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MONITORING OF HYDROCARBONS IN SHATT AL-ARAB RIVER BY USING SOME SPECIES OF MOLLUSCS

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ABSTRACT

This study comprises the monitoring of hydrocarbons in Shatt Al-Arab river water by using the native seven species of molluscs as bioindicators. These species are the snails, *Lymnaea auricularia*, *Theodoxus jordani*, *Physa acuta*, *Melanopsis nodosa*, *Melanoides tuberculata* and the bivalves, *Corbicula fluminea* and *Corbicula fluminalis*. The species of molluscs are 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 3500 adult of uniform size of individuals. The hydrocarbons which extracted from these species of molluscs were analyzed by spectroflurometer. The concentrations of total hydrocarbons in these species of Shatt Al–Arab river ranged from 1.93 µg/ g dry weight in the *T. jordani* to 26.56 µg/ g dry weight in the *C. fluminea.* The lowest fat content was in *T. jordani* (0.33 mg /g) and the highest was in *C. fluminea* (0.98 mg /g). Significant relationships were obtained between the fat contents and hydrocarbons concentrations in the tissues of these species of molluscs (r = 0.8 - 0.9).

INTRODUCTION

The industrial history of the area of the Shatt Al–Arab river is particularly useful factor in field monitoring work. Many large scales industries established in the areas around Shatt Al – Arab river for instance the paper mill, fertilizer mills, electrical power stations, refined oil plants, and other industries. Pollutants input has increased in the river from these industries and from a heavily populated areas located around the river (Al–Saad, 1995). Man’s activities have thus produced a pollution gradient in the Shatt Al–Arab river that overrides a physical variation as a controlling factor in the distribution of the resident aquatic species.

Elevated levels of metals and some organic chemicals have been found in the Shatt Al–Arab river sediments, in the water column, and in the tissues of the organisms that lives in river waters (Al–Saad, 1983, 1995). Stress in aquatic organisms exposed to even low levels of some of these pollutants has been repeatedly demonstrated, as for example petroleum hydrocarbons (Al-Saad, 1995), heavy metals (Abaychi and DouAbul, 1985, Farid, 2005), and pesticides (DouAbul *et al*., 1987).

The petroleum and its products have been identified as the major contaminants in the water of Shatt Al–Arab river (Al–Saad, 1995). Hydrocarbons are a major fraction of petroleum content and its products which may be used to detect thier presence in the environment. An important route is the uptake and assimilation of these components by marine organisms (NRC, 2003).

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 polluted by domestic sewage, petroleum by products and industrial waste products; tend to concentrate almost all noxious substances in the environment; they are relatively easy to locate and sample. It is possible to obtain or locate spat and thus, it is possible to monitor the rate of uptake and incorporation of various toxic chemicals 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 (UNEP, 1993; Cajaraville *et al*., 1995, NRC, 2003).

The aim of the present study is to monitor the hydrocarbons in Shatt Al–Arab river by using some species of molluscs {*L.auricularia*, *T. jordani*, *P. acuta*, *M. nodosa*, *M. tuberculata* (snails), *C. fluminea* and *C. fluminalis* (bivalves)}. The three principal aims of this monitoring involving:

- the establishment of present levels of hydrocarbons in molluscs of Shatt Al–Arab river (i.e., baseline).
- comparing the hydrocarbons concentrations in the molluscs of Shatt Al–Arab river with those from different geographical areas. Such measurement help to determine if current hydrocarbons are found to produce unacceptable contamination concentrations (i.e., they are causing or likely to cause, aquatic pollution problems).
- the measurement of hydrocarbons levels in the tissues of the molluscs of Shatt Al–Arab river in relation to public health (food chain).

MATERIALS AND METHODS

Specimens of seven species of molluscs, *L. auricularia*, *T. jordani*, *P. acuta*, *M. nodosa*, *M. tuberculata*, *C. fluminea* and *C. fluminalis* were collected from the Shatt Al–Arab river (along the region extended from Abu-Al-Khasib to Garmat - Ali) during 2004 and 2005 (Figure 1). At least 3500 adult individuals of uniform size of each species were collected.

