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ALIPHATIC AND AROMATIC COMPOUNDS IN ZOOPLANKTON AND BACTERIA SAMPLES FROM SHATT AL-ARAB ESTUARY

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ABSTRACT

Aliphatic and aromatic compounds were determined in zooplankton and bacteria samples taken from Shatt Al-Arab estuary. Concentration of hydrocarbon in zooplankton varied from 0.58 to 6.78 with a mean values of 4.45 Ug/g dry weight. These concentration reflect the normal habitat and / or feeding strategies of the organism. The present data is lower compared to that reported for the Arabian sea and this suggest that overall effect of oil pollution on marine zooplankton are neglible, and no detectable impact of recent acute pollution on the zooplankton community.

Bacteria also contain hydrocarbons range from 0.10 to 2.08 with a verage value of 1.4 Ug/g dry weight. These concentrations suggested that initially there were hydrocarbon degrading microbs in the water and sediment.

Zooplankton samples, show different alkanes patterns which may be a reflection of difference in metabolism, feeding patterns or maturity. Pristane and phytane were detected in zooplankton samples, while squalane was not detected and presumably it had metabolised. Zooplankton may accumulate hydrocarbons by grazing on particulate matter or organisms and the presence of some odd and

even carbon with UCM corroborate this. The high CPI value reflect the biogenic nature of n-alkanes.

The analysis of representative samples of heterotrophic bacteria showed that n-alkanes distribution between C13 to C30. There were two patterns of low and high molecular weight, the low molecular weight < C20 with predominance of C17, C18 and C19 and high molecular weight > C20 without carbon number predominance. The samples showed pristane and phytane with UCM and CPI greater than one indicating biogenic sources.

Several PAH were detected in zooplankton samples including Acenaphthene, Fluorene, B(ghi)P and B(a)P, while only five compounds of PAH were detected in bacterial samples including Acenaphtene, phenantherene, Anthracene, B(ghi)P and B(a)P.

INTRODUCTION

Organisms accumulate many contaminants from their environment (i.e., from seawater, suspended particulate matter, sediment, and food). Feild and laboratory studies have shown that contaminant concentrations in some marine organisms reflect concentrations in their enviroment (UNEP), 1993).

The incorporation of hydrocarbons from the environment into marine organisms has been shown to occur via adsorption of dissolved compounds from the water as well as by ingestion. Whether these compounds are then metabolized by the organisms, directly released, or subsequently transferred along the food web is known in very few cases (Cripps, 1989, 1990). There is some evidence that food web magnification dose not occur (Blumer et al., 1971). Marine organisms can synthesize hydrocarbons (Sallot, 1981) but it often difficult to distingulish petroleum pollutants from the natural (biogenic) hydrocarbons produced by the organisms themselves or transferred along the food web (Cripps, 1989).

Organisms produce their own hydrocarbons from their food sources. In both terrestrial and marine organisms, the synthesized normal alkanes are predominatly odd-numbered carbon chains (Saliot, 1981). Petroleum can be distingulshed from biogence hydrocarbons by their differences in distribution and compostion. Gas chromatography readily shows differences in n-alkanes distribution and is, in general, the most widely accepted technique used for analysis.

This work was undertaken to determined the distribution and concentrations of total petroleum hydrocarbons, normal alkanes and Polycyclic Aromatic Hydrocarbons (RAH) in bacteria and zooplankton samples taken from Shatt Al-Arab estuary and North-West region of the Arabian ulf using spectrofluorometry and high resoultion capillary gas chromatography.

MATERIALS AND METHODS·

Zooplankton and Bacteria samples were collected from Shatt Al-Arab estuary. Zooplankton were collected by pulling two nets (mesh 200 Um) at a speed of 3 knots under water surface for 15 min. The sample was transfered into a clean glass jar and stored at -20 C untill needed. The collected samples were passed on glass fiber filter paper (GF/F) and freeze-dried.

Water sample and 1 g of sediment sample were grown seperatelly on 100 ml Trypotone Soya broth (Oxoid) CM129 in 250 ml flasks. The flasks (20 in total) were incubated at 20-$^{+}$ C for 48 hours in a shaking incubator. The 48 hours growth was washed 6 times with sterile water and the pooled product was freeze-dried.

