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Levels of petroleum hydrocarbons and n-alkanes in different species of mollusca from Shatt Al-Arab Estuary North-West Arabian Gulf

H. T. Al-Saad; A. H M. Jawad and U. H. Yousif
Marine Science Centre , University of Basrah,
Basrah, Iraq

Abstract

This study concern a survey to determine the extent and degree of contamination by petroleum hydrocarbons in six mollusca species (*Neverita didyma, Trachycardium lacunosum , Tifia insulaechorab, Bassina callophyla, Paphia textile and Thais mutabilis*) collected from northwest Arabian Gulf region during 1998 .

The mean concentrations of total petroleum hydrocarbon which determined spectroflurometry ranged from 3.56 ug/g in *B. callophyla* to in 12.07 ug/g dry weight in *Tifia insulaechorab* while the n-alkane ranged from 2.19 to 4.95 ug/g dry weight respectively .

N-alkane distribution were characterized by the odd and even carbon range from C13 to C32 . A number of other hydrocabons were present in the aliphatic fraction , including pristane range (0.09-0.26)ug/g, phytane range (0.08-0.20). Values of odd/even n-alkanes from C13 to C34 were varied from 1.06 to 1.25 which indicated biogenic sources of hydrocarbons in these organism .

In general low levels of hydrocarbons and n-alkanes observed in mollusca samples However, these data indicates that hydrocarbon concentration in these organism are lower to those recorded in the Arabian Gulf region.

INTRODUCTION

Sedentary benthic organisms have been used for the past 20 years to monitor contaminant levels in aquatic systems (Prest *et al*, 1995) . The most commonly used organisms are those able to accumulate contaminants without adverse effects . It is generally held that the contaminant levels in the organisms represent a time-integrated picture of concentrations in the ambient environment . The monitoring of contaminant levels in organisms are therefore used as an alternative to water sampling , Which regular labour intensive, costly programmes to identify the most important trends (Naes *et al*, 1998).

The mollusca , because of its wide distribution , has been considered and used extensively as a most suitable and reliable organism for the monitoring of organic contaminants in estuarine and coastal environments (Granby and Spliid, 1995). Typically marine organisms, including bivalves, accumulate organic pollutants in their tissue, leading to concentration levels for above those found in the water column (Zhou *et al* , 1996) .

During the 1991 war , millions of barrels of Kuwait crude oil were released into the waters of the Arabian Gulf . In addition, hundreds of oil wells were set on fire and hydrocarbon compound resulting from the combusion of the petroleum may have been deposited into the water at the Arabian Gulf. (Readman *et al*, 1992).

In response to the growing need to assess the extent and degree of pollution emanating from the area after 7 years from the war . Marine Science Center at Basrah university was requested to undertake an initial survey of petroleum hydrocarbons in biota of N.W. Arabian Gulf which could be used to monitering pollution in this important area .

MATERIAL AND METHODS

Samples of mollusca were obtained through fishing trawling in Iraqi marine water during 1998 from North- West Arabian Gulf (Fig.1). After the collection Mollusca samples were classified according to smythe (1982) , Bosch and Bosch (1982) and Jones (1986) . The samples were dissected on board of the ship and edible tissues were separated . The tissues of individuals were wrapped separately in clean aluminium foil and stored frozen
(-4 c°) until analysis in the laboratory . For analysis of the samples , the tissues were thawed , weighed and freeze- dried for 48 hr . at (-45 c°) .

The samples were then ground to a fine powder and stored in amber glass bottles and kept in the refrigerator . The analysis method used was that reported by UNEP (1992) .