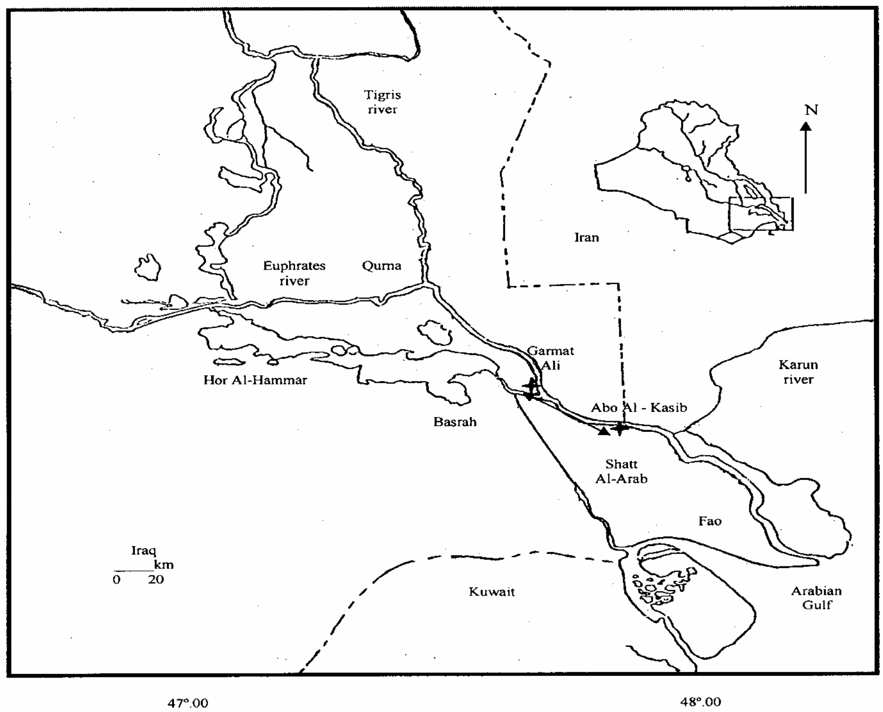

Figure (1): Map of sampling location.

Crude oils (Basrah regular - medium - API gravity between 28 – 34) was supplied by Iraqi South Oil Company.

Methanol, benzene, n – hexane, methylene chloride, petroleum ether and acetone were supplied from Burdick and Jackson laboratories, Inc.

Sodium sulphate and potassium hydroxide were supplied by Supelco SA. Sodium sulphate was extracted with methylene chloride for 36 hours in a soxhlet. Following clean up by extraction, It was dried in an oven at 130 ˚C for about 24 hours and deactivated with deionized water at the recommended percentage prior to use.

The tissues of the animals were pooled and macerated in a food liquidizer from which at least 3 replicates of 15 g 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 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 extraction of the unsaponified matter with hexane, the extract was dried over anhydrous Na_2SO_4 and concentrated by a stream of N_2 for UVF analysis.

A shimadzo RF–540 spectroflurometer equipped with a DR–3 data recorder was used to determine total petroleum hydrocarbons. The basis quantitative 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 by a stream of purified nitrogen. Ten µl of the concentrated extracts were taken by a Hamilton syringe and weighted after evaporation of the solvent.

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 fluorescence intensity of the sample analyzed is compared with the fluorescence of a reference solution (having almost the same concentration as the unknown extract) or to a series of reference solution (wherever, the measurement of fluorescence of the sample took more than one day). The fluorescence of reference solution was measured at least once a day under identical instrumental conditions.

The reference oil was used in the spectroflurometer obtained from Iraqi South Oil Company (Basrah regular crude oil) (Figure 2).

