

## Oil spill in the Río de la Plata estuary, Argentina: 2-hydrocarbon disappearance rates in sediments and soils

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*Hydrocarbon disappearance rates in Río de la Plata sediments and soils were determined.*

### Abstract

The 6-month assessment of the oil spill impact in the Río de la Plata described in the preceding paper [Colombo, J.C., Barreda, A., Bilos, C., Cappelletti, N., Demichelis, S., Lombardi, P., Migoya, M.C., Skorupka, C., Suárez, G., 2004. Oil spill in the Río de la Plata estuary, Argentina: 1 – biogeochemical assessment of waters, sediments, soils and biota. *Environmental Pollution*] was followed by a 13- and 42-month campaigns to evaluate the progress of hydrocarbon decay. Average sediment hydrocarbon concentrations in each sampling include high variability (85–260%) due to contrasting site conditions, but reflect a significant overall decrease after 3 years of the spill:  $17 \pm 27$ ,  $18 \pm 39$  to  $0.54 \pm 1.4 \mu\text{g g}^{-1}$  for aliphatics;  $0.44 \pm 0.49$ ,  $0.99 \pm 1.6$  to  $0.04 \pm 0.03 \mu\text{g g}^{-1}$  for aromatics at 6, 13 and 42 months, respectively. Average soil hydrocarbon levels are 100–1000 times higher and less variable (61–169%) than sediment values, but display a clear attenuation:  $3678 \pm 2369$ ,  $1880 \pm 1141$  to  $6.0 \pm 10 \mu\text{g g}^{-1}$  for aliphatics and  $38 \pm 26$ ,  $49 \pm 32$  to  $0.06 \pm 0.04 \mu\text{g g}^{-1}$  for aromatics. Hydrocarbon concentrations modeled to first-order rate equations yield average rate constants of total loss (biotic + abiotic) twice as higher in soils ( $k = 0.18$ – $0.19 \text{ month}^{-1}$ ) relative to sediments ( $0.08$ – $0.10 \text{ month}^{-1}$ ). Individual aliphatic rate constants decrease with increasing molecular weight from  $0.21 \pm 0.07 \text{ month}^{-1}$  for isoprenoids and  $<n\text{-C}22$  to  $0.10 \pm 0.08 \text{ month}^{-1}$  for  $>n\text{-C}27$ , similar to hopanes ( $0.10 \pm 0.05 \text{ month}^{-1}$ ). Aromatics disappearance rates were more homogeneous with higher values for methylated relative to unsubstituted species ( $0.17 \pm 0.05$  vs.  $0.12 \pm 0.05 \text{ months}^{-1}$ ). Continued hydrocarbon inputs, either from biogenic (algal  $n\text{-C}15,17$ ; vascular plant  $n\text{-C}27,29$ ) or combustion related sources (fluoranthene and pyrene), appear to contribute to reduced disappearance rate. According to the different loss rates, hydrocarbons showed clear compositional changes from 6–13 to 42 months. Aliphatics disappearance rates and compositional changes support an essentially microbiologically-mediated recovery of coastal sediments to pre-spill conditions in a 3–4 year period. The lower rates and more subtle compositional changes deduced for aromatic components, suggest a stronger incidence of physical removal processes.

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### 1. Introduction

The progress of oil disappearance in the aquatic environment is determined by the interaction of well-known petroleum- and environment-specific factors which control the effectiveness of physical, chemical

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and microbial removal of hydrocarbons, e.g. crude composition, hydro-dynamism, solar irradiation, temperature, particle abundance, sediment texture, microbiological composition and nutrient availability (e.g. Blumer and Sass, 1972; Atlas, 1981; Fusey and Oudot, 1984; Sugiura et al., 1997). The basic knowledge of petroleum geochemistry permitted the development of a solid framework in topics related to hydrocarbon fingerprinting, compositional alteration and weathering stages of oil components spilled in the environment (e.g. Hostettler and Kvenvolden, 1994; Wang et al., 1999, 2003; Pastor et al., 2001; Boehm et al., 2001). However, quantitative information on hydrocarbon removal rates in natural environments under different conditions is much more limited.

In the preceding paper (Colombo et al., 2004), the biogeochemical assessment of aliphatic and aromatic hydrocarbons in waters, sediments, soils and biota permitted to elucidate the magnitude of environmental impact produced by the spill of ~1000 tons oil in the coastal ecosystem of the Río de la Plata. Results indicated that offshore waters and sediments were little affected due to the rapid wind-driven transport of petroleum residues to the coast. Six months after the spill, coastal waters, sediments, soils and biota still presented very high hydrocarbon levels exceeding by 1–3 orders of magnitude baseline concentrations. Biotic and abiotic compartments consistently indicated that the most impacted area is the central sector close to Magdalena city, which presented the highest hydrocarbon levels, specially in low-energy stream embouchures and bays which acted as efficient oil traps. In order to follow the environmental evolution of oil residues, two additional campaigns were carried out 13 and 42 months after the spill. This paper presents results on the rates of hydrocarbon attenuation and changes of the aliphatic and aromatic composition during decay under different environmental conditions in most affected sediments and soils of this temperate, freshwater ecosystem.

## 2. Methods

Sampling was carried out on February 2000 and July 2002, 13 and 42 months after the spill, respectively, covering previously visited stations, the location and description of which are included in Table 1 of the preceding paper. An additional station (Alberdi, ALB) situated midway between LB and Be was included in these campaigns (Fig. 1). Sediments and soils were collected with stainless steel spatulas during low tide along 45 km shoreline. All samples contained in pre-cleaned glass jars were preserved in portable coolers until arrival to the laboratory. The analytical scheme included ultrasonic extraction, silica gel fractionation and HRGC-FID determination of individual

hydrocarbons as detailed in the previous paper (Colombo et al., 2004). In addition, selected samples from the three campaigns were re-analyzed for the determination of aliphatic hydrocarbons, including hopanes ( $C_{29}$  norhopane and  $C_{30}$  hopane), and aromatic components by HRGC-MSD using an Agilent 6850 gas chromatograph coupled to an Agilent 5973N MSD (EI 70 eV, 2.94 scans  $seg^{-1}$ , 50–550 amu). The column and chromatographic conditions were the same as those used for the HRGC-FID analyses. The presence of hopane biomarkers, together with terpanes was confirmed by selected ion monitoring at  $m/z$  191 (e.g. Wang and Fingas, 2003). Fig. 2 shows typical chromatograms of aliphatic and aromatic hydrocarbons and hopanes in soils at 6, 13 and 42 months after the spill.