Exactly , 10g of dried mollusca were placed in pre-extracted cellulose thimble and soxhlet extracted with 150 ml methanol : benzen (1:1) ratio for 24 hr . At the end of this period . the extract was transferred to astrong flask and the sample was further

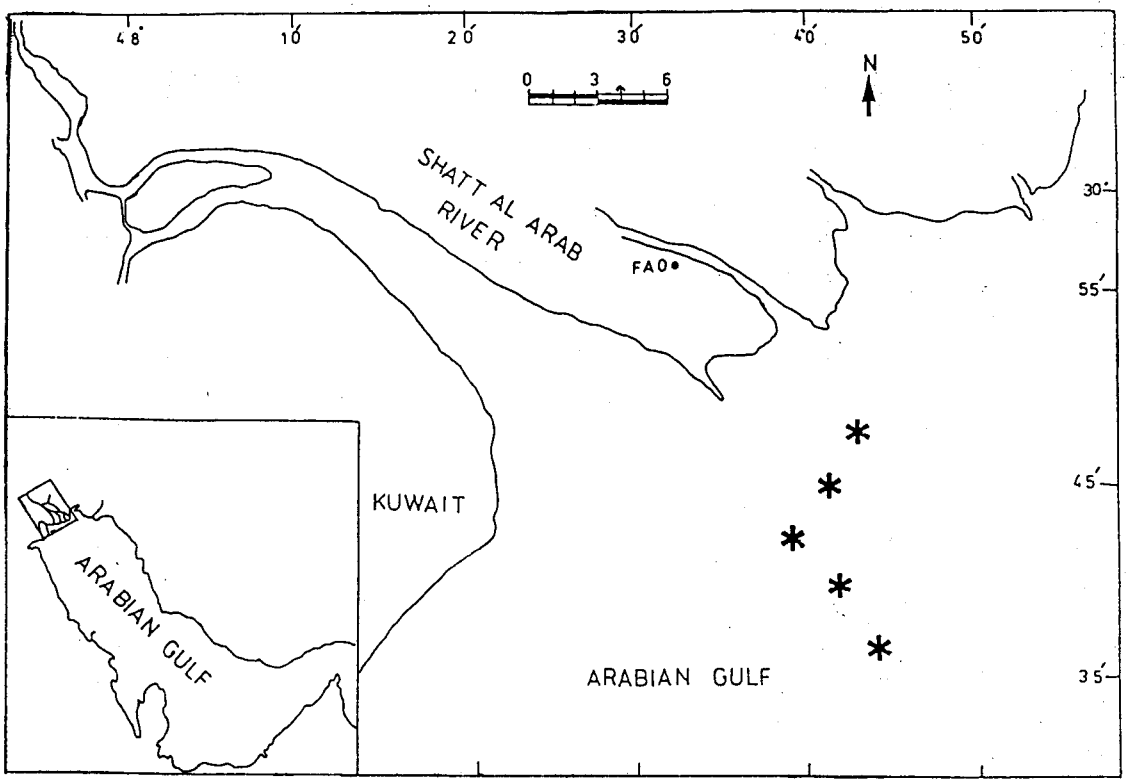
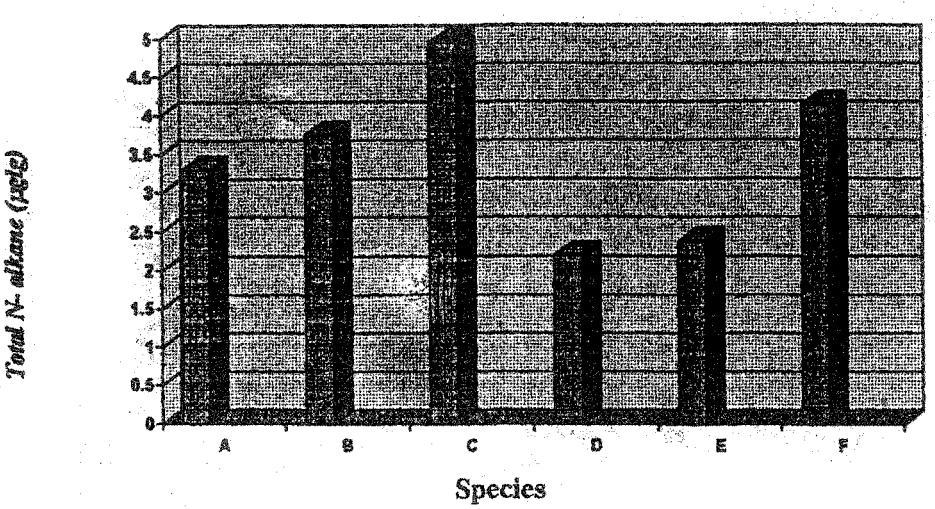