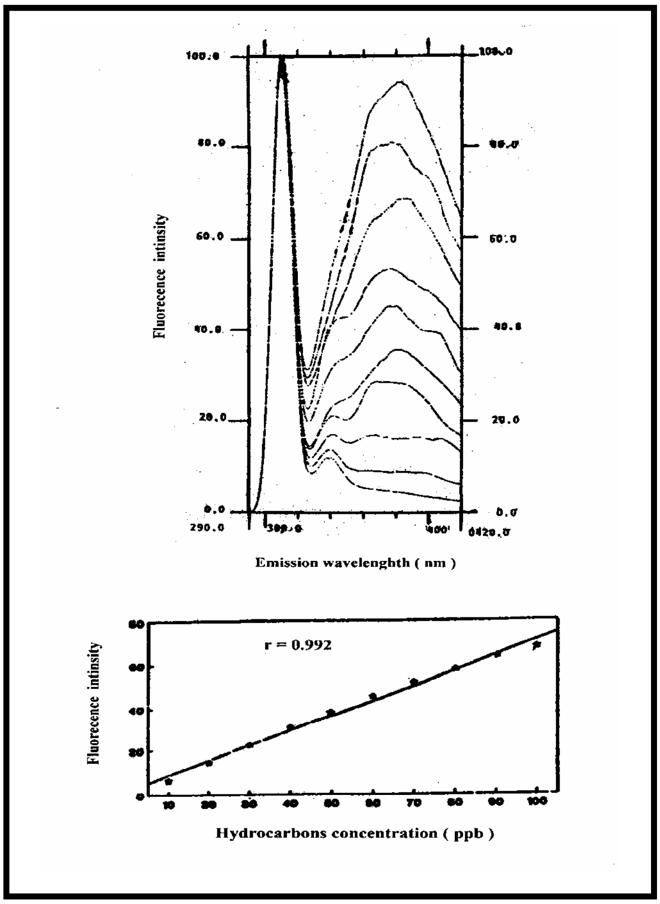

Figure (2): Fluorescence spectra with calibration curve of standard crude oil (Basrah regular).

RESULTS AND DISCUSSION

The biological monitoring programmes may use selected "indicator organisms" or whole communities. In this respect knowledge is rather rare concerning the best suitable indicator organisms to confidently arrange a marine monitoring programmes for the region.

In areas subjected to chronic input of petroleum hydrocarbons to water, the UNEP (1993), Phillips *et al*. (2003) and NRC (2003) reported that the analysis of species of molluscs tissues provides the means for the identification of identify input sources, types of hydrocarbons present in the system and the approximate average concentrations of hydrocarbons suspended in waters. The values obtained by species of molluscs analysis provide an integrated sample over more time than can be measured with an intermittent water sampling program me. The results of species of molluscs analysis are easier to interpret than water samples with low level petroleum contamination since the species of molluscs tend to show less interference from biogenic plant hydrocarbons. The concept of utilizing single species to monitor the contaminants in aquatic environment becomes increasingly popular (Bailey *et al*., 2004). This very concept has been extended by using several species to monitor rivers. We feel there are two primary advantages in using multiple species monitoring in the present study. First, it is possible to monitor the entire river which is not always possible when using one species. For example, the Shatt Al–Arab river bivalves *C. fluminea* and *C. fluminalis* are virtually dominant in the region of confluence of Tigris and Euphrates rivers and their dominance fall down toward the estuary; thus, we are able to monitor such areas with these species, which are generally not found in the nearby Al-Fao region. Second, by using molluscs that occupy a variety of habits, we hope to gather informations about the routes of movement, rates of transfer and reservoirs of hydrocarbons in the aquatic environment.

The concentrations of total hydrocarbons in the species of molluscs ranged from 1.93 µg /g dry weight (DW) in the *T. jordani* to 9.83 µg /g DW in the *C. fluminea* during Summer and from 2.90 µg / g DW to 22.37 µg /g dry weight during Autumn, whereas they range from 3.58 µg /g DW to 26.56 µg /g DW and from 2.60 µg /g DW to 14.13 µg /g DW during Winter and Spring, respectively (Table 1 and Figure 3). DouAbul and Al–Saad (1984) found that the concentrations of total hydrocarbons in *C. fluminalis* from Shatt Al–Arab river ranged from 13.1 µg /g wet weight to 34.6 µg /g wet weight. Talal (1999) found that the concentrations of total hydrocarbons in the species of molluscs of Shatt Al-Arab river were from 11.67 µg /g DW in *T. jordani* to 20.10 µg /g DW in the *C. fluminalis* during Summer and from 12.76 µg /g DW to 21.47 µg /g DW during Autumn, while they flocculate between 15.72 µg /g DW and 22.46 µg /g DW and from 17.25 µg /g DW to 23.90 µg /g DW during Winter and Spring. However, Al–Saad (1995) found that the concentrations of total hydrocarbons in the plants of Shatt Al–Arab river ranged from 0.06 µg /g DW to 0.59 µg /g DW. While the concentrations of total hydrocarbons in the zooplankton and bacteria changed from 0.58 µg /g DW to 6.78µg /g DW and form 0.1 µg /g DW to 2.08 µg /g DW, respectively. Moreover fishes contain concentrations of total hydrocarbons varied from 1.70 µg /g DW to 10.91 µg /g DW.

