanoides tuberculata* while the bivalves were *Corbicula fluminea* and *Corbicula fluminalis*. The molluscs were collected from different locations of Shatt Al-Arab river (along the region extended from Abu-Al-Khasib to Garmat-Ali) during 2004 and 2005. Each species consisted of at least 350 adults of uniform size of individuals. The n-alkanes compounds from species of molluscs were extracted and analyzed by high resolution capillary gas chromatography. The total concentrations of n-alkanes in the molluscs varied from 1.50 µg/g dry weight in the *T. jordani* to 8.78 µg/g dry weight in the *C. fluminea* during summer and from 2.26 µg/g dry weight to 12.37 µg/g dry weight during autumn. While ranged from 3.15 µg/g dry weight to 12.44 µg/g dry weight and from 1.78 µg/g dry weight to 5.31 µg/g dry weight during winter and spring. The study confirmed lower concentrations of n-alkanes compounds in the molluscs of Shatt Al-Arab river during summer and spring while higher concentrations were recorded during winter and autumn. This is due to several factors which could act to produce such seasonal variations.

Introduction

Petroleum hydrocarbons are considered as important pollutants in marine and estuarine environments, and therefore their biological effects need to be established at each level of biological organization (El-Nemr *et al.*, 2004). 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 dominate members of coastal and estuarine communities, accumulate different toxicants in their tissues, are responsive to many environment pollutants, and have a wide geographical distribution (Farid *et al.*, 2008).

The Shatt Al-Arab river in the Basrah city, southern of Iraq are known to be severely polluted due to entry of both domestic sewage and industrial wastewater. The industrial effluents are derived from paper and fertilizer mills, electrical power stations, refined oil plants, petrochemical manufacture and other industries. Bedair and Al-Saad (1992) reported that the petroleum hydrocarbons in Shatt Al-Arab river were likely originated from boating activities, runoff from land and introduction via sewage outfalls. DouAbul and Al-Saad (1985) indicated that oil pollution in Shatt

Al-Arab river was possibly originated from diverse sources such as; oil refineries, rural runoff, electricity generating stations, sewage discharges and river transportation activities. But they considered sewage discharge and urban runoff as the most significant sources of oil entering Shatt Al-Arab river. Al-Saad (1995) also reported that many aquatic organisms of Shatt Al-Arab river including plants, algae, zooplankton, bacteria and fish were capable to synthesize biogenic hydrocarbons.

The snails, *Lymnaea auricularia* (Linneus), *Theodoxus jordani* (Soweby), *Physa acuta* (Draparnaud), *Melanopsis nodosa* (Ferussac), *Melanoides tuberculata* (Muller) and the bivalves, *Corbicula fluminea* (Muller) and *Corbicula fluminalis* (Muller) are a common molluscs in the water of Shatt Al-Arab river and are dominant members of its benthic macrofauna. As with many molluscs species their growth rates are slow and the animals are long lived. This molluscs occur frequently in the areas receiving acute and chronic oil exposures and are often considered as an important part of food web in the stream. Farid *et al.* (2008 and 2010) used the above species of molluscs in the monitoring of biogenic and anthropogenic hydrocarbons in Shatt Al-Arab river.

The objective of the present study was to determine the distribution and seasonal variations of n-alkanes in the several species of molluscs collected from different location of Shatt Al-Arab river. These species of molluscs are *L. auricularia*, *T. jordani*, *P. acuta*, *M. nodosa*, *M. tuberculata* (snails), *C. fluminea* and *C. fluminalis* (bivalves).

Materials and Methods

Specimens of species of molluscs, *L. auricularia*, *T. jordani*, *P. acuta*, *M. nodosa*, *M. tuberculata*, *C. fluminea* and *C. fluminalis* were collected from Shatt Al-Arab river (along the region extended from Abu Al-Khasib to Garmat-Ali) during 2004 and 2005 (Figure 1). At least 350 adult individuals of uniform size of each species were collected.

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.

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 150ml 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 10ml in a rotary vacuum evaporator. They were then saponified for 2 hours with a solution of 4N KOH in 1:1 methanol:benzene. After extraction of the unsaponified matter with hexane, the extract was dried over anhydrous Na_2SO_4 and concentrated by a stream of N_2 .

The concentrated extract was cleaned up by column chromatography. A column filled with 8g each of 5% water deactivated alumina (100-200 mesh) is placed at the top and silica gel (100-200 mesh) at the bottom. The extract was then applied to the head of the column and eluted with 50 ml of n-hexane to isolate the aliphatic fraction. The fraction was reduced to a suitable volume prior to analysis by capillary gas chromatography.

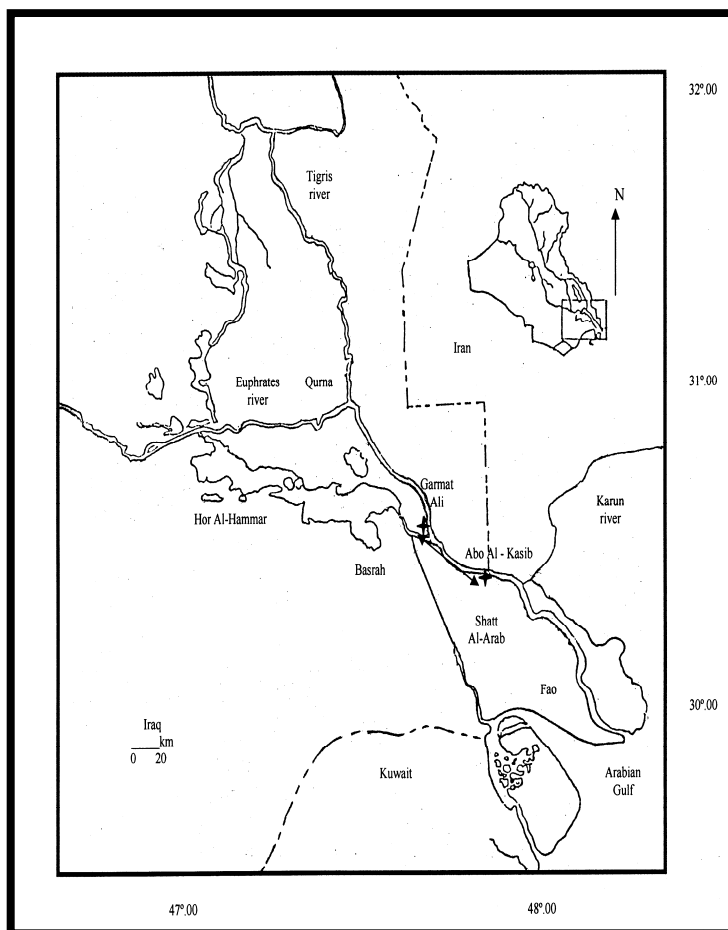


Figure 1. Map of sampling location.