Extraction procedure employed was based upon that of Grimalt and Olive (1993). Exactly 3g of dried zooplankton and 1 g of dried bacteria were placed in a preextracted cellulose thimble and soxhlet extracted with 150 ml methanol: benzene (1:1 ratio) for 24 hours. At the end of this period the extract was transferred to a storage flask and the sample was further extracted with fresh solvent. The combined extracts were reduced in volume to ca 10 ml in a rotary vacuum evaporator and

was then saponified for 2 hours with a solution of 4N KOH in 1:1 methanol:benzene.

After extraction the unsaponified matter with hexane, the extract was dried over anhydrous Na_2So_4, concentrated by stream of N_2 for UVF analysis.

The concentrated extract was cleaned up by column chromatography. A column filled with 8 g each of 5% water deactivated alumina (100 - 200 mesh) top. and silica (100 - 200 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 fraction and 50 ml of benzene to isolated the aromatic one. Both fractions were reduced to a suitale volume prior to analysis by capillary gas chromatography.

Fluoresence measurements were made by measuring emission intensity at 360 nm with exciatation set at 310 nm and monochrometer slit of 10 nm. All blanks standards and samples were run at identical instrumental conditions. For this work a shimadzu RF-540 spectrofluorometer equipped a DR-3 data recorded was used.

For this study a Perkin-Elmer sigma 300 capillary gas chromatograph, equipped with Flame Ionization Detector (FID) and splitless mode injection port was used. Quantification of peaks and identification of hydrocarbons in chromatograms was achieved by Perkin-Elmer computing integrator model LC-100. The fused silica capillary column used was a wall Coated Open Tubular (WCOT) 50 m X 0.25 mm i.d. SE-30 (methylsilicone). Helium was used as a carrier gas with a linear velocity of 1.5 ml/min. Operating temperatures for detector and injector were 350 and 320 C respectively. The column was operated under temperature programmed conditions (4 C/min) for 60 C to 280 C with an isothermal period of 30 min at the end for aliphatic fraction, while for aromatic fractions (4 C/min) for 70 C at 0 min to 280 C with an isothermal period of 30 min at the end. The Unresolved Complex Mixture (UCM), which also called unresolved envelop was measured by planimetry.

RESULTS AND DISCUSSION

Petroleum hydrocarbons concentrations in zooplankton varied from 0.58 to 6.78 Ug/g dry weight expressed as Kuwait crude oil equivalents with a mean concentration of 4.45 Ug/g (Fig. 1), while hydrocarbons concentration in bacteria ranged from 0.16 to 2.98 Ug/g with a mean values Of 1.41 Ug/g (Fig.1).

Zooplankton contained n-alkanes in the carbon number range from C13 to C32 with a total of 13.16 Ug/g dry weight (Table 1). The Carbon perference index (CPI) values equaled to 1.31, an indicative of biogenic sources. The distribution of n-alkanes showed a predominance of odd and even carbon number (Fig.2) Pristane concentration was 1.02 Ug/g, while that of phytane was 0.97 Ug/g. Bacteria also contained n-alkanes ranging in carbon number from C13 to C30 (Table 1 and Fig.2) with a total of 38.28 Ug/g dry weight. Isoprenoid hydrocarbons such as pristane and phytane were present in bacteria samples with values of 2.56 and 1.08 Ug/g respectively (Fig.2), while high value of CPI (1.15) indicating a biogenic origin. Both chromatograms of zooplankton and bacteria samples contain UCM as shown in Fig.2 . Zooplankton were observed to contain few polycyclic Aromatic Hydrocarbons (PAH) compounds (Fig. 3). The concentrations of Acenaphthene, phenanthrene, Anthracene, and Benzo (ghi) perylene were 2.24, 3.62, 6.13 and 1.92 ng/g dry weight respectively. The total PAH was 13.21 ng/g.

Anumber of PAH compounds were derected in bacteria (Fig. 3). Those were Acenaphthene phenanthrene, Anthracene, B(ghi)P and B(a)P with concentrations of 3.25, 2.68, 5.27 and 4.12 ng/g dry weight respectively. The total PAH in bacteria was 19.04 ng/g.

The zooplankton community was dominated by copepods, Cladocera, Chaetognaths and many invertebrate larvae which were available during the sampling period.