Table (1): Concentrations of total hydrocarbons (µg /g dry weight) and fat contents (mg /g) in the species of molluscs tissues from the Shatt Al–Arab river during 2004 - 2005.

Species	Concentrations of Total Petroleum Hydrocarbons				Fat Content (mg /g)
	Summer	Autumn	Winter	Spring	
L . auricularia	2.82 ± 0.04	3.53 ± 0.06	5.27 ± 0.07	3.72 ± 0.04	0.47 ± 0.02
T . jordani	1.93 ± 0.06	2.90 ± 0.04	3.58 ± 0.08	2.60 ± 0.02	0.33 ± 0.04
P . acuta	2.60 ± 0.08	3.06 ± 0.04	3.91 ± 0.05	3.33 ± 0.01	0.43 ± 0.02
M . nodosa	4.28 ± 0.04	6.27 ± 0.04	8.16 ± 0.05	5.55 ± 0.03	0.68 ± 0.03
M .tuberculata	3.21 ± 0.03	5.15 ± 0.05	6.85 ± 0.04	4.23 ± 0.06	0.57 ± 0.03
C . fluminea	9.83 ± 0.06	22.37 ± 0.05	26.56 ± 0.02	14.13 ± 0.06	0.98 ± 0.01
C . fluminalis	5.49 ± 0.08	7.25 ± 0.05	9.30 ± 0.05	6.54 ± 0.08	0.86 ± 0.04