A perkin-Elmer Sigma 300 capillary gas chromatography equipped with flame ionization detector and splitless mode injection part was used to determine aliphatic compounds.

Quantification of peaks and identification of hydrocarbons in the chromatograms was achieved by A Perkin-Elmer computing injection model LC-100. The fused silica capillary column used was a wall coated open tubular (WCOT) of 50 m x 0.25 mm i.d. SE (methyl silicone) (Perkin-Elmer). Helium was used as a carrier gas with a linear velocity of 1.5 ml/min. The operating temperatures for detector and injector were 350 and 320°C respectively. The column was operated under temperature programmed as follows: Initial temperature=60°C, initial time=4 minutes, final temperature=280°C, final time=30 minutes and rate=4°C/minute. The Unresolved Complex Mixture (UCM) was measured by using planimetry.

Strenuous efforts are made to minimize the contamination of the samples; for such contamination would otherwise yield in erroneous results. Throughout the procedure, a great care is taken to ensure that samples are not contaminated; it is very important to avoid an unnecessary exposure of the samples (whether the solvent or the final extract) to the atmosphere or other potential contamination sources. However, procedural blanks of all reagents and glassware that were used during the analysis are periodically determined. It is preferred to eliminate contamination sources rather than adjusting or correcting the data that were actually obtained according to the blank value.

The Standards of n-alkanes compounds were supplied by Supelico and Chrompack which were used in capillary gas chromatography for calibration (Figure 2).

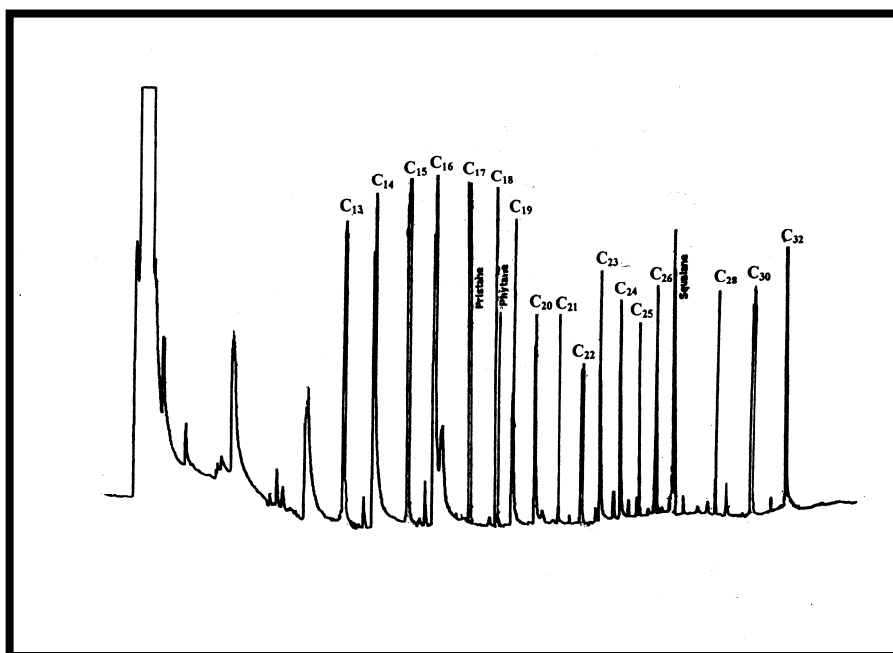


Figure 2. Capillary gas chromatography of n-alkanes standard.

Results and Discussion

In the present study, the concentrations of n-alkanes compounds were measured seasonally in species of molluscs of Shatt Al-Arab river. the concentrations of n-alkanes compounds varied from 1.50 $\mu\text{g/g}$ dry weight in the *T. jordani* to 8.78 $\mu\text{g/g}$ dry weight in the *C. fluminea* during summer and from 2.26 $\mu\text{g/g}$ dry weight to 12.37 $\mu\text{g/g}$ dry weight during autumn, whereas they range from 3.15 $\mu\text{g/g}$ dry weight to 12.44 $\mu\text{g/g}$ dry weight and from 1.78 $\mu\text{g/g}$ dry weight to 5.31 $\mu\text{g/g}$ dry weight during winter and spring (Table 1) and (Figure 3).

It is clear that the higher concentrations of n-alkanes compounds were recorded in all species of molluscs during winter and autumn. The lowest levels were recorded during summer and spring (Table 1) and (Figure 4). The n-alkanes compounds were probably biogenic or anthropogenic accumulated into species of molluscs tissues from phytoplankton or through water, either from solution or absorbed to suspended particles, while feeding, and retained as part of the lipid energy store and utilized during the winter. Thus the lowest levels were recorded in summer and spring. The same conclusion has been arrived by (Cripps and Priddle, 1995).

