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Hydrocarbon Pollution in the Sediment from the Jarzouna-Bizerte Coastal Area of Tunisia (Mediterranean Sea)

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Abstract This study investigated the presence and origin of hydrocarbon pollution in industrial waste water sediments found near the Jarzouna (Bizerte, Tunisia) oil refinery. Analyses of surface sediments (layer 1) and deep sediments (layer 2) showed that Total Hydrocarbon (TH) concentrations ranged from 602 ± 7.638 μg/g in layer-1 to 1270 ± 2.176 μg/g in layer-2. The results suggest that the deeper the sediment, the higher the level of total hydrocarbon found. The sedimentary Non Aromatic Hydrocarbon (NAH) and Aromatic Hydrocarbon (AH) concentrations ranged from 66.22 ± 1.516 to 211.82 ± 10.670 μg/g for NAH, and from 13.84 ± 0.180 to 115.60 ± 2.479 μg/g for AH. The high variability of these concentrations was associated with the location of the sediment collection sites. Aliphatic biomarker analysis revealed petroleum contamination close to the refinery rejection site, and biogenic sources further away. Petroleum contamination may be associated with increased industrial activity in the area of Jarzouna-Bizerte in the Mediterranean Sea.

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Evidence has shown that the environment has its limits and cannot continue to serve as the repository for refuse from different sources, including industrial waste (Stuart 1996; Jovandićević et al. 2005; Trably and Patureau 2006; Brack et al. 2007; Hu et al. 2007; Rao et al. 2007). Oil exploration and related energy sources have markedly increased over the years. Since early 1960, an estimated 500 million gallons of oil have spilled from tankers in Europe and in Pacific Asia. Contamination of the Mediterranean Sea by petroleum hydrocarbons is known to be caused by marine operations, run-offs by refineries and industry, and tanker accidents. The Mediterranean Sea is considered to be a representative model of the world's ocean. Being semi-enclosed, it also records various signals of high anthropic pressures from surrounding countries, as well as industrialized North European countries (Zaghden et al. 2005). Little is known, however, regarding the southern region of the Mediterranean Sea, which explains the lack of marine pollution evaluation studies in Tunisia. Fossil fuel storage installations are ensured by the marine transport of oil to the Tunisian Refining Industry Company (Société Tunisienne des Industries de Raffinage) (STIR) facility, Tunisia's only refinery. Due to its geographical location, environmental protection and its integration within operational activities have become STIR's major concerns. Hydrocarbon accumulations in marine sediment may be of biogenic or anthropogenic origin (Stuart 1996). It has been recognized (Blumer and Youngblood 1975) that both petroleum and biogenic hydrocarbons have different chemical

characteristics which are easy to distinguish; making the distinction between hydrocarbons and other sources is an important part of any objective petroleum contamination study. This study examined the aliphatic and aromatic contamination levels from superficial marine sediments in the Jarzouna coastal area of Tunisia. The eventual contamination and sources were determined by analysing hydrocarbon levels, resolved (n-alkanes) and unresolved complex mixtures (UCM), and aliphatic biomarkers.

Materials and Methods

The STIR refinery is situated in Jarzouna (Bizerte) in northern Tunisia. The sampling sites were located in the vicinity of the rejection site of seawater used in the treatment of the petroleum effluent, the refining process, and the cleaning of the oil reservoirs. The treatment consists of successive decantation basins to recover the oil, with the used seawater rejected into the sampling area. The area under study was 37°17′57.10″ N, 9°51′53.61″ E of Tunisia using the Global Positioning System (GPS) (Fig. 1). Sample collection occurred in March 2002. Thirty-five sediment samples were collected from two layer depths at four different sampling sites (SMTB-1, SMTB-2, SMTB-3, and SMTB-4), as described in Fig. 1. The sediments were stored in aluminum boxes at 4°C during transportation to the laboratory. Layer-1 refers to surface sediment (0–15 cm in thickness), while layer-2 refers to the deeper part of the sediment (15–30 cm). The samples were manually homogenized and lyophilized, and the total hydrocarbons (TH) present in 20 g of dry weight (dw) sediment were extracted in a Soxhlet apparatus with chloroform for 16 h. Recovery