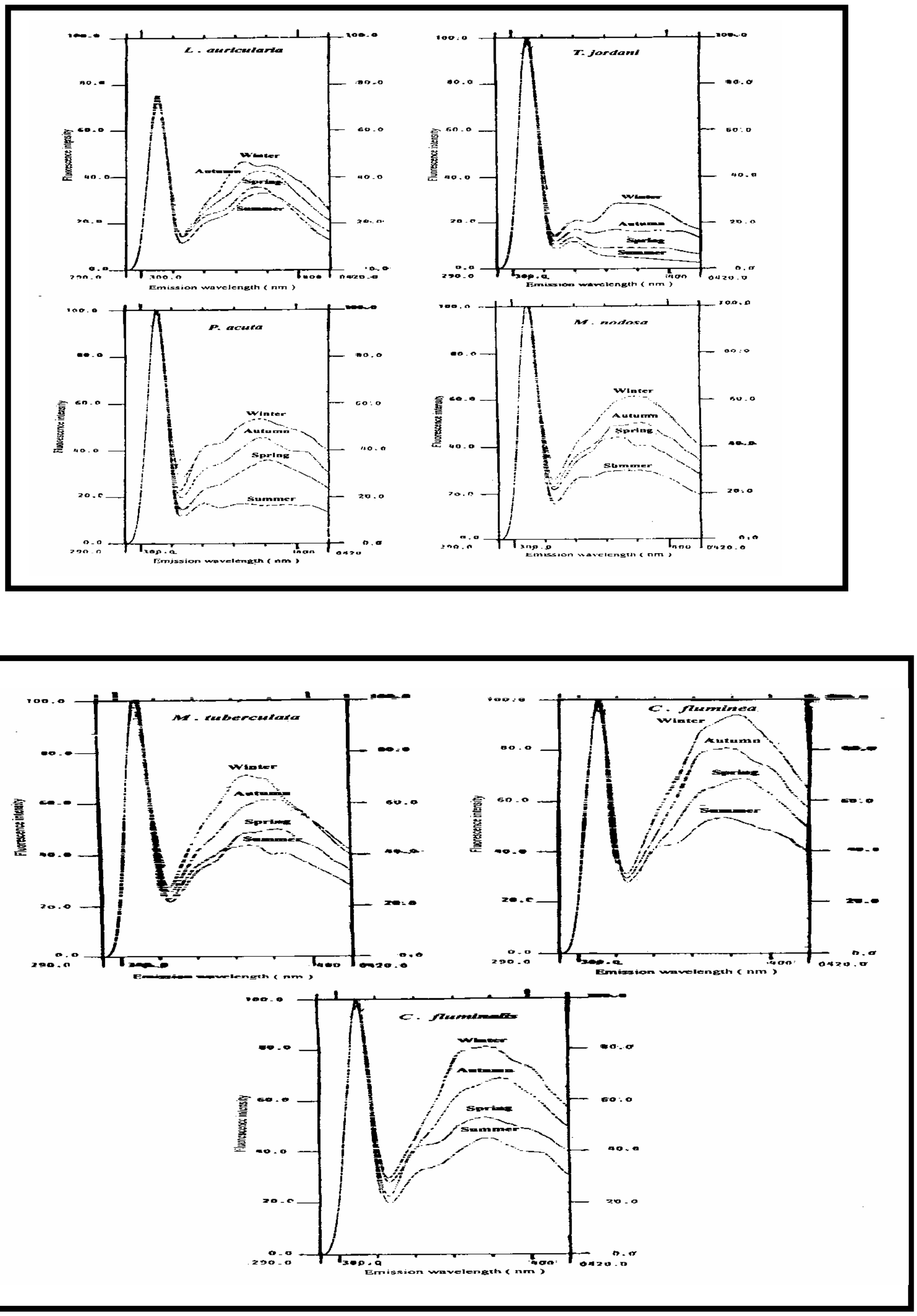

Figure (3): Fluoresence spectra of total petroleum hydrocarbons concentrations in the molluscs tissues from Shatt Al–Arab river during 2004 - 2005.

The lower fat content is found in *T. jordani* (0.33 mg /g) and the higher is in *C. fluminea* (0.98 mg/g) (Table 1). Based on the different concentrations of total hydrocarbons observed in the species of molluscs of Shatt Al–Arab river, significant relationships found between the concentrations of total hydrocarbons and fat content of species of molluscs (r = 0.8– 0.9) (Figure 4). Many studies conducted using species of molluscs indicated that the accumulation of hydrocarbons in their tissues increased with the increase of their lipid content (Zhou *et al.*, 1996; Allen *et al*., 2002).