In addition, several factors acting as either single or combination could produce the seasonal variation in concentrations of n-alkanes compounds in Shatt Al-Arab river (Al-Saad, 1995) which will accumulate later in tissues of the river organisms (include molluscs). It was found that the concentrations of n-alkanes compounds in Shatt Al-Arab river water vary inversely in relation to water temperature (Al-Saad, 1995). Al-Saad and Al-Timari (1993) and Al-Saad (1995) documented that temperature is the most important factor governing the removal of n-alkanes compounds in the water of Shatt Al-Arab river by evaporation. In addition to the direct effect of temperature on the evaporation of n-alkanes compounds from water of Shatt Al-Arab river, increase in temperature favors bacterial degradation process (Abdul-Retha, 1997). Abdul-Retha (1997) reported that the activity of oil degrading bacteria of Shatt Al-Arab river water is controlled by temperature of water. Shamsboom *et al.* (1990) found that oil degrading bacteria was more active in summer than in winter in Shatt Al-Arab river. The photo-oxidation may also degrade the components of oil in water environment (Ehrhardt and Burns, 1993). The intense solar radiation coupled with relatively high water temperature are the characteristic features of the climate of the subtropical regions of Iraq. These two factors could account for rather low levels of n-alkanes compounds encountered in Shatt Al-Arab river water (Bedair and Al-Saad, 1992; Al-Saad and Al-Timari, 1993; Al-Saad, 1995).

Table 1. Concentrations of total n-alkanes ($\mu\text{g/g}$ dry weight) in the species of molluscs tissues from The Shatt Al-Arab river during 2004-2005.

Species	Concentrations of total n-alkane hydrocarbons			
	Summer	Autumn	Winter	Spring
<i>L. auricularia</i>	2.02 \pm 0.05	3.42 \pm 0.07	4.87 \pm 0.07	2.22 \pm 0.02
<i>T. jordani</i>	1.50 \pm 0.04	2.26 \pm 0.04	3.15 \pm 0.06	1.78 \pm 0.06
<i>P. acuta</i>	1.99 \pm 0.08	3.40 \pm 0.05	4.64 \pm 0.05	2.00 \pm 0.01
<i>M. nodosa</i>	3.11 \pm 0.04	6.80 \pm 0.08	7.57 \pm 0.07	2.96 \pm 0.07
<i>M. tuberculata</i>	2.33 \pm 0.05	3.87 \pm 0.05	6.25 \pm 0.03	2.41 \pm 0.06
<i>C. fluminea</i>	8.78 \pm 0.06	12.37 \pm 0.03	12.44 \pm 0.03	5.31 \pm 0.03
<i>C. fluminalis</i>	3.36 \pm 0.05	8.32 \pm 0.04	8.38 \pm 0.02	3.19 \pm 0.07

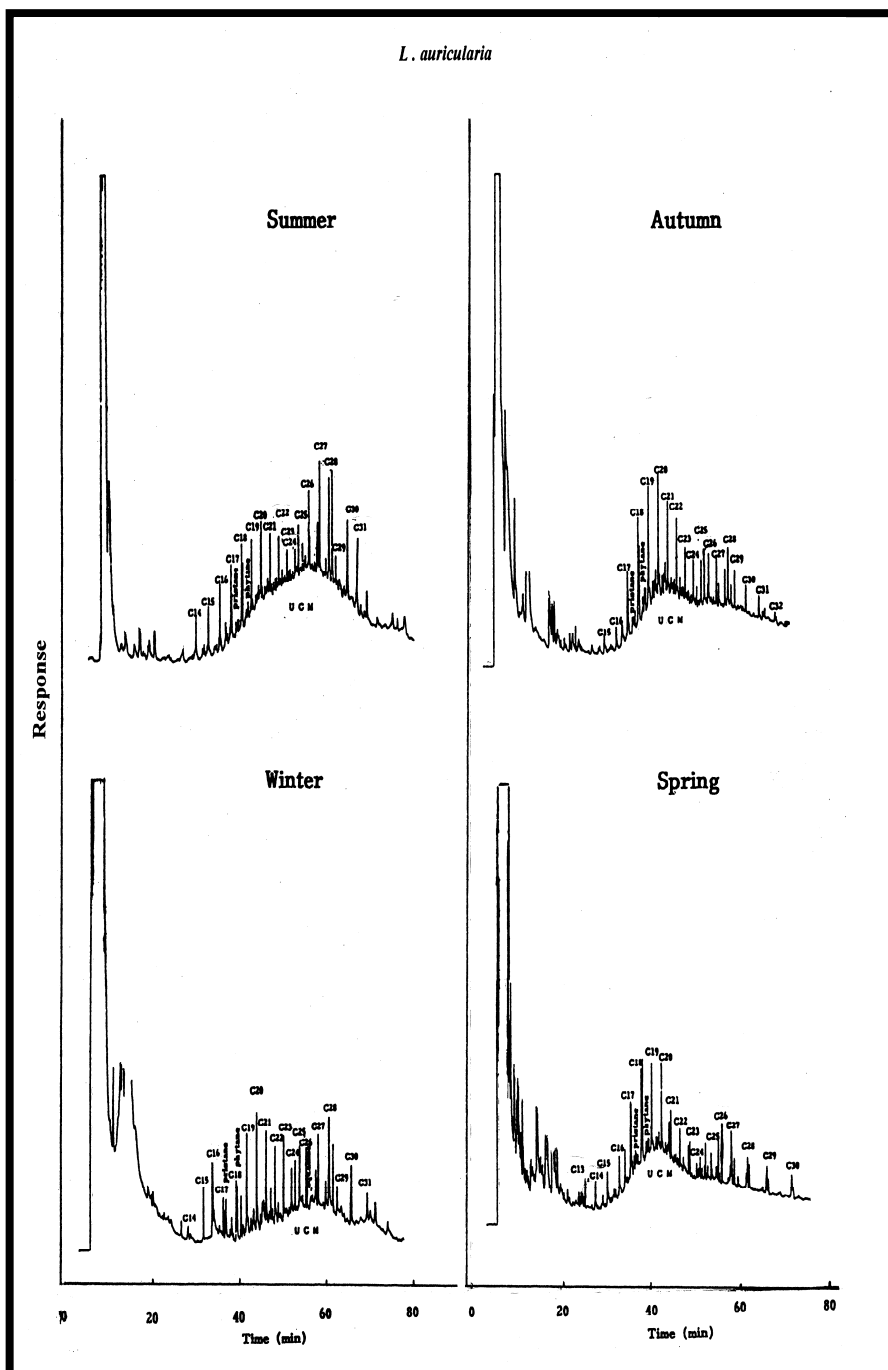


Figure 3. Capillary gas chromatograms of n-alkanes concentrations in *L. auricularia* tissues from the Shatt Al-Arab river during 2004–2005.