Price et al., (1993) reported that following the oil spill in the North-West Arabian Gulf during 1991, number of biomass of zooplankton were considerably reduce within and near the boundaries of slick. Many

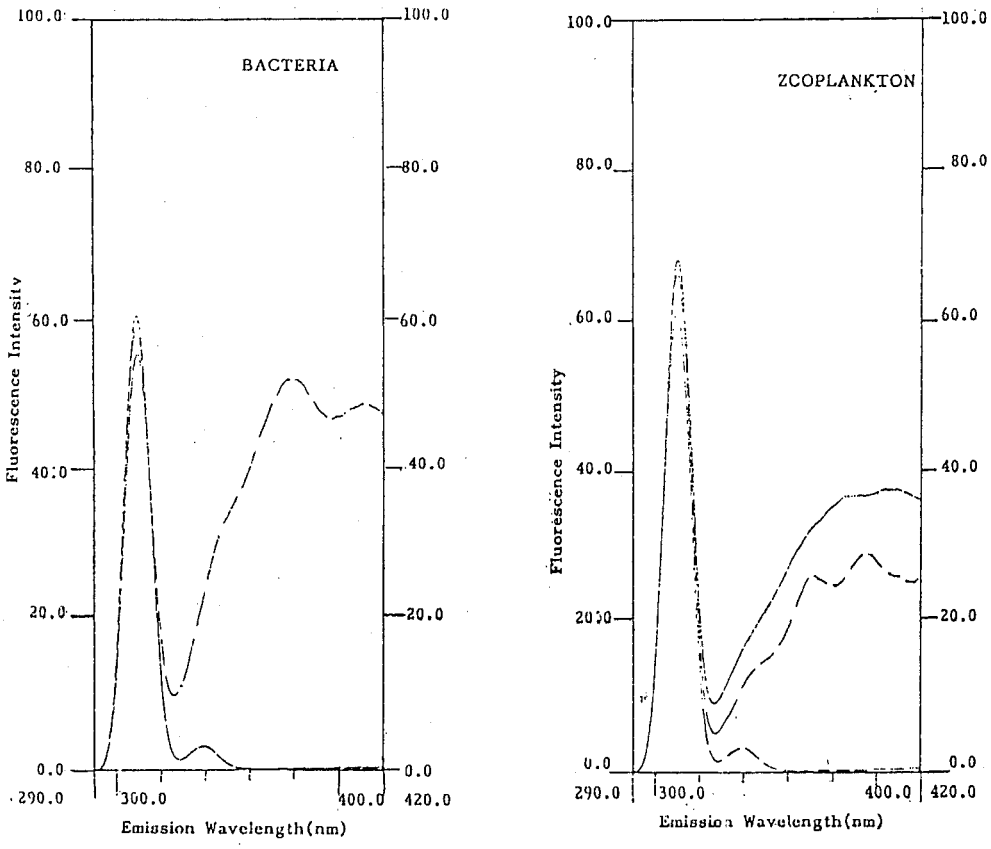

Fig. 1. Fluorescence spectra of hydrocarbons extract from Bacteia and Zooplankton samples

Table-1-

Concentrations of n-alkanes in Zooplankton and Bacteria samples (µg/g dry weight) from Shatt Al-Arab estuary and NW Arabian Gulf

Carbon Number	N-alkanes Concentrations	
	Zooplankton	Bacteria
C13	0.04	0.25
C14	0.06	0.29
C15	0.08	2.14
C16	0.09	2.22
C17	1.65	3.97
C18	1.03	3.85
C19	1.44	2.25
C20	0.98	3.16
C21	1.21	3.20
C22	0.93	2.95
C23	1.16	3.57
C24	0.85	2.09
C25	0.93	2.12
C26	0.74	2.03
C27	0.63	2.01
C28	0.36	1.06
C29	0.23	1.03
C30	0.55	0.09
C31	0.11	—
C32	0.09	—
Total	**13.16**	**38.28**
ODD	**7.48**	**20.54**
EVEN	**5.68**	**17.74**
CPI	**1.31**	**1.15**
PRI	**1.02**	**2.56**
PHY	**0.97**	**1.08**
PRI?PHY	**1.05**	**2.37**
C17/PRI	**1.61**	**1.55**
C18/PHY	**1.06**	**3.56**
UCM	**4.58**	**13.08**

CPI = Carbon Perference Index.

UCM ≙ Unresolved Complex Mixture.

