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THE USE OF HOPANES TO TRACK *IN SITU* VARIATIONS IN PETROLEUM COMPOSITION IN SURFACE SEDIMENTS

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Abstract: Alterations in the composition of a Light Arabian crude oil has been studied *in situ* during 12 months in an artificially contaminated site. After 12 months of experimentation, C₁₇/Pr and C₁₈/Ph ratios could not be used, because the isoprenoids were no longer stable. In contrast, a high degree of stability of biomarkers, especially hopanes, was observed. These are very useful tools to assess alterations in other less stable classes of saturated compounds (*n*-alkanes and isoprenoids) contained in petroleum. Marked degradation of both *n*-alkanes and isoprenoids (using 17 α 21 β C₃₀ hopane as "internal standard") has been evidenced. The use of biomarkers also made it possible to observe a burying process which occurred down to 6 cm deep after 12 months of experimentation.

INTRODUCTION

Oil pollution of marine environments as the result of human activity is on the increase and causes serious damage to the marine habitat and its wildlife; many adverse effects, invisible to the human eye, remain many years later. To successfully assess sedimentary petroleum pollution, we can further differentiate between natural hydrocarbons and anthropogenic hydrocarbons contained in marine organic matter by reference to their composition, since hydrocarbon composition varies according to its origin. Oil spills can continue to contaminate marine sediments for many years even if they undergo gradual alteration.

In vitro biodegradation studies are straightforward because the experimental parameters are controlled and the quantities of petroleum subjected to biodegradation are precise and not altered by other processes.

Numerous *in vitro* studies have shown that a selective removal of hydrocarbons by bacteria seems to occur roughly in the following sequence : *n*-alkanes (<C₂₅), isoprenoid alkanes, alkyl- cycloalkanes and aromatic compounds^{1, 2}.

In natural environments, it is virtually impossible to assess the sole effect of biodegradation on petroleum, because other physical and / or chemical processes can simultaneously eliminate some individual petroleum compounds or part of the oil without changing its composition. That is why *in situ* studies on patterns of change in hydrocarbons are less developed. In previous works³⁻⁵, in order to study these alterations, concentrations of certain classes of compounds were compared to other products supposed to be more recalcitrant to alteration. By comparing alkane concentrations to those of isoprenoids, an indication of the *in situ* degradation rate can be obtained. C₁₇/Pr (*n*-heptadecane / pristane), C₁₈/Ph (*n*-octadecane / phytane) and ALK/ISO (*n*-alkanes from 14 to 18 carbon atoms / homologue isoprenoids) ratios can provide information on patterns of hydrocarbon alterations^{3, 6}. These ratios have been widely used. In a recent work, Gilbert *et al.*⁷ studied the effect of the *in situ* bioturbation on petroleum alteration patterns over time in a natural environment, and they used these ratios to assess alterations in the saturated fraction of a Light Arabian crude oil artificially added to the water-sediment interface in a Mediterranean coastal area. If these ratios are adequate tools over a short period of time to study the beginning of biodegradation (in bioremediation studies for example), they become meaningless over a longer period. It is now known that isoprenoid alkanes, in some natural conditions, can also be consumed by bacteria⁸⁻¹⁰. Moreover, pristane and phytane are isoprenoids that are commonly detected in coastal marine sediments¹¹ with an abundance depending on the local environment. Some biogenic pristane and phytane can be naturally produced¹² or come from the alteration of chlorophyll¹³, which is why these ratios can be distorted. Besides, isoprenoid alkanes cannot be used to assess the degradation of other more resistant classes of compounds, e.g. polyaromatic compounds.

In some recent studies¹⁴⁻²⁰, authors have begun to look for trace components in crude oil such as hopanes that have previously been of interest as unique biomarkers useable for petroleum exploration. In pollution studies, these compounds are of interest for petroleum fingerprinting. If the inalterability of these compounds can be proved²¹⁻²², under natural environmental conditions, they will be useful for determining the rate of alteration of other more easily degradable petroleum hydrocarbons.