## 3. Results and discussion

### 3.1. Total hydrocarbon averages in surface sediments and soils

Table 1 and Fig. 3 present total aliphatic and aromatic hydrocarbon concentrations in sediment and soils collected at 6, 13 and 42 months after the spill.

General sediment averages include a 85–260% variability during each sampling due to contrasting site conditions, but reflect a significant overall decrease after 3 years of the spill:  $17 \pm 27$ ,  $18 \pm 39$  to  $0.54 \pm 1.4 \mu g g^{-1}$  for resolved aliphatics;  $0.44 \pm 0.49$ ,  $0.99 \pm 1.6$  to  $0.04 \pm 0.03 \mu g g^{-1}$  for aromatics at 6, 13 and 42 months, respectively. These general averages suggest stable hydrocarbon levels during the first and second campaign and a strong decrease at 42 months. However, hydrocarbon evolution shows great site to site variations and most contaminated stations show more consistent 1–3 orders of magnitude decrease along the three samplings, i.e. GGi, GGe, JBi. In agreement with the attenuation of hydrocarbon levels, the spatial extension of the impacted area is clearly reduced 42 months after the spill. Except some low-energy, heavily polluted sites such as GGi, Ri, JBi, most stations have declined to previous background hydrocarbon concentrations during the third sampling (Fig. 3). Average soil hydrocarbon levels are 100–1000 times higher and more homogeneous (61–169%) than sediment values, but show a consistent 2–4 orders of magnitude attenuation along the 3 campaigns:  $3678 \pm 2369$ ,  $1880 \pm 1141$  to  $6.0 \pm 10 \mu g g^{-1}$  for aliphatics and  $38 \pm 26$ ,  $49 \pm 32$  to  $0.06 \pm 0.04 \mu g g^{-1}$  for aromatics.

In spite of the large variability of these general means, they suggest a faster drop of aliphatic hydrocarbons relative to aromatic compounds which show comparable or even higher concentrations during the second sampling campaign. Some degree of residue remobilization from heavily polluted sites can be expected in this

Table 1

Total resolved and UCM aliphatic and aromatic hydrocarbons in sediments and soils after 6, 13 and 42 months of the spill

	Sediments ( $\mu\text{g g}^{-1}$ )						Soils ( $\mu\text{g g}^{-1}$ )					
	ALI	Petro/biog	ALI UCM	ARO	Petro/pirog	ARO UCM	ALI	Petro/biog	ALI UCM	ARO	Petro/pirog	ARO UCM
LB6	0.1	0.3	1	–	–	–						
LB13	0.03	0.0	–	0.14	0.6	–						
LB42	0.1	0.1	–	–	–	–						
ALB6	na	na	na	na	na	na	na	na	na	na	na	na
ALB13	0.3	1.6	–	0.14	0.4	–	2729	28.7	63 590	101	0.4	4401
ALB42	0.03	0.6	–	–	–	–	0.4	0.3		0.03	0.6	
Be6	1.0	0.7	20	0.43	0.3	8.9						
Be13	0.7	0.1	–	0.19	0.8	–						
Be42	0.01	1.5	–	–	–	–						
JI6	0.3	0.7	9	0.01	1.0	0.6						
JI13	0.1	0.2	–	0.24	0.1	–						
JI42	0.2	0.3	–	–	–	–						
JiIC6							948	1.9	15 725	22	0.2	966
JiIC13							518	2.8	11 154	18	0.6	528
JiIC42							27	0.03	21	0.06	0.1	
JiIS6	13.1	1.9	220	0.23	0.6	36.6						
JiIS13	133.7	6.3	3949	3.76	0.7	200.2	2867	2.5	127 148	69	0.5	7480
JiIS42	0.1	0.5	–	–	–	–	5	0.1	247	0.15	0.2	
PN6	1.8	0.6	24	0.02	0.2	2.0						
PN13	0.1	1.5	–	0.03	1.2	–						
PN42	0.1	0.7	–	–	–	–						
GGi6	86.6	1.9	1445	1.34	0.4	205.7						
GGi13	37.4	3.1	1256	2.87	0.2	84.0						
GGi42	5.3	0.1	43	0.05	0.1	–						
GGe6	58.9	1.5	311	0.64	1.1	20.7	5187	1.1	20 218	25	0.6	4130
GGe13	1.6	0.7	155	0.10	0.4	5.2	3073	1.0	56 076	21	0.9	1223
GGe42	0.1	0.7	–	0.004	0.2	–	1	0.1	18	0.03	0.2	
Ri6	16.4	1.6	689	1.18	0.2	139.1	4900	1.6	37 178	69	0.6	5751
Ri13	71.6	2.5	3553	4.90	0.5	116.4	775	19.9	37 199	30	1.0	2890
Ri42	0.9	0.3	14	0.08	0.1	–	2	0.1	15	0.05	0.1	
Re6	1.0	1.7	27	0.04	0.5	4.2						
Re13	0.4	0.4	–	0.16	0.5	–						
Re42	0.1	0.3	–	–	–	–						
AL6	5.1	1.5	58	0.08	0.7	7.3						
AL13	0.7	0.9	46	0.11	0.5	–	1316	2.0	38 910	53	0.3	2414
AL42	0.1	0.5	–	–	–	–	1	0.1		0.05	0.1	
JBi6	34.2	1.6	310	0.78	0.5	30.6						
JBi13	2.4	0.3	62	0.90	–	–						
JBi42	0.4	0.2	–	0.02	0.1	–						
Jbe6	6.6	1.9	68	0.06	0.1	0.9						
Jbe13	0.2	0.3	–	0.14	0.2	–						
Jbe42	0.1	0.2	–	–	–	–						
PE6	0.02	–	–	–	–	–						
PE13	0.4	0.2	–	0.23	0.1	–						
PE42	0.04	0.1	–	–	–	–						
AVG6	17.3	1.3	265	0.44	0.59	41.5	3678	1.5	24 374	38	0.47	3616
SD6	27.0	0.6	423	0.49	0.34	67.5	2369	0.4	11 314	26	0.20	2434
AVG13	17.8	1.3	1504	0.99	0.47	101.5	1880	9.5	55 680	49	0.61	3156
SD13	39.2	1.7	1803	1.61	0.30	80.7	1141	11.8	39 435	32	0.29	2510
AVG42	0.54	0.4	29	0.04	0.13	–	6.0	0.1	75.2	0.06	0.22	–
SD42	1.40	0.4	20	0.03	0.08	–	10.2	0.1	114.9	0.04	0.21	–