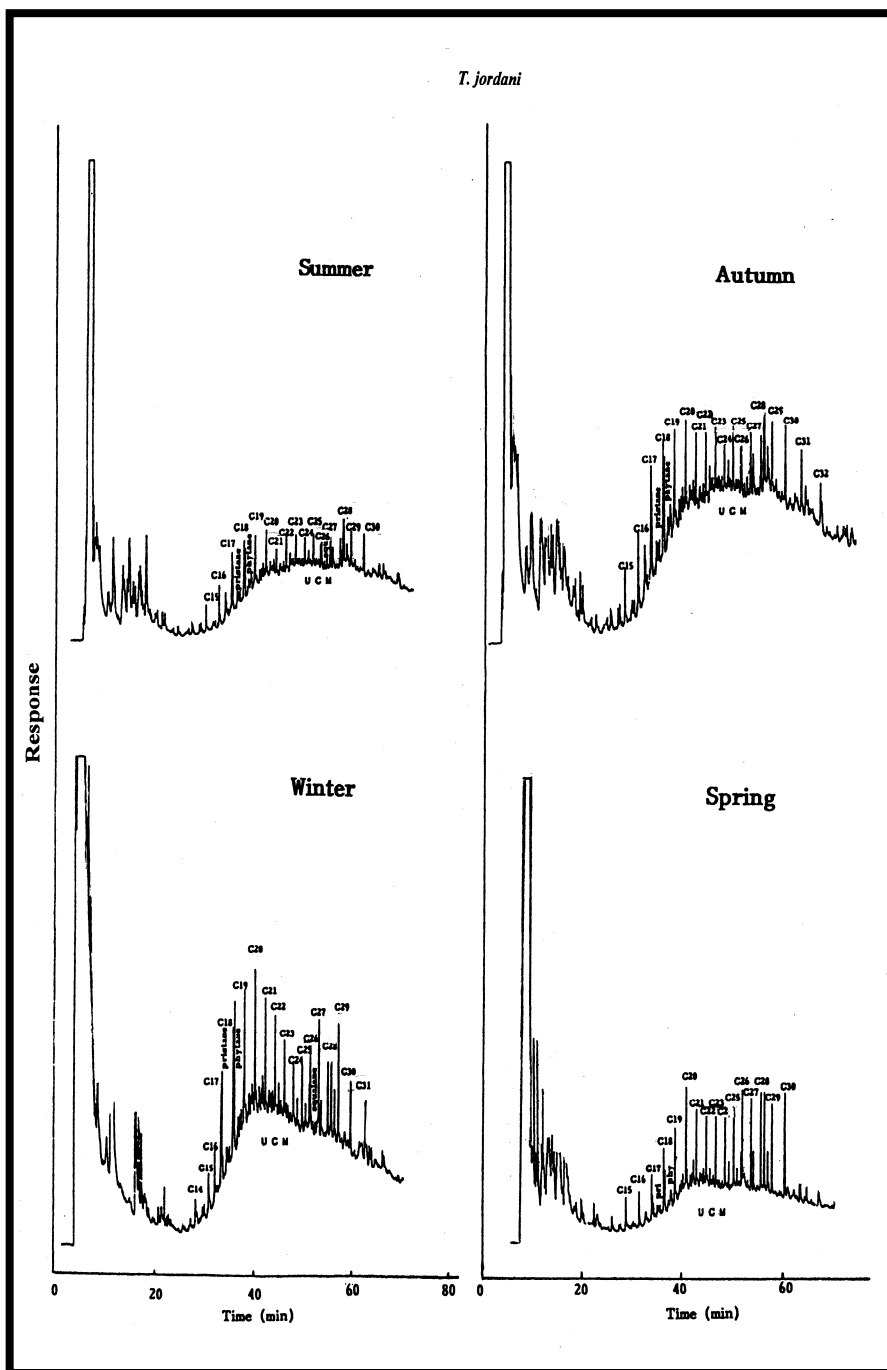


Figure 3 (continued). Capillary gas chromatograms of n-alkanes concentrations in *T. jordani* tissues from the Shatt Al-Arab river during 2004–2005.