A molecular approach was adopted in order to identify unalterable compounds (over at least a limited period). The aim of the present work was to study simultaneously the *in situ* alteration patterns of *n*-alkanes and isoprenoid alkanes using different ratios and to compare the data provided by the two approaches.

- Experimental strategy: An *in situ* experimental program to determine the patterns of alteration over time of different saturated petroleum compound classes in sediments was conducted at the site chosen by Gilbert *et al.*⁷ for their *in situ* experiment (Figure 1); the experimental strategy was similar to the one they described. In December 1994, PVC cores (11 cm in diameter, 25 cm in length) divided into two series were inserted into sediments. A frozen disk of sediment, 1 cm thick, was placed on the surface of the cores. Half of these disks were contaminated by Light Arabian crude oil at a concentration of 50 g. kg⁻¹ dry weight sediment. The other half were not contaminated and served as controls. A core from each series (contaminated and control) was removed every six months (at 0, 6 and 12 months) and stored frozen (-18°C) until analysis. Each core was sectioned into 2 cm segments and each segment was analysed.

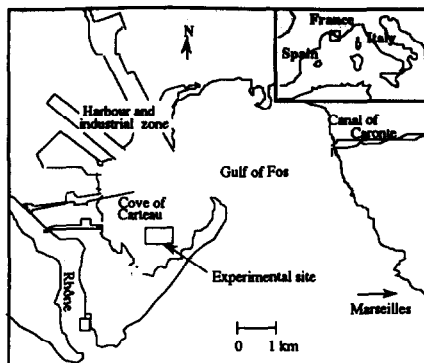


Figure 1: Location of the experimental site in Carteau cove (Gulf of Fos, Mediterranean Sea)

- **Chemical analyses:** Hydrocarbons were extracted from wet sediment (about 50 g accurately weighed) by direct saponification via alkaline hydrolysis. Samples were heated under reflux for 4 hours in a mixture of 0.5 N KOH in 95% methanol/toluene 2/1. After filtration on a glass fibre filter (Whatman GF/C) and separation of the two phases, the aqueous phase was extracted with 3x50 ml of toluene. All toluenic phases containing hydrocarbon compounds were combined, dried on anhydrous MgSO₄, evaporated to a residue and weighed to obtain the total extractable organic matter (EOM).

All or part of the EOM was dissolved in a minimum of *n*-hexane and applied to a half silica (8 g) and half alumina (8 g) (both of these 5% deactivated with water) chromatography column (30 x 1 cm). The aliphatic fraction (F1) was eluted with 30 ml of *n*-hexane on the column. Elutions with 20 ml of *n*-hexane / dichloromethane (9/1) and 40 ml of *n*-hexane/dichloromethane (4/1) yielded an aromatic fraction (F2). Each fraction was evaporated on a rotary evaporator and under a stream of nitrogen and residues were quantified gravimetrically. The sum of the aliphatic fraction and the aromatic fraction gave the total hydrocarbon content. Procedural blanks were carried out between each batch of four samples and no contamination was found.

F1 fractions were diluted in an adequate quantity of *n*-hexane and were analysed by capillary gas chromatography coupled to a mass spectrometer (5890 II HP gas chromatograph and 5989 A HP mass spectrometer) in full scan and selected ion recording (SIR) modes under the following conditions: splitless injection (45 s), HP5-MS (low bleed) capillary column, 30 m x 0.25 mm (i.d) x 0.25 mm, helium as carrier gas, temperature program from 70 to 110°C at 50°C min⁻¹ then from 110°C to 295°C at 5°C min⁻¹ and 295°C for 30 min.

RESULTS AND DISCUSSION

Hydrocarbons were present in all samples. The differences of composition between autochthonous and petroleum hydrocarbons allowed their individual differentiation by GC/MS.