ALI: total resolved aliphatic hydrocarbons; petro/biog: <C22 + isopr/C15 + C17 + >C23; ALIUCM: aliphatic unresolved complex mixture; ARO: total resolved aromatic hydrocarbons; petro/pirog: methylated/unsubstituted ARO; AROUCM: aromatic unresolved complex mixture; stations as in table 1 of Colombo et al., 2004; sampled at 6, 13 and 42 months after the spill; –: below quantification limits.

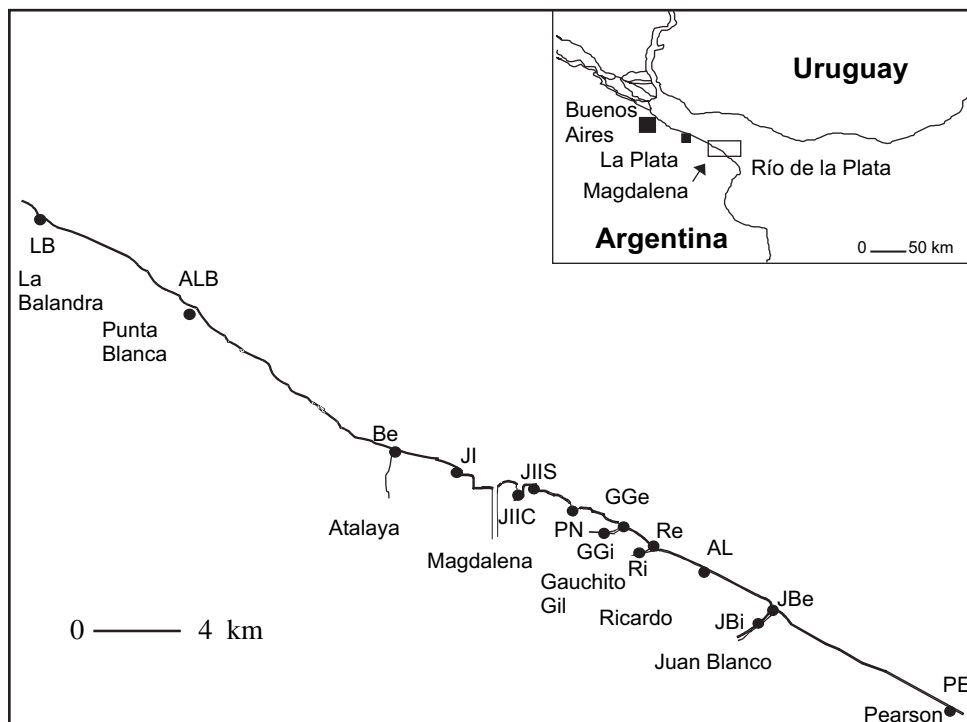


Fig. 1. Study area and station location along Río de la Plata coast.

dynamic coastal area where tides and specially wind-driven currents facilitate sediment transport. This physical remobilization would also explain the consistent 130–470% increase of the ALIUCM from 6 to 13 months ( $265 \pm 423$  to  $1504 \pm 1803 \mu\text{g g}^{-1}$  in sediments and  $24 \pm 11$  to  $56 \pm 39 \text{mg g}^{-1}$  in soils). Since this unresolved hump has been long recognized as indicative of degraded petrogenic residues (e.g. Volkman et al., 1984; Killops and Al-Juboori, 1990), the UCM increase suggests remobilization of heavily biodegraded aliphatic residues. Aromatic hydrocarbons show more conservative levels for both the resolved and UCM fractions (Table 1). The more drastic changes of aliphatic hydrocarbons are consistent with their known higher susceptibility to microbial attack relative to aromatic components (e.g. Atlas, 1981; Colombo et al., 1996; Sugiura et al., 1997). In the last campaign all hydrocarbon fractions, including the UCM are consistently reduced, reflecting the combined effect of biodegradation and physical removal.

### 3.2. Modeling of total average hydrocarbon loss rates

The disappearance of oil residues in coastal environments has been modeled as an exponential decrease characterized by first-order rate constants (e.g. Page et al., 2002; Grossi et al., 2002). However, in contrast with controlled laboratory or field experiments, in real spill situations possible confounding factors such as

continuous inputs from commercial ships, boating, coastal discharges or biogenic sources introduce some uncertainty in the calculations. The oiled area around Magdalena is relatively remote from large urban centers and is not subjected to heavy chronic impact, thus providing a good opportunity to study oil disappearance rates in a natural environment without intensive human intervention. To obtain a first estimation of the rates of hydrocarbon attenuation in this coastal ecosystem, average levels in sediments and soils for the three campaigns were modeled to a first-order rate equation (exponential decrease):

$$\text{HC} = \text{HC}_0 e^{-kt}$$

where HC is the hydrocarbon concentration,  $\text{HC}_0$  is the initial concentration,  $k$  is the first-order rate coefficient (degradation constant:  $\text{month}^{-1}$ ) and  $t$  is time (months). Fig. 4 presents the results for average sediment and soil hydrocarbon concentrations along the three campaigns (note the logarithmic scale). Three rate equations were obtained since the AROUCM was not quantifiable in the third sampling. Exponential fittings are in general good ( $R^2 = 0.65\text{--}0.99$ ), but results should be interpreted with caution since 3 sampling times is the lowest resolution needed for these models. Also the exponential fit of the ALIUCM is somewhat poorer due to its remobilization-mediated increase in the second campaign. The average rate constants which represent total losses (both biotic and abiotic) are comparable for