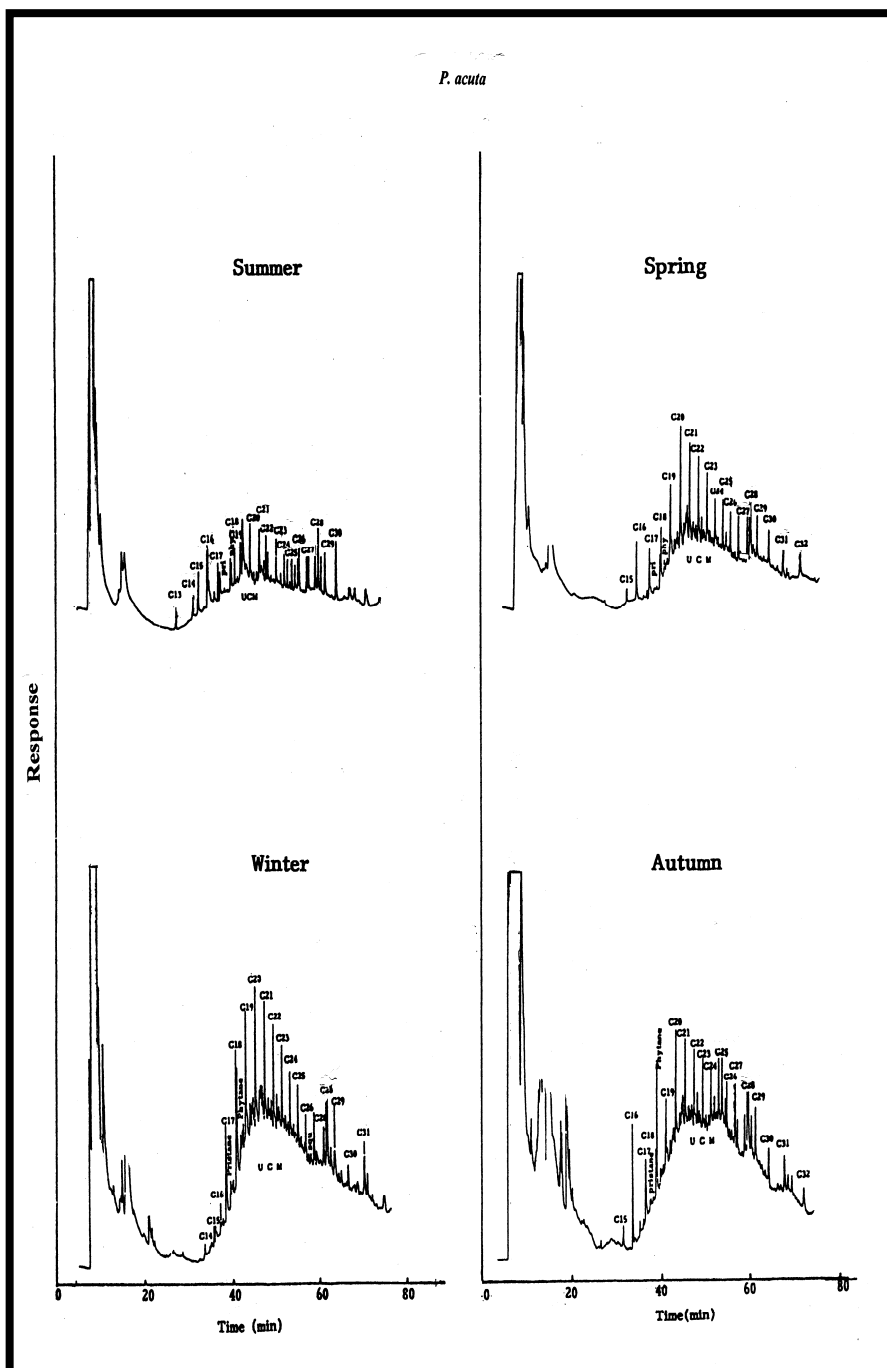


Figure 3 (continued). Capillary gas chromatograms of n-alkanes concentrations in *P. acuta* tissues from the Shatt Al-Arab river during 2004–2005.

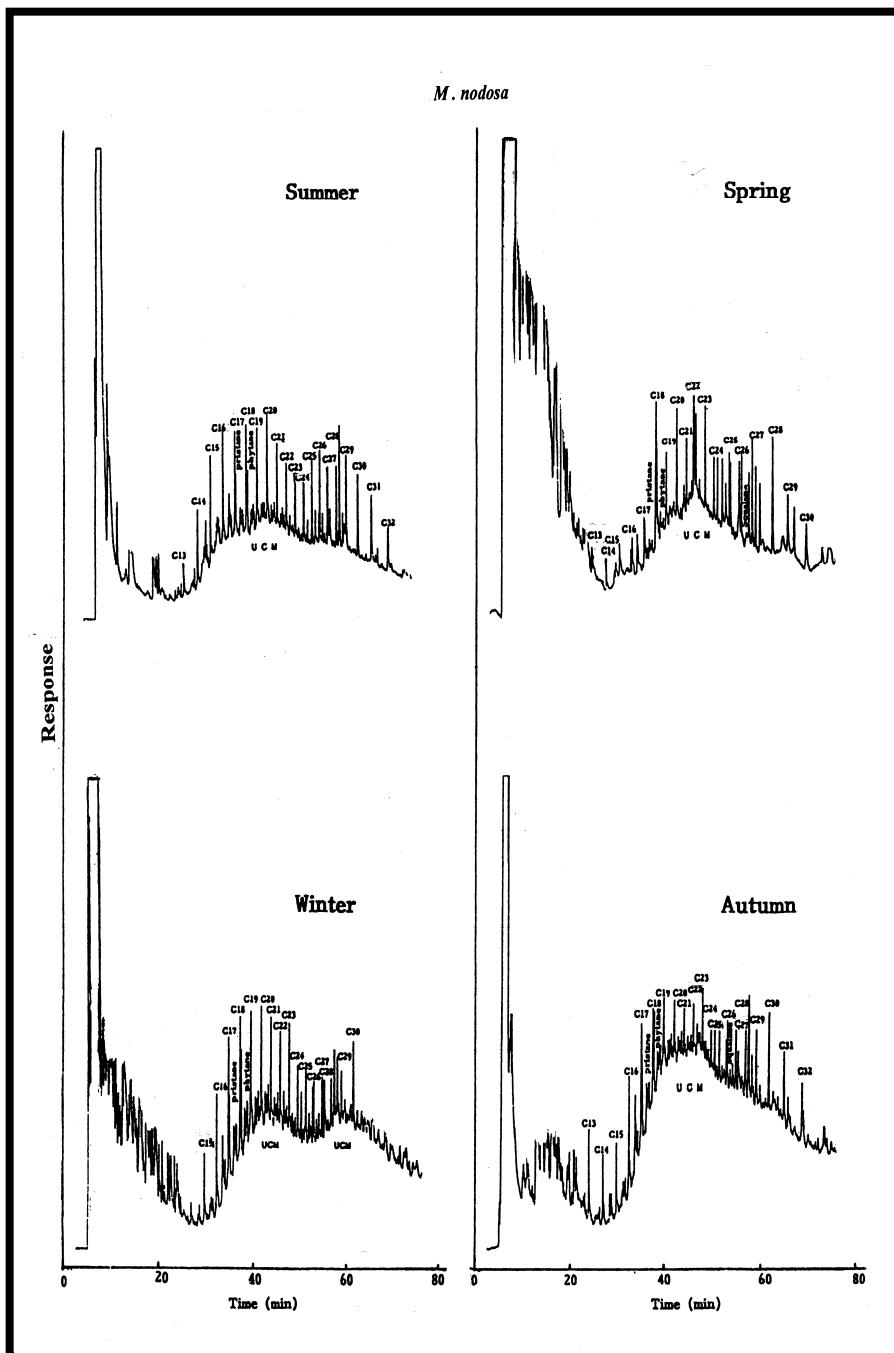


Figure 3 (continued). Capillary gas chromatograms of n-alkanes concentrations in *M. nodosa* tissues from the Shatt Al-Arab river during 2004–2005.