The *m/z* = 71 chromatograms (characteristic of linear and branched alkanes) of the saturated hydrocarbon fractions from upper layers (0-2 and 2-4 cm) showed a more or less important Unresolved Complex Mixture (UCM). At 6 months, down to 4 cm depth, they showed a regular wide range of alkanes (from C₁₂ to C₃₀) in lesser abundances than for the original petroleum. No predominance of odd or even *n*-alkanes was observed.

The alkane abundances decreased as the carbon number increased. The presence of pristane, phytane and other branched alkanes such as norfarnesane, farnesane, norpristane was detected. These chromatographic characteristics correspond to criteria specific to petroleum hydrocarbons mentioned by Requejo and Boehm⁴ and by Kennicutt *et al.*⁵. Chromatograms relative to 12 months down to 6 cm deep showed less clear peaks corresponding to *n*-alkanes. Peaks corresponding to isoprenoid compounds (pristane and phytane especially) clearly emerged from a large UCM. The $m/z = 71$ chromatograms from deeper layers (6-10 cm) were notably different from the upper layers and similar to controls. They showed a strong predominance of odd *n*-alkanes between C₁₅ to C₁₉ which is characteristic of a marine biogenic origin^{23, 24}.

Table I contains diagnostic ratios calculated from contaminated samples over time. These ratios, usually used to determine petroleum degradation in sediments, are based on relative concentrations (assessed using squalane for noncyclic alkanes and androstane for biomarkers). For contaminated samples, the calculated concentrations of individual hydrocarbons are the quantities found in these samples minus those present in the control samples.

Table I
Indices relative to alkanes (linear and branched). See text for signification of these indices .

		Alkanes			CPI
		$\frac{C_{17}}{Pr}$	$\frac{C_{18}}{Ph}$	$\frac{ALC}{ISO}$	
initial deposit layer		2.7	1.8	4.7	1.0
6 months	0-2 cm	2.3	1.1	1.8	1.1
	2-4 cm	2.5	1.1	2.6	1.0
	4-6 cm	1.6	1.1	2.3	3.0
	6-8 cm	1.8	1.7	3.2	3.1
	8-10 cm	2.0	2.1	4.4	2.2
12 months	0-2 cm	3.5	1.4	2.6	1.3
	2-4 cm	2.7	1.1	2.1	1.1
	4-6 cm	1.7	1.4	1.8	1.3
	6-8 cm	1.9	1.7	5.4	3.2
	8-10 cm	2.0	1.8	5.6	2.6

Six months after the petroleum input, while the CPI²⁵ values (relative abundance between odd numbered linear alkanes and even numbered linear alkanes, around 1 for petroleum hydrocarbons²⁶) were the same as the deposited crude oil values, the contaminated samples showed a decrease in the C₁₇/Pr and C₁₈/Ph ratios in the deposit layer and in the 2-4 cm layer compared to the initial values of the deposited petroleum. This corresponds to a selective removal of hydrocarbons, perhaps by bacteria, which seems to occur roughly in favour of *n*-alkanes compared to isoprenoid alkanes^{27, 28}. Twelve months after the hydrocarbon input, the CPI values were still constant and the C₁₇/Pr and C₁₈/Ph ratios were still lower than the initial values, but had increased compared to the corresponding values obtained 6 months earlier. These results mean that isoprenoid alkanes began to be altered at the same time as *n*-alkanes. Thus, isoprenoid alkanes can be used during the first 6 months of experimentation to give an indication of the rate of degradation, but become useless thereafter because they are partially degraded as well.

Some hydrocarbons (hopanes, steranes...) known as biomarkers are often used in petroleum geochemistry to determine the degrees of alteration and maturity characteristic of petroleum in the deposition basins^{29, 30}. Parameters based on these compounds are also the discriminating indices which contribute to the identification

of crude oil spilled in the natural environment and are used in this way in some environmental studies¹⁴⁻²⁰. On the basis of these works, these compounds have also been analysed by GC/MS in SIR mode to obtain fingerprints characteristic of hopanes (Figure 2) and steranes. Attributions have been accomplished by comparison with published chromatograms^{2,16-19}.