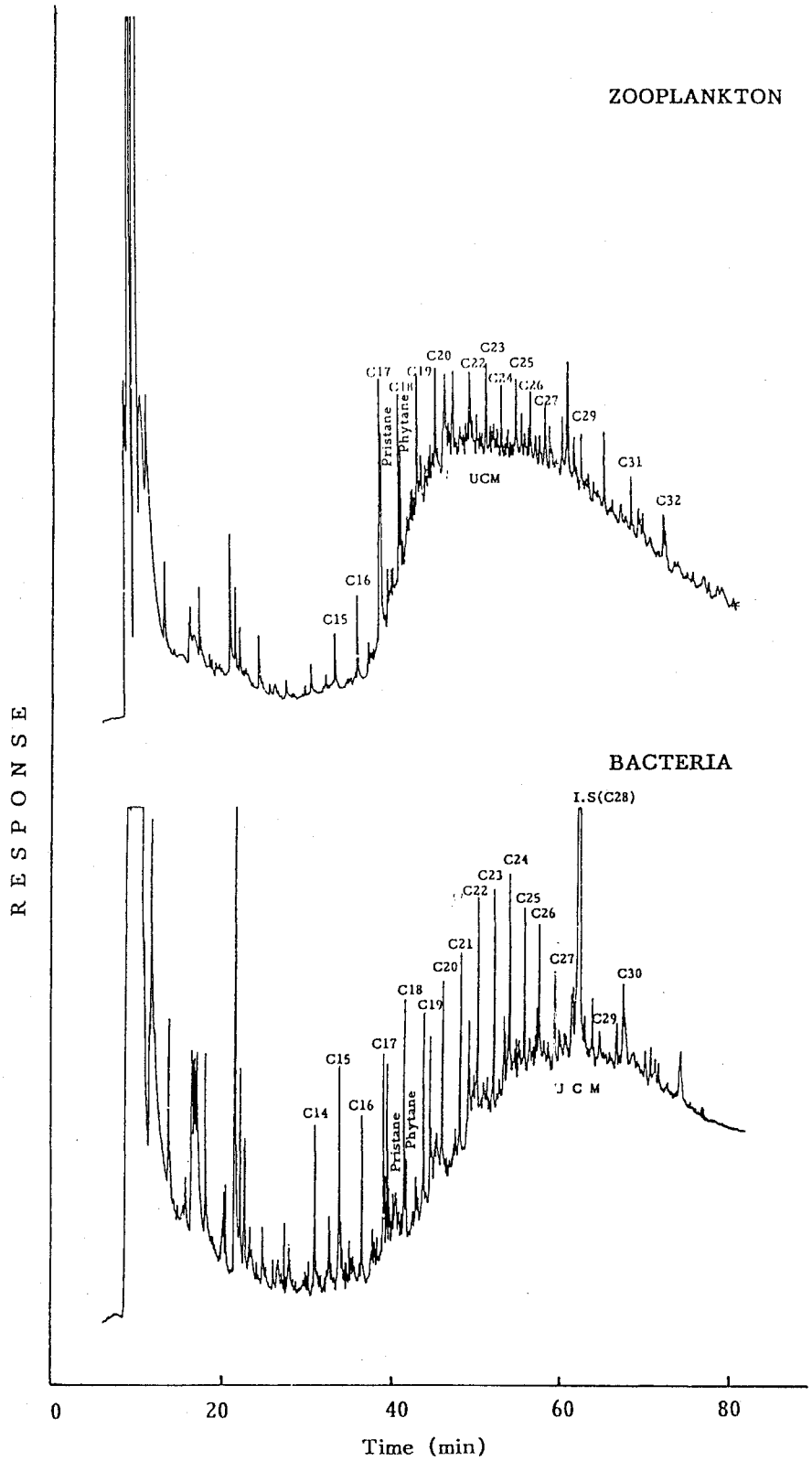

Fig. 2, Representative capillary gas chromatograms (GC/FID) of n-alkanes in Zooplankton and Bacteria samples.