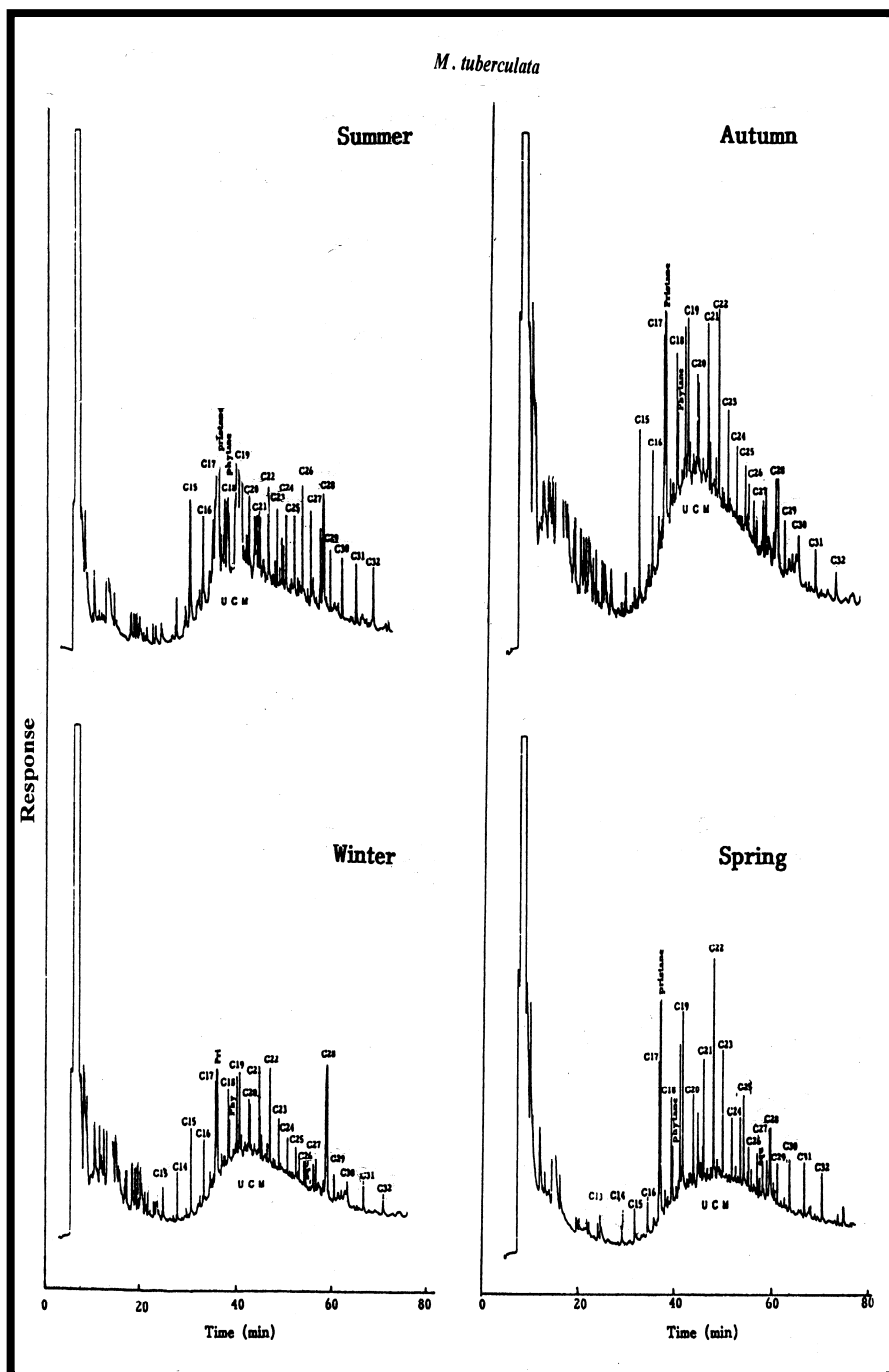


Figure 3 (continued). Capillary gas chromatograms of n-alkanes concentrations in *M. tuberculata* tissues from the Shatt Al-Arab river during 2004–2005.