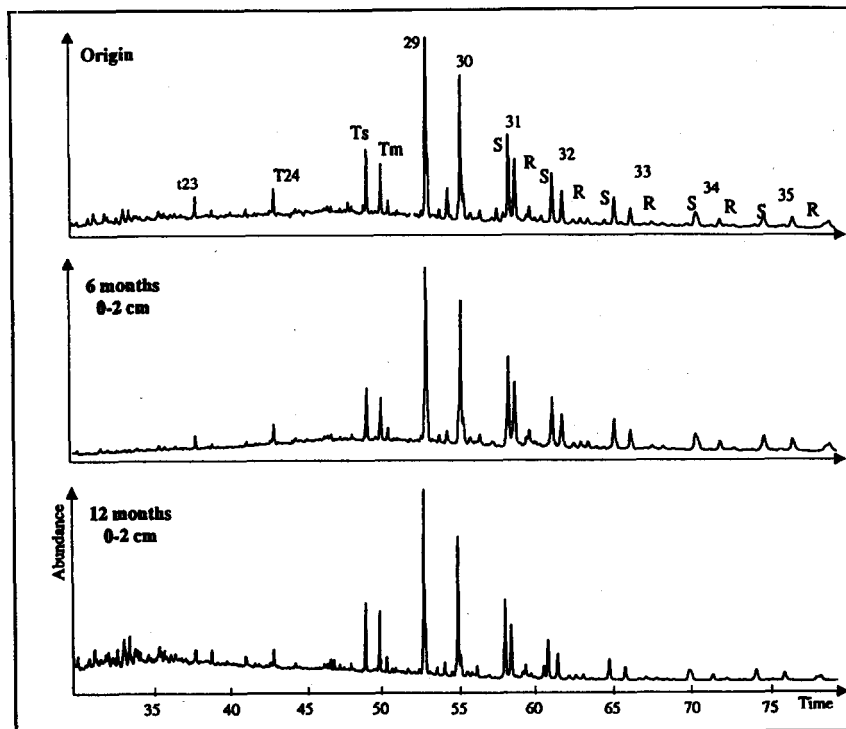


Figure 2: Fragmentograms at ion $m/z=191$ for the 0-2 cm layer at the origin, 6 months and 12 months, respectively. $C_n = C_n$ hopane with $n =$ number of carbon atoms constitutive of the molecules; $t_{23} = C_{23}$ tricyclic terpane; $T_{24} = C_{24}$ tetracyclic terpane; $T_s = C_{27} 18\alpha(H) 22,29,30$ trinormehopane; $T_m = C_{27} 17\alpha (H) 22,29,30$ trinorhopane

Figure 2 represents the fragmentograms obtained for ion $m/z=191$ characteristic of hopanes for the initial deposit layer over time for contaminated cores. For the contaminated samples, analysed after 6 and 12 months, the same patterns as those obtained for the original petroleum were observed.

The indices generally used in geochemistry and in environmental studies are based on comparison of the abundance of 2 or more compounds which are supposed to behave differently, when exposed to environmental conditions. These indices^{2,16-19} were calculated for all contaminated samples and varied with depth (Table II). The values of these indices obtained throughout the duration of the experiment were generally very similar for the upper layer samples (down to 4 cm after 6 months and 6 cm after 12 months) and identical to those obtained for the contaminating petroleum. This result demonstrates that hopanes, steranes and drimanes, have either been altered in the same way during this period or have not been altered. The second hypothesis seems the most probable because these components are known to be very stable. Even if *in vitro* biodegradation studies have shown that these polycyclic alkanes can be biodegraded, in optimal conditions of bacterial

growth³¹, the degradation rate is extremely slow compared to the that of most other saturated hydrocarbons. Moreover, a preferential order of alteration concerning steranes and hopanes has been described²: e.g. for hopanes, 22R isomers are altered before 22S isomers and C₃₄ before C₃₁. This result was not observed after 12 months of our experiment. So hopanes may be considered to be stable for the purpose of this experiment. For the three deeper layers (4-6, 6-8, 8-10 cm), the molecular ratios were significantly different from the ratios found for the deposited petroleum and for the upper layers of the sediment, indicating that these zones had not been contaminated.