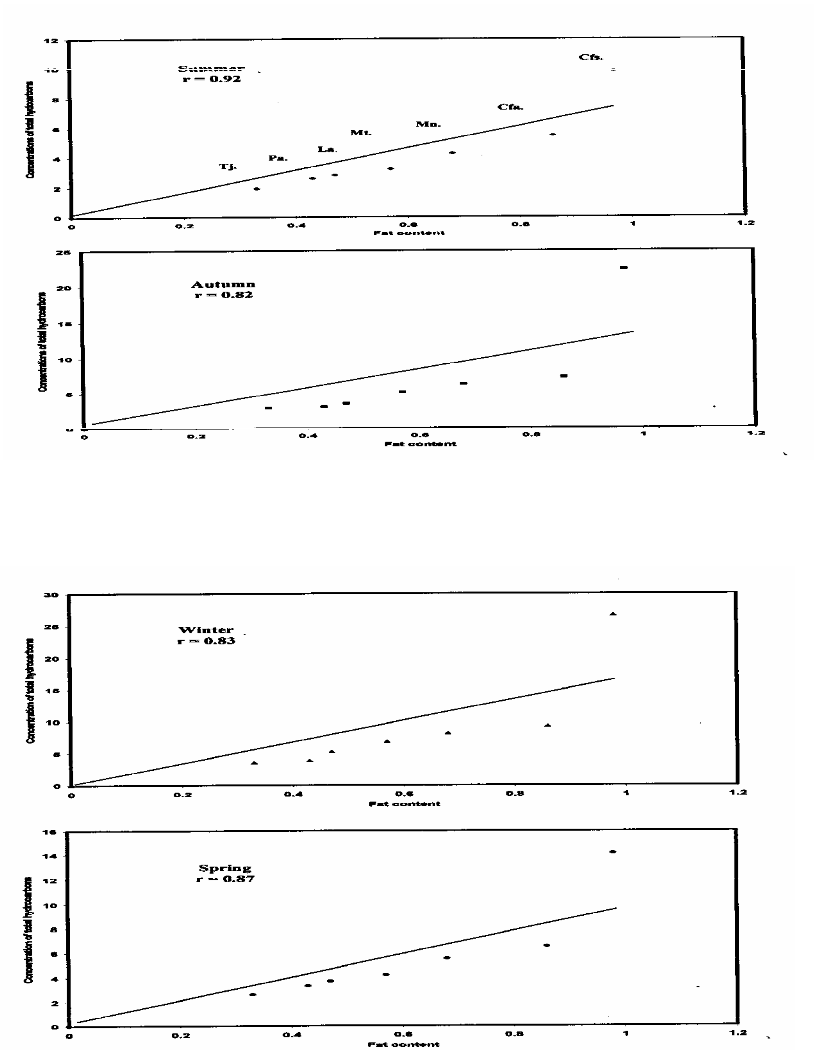

Figure (4): The relationship between fat contents (mg/g) and concentrations of total hydrocarbons (µg /g dry weight) in the molluscs of Shatt Al-Arab river.

The sources of hydrocarbons which accumulated in the tissues of Shatt Al–Arab river molluscs may be from two different origins, anthropogenic and biogenic. Phillips *et al*. (2003) reported that the hydrocarbons can enter the tissues of the mollusc from phytoplankton and through water, either from solution or absorbed to suspended particles, while feeding. Bedair and Al–Saad (1992) reported that the petroleum hydrocarbons in Shatt Al–Arab estuary were likely originated from boating activities, runoff from land and introduction via sewage outfalls. Boats with outboard engines using mixture of gasoline and lubricating oil as fuel is discharged most of the oil through thier exhaust into the water. The storm water runoff from the urban areas was found to be the primary source of petroleum hydrocarbons in the area (Al–Mudaffer *et al*., 1990). The storm water contained higher levels of hydrocarbons derived from lubricating oils as pyrolytic products released by automobile traffic. This may also constitute a major source of hydrocarbons found in the river, since the used motor oil from automobiles may be discharged discriminately into the environment and so reaches the river via runoff. DouAbul and Al–Saad (1985) indicated that oil pollution in the 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 the Shatt Al–Arab river. Al–Saad (1995) reported that many aquatic organisms of Shatt Al–Arab river including plants, algae, zooplankton, bacteria and fish were capable to synthesize biogenic hydrocarbons.

There are many factors that affect the distribution of hydrocarbons in Shatt Al–Arab river (which accumulated later in the tissues of molluscs) such as volatilization, mixing, flushing, adsorption, chemical oxidation, photo – decomposition, sedimentation, and biodegradation. These factors collectively reduce the concentration of hydrocarbons compounds (Al-Saad, 1995). Lee *et al*. (1978) in his experiments on the fate of some hydrocarbons in a control ecosystem indicated that hydrocarbons in shallow marine water may have a residence time for a few days for low molecular weight aromatics. The results of experiments suggested that microbial degradation and volatilization were the primary removal processes. In contrast the concentrations of higher molecular weight hydrocarbons were shown to be affected primarily by sedimentation and photo – chemical oxidation.

The concentrations values of total hydrocarbons in the species of molluscs of Shatt Al-Arab river of the present study were within the values obtained elsewhere (Table 2).

CONCLUSION AND RECOMMENDATIONS

The species of molluscs from Shatt Al-Arab river were found to contain measurable amount of hydrocarbons. The compounds seem to be derived from both biogenic and anthropogenic sources. The hydrocarbons found in the species of molluscs of Shatt Al–Arab river are due to food and water sources, type of habitat, environmental factors, and lipid content.