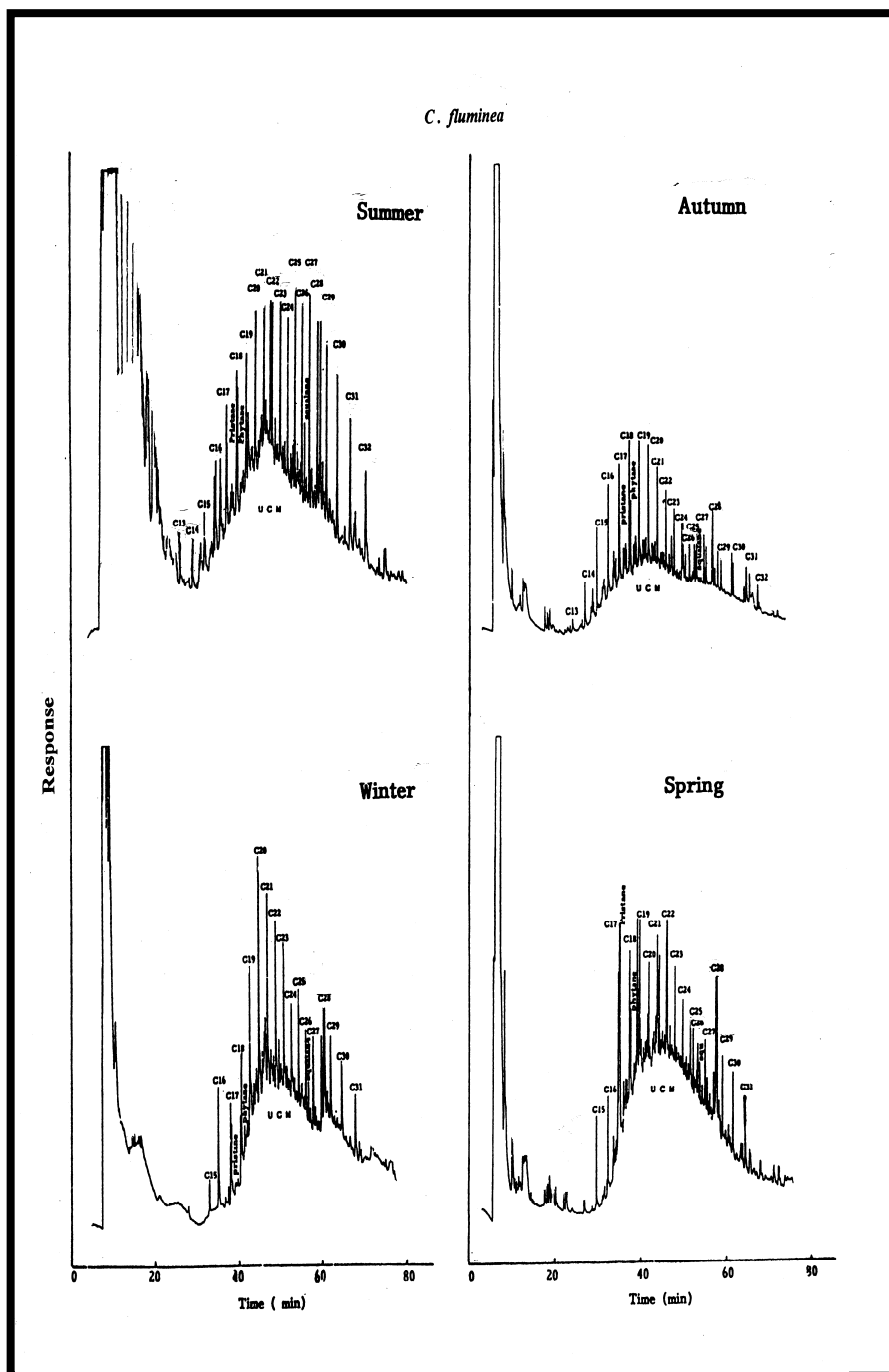


Figure 3 (continued). Capillary gas chromatograms of n-alkanes concentrations in *C. fluminea* tissues from the Shatt Al-Arab river during 2004–2005.

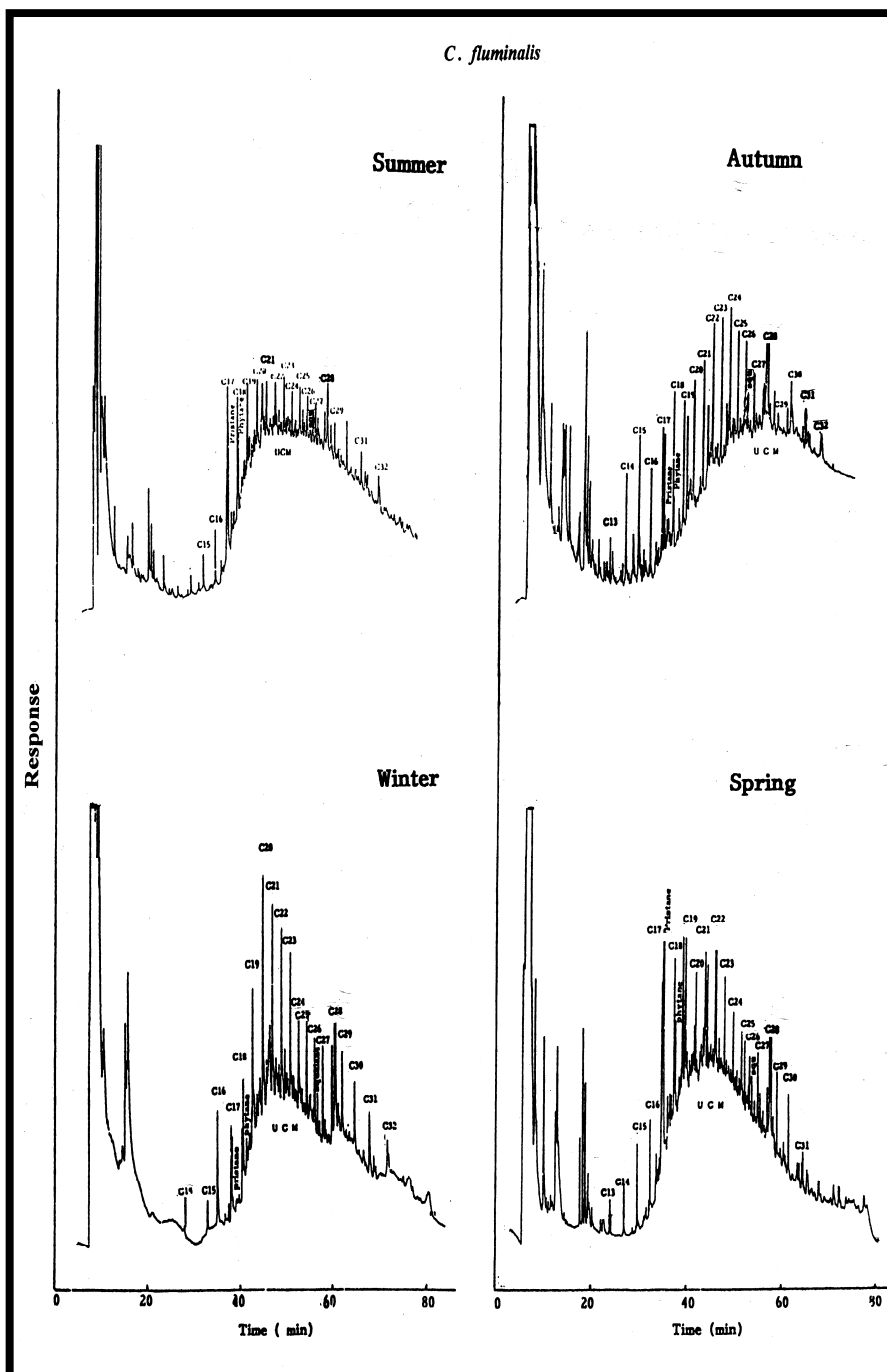


Figure 3 (continued). Capillary gas chromatograms of n-alkanes concentrations in *C. fluminalis* tissues from the Shatt Al-Arab river during 2004–2005.