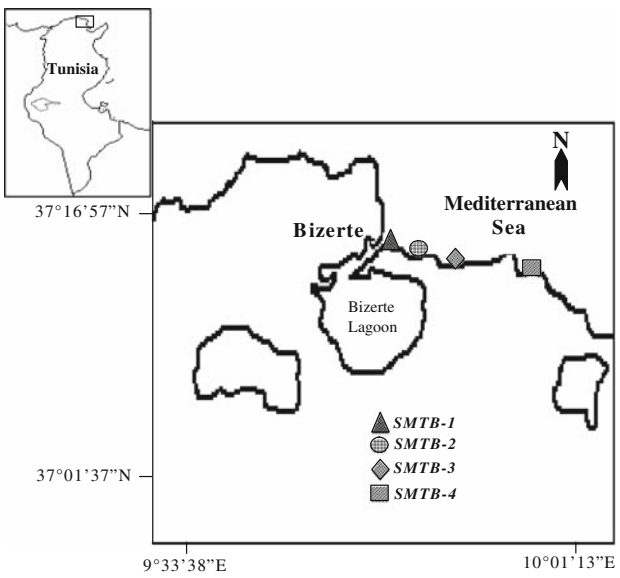

Fig. 1 Map of the Bizerte area showing the four collection sites

ranged from 97.4% to 98.9% for the n-eicosene. Following chloroform evaporation, the extract was fractionated into aliphatic and aromatic hydrocarbons by adsorption liquid chromatography using a column of alumina and silica-gel, and gradient solvents as eluent: n-hexane and 2:1 n-hexane/chloroform for non-aromatic hydrocarbon (NAH) (F1) and aromatic hydrocarbon (AH) fractions, respectively. The polar fraction (PF) was not eluted. The total petroleum hydrocarbon (TPH) represents the sum of the NAH and AH fractions. Fractionation was performed in a silica microcolumn (silica gel 60 of 63–200 μm). Prior to use, the silica was cleaned with the chloroform, subjected to a one-hour wash at 50°C under magnetic agitation, and filtered through a glass fiber filter (type GF/A, 47 mm diam., 1.6 μm retention) (Whatman International, Kent, UK). The silica was then conditioned overnight at 110°C. Following solvent evaporation, the NAH and AH were weighed. The NAH were analysed with a Hewlett-Packard 5890 gas chromatograph equipped with a temperature-controlled injector, a flame ionization detector (GC/FID), and a capillary column HP5: 5% diphenyl, 95% dimethylpolisiloxane (25 m × 0.32 mm × 0.52 μm). The oven temperature program was as follow: 1 min at 80°C, from 80 to 280°C at 4°C/min and 10 min at 280°C. The injector and detector temperatures were 250°C and 280°C, respectively. The samples were solubilised in cyclohexane and 1 μl of this sample was injected following the addition of external standard (n-eicosene). Quantification of the total resolved (TR) hydrocarbon (n-alkanes) and unresolved complex mixtures (UCM) were calculated using the mean response factors of n-alkanes. Each hydrocarbon concentration is expressed on a dry weight (dw) basis. Recovery assays ranged from 94.50% to 98.12% for C18 n-alkane and from 87.0% to 92.3% for a mixture of C10–C34 n-alkanes. Concentrations of individual n-alkanes (n-C12 to n-C32), isoprenoids pristane and phytane, TR, UCM, and total aliphatic (TA) fraction (sum of identifiable aliphatic peaks + UCM) were calculated. The detection limit was determined at 0.001 μg/g for the C18 n-alkanes. Data of the TH, NAH, and AH concentrations are given as means ± SE. Comparisons between multiple groups were achieved by one-way ANOVA analysis followed by Newman-Keuls Multiple Comparison Test. The major errors found during sampling and extractions were determined to ensure the significance of the different values. The $p < 0.5$ was considered significant.

Results and Discussion

The sediment samples from layer-1 and layer-2 were subjected to an organic study to determine the biogenic and anthropogenic hydrocarbon input in the region. Table 1 and Fig. 2 show that TH concentrations differed from one site