In general the concentrations of hydrocarbons in the species of molluscs of Shatt Al-Arab river are within the values obtained elsewhere in the world.

Table (2): Comparison of concentrations of total hydrocarbons in the molluscs species of Shatt Al-Arab river with other parts of the world.

Location	Concentration µg / g	Species and Reference
Galveston Bay	2 - 34	*Crossostrea virginosa* Ehrhardt (1972)
Narragansett Bay	0 - 25	*Saxidomus giganteus* Farrington and Quinn (1973)
Kiel Bight	0.7 - 33.2	*Mytilus edulis* Ehrhardt and Heinemann (1975)
Western Port Bay (Australia)	0.4 – 7.5	*Nassarius vibex* Burns and Smith (1977)
Southren Baltic Sea	0 – 89	*Mytilus edulis* Law and Andrulewicz (1983)
Gulf of Mexico	1.4 – 20.7	*Ostero lurida* Wade *et al*. (1991)
Signy Island , UK.	0.18 – 0.56	*Yoldia eightsi* Cripps and Priddle (1995)
Canada	0.11 – 15.66	*Mytilus edulis* Zhou *et al*. (1996)
British Columbia	23 - 98	*Maya arenoria and This haemastoma* Birtwell and McAllister (2000)
San Francisco Bay, California	0.55 – 77.8	*Mytilus galloprovincialis* Phillips *et al*. (2003)
Shatt Al – Arab River	1.50 – 12.44	Present study

There are many biological and environmental factors (temperature, evaporation, biodegradation, chemical oxidation, and sedimentation), may produce pronounce variations in the concentrations of hydrocarbons in the molluscs of Shatt Al–Arab river.

In an area subjected to a chronic input of petroleum into water, the analysis of these species of molluscs provides the means of identification input sources, types of hydrocarbons present in the stream, and approximate average concentrations of hydrocarbons in water. Petroleum projects in the water system should emphasize on indicator species such as molluscs. Every possible effort should be made to minimize petroleum input into the Shatt Al-Arab river environment. Outfall licenses should be strictly enforced and should be amended to specify permissible levels on the basis of the most toxic fractions of petroleum released into the river environment. Oil pollution associated with boating activities could be controlled by enforcing stringent regulations on oil discharge and providing pump stations similar or superior to the facilities for tanker ballast water treatment. A monitoring project using species of molluscs in areas subjected to pollution hydrocarbons for later has already commenced.

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مراقبة الهيدروكاربونات في نهر شط العرب باستخدام بعض انواع النواعم

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المستخلص

تضمنت الدراسة الحالية مراقبة الهايدروكاربونات في شط العرب باستخدام سبعة انواع من النواعم وهي خمسة أنواع من القواقع *(Lymnaea auricularia* و*Theodoxus jordani* و*Physa acuta* و*Melanopsis nodosa* و *Melanoides tuberculata*) ونوعين من المحار (*Corbicula fluminea* و *Corbicula fluminalis*) كمؤشرات حيوية. جمعت عينات النواعم من مناطق مختلفة من شط العرب (على طول المنطقة الممتدة من أبو الخصيب إلى كرمة علي) خلال عام 2004 - 2005. يتكون كل نوع من النواعم من 3500 من الأفراد البالغة المتشابه بالحجم على الاقل. استخلصت الهايدروكاربونات من هذه العينات وقيست بواسطة جهاز التفلور. تراوح تركيز الهايدروكاربونات الكلية في نواعم نهر شط العرب من 1.93 مايكروغرام/ الغرام وزن جاف في القوقع *T. jordani* إلى 26.56 مايكروغرام/ الغرام وزن جاف في المحار *C. fluminea*. وكان اوطأ محتوى دهني في القوقع *T. jordani* (0.33 مليغرام/الغرام) في حين كان أعلى محتوى دهني في المحار *C. fluminea* (0.98 مليغرام/ الغرام). وجدت علاقة معنوية (معامل الارتباط = 0.8 – 0.9) بين المحتوى الدهني وتركيز الهايدروكاربونات في أنسجة نواعم منطقة شط العرب.