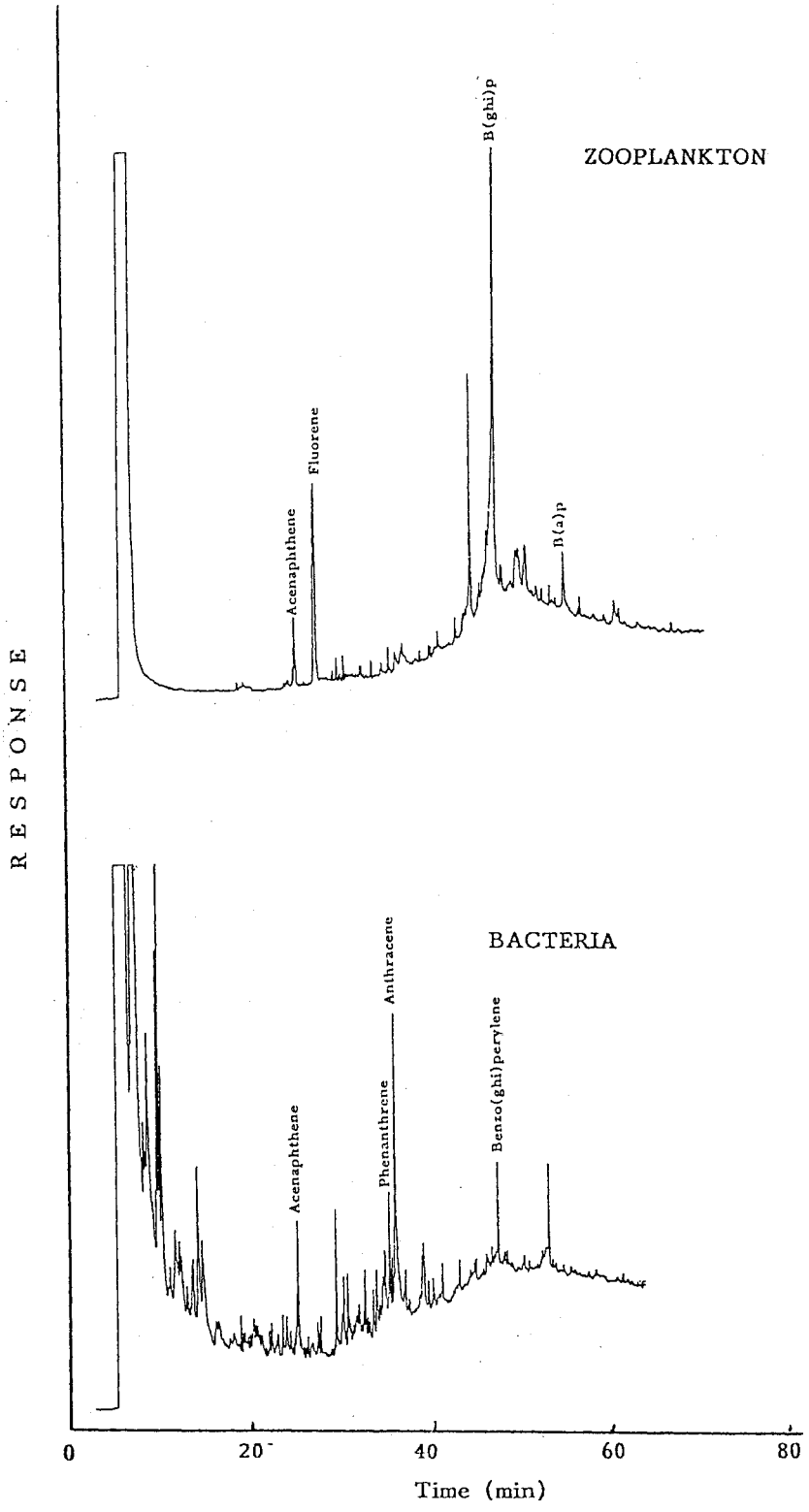

Fig. 3. Representative capillary gas chromatograms (GC/FID) of PAH in Zoopalnkton and Bacteria samples

zooplankton take up a variety of petroleum hydrocarbons from either food and/or water. Thus the total hydrocarbons in the zooplankton reflect the normal habitat and/or feeding strategies of the organisms. Zooplankton are effective in packaging both soluble and particulate hydrocarbons residues into fast sinking fecal pellets. Therefore Burns et al., (1985) speculated that the pattern of petroleum hydrocarbons seen in particulate samples is depended on the type and relative abandance of predominant zooplankton in the water column. Zooplankton can metabolize and extract hydrocarbons. It is observed that they can ingest large quantities of small droplets of oil and eliminate them in from of fecal matter (up to 7 % by weight, Conover, 1971). Zooplankton also take up dissolved hydrocarbons from the water. The zooplankton posses enzyme systems which metabolize the hydrocarbons to various hydroxylated metabolites which are later excreted (Lee, 1975). This mechanism of metabolization and excretion might be responsible for the significant differences in petroleum hydrocarbons concentration in zooplankton. However the present data lower compared to that reported for the Arabian Sea (Sen Gupta et al., 1993) and this suggest that the overall effect of oil pollution on marine zooplankton are negligible.