Fig.1) Sample Locations

Table -2- N - alkanes concentrations in mollusca samples (µg/g dry weight) from North - West Arabian Gulf

Carbon number	*Neverita didyma*	*Trachycardium lacunosum*	*Tibia insulaechorab*	*Bassina calophyla*	*Paphia textile*	*Thais mutabilis*
C13	0.01	0.01	0.06	-	0.01	0.04
C14	0.02	0.02	0.09	-	0.01	0.06
C15	0.05	0.03	0.12	0.03	0.02	0.09
C16	0.09	0.05	0.13	0.08	0.04	0.15
C17	0.16	0.28	0.26	0.10	0.13	0.18
C18	0.14	0.20	0.24	0.11	0.12	0.14
C19	0.20	0.30	0.28	0.16	0.14	0.18
C20	0.18	0.28	0.30	0.14	0.18	0.20
C21	0.22	0.32	0.32	0.16	0.22	0.25
C22	0.23	0.26	0.28	0.12	0.16	0.28
C23	0.25	0.25	0.35	0.10	0.18	0.35
C24	0.23	0.20	0.30	0.13	0.16	0.28
C25	0.28	0.22	0.28	0.23	0.14	0.38
C26	0.20	0.23	0.36	0.18	0.16	0.30
C27	0.31	0.29	0.49	0.23	0.20	0.36
C28	0.21	0.22	0.26	0.13	0.18	0.24
C29	0.18	0.24	0.30	0.16	0.13	0.22
C30	0.16	0.18	0.22	0.06	0.09	0.18
C31	0.10	0.10	0.16	0.03	0.06	0.16
C32	0.09	0.07	0.10	0.02	0.05	0.09
Total	3.31	3.75	4.95	2.19	2.38	4.13
Pristane	0.13	0.26	0.22	0.09	0.10	0.16
Phytane	0.12	0.20	0.18	0.08	0.08	0.12
Odd	1.76	2.04	2.62	1.22	1.23	2.21
Even	1.55	1.71	2.33	0.97	1.15	1.92
CPI	1.13	1.19	1.12	1.25	1.06	1.15

- = Not detected



(Fig.3) Total n- alkanes (µg/g) in different mollusca species from NW Arabian Gulf.

A-Neverita didyma
B-Trachycardium lacunosu
C-Tibia insulaechorab
D- Bassina callophyla
E-PaPhia textile
F- Thais mutabilis

Table -3- Comparison of total hydrocarbons in mollusca samples from the Arabian Gulf region •

Country location	Bivalve species	Kuwait crude oil equivlants (µg/g-1)	Total aliphatic hydrocarbons (µg/g-1)
Saudi Arabia Ras Al- Mishab	*Mertrix meretrix (clam)*	570	184
Ras Al- Tanajib	*Mertrix mertrix*	1040	234
Ras Al-Tanajib	*tapes sulcarius (clam)*	2600	475
Ras Al Qurrayyah	*Trachycardium lacunosum (cockle)*	86	143
Bahrain Al Malikiyah	*Pinctada radiata (Pearl oyster)*	3	40
Askar	*Spondylus exilis*	27	124
Askar	*Pinctada radiata*	33	108
UAE Jebel Ali	*Pinctada radiata*	15	59
Oman Al - Gurum	*Saccostrea cucullata (Rock oyster)*	34	88
Masirah (Ras Al Yei)	*Saccostrea cucullata*	30	40
Salalah (Raysut)	*Saccastrea cucullata*	31	27
Iraq North - West Arabian Gulf	as cited in the text	3.56-12.07	2.19- 4.95

•Adopted from fowler *et.al* (1993)

Table -1- Concentration of total petroleum hydrocarbons (µg/g dryweight) in different mollusca sample from North - West Arabian Gulf .