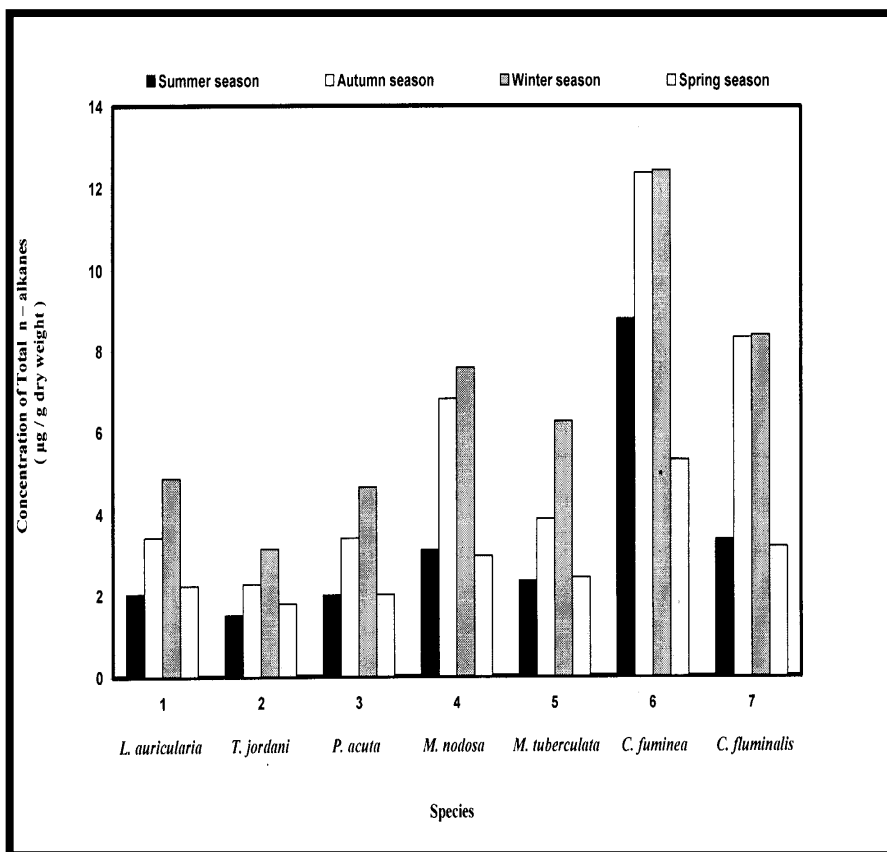


Figure 4. Seasonal variations of total n-alkanes in the species of molluscs from the Shatt Al-Arab river during 2004–2005.

Accordingly, one expects that the levels of n-alkanes compounds that accumulated in the tissues of the Shatt Al-Arab river organisms (include molluscs) will be considerably less in summer and spring than in winter and autumn.

Menon and Menon (1999) documented that the n-alkanes compounds have a tendency to adsorb onto particulate matter. This may come into conclusion that adsorption of n-alkanes compounds to Shatt Al-Arab river sediment is the principle mechanism for their removal from water. Al-Saad (1995) showed pronounced seasonal variations in the average concentrations of n-alkanes compounds in suspended matter along Shatt Al-Arab river being minimum in summer. Accordingly lower concentrations of n-alkanes compounds in Shatt Al-Arab river water during summer season could be caused in part by the increased sedimentation of adsorbed n-alkanes compounds. The same conclusion arrived by (DouAbul and Al-Saad, 1985; Al-Saad and Al-Timari, 1993). The

difference in the discharge of n-alkanes compounds into Shatt Al-Arab river water during the different seasons could produce in seasonal variations in the concentrations of n-alkanes compounds in the river. 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 with large amount of fossil fuel used in household heating during the cold season as well as the higher association of these n-alkanes compounds with atmospheric particles at lower ambient temperature. Furthermore, the n-alkanes compounds deposited on land during summer would not reach the aquatic environment in the same extent as during winter when runoff from land is much more extensive due to rain-storms in the region.

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التغيرات الفصلية وتوزيع الالكانات الاعتيادية في بعض أنواع النواع المتواجدة في نهر شط العرب

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المستخلص - تم دراسة التغيرات الموسمية للالكانات الاعتيادية في نواع نهر شط العرب وتشمل خمسة أنواع من القواقع ونوعين من المحار. جمعت عينات النواع من مناطق مختلفة من نهر شط العرب (على طول المنطقة الممتدة من أبو الخصيب إلى كرامة علي) خلال عام 2004–2005. كل نوع من النواع يتكون على الأقل من 350 من الأفراد البالغة المتشابه بالحجم. استخلصت الالكانات الاعتيادية من هذه العينات وقيست بواسطة جهاز الغاز الكروماتوغرافي المزود بالعمود الشعري ذات التقنية الفصل العالية تراوح تركيز الالكانات الاعتيادية الكلية في نواع نهر شط العرب من 1.50 مايكروغرام/غرام وزن جاف في قوقع *T. jordani* إلى 8.78 مايكروغرام/غرام وزن جاف في محار *C. fluminea* خلال فصل الصيف ومن 2,26 مايكروغرام/غرام وزن جاف إلى 12.27 مايكروغرام/غرام وزن جاف خلال فصل الخريف ومن 3.15 مايكروغرام/غرام وزن جاف إلى 12.44 مايكروغرام/غرام وزن جاف خلال فصل الشتاء ومن 1.78 مايكروغرام/غرام وزن جاف إلى 5.31 مايكروغرام/غرام وزن جاف خلال فصل الربيع. بينت الدراسة الحالية بان هناك تغير فصلي بارز في تركيز الالكانات الاعتيادية في نواع نهر شط العرب. إذ كان أوطئ تركيز للالكانات الاعتيادية في فصلي الصيف والربيع وأعلى تركيز في فصلي الشتاء والخريف وتغزى التغيرات الموسمية في تركيز الالكانات الاعتيادية في نواع نهر شط العرب إلى عوامل مختلفة.