These finding agree with the result of ROPME (1991) and Al-Yamani et al., (1993) in the Arabian Gulf, they found no detectable impact of recent acute pollution of 1991 of the Arabian ulf on zooplankton community.

The concentrations of hydrocarbons in bacteria suggest that initially there were hydrocarbon-degrading microbs in the water and sediment, and growth of these microbs was inhanced after the addition of petroleum hydrocarbons (Leahy and Colwell, 1990). In aquatic environment, bacteria are generally considered to represent the predominant hydrocarbon-degrading element of the microbial community (Leahy and Colwell, 1990). Adaptation of microbial communities of hydrocarbons, i.e., increase in rates of transformation of hydrocarbons associated with oil-contaminated environments, has been reported in several studies. Walker et al., (1975) studies South-Louisiana crude oil

and observed greater degradation of mixed-hydrocarbon substrate by sediment bacteria from oil-polluted harbor than bacteria from relatively unpolluted environment.

The physical form of petroleum greatly affects the rate of breakdown of hydrocarbons, mainly through a simple surface area relationship. Temperature, oxygen concentration, nutrient, salinity, pressure, water activity of sediment and PH are all effective on the rate of biodegradation (Leahy and Colwell, 1990).

Delille and Jillant (1990) had reported that the introduction of hydrocarbons to marine environment induces rapaid increases in bacterial number. The rate of degradation on hydrocarbons depends on the amount of bacteria present which essentially variable. There are more than 25 genera of hydrocarbon degrading bacteria which has been isolated from marine environment (Floodgate, 1984).

There has been many reports on zooplankton capable of producing n-alkanes (Saliot, 1981; Saliot, et al., 1982 and Cripps, 1990). Zooplankton samples show different alkane patterns which may be a reflection of difference in mmetabolism, feeding patterns or maturity (Saliot, 1981). The total n-alknes obtained in the present study were calculated for C13 to C32 compounds (Table. 1). C15, C16, C17 and C18 probably originated from these zooplanktons (Avigan and Blumer, 1968).

The. predominance of isoprenoids pristane which usualy occures in high concentration (1-3 % of total body lipid) in different zooplankton species (Saliot et al., 1982) were commonly encountered in the present samples, and their abundance could be related to the presences of copepoda and other zooplankton species (Han and Calvin, 1969; Saliot, 1981 and Saliot et al., 1982). Pristane is widely spread in the food web and occurs as a major component of some copepod, where it is known to be derived from the phytyl moiety of chlorophyll present in algal diet of copepods (Burns et al., 1985). Squalane was not detected in zooplankton samples and presumably it had metabolised, at it is a precursor in the biosynthesis of cholestrol (Cripps, 1990). Zooplankton may accumulate hydrocarbons by grazing on particulate matter or organisms)Cripps,

1990), and the presence of some of odd and even carbon with UCM corroborates this (fig. 3). The high carbon perference index (CIP) value reflect the biogenic nature of n-alkanes in these zooplanton samples.

Bacterial communities contributes considerably into the different biogenic sources of n-alkanes in the aquatic environment. The analysis of representative samples of total heterotrophic bacteria from the study area showed that n-alkanes distribution was between C13 to C30 (table 1 and fig. 3). There were two patterns of low and high molecular weight , C20 with a predominance of C17, C18 and C19 and the high molecular weight > C20 without carbon number predominance. The samples showed pristane and phytane with UCM and CPI greater than one indicating biogenic souces.

In the present study several PAH were detected in zooplankton samples, these PAH have also been detected in zooplankton samples from Antarctic and North Sea by Cripps (1990). The higher concentrations of PAH (13.21 ng/g) dry weight in zooplankton sample may be an indicator of accumulation from prey. Zooplankton may accumulate PAH by grazing on particulates (Cripps, 1990). Many species of these zooplankton are known as grazers or appotunistic particle feeders (Tissier and Saliot, 1983). This will lead to enrichment of PAH in their body, which would be without selection of individual PAH. Those PAH which are not found in zooplankton must have been either metabolised or rejected as fecal matter.