Species	Hydrocarbons range	Mean	Standard deviation	+Standard - error	Fat %
Neverita didyma	7.22 - 8.53	8.09	0.492	0.246	2.60
Trachycardium lacunosum	4.69 - 6.83	5.55	0.679	0.339	1.48
Tibia insulaechorab	10.56 - 12.98	12.07	0.816	0.408	3.85
Bassina callophyla	2.98 - 4.58	3.56	0.684	0.342	0.38
Paphia textile	4.18 - 6.89	5.45	0.890	0.445	1.48
Thias mutabilis	7.96 - 9.51	8.39	0.565	0.282	2.74

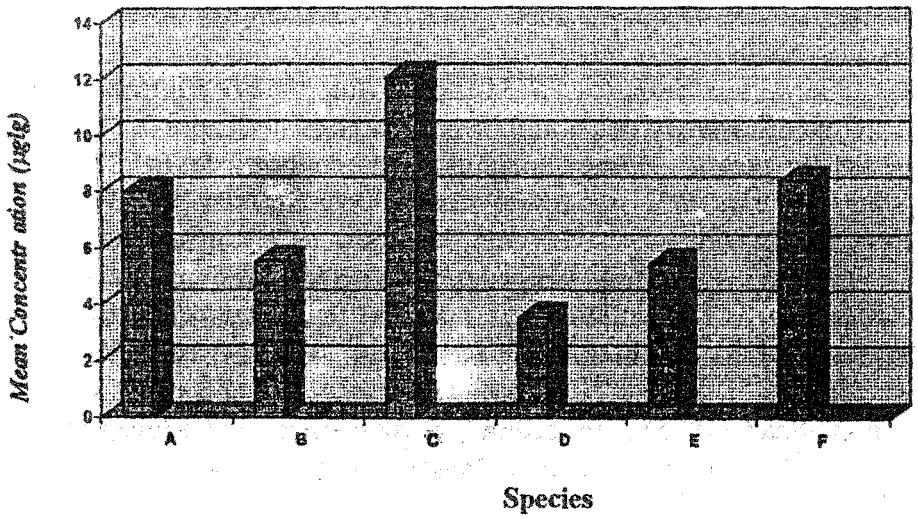


Fig.2.Mean Concentrations (µg/g) of total hydrocarbons in different mollusca species from NW Arabian Gulf.

A-Neverita didyma
B-Trachycardium lacunosu
C-Tibia insulaechorab
D- Bassina callophyla
E-PaPhia textile
F- Thais mutabilis

extracted with fresh solvent. The combined extract were reduce in volume to 10 ml in a rotary vaccum evaporater and was then saponified for 2 hr. with a solution of 4N KOH in (1:1) methanol : benzene . After extraction , the unsaponified matter were separated with hexane and the extract was dried over anhydrous Na_2So_4 concentrated by steam of N_2 for spectroflurometric analysis .

The concentrated extract was cleaned up by column chromatograph . A column filled with 8g each of 5% water deactivated alumina (100-200 mesh) up and silica (100-210 mesh) bottom was used. The extract was then applied to the head of the column and eluted with 50 ml of n-hexane to isolate the aliphatic fractions , then 50 ml of benzen were added to isolate the aromatic fractions . Then the two fractions were reduced to a suitable volume prior to analysis by spectrofluorometer and capillary gas chromatography .

The basic quantitative measurement of total hydrocarbons were made by measuring emission intensity of 360 nm with excitation set at 310 nm and monochrometer slits of 10 nm, All blanks, standard and samples were run at identical instrumental conditions .

