Distribution of N-Paraffins in Sea-Grasses, Benthic Algae, Oysters and Recent Sediments from Terminos Lagoon, Campeche, Mexico

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INTRODUCTION

To determine the presence of petroleum hydrocarbons in the components of a marine ecosystem one must first evaluate the relative quality of the hydrocarbons biosynthesized in the system. In this way it is possible to distinguish some of the biogenic hydrocarbons series occurring in marine organisms and sediments from those of fossil origin (CLARK and FINLEY, 1973).

Organisms possess specific biosynthetic pathways which favor the production of hydrocarbons in preferred size ranges. Crude oils and oil products, on the other hand, are wide range mixtures that contain molecules of different sizes in fairly even distribution.

The primary purpose of this study is to establish the distribution of biogenic n-paraffins in marine "sea-grasses", benthic algae, invertebrates and recent sediments from Terminos Lagoon, Campeche, Mexico. Considering the uncontaminated nature of this coastal region the results of this study will provide a basis for the assessment of future man-induced alterations.

METHODS AND MATERIALS

The sampling sites are presented in Fig. 1.

Marine grass-like plants, Thalassia testudinum, Halodule beaudetii, Syringodium filiforme, Ruppia maritima and the benthic algae Acanthophora spicifera, were collected by hand in one to three feet of water in the area adjacent to Real Inlet. Oyster samples were obtained from banks located at Boca de Atasta, Palizada vieja and Panlau. Sediment

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samples were collected with a dredge which sampled the upper 10 cm of the sediments.

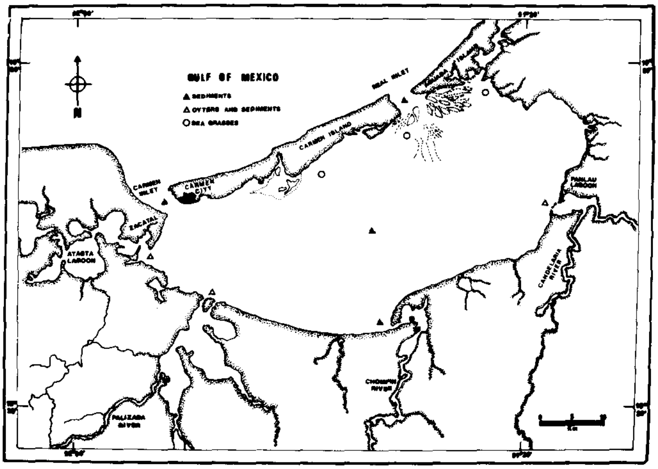

Fig. 1: Terminos Lagoon, Campeche, Mexico, sampling stations.

After collection the samples were placed in glass jars properly washed for hydrocarbon analysis, and stored at -20 °C.

The extraction procedure was conducted on 5 g of the dried material in two stages using 200 ml of methanol and 200 ml of benzene. All the solvents (methanol, benzene, and hexane) were distilled twice and passed through a silica-packed glass column (CLARK and BLUMER, 1967).

All-glass soxhlet extraction apparatus was used throughout. Each extraction was carried out for about 12 hours and the two organic phases were combined. The solvent was then removed using a rotary evaporator under a stream of nitrogen to a final volumen of 100 ml. Lipids were saponified in 5% methanolic KOH for about two hours. Nonsaponifiables were removed from saponification mixtures by extraction with hexane.

N-paraffins were separated from aromatic hydrocarbons by column chromatography on silica gel (Woelm) 200 mesh by elution with 50 ml of hexane. The solvent was evaporated first to near dryness and completed in a weighted vial

TABLE 1

Percent composition of the alkane fractions of four "sea-grasses" and one benthic algae, from Terminos Lagoon.

	Acanthophora spicifera	*Halodule beaudetii*	*Thalassia testudinum*	*Syringodium filiforme*	*Ruppia maritima*
CPI	1.40	1.24	1.19	1.26	1.09
ppm n-paraffins (1)					
Dry weight	87	103	110	97	127
% n-paraffins					
C14		0.15		0.69	
C15	0.13	6.45		0.50	
C16	2.10	2.13	3.14	1.24	
Pristane	0.44				
C17	37.35	2.77	24.86	7.11	
Phytane					
C18	2.40	9.29	2.20	2.22	
C19	2.69	4.19	3.29	4.29	
C20	2.97	9.77	5.56	5.33	
C21	1.23	10.09	3.54	2.63	
C22	16.47	27.22	4.46	15.27	
C23	2.64	1.94	17.11	14.52	2.89
C24	4.37	22.99	6.90	20.03	6.58
C25	3.42		4.56	1.79	8.70
C26	1.86	2.91		5.69	1.97
C27	2.08	0.04		0.40	57.27
C28				0.22	7.28
C29					10.43
C30				0.14	3.51
C31					0.11
C32				0.50	
C33					
C34					1.13

(1) These values correspond to one sample of 1 kg of freeze dried homogenized plant material. They are mean values of replicate analysis.

under a stream of nitrogen. Gas chromatography was done on a Perkin Elmer 900 gas chromatograph equipped with hydrogen-flame detector. Dual columns (1/4 in x 6 ft) packed with 5% FFAP on Gas Chrom-Q (80/100 mesh) were used.