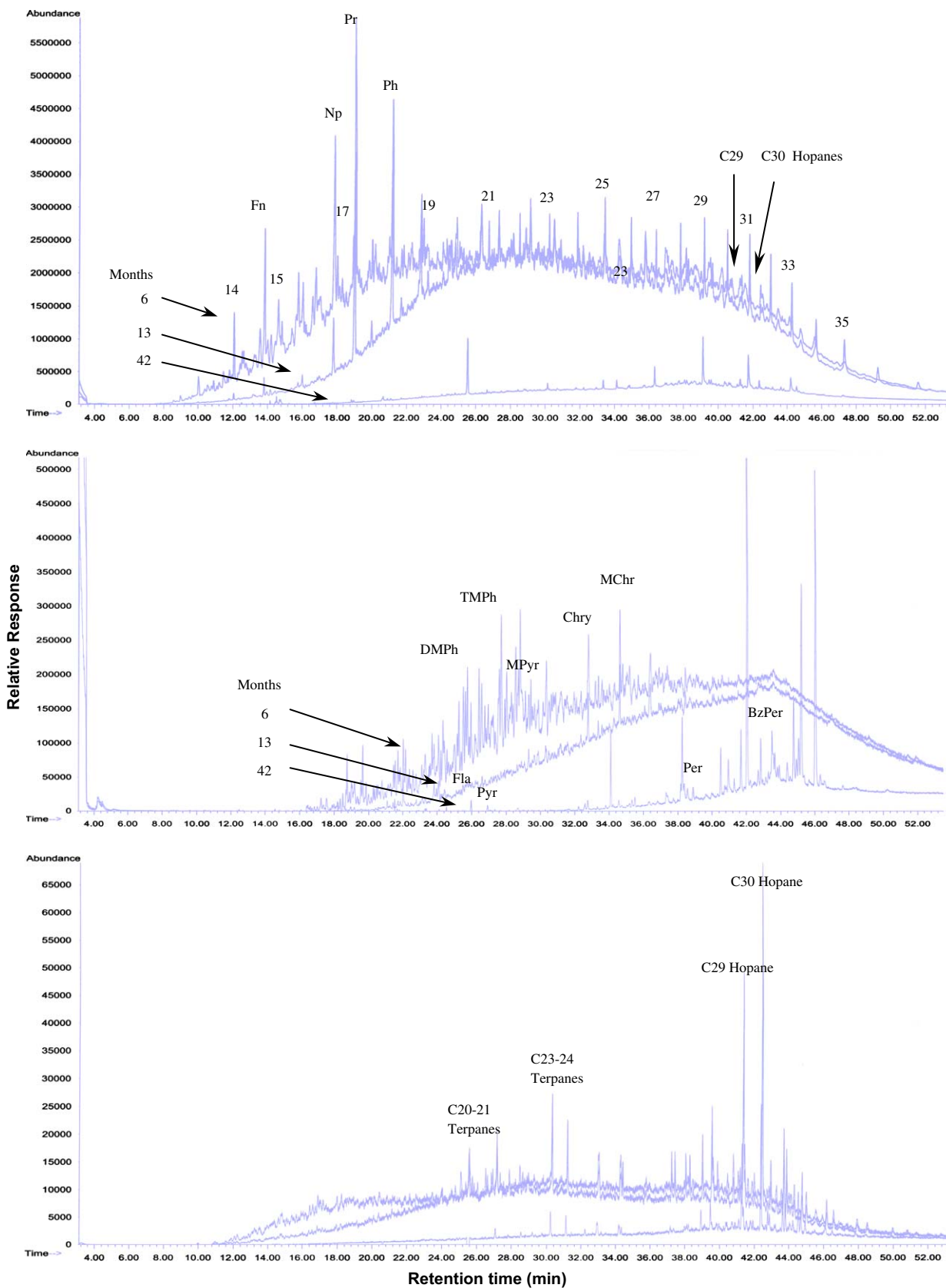


Fig. 2. Chromatograms of aliphatic, aromatic and hopane ( $m/z = 191$ ) hydrocarbons in a soil sample (Ricardo) collected after 6, 13 and 42 months of the spill. Fn: farnesane, Np: norpristane, Pr: pristane, Ph: phytane, 14–35: C14–35 *n*-alkanes, Fla: fluoranthene, Pyr: pyrene, DMPH and TMPH: di and trimethylphenanthrenes, MPYR: methylpyrene, Chry: chrysene, MCHR: methylchrysene, Per: perylene, BzPer: benzo(*ghi*)perylene.