For this work, a shimadzu RF-540 spectrofluorometer equipped with a DR-3 was used . Quantification of peaks and identification of n-alkanes in chromatograms was a chieved by perkin - Elmer computing integrator model LC-100 . The fused silica capillary column used was a Wall Coated Open Tubular (WCOT) 50 m xo.25 mm.i.d.SE-30 (methylsilicon) . Heliume was used as a carrier gas with a linear velocity 1.5 ml/min . Operating temperature for detector and injector were 350 and 320c° respectively . The column was operated under temperature programmed conditions from 60c° for 4 min. to 280c° for 30 min . with rtate of 4c°/min .

Fat contents in all mollusca samples determined according to the method presented in Al-Saad (1995) . Procedural blank consisting of all reagents and glass were used during the analysis , were periodically determined .

RESULTS & DISCUSSION

The total petroleum hydrocarbons and fat percentage studied are summarized in Table (1) and (fig-2) . The mean concentration ranged from 3.56 µg/g in *Bassina callophyla* to 12.07 µg/g dry weight in *Tibia insulaechorab* (Basrah crude oil equivalent). Fat content observed was lower in *Bassina callophla* (0.38%) and high in *Tibia insulaechorab* (3.85%). The distribution of n-alkanes are listed in Table (2) with (fig-3) . The range of carbon chain length of n-alkanes for these samples are C_{13}-C_{32} . The concentrations of n-alkanes in these mollusca varied from 2.19 µg/g in *Bassina callophyla* to 4.95 µg/g dry weight in *Tibia insulaechorab*. Anumber of other hydrocarbons were present range (0.09-0.26 µg/g) phytane, range

(0.08-0.20). The Carbon Performance Index (CPI) from C_{13} to C_{32} were calculated separately for each species, and they varied from 1.06 to 1.25 .

Generally , monitoring programmes are established to describe spatial gradients and variation within and between areas of interest , and to registor temporal changes . it is therefore, a basic assumption that the contaminant levels do not vary due to the activity and metabolism of the organism to a degree that skews the patterns at tissue . Attention should therefore be devoted to sampling design and biotic factors , such as metabolic regulation and the ability of the indicator organisms to integrate the contaminant in question over time . The use of different species could hamper comparison between areas because the species could have different accumulation characteristics (Naes *et al*, 1998).

The general profile differences between the species may be associated with their depth and habitat preferences .

All the indicator species are microphagous feeder which can ingest hydrocarbons with food particles , While mussels collected food particles from the water by filter feeding . Although organisms may metabolise hydrocarbon compounds, their metabolic capacity dependes on specific enzymatic systems which differ among groups of aquatic organism (Means, 1998) .

Comparing patterns of hydrocarbons from samples of biota provides useful information a bout possible sources of contamination ; however, certain limitation become appearent when comparing patterns of hydrocarbone in tissue sample patterns in sources . hydrocarbons patterns may come from a variety of sources , including petroleum , different fuel oils, and products of combustion , and there are distinct differences in patterns of hydrocarbons in these material . In addition when released into the environments, all of these materials undergo avariety of changes (eg., evaporation , dissolution in water, bacterial degradation, photo chemicle oxidation) . Moreover biogenic processes such as bioaccumulation , metabolism and depuration of alkanes can influence both the levels and patterns of alkanes present in marine organisms . For this reasons certain differences in patterns of alkanes in different samples are expacted (Brown *et al*, 1996). Thus , relating of hydrocarbons in biota such as mollusca to sources is complicated .

Bivalve mollusca feed by removing fine suspended particles frome large quantities of water (Gosling, 1992) and therefore are exposed to both the water-soluble and the particle associated organic pollutants .

Straight chain alkanes are commonly present in almost all marine organisms (Snedaker *et al*, 1995). The most predominant n- alkanes reported in marine organisms are odd-number carbon chains such as C_{15} and C_{17}, which mainly came from the biosynthesis processes of ingested algae and /or microbial precursors from the marine food web, and propably originated from the decarboxylation of even carbon numbered fatty acids (Zhou *et al*, 1996). Most biogenic n-alkanes of other chain lengths (both odd- and even numbered chains) are usually found only in specific marine organisms . It is interesting to note that very long , even - chain aliphatic n-alkanes from C_{16} to C_{36} peaking at C_{24} to C_{28} , were found to be the major aliphatic hydrocarbons in both formed and wild mussels from Galicia spain (Hermida Amejeiras *et al*, 1994).