Table II

Indices relative to drimanes, steranes and hopanes from the origin to 6 months and 12 months in function of the depth. See text for signification of these indices.

		Drimanes		Steranes				Hopanes				
		H (H+D)	88H 88D	C ₂₇ D C ₂₉ St	C ₂₇ C ₂₉	C ₂₉ S (S+R)	ααα αββ	C ₂₃ C ₃₀	Ts Tm	C ₂₉ C ₃₀ H	C ₃₁ S (S+R)	C ₃₁ C ₃₄
initial deposit layer		0.63	0.88	0.30	0.41	0.43	0.43	0.08	0.54	0.54	0.57	0.82
6 months	0-2 cm	0.64	0.88	0.31	0.40	0.43	0.46	0.08	0.55	0.55	0.57	0.82
	2-4 cm	0.62	0.87	0.30	0.41	0.42	0.43	0.10	0.53	0.56	0.57	0.82
	4-6 cm	0.73	0.90	0.27	0.47	0.44	0.52	0.22	0.45	0.51	0.59	0.92
	6-8 cm	0.81	0.94	0.22	0.45	0.44	0.29	0.23	0.44	0.50	0.59	0.91
	8-10 cm	0.70	0.82	0.28	0.44	0.45	0.46	0.24	0.47	0.48	0.61	0.95
12 months	0-2 cm	0.64	0.88	0.29	0.41	0.42	0.43	0.10	0.54	0.54	0.57	0.84
	2-4 cm	0.61	0.90	0.30	0.42	0.44	0.37	0.08	0.55	0.55	0.56	0.82
	4-6 cm	0.72	0.94	0.27	0.48	0.44	0.42	0.21	0.45	0.51	0.57	0.94
	6-8 cm	0.81	0.97	0.26	0.47	0.41	0.45	0.26	0.44	0.49	0.59	0.96
	8-10 cm	0.71	0.93	0.31	0.49	0.44	0.48	0.25	0.44	0.45	0.60	0.88

H/(H+D) = Σ homodrimanes / (Σ homodrimanes + Σ drimanes); 88H/88D = 88 homodrimane / (88 drimane + 88 homodrimane); C₂₇D/C₂₉St = Σ C₂₇ diasteranes / (Σ C₂₇ diasteranes + Σ C₂₉ steranes); C₂₇/C₂₉ = Σ C₂₇αββ steranes / (Σ C₂₇αββ steranes + Σ C₂₉αββ steranes); C₂₉S/(S+R) = C₂₉S sterane (C₂₉S sterane + C₂₉R sterane); ααα/αββ = Σ C₂₉ ααα steranes / (Σ C₂₉ ααα steranes + Σ C₂₉αββ steranes); C₂₃/C₃₀ = C₂₃ tricyclic terpene / (C₂₃ tricyclic terpene + C₃₀ hopane); Ts/Tm = C₂₇18α(H)22,29,30 trinorneohopane / (C₂₇18α(H)22,29,30 trinorneohopane + C₂₇17α(H)22,29,30 trinorhopane); C₂₉H/C₃₀H = C₂₉ hopane / (C₂₉ hopane + C₃₀ hopane); C₃₁ S/(S+R) = C₃₁S hopane / (C₃₁S hopane + C₃₁R hopane); C₃₁/C₃₄ = Σ C₃₁ hopanes / (Σ C₃₁ hopanes + Σ C₃₄ hopanes).