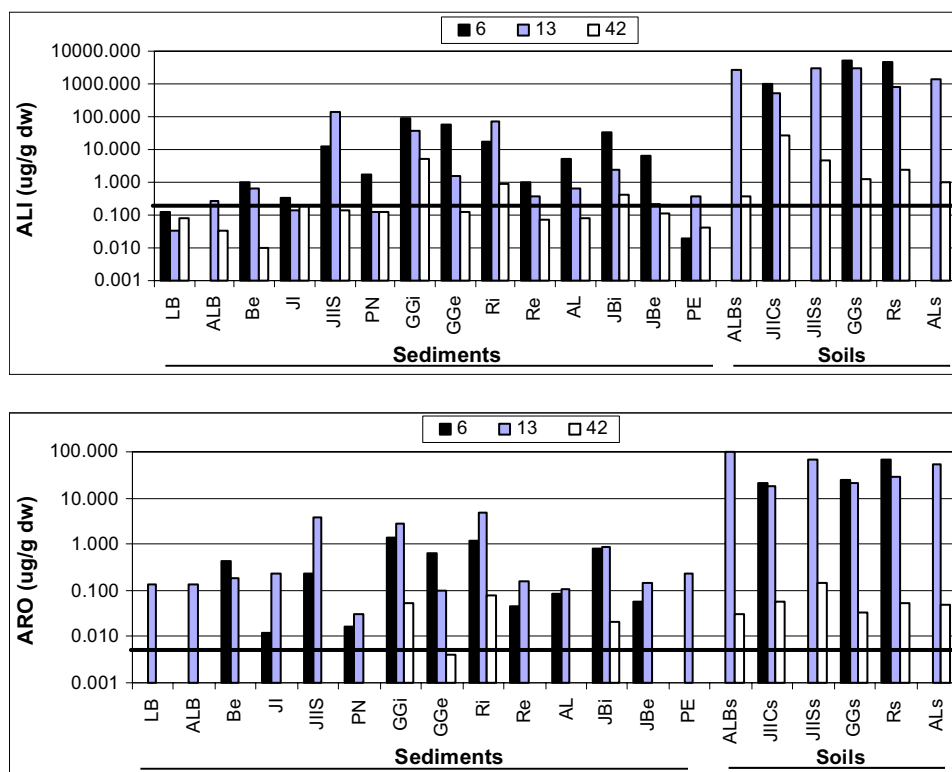


Fig. 3. Total resolved aliphatic and aromatic hydrocarbon concentrations in sediments and soils after 6, 13 and 42 months of the spill.

aliphatics and aromatics, but they are twice as high in soils relative to sediments ( $k = 0.18\text{--}0.19$  vs.  $0.08\text{--}0.10$  month<sup>-1</sup> or  $0.006$  vs.  $0.003$  day<sup>-1</sup>). Due to differences in the degree of oiling and environmental factors, the rate constants of individual sites show large variability ranging from  $0.07\text{--}0.16$  month<sup>-1</sup> in sediments to  $0.10\text{--}0.31$  month<sup>-1</sup> in soils.

These rate constants are comparable or lower than those reported in the literature for field or laboratory experiments. For example, a field experiment with Arabian light crude oil spread on surface seashore sediments and treated with different fertilizers yielded rate constants of  $0.11\text{--}0.27$  month<sup>-1</sup> for saturated and aromatic components (Fusey and Oudot, 1984). Shorter term field experiments performed with chemically dispersed oils in a wetland environment report 2–10 times higher rate constants, i.e.  $0.012\text{--}0.052$  day<sup>-1</sup> (Page et al., 2002). Another field experiment performed in bioturbated Mediterranean coastal sediments treated with Arabian light crude oil yielded rate constants of  $0.12\text{--}0.39$  month<sup>-1</sup> for C17–30 *n*-alkanes and isoprenoids (Grossi et al., 2002). In a 3-month biodegradation experiment of 10% crude oil-contaminated soils inoculated with different microflora, the rate constants ranged from  $0.09$  to  $0.49$  month<sup>-1</sup> for aliphatic hydrocarbons (Colombo et al., 1996).

According to the intercept values of the model, the average initial (time 0) sediment hydrocarbon

concentrations would be  $1.3$  and  $45$   $\mu\text{g g}^{-1}$  for ARO and ALI, respectively. These estimated initial levels are about 3 times higher than average values measured 6 months later. The corresponding initial concentrations in soils, are  $251$  and  $14610$   $\mu\text{g g}^{-1}$ , respectively, which are 4–7 times higher than the 6-month levels. The average percent loss from initial concentrations determined from the model equations (Fig. 3c) indicate that more than 40% of sediment hydrocarbon residues were lost 6 months after the spill with an almost complete recovery at about 4 years. The more rapid recovery in soils appears to occur at 3 months and less than 3 years, respectively.

### 3.3. Modeling of individual hydrocarbon loss rates

The total average loss rates calculated previously reflect the mean disappearance of aliphatic and aromatic hydrocarbons, but the individual components show different behaviors. The detailed analysis of each fraction by HRGC-MSD in selected soils (Rs, JIICs, GGS) and sediments (GGi) permitted to model compound-specific rates, including hopane biomarkers. Fig. 5 shows the rate constants of some aliphatic and aromatic hydrocarbons grouped according to expected sources and reactivity. The results indicate lower disappearance rates in GGi sediments relative to soils, and contrasted differences between compounds. Lower

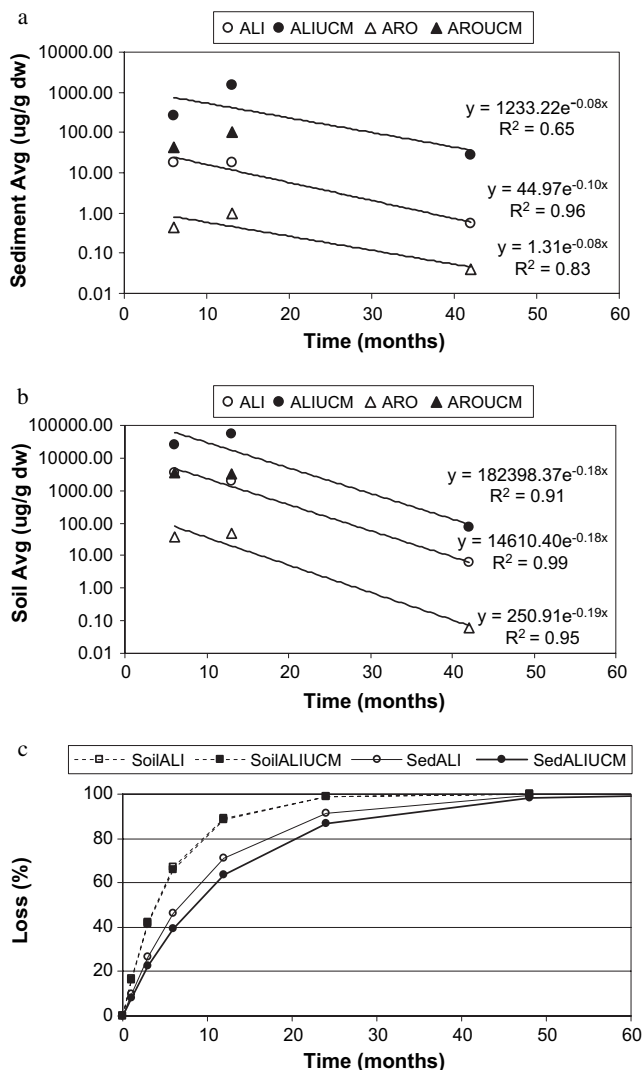


Fig. 4. Rate loss modeling of average aliphatic and aromatic hydrocarbon concentrations in sediments (a), soils (b) and estimated aliphatic hydrocarbon disappearance (c).

molecular weight petrogenic *n*-alkanes and isoprenoids show the highest rate constants whereas heavier *n*-alkanes and hopanes disappear 2–3 times more slowly. Aromatic hydrocarbons show more homogeneous, intermediate rates.