The temperature was programmed from 60 °C to 270 °C at 6 °C per minute. Helium, flowing at 25 ml/min under 50 psi, was the carrier gas. The chromatography was repeated on the same instrument using the same flow rate and columns of the same size packed with 4% Apiezon L on Gas Chrom-Q (80/100) mesh.

Each hydrocarbon fraction was coinjected with a standard hydrocarbon mixture containing nine normal alkanes from C14 to C28 plus pristane and phytane. The abundance of each component was determined by its corresponding peak area in the chromatograms.

A Model 21-491 Dupont mass spectrometer interfaced to a Varian Aerograph 2700 gas chromatograph was used to identify some of the n-paraffins in the samples.

RESULTS AND DISCUSSION

The n-alkanes of four marine grass-like plants and a benthic algae which occur in Terminos Lagoon were characterized and identified, (Table 1).

Syringodium and Halodule (Diplanthera) showed a broader range of hydrocarbons. These two "grasses" share peaks of 22 and 24 carbons, the main difference being the stronger peaks at C17 and C23 in Syringodium. The ranges and average hydrocarbon lengths were very similar. In addition, there was a slight odd-carbon predominance in the n-C20 to n-C23 region for both plants, Syringodium showed a CPI = 1.26 and Halodule a CPI = 1.24. Total concentration of n-paraffins was also very similar, 97 and 103 ppm dry weight, respectively. The CPI was calculated according to COOPER and BRAY (1963).

The hydrocarbons of Ruppia, on the other hand, were restricted to 23 - 24 carbon range, and the predominant component was a C27 hydrocarbon (57% of the paraffins) with secondary peaks at C25 and C29 and the absence of short chain hydrocarbons. The total concentration of n-paraffins in Ruppia samples averaged 127 ppm dry weight with an odd-carbon predominance (CPI = 1.19).

Thalassia grass exhibited a hydrocarbon dominance of n-heptadecane and n-triacontane, 25% and 17%, respectively, with an odd-carbon predominance (CPI = 1.45) and a total n-paraffin concentration averaging 110 ppm dry weight.

The only benthic algae studied, the rodophycean *Acanthophora spicifera*, had a high content of n-heptadecane (37% of the paraffins) and a secondary maximum at n-C22. The total n-paraffins content of this algae was slightly lower than those determined in the "sea-grasses", with 87 ppm dry weight. The odd-carbon predominance of *A. spicifera* (CPI = 1.40) was within the range determined in the "sea-grasses".

In contrast to the results reported by ATTAWAY *et al.* (1970), for *Syringodium filiforme* and *Halodule* sp. (*Diplanthera* sp.) from the Texas coast, the plants from Terminos Lagoon showed major peaks of 22 and 24 carbons. In the Texas plants major peaks corresponded to C21 and C23 n-paraffins.

Thalassia plants from Terminos Lagoon also showed slight differences in their n-paraffins distribution, as compared to the Texas plants. Among the 17, 19, 21 and 23 carbon peaks of the Texas plants only two, C17 and C23, were dominant hydrocarbons in the Terminos Lagoon plants. The n-paraffins pattern in *Ruppia* sp. was very similar in both regions.

In general, hydrocarbons isolated from marine organisms uncontaminated by petroleum hydrocarbons are not very complex mixtures and usually have a limited boiling range (carbon chain length C12 to C30) with an odd chain predominance. This is particularly true for benthic organisms, such as oysters and clams (FARRINGTON and QUINN, 1973). Analysis of n-paraffins in oysters from three productive areas, presumably free of petroleum contamination, located in Terminos Lagoon were also performed. The GC pattern of hydrocarborns extracted from the oysters showed that n-paraffins were present within the C14 to C32 range with a predominance of C23 in all the samples analyzed, (Table 2).

Total concentration of n-paraffins in *C. virginica* oysters from Terminos Lagoon ranged from 22 to 56 ppm (dry weight) - 3.3 to 8.4 ppm (wet weight). The highest values were determined in oysters from the eastern end of the lagoon.

TABLE 2

Percent composition of the alkane fractions in *C. virginica* from three locations in Terminos Lagoon

	Location		
	Boca Atasta	Palizada vieja	Panlau
CPI	1.2	1.1	1.3
ppm n-paraffins (Dry weight) (1)	37	22	56
% n-paraffins			
C14	0.43	0.02	
C15	2.24	2.63	1.93
C16	0.48	1.49	1.87
Pristane	1.27	1.32	1.58
C17		6.78	2.14
Phytane	1.77	2.80	
C18	0.73	5.07	2.46
C19	1.92	6.05	2.31
C20	2.98	6.57	3.12
C21	12.15	15.68	11.54
C22	2.79	8.69	4.41
C23	20.62	13.66	25.25
C24	1.79	2.56	3.60
C25	4.95	0.86	1.98
C26	0.93	3.27	3.14
C27	0.98	0.75	2.23
C29	0.07	1.63	1.84
C30	1.87		1.21
C31	13.27	9.57	4.06
C32	3.19		0.88

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(1) These values correspond to one sample for each site, and each sample consists of the homogenized tissues of one hundred oysters. They are mean values of replicate analysis.