Hopanes have enabled us to track the burying process, which reached 4 cm after 6 months and 6 cm after 12 months, and was probably induced by bioturbation. These observations corroborate the results of Gilbert *et al* 7. This stability of hopanes also means that they can be used as standards^{21,22} to assess possible alterations in the other less stable hydrocarbons. Using 17α 21β C₃₀ hopane (C₃₀H), which is the most abundant compound of the hopane series, the patterns of alteration in linear alkanes and isoprenoid alkanes were monitored during the 12 months of experimentation.

Table III presents the abundance ratios between several hydrocarbons (obtained at m/z = 71 for linear alkanes and isoprenoids, at m/z = 217 for C₂₇ αββ R sterane and at m/z = 123 for 8β homodrimane) compared to that of 17α 21β C₃₀ hopane (m/z = 191 chromatogram) and all the different hydrocarbon group areas compared to that of the total hopane family. If these ratios do not represent the absolute concentrations of these 3 classes of

hydrocarbons, they are nevertheless of use in obtaining information about their patterns of alteration. The method using 17α 21β C_{30} hopane as reference confirms that no variation of sterane and drimane has occurred during the experiment.

Table III

Indices relative to alkanes, drimanes, steranes and to selected individual hydrocarbons.

		$\frac{\Sigma Alc}{\Sigma H}$	$\frac{\Sigma D}{\Sigma H}$	$\frac{\Sigma S}{\Sigma H}$	$\frac{C_{17}}{C_{30H}}$	$\frac{C_{18}}{C_{30H}}$	$\frac{Pr}{C_{30H}}$	$\frac{Ph}{C_{30H}}$	$\frac{8\beta H}{C_{30H}}$	$\frac{C_{27\alpha\beta\beta R}}{C_{30H}}$
initial deposit layer		294	2.79	0.09	186	168	69	93	5.88	0.07
6 months	0-2 cm	135	2.55	0.09	71	49	35	45	5.24	0.07
	2-4 cm	124	2.49	0.09	67	54	29	48	5.51	0.07
	4-6 cm	26	0.04	0.12	18	11	12	10	2.74	0.07
	6-8 cm	20	0.03	0.22	15	8	7	8	0.97	0.10
	8-10 cm	20	0.04	0.29	19	16	9	10	1.05	0.13
12 months	0-2 cm	18	2.48	0.09	7	7	2	5	5.35	0.07
	2-4 cm	18	2.47	0.07	5	2	3	2	4.81	0.08
	4-6 cm	17	0.06	0.10	13	15	8	12	0.98	0.12
	6-8 cm	13	0.04	0.14	17	15	8	10	0.93	0.11
	8-10 cm	13	0.07	0.15	15	14	7	7	1.08	0.16

ΣAlc = sum of linear alkanes from C_{14} to C_{30} , ΣD = sum of drimanes, ΣS = sum of steranes, ΣH = sum of hopanes, $8\beta H$ = 8β homodrimane, $C_{27\alpha\beta\beta R}$ = $C_{27\alpha\beta\beta R}$ sterane, C_{30H} = 17α 21β C_{30} hopane.

In contrast, this method shows that both *n*-alkanes and isoprenoïds significantly decreased in the 2 upper layers although a strong similarity of the corresponding values obtained for the deeper layers can be observed. These hydrocarbons have both been altered during the same period of time in the surface layer but at a different rate. The decrease of linear and branched alkanes corresponds to microbial degradation which is one of the major processes for oil removal from the environment²². Hydrocarbon metabolising bacteria have been found almost everywhere^{32,33} and are known to grow on various hydrocarbons and to mineralise them at different rates. Differential metabolic regulations in various organisms allow the degradation of only one class of molecules at a time. For example, a *Brevibacterium* known to grow on normal and branched alkanes does not begin to metabolise pristane until *n*-hexadecane is < 5% in a *n*-hexadecane + pristane mixture³⁴.