Fig. 6 shows the relationship of rate constants and molecular weight of individual aliphatic hydrocarbons. The rates show significant inverse correlations with molecular weight ( $R^2 = 0.47$ – $0.84$ ), and consistently lower values for GGi sediments relative to soils ( $0.08 \pm 0.05$  vs.  $0.19 \pm 0.08$  month $^{-1}$ ). Norpristane, pristane and C15–19 *n*-alkanes show the highest rates ( $0.22 \pm 0.07$  month $^{-1}$ ) whereas higher molecular weight C27–29 *n*-alkanes present very low values ( $0.10 \pm 0.08$  month $^{-1}$ ), similar to hopanes ( $0.10 \pm 0.05$  month $^{-1}$ ). The decrease of biodegradation with increasing

molecular weight of aliphatics has been reported for laboratory and field experiments (e.g. Colombo et al., 1996; Grossi et al., 2002), and probably reflects the reduced membrane permeability of heavier molecules (Sugiura et al., 1997).

The intense biodegradation of isoprenoids in the Río de la Plata suggests the existence of well-acclimated microbes using hydrocarbons as the sole carbon source. This is favored by the 6-month period elapsed since the spill. In the Aegean Sea spill in Galicia coast, alkanes and isoprenoids were also degraded within 6 months of the accident (Pastor et al., 2001). The continued inputs of biogenic *n*-C15–17 from periphytic algae growing on macrophytes, sands and coastal soils are the most probable explanation for the apparent lower rates of these compounds (Fig. 6). A similar interference from terrestrial vegetation cuticular wax inputs would explain the low rates of C27,29 *n*-alkanes compared with *n*-C26–28. This highlights the importance of characterizing other natural and anthropogenic hydrocarbon sources to estimate oil disappearance rates. In this context, isoprenoid rates may be considered as a more precise estimation of bulk oil residue attenuation.

As hopanes are well known, very slowly-degrading compounds, they are used as “internal standards” to discriminate the relative importance of biodegradation from physical removal of oil residues (e.g. Grossi et al., 2002; Page et al., 2002; Wang and Fingas, 2003). Following the decreasing trend of rate constants with molecular weight (Fig. 6), the importance of biodegradation calculated as the difference with hopane values decreases from  $58 \pm 14\%$  for isoprenoids and lower molecular weight *n*-alkanes to  $31 \pm 14\%$  for *n*-C23–26. There is no apparent biodegradation of higher molecular weight *n*-alkanes ( $9.2 \pm 15\%$ ) which disappear at similar rates than hopanes. In GGi sediments, where overall hydrocarbon reduction is lower, the relative importance of biodegradation is higher (60–75% for isoprenoids to *n*-C21). In contrast, biodegradation is responsible for only 41–64% average of the stronger hydrocarbon decrease observed in soils, indicating enhanced physical removal.

The rate modeling of individual aromatic hydrocarbons is limited by the reduced detections in the third campaign. In general, aromatics show more homogeneous lower values relative to aliphatics ( $0.04$ – $0.26$  month $^{-1}$ ), and confirm the lower rates at GGi sediments. Methylated aromatics disappearance rates are somewhat higher relative to unsubstituted compounds ( $0.17 \pm 0.05$  vs.  $0.12 \pm 0.05$  month $^{-1}$ ) but the differences are small, perhaps reflecting the slow disappearance of heavy aromatics summed to confounding factors such as continued atmospheric inputs of pyrogenic PAHs. Petrogenic chrysene and methylchrysene present homogenous higher rates ( $0.17 \pm 0.04$  month $^{-1}$ ) relative to pyrogenic fluoranthene and pyrene ( $0.10 \pm 0.02$