ANDERSON et al. (1974) reported on the concentration ranges of n-paraffins in C. virginia from uncontaminated and petroleum contaminated areas of the USA. These were 1 to 2 ppm and 10 to 160 ppm wet weight for the respective areas. The oysters from Terminos Lagoon showed n-paraffins contents slightly higher than those reported for oysters from uncontaminated areas of the USA.

In order to determine the possibility of petroleum uptake by oysters from Terminos Lagoon the recomendations of CLARK and FINLEY (1974) were followed. The chromatogram of the sample extracts did not show any unresolved peak envelope below the discrete n-paraffin peaks in the range C12-C22. The carbon preference indices (CPIs), which ranged from 1.1 to 1.3, indicated a slight odd-carbon predominance.

The plot of the dry extracted weight of each n-paraffins against the carbon atom per molecule on a semi-log paper exhibited the saw-toothed odd-carbon pattern representative of biogenic n-paraffins (Fig. 2).

In contrast to the samples of water and biota, sediments can be considered reflective of local environmental conditions in a finite period of time.

The analysis of Terminos sediments confirmed the predominance of odd-carbon number n-paraffins in a area for which no analysis was available. The odd-carbon predominance (CPI = 3.5 to 4.5) was well within the range reported by COOPER and BRAY (1963), (CPI = 3.5 to 5.3).

The predominance of odd-carbon number paraffins in recent sediments of Terminos Lagoon suggests that the source of a large fraction of the paraffins may be through organisms indigenous to the sediments or introduced by terrestrial plant detritus.

The concentration of n-paraffins in the surface sediments of Terminos Lagoon ranged from 12 to 56 ppm (dry weight). These figures are within the values recorded ($<$70 ppm) for unpolluted coastal areas and deep marginal seas and basins (NATIONAL ACADEMY OF SCIENCE, 1975).

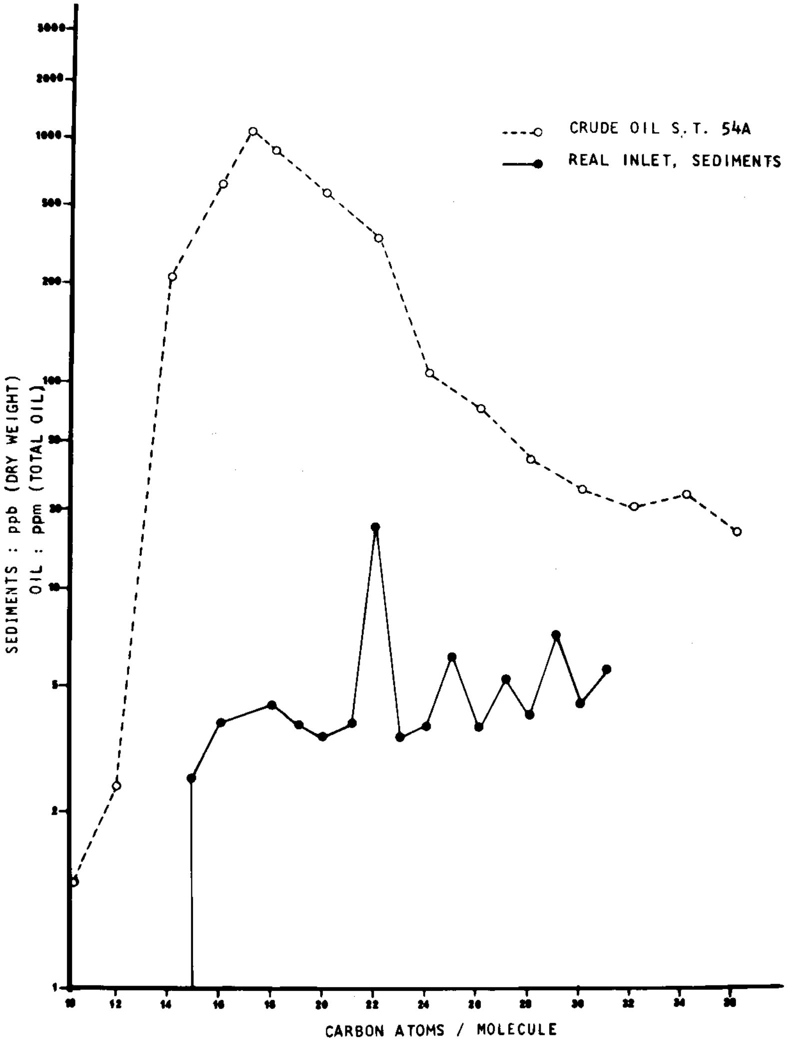

Fig. 2: Line graph used for comparing n-paraffin hydrocarbon patterns, in sediments from Real Inlet, Terminos Lagoon, Campeche, Mexico.

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