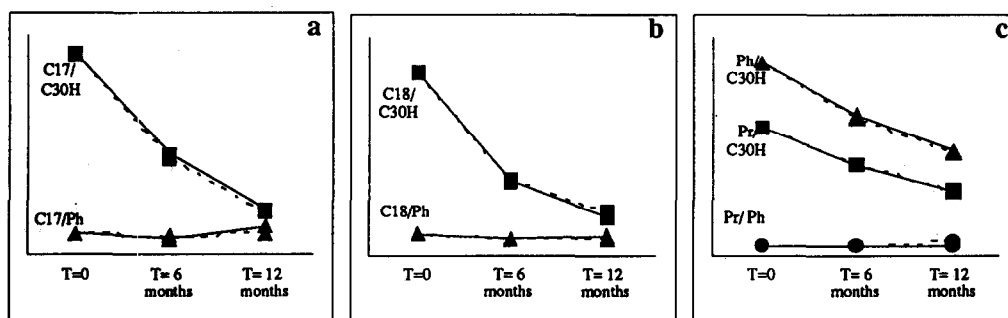


Figure 3: Comparative representation of different ratios versus both C_{30} hopane and respective isoprenoïd homologues of C_{17} (a), C_{18} (b), Pr. and Ph (c) for the 0-2 cm (—) and 2-4 cm (----) layers respectively at origin, 6 months and 12 months.

Figure 3 shows the patterns of alteration in *n*-heptadecane (C₁₇) (a), *n*-octadecane (C₁₈) (b), pristane (Pr) and phytane (Ph) (c) versus both C₃₀ hopane and their isoprenoid homologues (a and b) for the first 2 layers where modifications have occurred because of the burying processes of the contaminating petroleum. The deeper layers, which have not been contaminated by the deposited petroleum, are not represented. A strong alteration of *n*-heptadecane and *n*-octadecane was observed. This alteration is not obvious when the comparison is made with isoprenoid hydrocarbons such as pristane and phytane. During the first 6 months, C₁₇ and C₁₈ were degraded at nearly the same rate but thereafter alteration of C₁₇ occurred faster than C₁₈ according to *in vitro* studies^{1,31,35} which have demonstrated a preferential alteration of light weight hydrocarbons compared to heavier ones. On the other hand, pristane and phytane were both altered at approximately the same rate. This explains why the Pr / Ph ratio hardly changed during the 12 months of experimentation. During the first 6 months, *n*-alkanes were altered faster than branched alkanes. After 6 months, branched alkanes were degraded at the same speed or faster than *n*-alkanes. This phenomenon explains the increase of the C₁₇/Pr ratio after 12 months.

In the 2-4 cm layer (Figure 3), similar alterations of the relative concentrations of both linear and isoprenoid alkanes versus C₃₀ Hopane was observed. Either burying of the altered petroleum or burying of the original petroleum followed by degradation in the zone which led to the same level of degradation as at the surface might have occurred. At the beginning of the experiment, crude oil input was restricted to the surface layer as may be the case with an accidental oil spill. Deposited and buried hydrocarbons may be degraded over time at the surface and in the sedimentary column in aerobic and / or anaerobic conditions³⁶⁻³⁹.

This *in situ* experiment was designed to study simultaneously the patterns of change in different hydrocarbons classes. After 12 months, the weathered petroleum residues showed no alterations in of biomarkers but a severe decrease of *n*-alkanes and a depletion of isoprenoid alkanes. These observations were obtained using hopanes (especially 17 α 21 β C₃₀ hopane) as stable reference compounds. On the other hand, because of their own alteration, the use of isoprenoids as reference showed only a weak alteration of *n*-alkanes. Such *in situ* experiments add a new dimension to environmental studies by studying the *in situ* evolution of hydrocarbons, by comparing alterable compounds to more recalcitrant ones such as hopanes. Further experiments of this type are required in order to validate the method and to find other indices for improved monitoring of patterns of hydrocarbon change in marine sediments.

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