Several species of bacteria have been shown to synthesis PAH (Zobell, 1971 and Zitko, 1975). Benzo(a) pyrene is one of the compound which can be synthesised by Bacillus sp. (Saliot, 1981). The possible biosynthesis of PAH in recent sediment was studied by Hase and Hites (1976); a mixed culture of anaerobic bacteria taken from charles river sediments showed no evidence to suggest biosynthesis of PAH, although a bioaccumulation of PAH in the medium was established.

Farrinhton et al., (1986) found that microbial processes in sewage sludge being a likely sources of some PAH. In the present study the role of bacteria in providing a biogenic source of PAH to the environment was apparent.

As a conclusion: both bacteria and zooplankton samples contained hydrocarbons. Zooplankton contained n-alkanes in the carbon number range from C13 to C32. These alkanes could be a result of the different biosynthetic hydrocarbons from different zooplankton species, or taking-up from different food sources or water masses and different biosynthetic hydrocarbons resulting from environmental variations. Also bacteria contained n-alkanes in the carbon number range from C13 to C30. The presences of odd and even carbon with UCM and high CPI values reflect the biogenic nature of this alkanes. PAH were also detected in zooplankton and bacteria samples.

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المركبات الالفاتية والاروماتية لعينات الهائمات الحيوانية والبكتريا المعزولة من مصب شط العرب

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المستخلص

قيست كمية ونوعية المركبات الالفاتية والاروماتية في عينات الهائمات الحيوانية والبكتريا المتواجدة في مصب شط العرب. تراوحت تراكيز الهايدروكاربونات المتواجدة في الهائمات الحيوانية من ٥٨ ,٠ الى ٦, ٧٨ مع معدل بلغ ٤٥ ,٤ مايكرغرام بالغرام كوزن جاف، هذه التراكيز تعكس طبيعة المعيشة او/و استراتيجية التغذية لهذه الكائنات. اوضحت الدراسة بان القيم الحالية قليلة مقارنة مع ما مسجل في البحر العربي، وهذا يعكس بان التلوث الذي حصل في المنطقة على الهائمات الحيوانية قليل وان مجاميع الهائمات الحيوانية غير متاثرة بحادثة التلوث النفطي لعام ١٩٩١ .

البكتريا احتوت على هايدروكاربونات تراوحت من ١٠ ,٠ الى ٨ ,٠ ٢, مع معدل بلغ ٤ ,١ مايكروغرام بالغرام كوزن جاف . هذه التراكيز اوضحت بان هنالك احياء دقيقة في الماء والرواسب قادرة على تكسير مركبات النفط .

بينت الدراسة وجود مركبات الالكانات الاعتيادية في الهائمات الحيوانية والتي قد تتأتى من عدة عمليات كاختلاف في الفعاليات الفسيولوجية ، طبيعة التغذية او النضج كذلك امكن الكشف عن مركبين البرستين والفايتين ، اما السكوالين فلم يتم الكشف عنه، وان الهائمات الحيوانية تجمع الهايدروكاربونات عن طريق التغذية على المواد العالقة او على الكائنات ويدعم هذا الاستنتاج وجود ذرات الكاربون المفردة والمزدوجة مع وجود المواد غير الذائبة وان القيم العالية لمعامل تفضيل الكاربون يعكس الطبيعة الحياتية لمركبات الالكانات الاعتيادية .

تحليل عينات البكتريا اوضحت بان توزيع الالكانات الاعتيادية تراوح من كاربون ١٣ الى كاربون ٣٠ وان هنالك نموذجين من الاوزان الجزئية القليلة والعالية، فالاوزان الجزئية القليلة التي هي اقل من ذرة كاربون ٢٠ والتي فيها سيادة لذرات الكاربون ١٧ و ١٨ و ١٩ ، والاوزان الجزئية العالية التي هي اكثر من ذرة كاربون ٢٠ مع عدم وجود سيادة فيها لاي من ذرات الكاربون وان وجود مركبات البرستين والفايتين والمواد غير الذائبة وقيمة معامل تفضيل الكاربون العالي دليلا" على المصادر الطبيعية لهذه المركبات .

تم الكشف عن المركبات الاروماتية المتعددة الانوية في الهائمات الحيوانية وتضمنت الاسينفثالين ، الفلورين ، البنزو(كاي) بريلين والبنزو(اي) بايرين بينما خمس مركبات فقط وجدت في عينات البكتريا تضمنت الاسنفثين ، الفينانثرين ، الانثراسين ، البنزو(كاي) بريلين والبنزو(اي) بايرين .