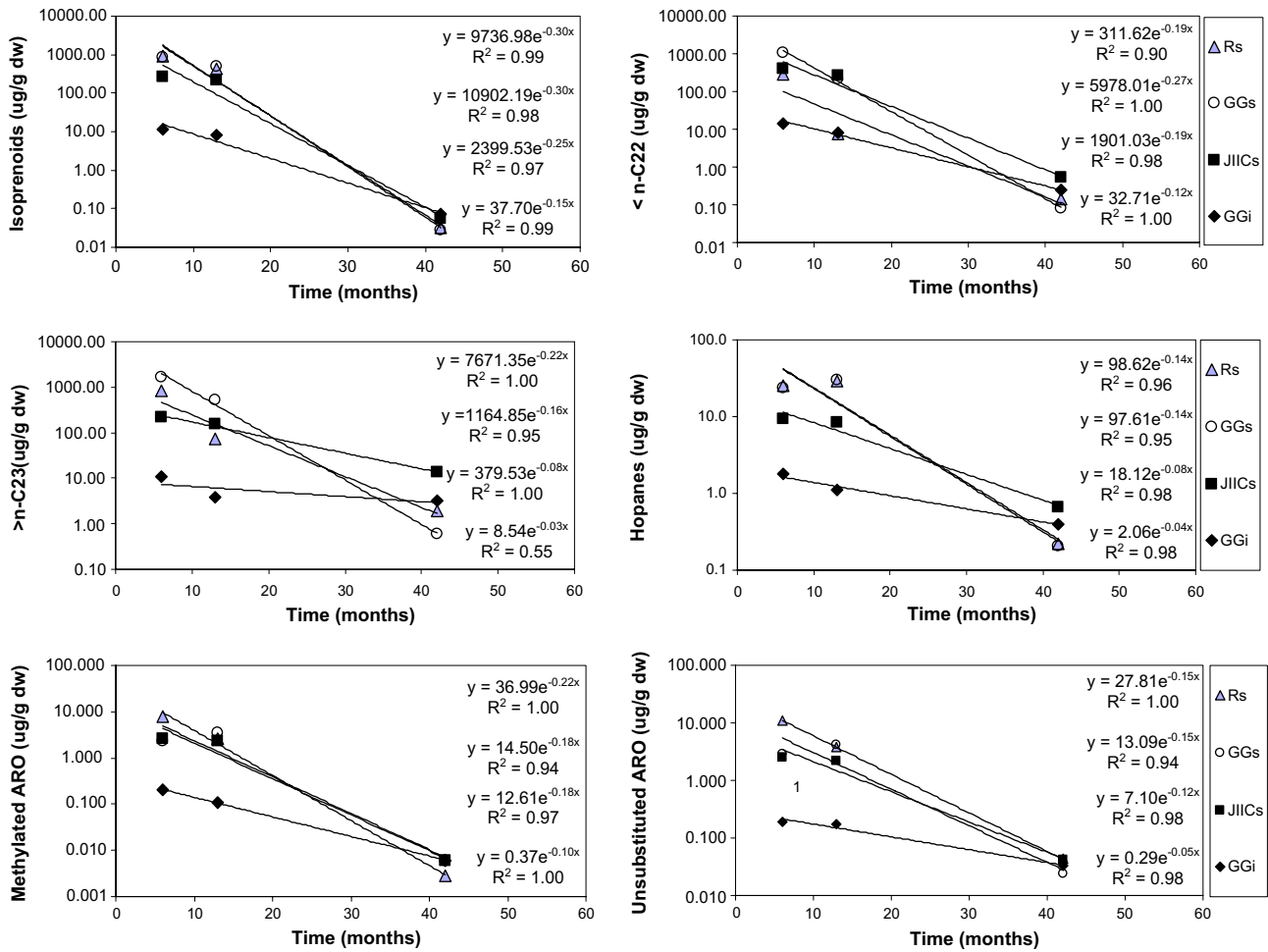


Fig. 5. Rate loss modeling of aliphatic and aromatic components: isoprenoids (farnesane, norpristane, pristane and phytane), <n-C22 alkanes, >n-C23 alkanes, hopanes, methylated and unsubstituted aromatic hydrocarbons.

month<sup>-1</sup>). The pentacyclic aromatic perylene shows the lowest rate constants (0.06–0.08 month<sup>-1</sup>) probably reflecting its major natural, continuous diagenetic input.

The comparison with hopane disappearance rates, supports the lower importance of biodegradation for

aromatics. For methylated aromatics and chrysene the relevance of biotic removal is higher (22–64%), compared to pyrene (0–33%), suggesting a prevailing physical attenuation. At JIIC the percentages of biodegradation are very homogenous for chrysene and

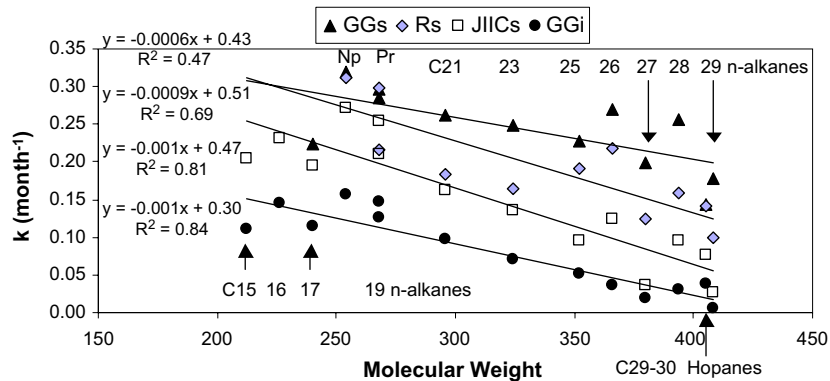


Fig. 6. Relationship between disappearance rate constants and molecular weight of individual aliphatic hydrocarbons in soils (GGs, Rs, JIIC) and sediments (GGi).

methylated phenanthrene, pyrene and chrysenes (52–58%). As observed for aliphatic hydrocarbons, the highest biodegradation corresponds to chrysene and its methylated species in GGi sediments (60–65%), which present the lower overall hydrocarbon losses.

The observed trend in disappearance rate constants is broadly consistent with reported patterns for field or laboratory experiments (e.g. Hostettler and Kvenvolden, 1994; Colombo et al., 1996). Major differences as high isoprenoid rates and low aromatic disappearance, appear to be related to the already advanced alteration of oil residues in the first 6-month sampling, i.e. the rapid attenuation of lighter *n*-alkanes (<*n*-C17) and aromatics (i.e. naphthalene, phenanthrene and methylated species) was basically missed and results reflect advanced alteration stages of more persistent oil components.

### 3.4. Changes of hydrocarbon composition

According to the different loss rates of individual components and the stage of microbiological alteration, hydrocarbon composition of sediments and soils showed compositional changes over the 42 months covered by the study (Fig. 2).

The aliphatic fraction showed a consistent shift from higher isoprenoid abundance at 6 and 13 months of the spill to a higher molecular weight *n*-alkane and hopane predominance at 42 months. Aromatic composition was more conservative but showed a significant reduction of methylated species over time.

The average relative abundance of isoprenoids in sediments along the three samplings decrease from  $24 \pm 15$ ,  $25 \pm 22$  to  $7.6 \pm 6.2\%$ , whereas  $>n$ -C23 alkanes increase from  $34 \pm 16$ ,  $42 \pm 22$  to  $58 \pm 17\%$ , at 6, 13 and 42 months, respectively. The higher hydrocarbon disappearance rates and direct inputs of terrestrial plant material in soils, produce more contrasted changes for isoprenoids ( $34 \pm 12$ ,  $57 \pm 20$  to  $3.2 \pm 3.2\%$ ) and higher molecular weight *n*-alkanes ( $33 \pm 7.3$ ,  $21 \pm 17\%$  to  $84 \pm 12\%$ ). The intense weathering in soils is also reflected by a decrease in the proportions of  $<n$ -C22 alkanes ( $26 \pm 5.4$ ,  $19 \pm 5.8$  to  $10 \pm 6.4\%$ ). An intermediate enrichment in isoprenoids at 13 months is also indicated by these general means.