The detection of long - chain n-alkanes (C_{13}-C_{32}) in the mussles probably reffect the very intensive ingestion of phytoplankton from the area. An identical range of long -chain n-alkanes was observed by Napolitano and Ackman (1989) in the lipids of the amphipod *Corophium volutator* from Minas Basin Canada, and also occurred in the mussels of dabs (*Limanda limando*) caught in the North sea (McGill *et al*, 1987). Those particular long chain n- alkanes have also been found in the water column, and in few marine organism in the North- west Arabian Gulf (Al- Saad,1995)

The Carbon Performance Index (CPI) greater than one indicating biogenic sources of hydrocarbons in these mollusca (Al-Saad *et al*, 1997). The presence of pristane and phytane in significant concentration support the biogenic of n-alkanes in these organism . The pristane can be a derivative of the phytol in chlorophyll, but is also one of the major hydrocarbon components present in some phytoplankton and various other algal species (Zhou *et al*,1996) other evidence on aquatic biogenic origin of n - alkanes may be derived from diatoms populations which contain a range of n - alkanes from C13 to C31 . Diatoms have been found to be the most dominant group of phytoplankton in North - West regian of the Arabian Gulf (Al - Saad 1995); Therefore, it may be concluded that phytoplankton are the most likely source of n - alkanes in this region .Either way , the high concentration of pristane is a clear indication of intensive feeding on phytoplankton by the mollusca .

The present survey had shown that these organism contain a measurable amount of petroleum hydrocarbons . The components of n-alkanes seem to be derived from biogenic source, moreover, these concentration were very low in comparison with those reported from Arabian Gulf (Table 3).

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مستويات الهيدروكاربونات النفطية والالكانات لإنواع مختلفة من النواعم المتواجدة في شمال غرب الخليج العربي

أ.د. حامد طالب السعد ، عبدالحميد جواد ، اسامة حامد يوسف
مركز علوم البحار – جامعة البصرة
البصرة – العراق

الخلاصة

تتضمن الدراسة الحالية مسح لتعين مدى ومقدار التلوث النفطي لستة انواع من النواعم (Neverita didyma, Trachycardium lacunosum, Paphia textile, Bassina callophyla, Tibia insulaechorab, Thais mutabilis) التي تم جمعها من منطقة شمال غرب الخليج العربي خلال العام ١٩٩٨.

أظهرت هذه الدراسة بان مقدار تركيز الهيدروكاربونات النفطية الكلية التي قيست بجهاز التفلور تراوحت بين 356 مايكروغرام بالغرام في B. callophyla الى 12.07 مايكروغرام بالغرام كوزن جاف في T.insulaechorab بينما تراوحت من 2.19 الى 4.95 مايكرو غرام بالغرام كوزن جاف على التوالي.

توزيع الالكانات اظهر وجود ذرات الكاربون المفردة والمزدوجة من ذرات كاربون 13 الى 32 مع وجود بعض مركبات الهيدروكاربون في هذا الجزء الالفاتي متضمنة البرستان حيث تراوحت تراكيزه من 0.26 – 009 مايكروغرام بالغرام والفايتان من 0.20 – 0.08 . اما قيم ذرات الكاربون المفردة الى المزدوجة للالكانات من كاربون 13 الى 32 فتراوحت من 1.0 الى 1.25 والتي اعطت دليلاً على المصادر الحيوية لهذه المركبات في هذه الكائنات الحية.

وبصورة عامة اوضحت الدراسة بان مستويات الهايدروكاربون والالكانات في هذه النواعم وهي اقل مما هو مسجل لمناطق اخرى في الخليج العربي.