Fig. 7 presents the percent composition of aliphatic and aromatic hydrocarbons and total concentrations in selected soil and sediment samples. The initial reduction of total aliphatic concentrations between 6 and 13 months, is paralleled by a decrease of  $<n$ -C22 *n*-alkanes and relative enrichment in isoprenoids, reflecting their selective preservation during biodegradation. This intermediate isoprenoid enrichment is consistent with the interpretation of heavy biodegradation indicated by the UCM increase at 13 months. During the 42 month

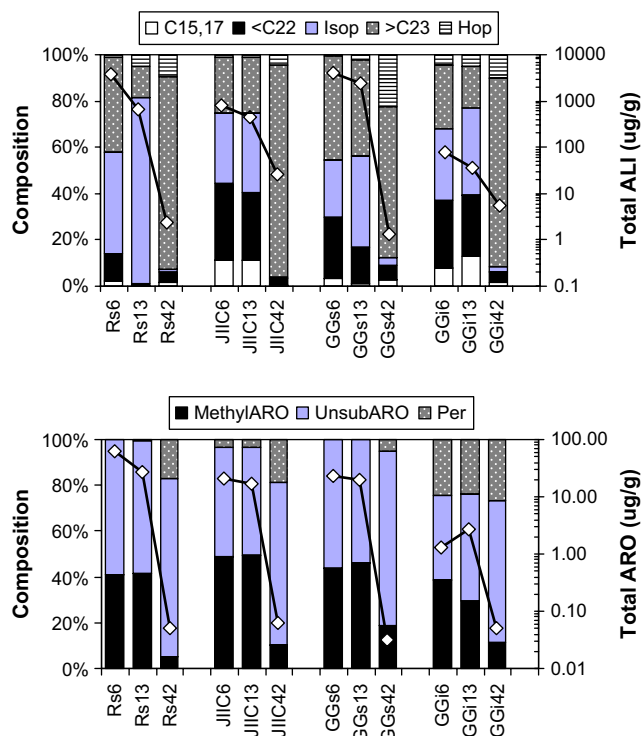


Fig. 7. Changes in aliphatic (a) and aromatic (b) hydrocarbon composition after 6, 13 and 42 months of the spill in soils (GGs, Rs, JIIC) and sediments (GGi). Total aliphatic and aromatic concentrations are also shown.

attenuation to basal levels, isoprenoids disappear almost completely and higher molecular weight *n*-alkanes from plant waxes predominate together with residual petrogenic hopanes.

The petrogenic/biogenic ratio  $<n$ -C22 + isoprenoids/ $n$ -C15 +  $n$ -C17 +  $>n$ -C23 (Table 1), reflects the shift of the aliphatic composition along the three campaigns. In the first sampling, the general average ratio in sediments is high ( $1.3 \pm 0.57$ ) close to a fresh crude oil value (1.6) reflecting the impact of the spill, but non-affected sites such as LB show baseline values (0.3). At 13 months, the general average of the ratio continues high but more variable ( $1.3 \pm 1.7$ ) because in polluted sites the ratios increased (2.5–6.3) due to the relative enrichment of isoprenoids. After 42 months, the biotic and abiotic removal of oil residues and selective preservation in addition to continued inputs of vascular plant waxes reduce the ratios to background values in nearly all stations ( $0.44 \pm 0.37$ ). In soils, the shift of petrogenic/biogenic ratios is more drastic. They change from intermediate values similar to a crude in the first sampling ( $1.5 \pm 0.4$ ), to high variable ratios in the second campaign due to the isoprenoid enrichment ( $9.5 \pm 12$ ), and very low background values in the third sampling ( $0.13 \pm 0.10$ ).

In contrast to the aliphatic results, the chemical composition of the aromatic fraction is more conservative, specially during the first and second campaign. Effectively, following the almost constant levels at 6 and 13 months (Fig. 7), the aromatic composition in soils and GGi sediments show essentially no changes showing a 40–50% abundance of methylated species, basically phenanthrene, chrysenes and pyrenes. Only after 42 months of the spill, with the drastic reduction of total aromatic levels, there is a significant decrease in the relative abundance of methylated species in favor of unsubstituted PAHs, including perylene.

The methylated/unsubstituted ratios indicative of petrogenic versus pyrogenic inputs are thus higher and variable during the first and second campaigns and decrease to lower background values in the third sampling. Average ratios in sediments after 6 and 13 months of the spill are high ( $0.6 \pm 0.3$  and  $0.5 \pm 0.3$ ), comparable to fresh crude oil values (0.75). The ratios decrease sharply at 42 months ( $0.1 \pm 0.1$ ) due to the selective elimination of methylated species relative to pyrogenic PAHs and natural perylene. The relationship in soils shows a similar decrease along the three samplings ( $0.5 \pm 0.2$ ,  $0.6 \pm 0.3$  to  $0.2 \pm 0.2$ ).

Taking together the information on disappearance rates and compositional changes, the aliphatic data support an essentially microbiologically-mediated recovery of coastal sediments to pre-spill conditions in a 3–4 year period. Isoprenoids and shorter chain *n*-alkanes decrease over time whereas higher molecular weight *n*-alkanes are as persistent as hopanes. In contrast, the more subtle compositional changes deduced for aromatic components, support the interpretation of a more efficient physical removal process. Methylated aromatics decrease over time whereas pyrogenic components disappear at rates similar to hopanes. For both, the aliphatic and aromatic fractions, soil disappearance rates are consistently higher, mainly due to enhanced physical removal. The presence of continued inputs, either from biogenic or combustion related sources, appear to contribute to decreased rates.

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