Table 1 Hydrocarbon concentration in sediments from the Jarzouna coastal region

Site	Layer depth (cm)	TH (μg/g)	NAH (μg/g)	AH (μg/g)	NAH/TH %	AH/TH %	TPH %	NAH/AH
SMTB-1-C1	0–15	780 ± 34.65	166.14 ± 5.633	115.6 ± 2.479	21.3	14.8	36.1	1.4
SMTB-1-C2	15–30	890 ± 7.024	136.17 ± 3.305	113.92 ± 1.882	15.3	12.8	28.1	1.2
SMTB-2-C1	0–15	632 ± 6.557	105.8 ± 4.976	13.9 ± 0.1079	16.7	2.2	18.9	7.6
SMTB-2-C2	15–30	646 ± 10.54	107.52 ± 3.675	21.96 ± 0.4329	16.6	3.4	20	4.9
SMTB-3-C1	0–15	602 ± 7.638	66.22 ± 1.516	13.84 ± 0.18	11	2.3	13.3	4.8
SMTB-3-C2	15–30	636 ± 6.658	68.05 ± 1.921	21.62 ± 1.259	10.7	3.4	14.1	3.15
SMTB-4-C1	0–15	1190 ± 24.09	211.82 ± 10.67	18.65 ± 0.4986	17.8	1.5	19.3	11.3
SMTB-4-C2	15–30	1270 ± 22.34	81.66 ± 2.176	19.36 ± 0.6255	6.4	1.5	7.9	4.2

C1, layer-1 (0–15 cm); C2, layer-2 (15–30 cm); TH, total hydrocarbon; NAH, non aromatic hydrocarbon; AH, aromatic hydrocarbon; TPH, total petroleum hydrocarbon. These are expressed as μg/g dwt of sediment by using gravinemetry analyses

to another. Indeed, a decrease in TH concentrations was observed between SMTB-1 and SMTB-3, which suggests that TH absorption occurred upon discharge from the refinery (Fig. 1). It should be noted that the TH concentrations were greater in the deeper layers than in the surface layers. This may be related to sediment characteristics, seawater flux, and other physical parameters, as previously reported by Liu et al. (1999) and confirmed for TPH and PAHs hydrocarbons by Hartmann et al. (2005). These authors demonstrated that hydrocarbon concentrations are relatively low in surface levels, which is in agreement with our findings. On the other hand, TH values were lower at SMTB-1, SMTB-2, and SMTB-3 than at SMTB-4. The latter was located in a fishing area sufficiently far away from the refinery's discharge site, which suggests the presence of other sources of hydrocarbon contamination. Our findings indicate that the total hydrocarbon levels in the Jarzouna Mediterranean coastal area ranged from 602 ± 7.638 μg/g dw at SMTB-3-C1 to 1270 ± 2.176 μg/g dw at SMTB-4-C2. These levels were higher than those found in the Mangrove Estuary (0.36–339.84 μg/g) (Bernard et al. 1995) and in the Gulf of Fos area of France (10.3–260.0 μg/g) (Mille et al. 2007). However, TH concentrations found off the Jarzouna coast were found to be lower than those found in surface sediments in Saudi Arabia (Ehrhardt and Burns 1993; Redman et al. 1996) and those off the northern coast of Sfax-Tunisia (1127 μg/g and 5217 μg/g) (Louati et al. 2001). These results suggest that the contamination impact of the STIR Jarzouna refinery appears to be under better control than are other sources of pollution, such as that found in the Sfax region. In the Bizerte coastal area, only one potential source of pollution exists, whereas the Sfax region supports significant industrial activity and thus, other possible sources. Crude oil is made up of hundreds of compounds and primarily NAH fraction composites, which include n-alkane hydrocarbons, branched isoalkanes, such as pristane and phytane, and cyclo-alkane represented by an unresolved complex mixture. Crude oil also contains multiple AH, including polycyclic aromatic hydrocarbons (PAHs), and is composed of polar fraction (PF) containing resin and asphalt, with an unknown contribution in petroleum hydrocarbon pollution. Among all of the compounds found in crude oil, NAH and AH are considered to be the most important pollutants. In the present study, NAH and AH levels showed a variation among the collection sites, with an average of 66.22–211.82 μg/g for NAH and 13.84–115.60 μg/g for AH (Table 1). Figure 2 shows that NAH was predominant in the TPH fraction in the two layers of sediment from each site. NAH levels varied from 6.4% to 21.3%, while the aromatic levels varied from 1.5% to 14.8%. Elevated levels were found at SMTB-1, located nearest to the refinery's rejection basin. Both NAH and AH levels at SMTB-4 were not significantly elevated, compared to those at the other collection sites. The NAH/AH ratio (Table 1) confirms the predominance of NAH in the sediment, with a TPH value of less than 50%. This signifies an accumulation of high molecular weight compounds in the sediment which may be due to the degradation of petroleum hydrocarbons or the presence of biogenic high compounds. Our results show that the TPH levels found in the Jarzouna coastal region were relatively low compared to those of locations around the world reported to be chronically contaminated by oil, such as, the 60–646 μg/g reported in the Hong Kong area (Hong et al. 1995), 11–6900 μg/g along the Saudi Arabian coast (Readman et al. 1996), and 779 μg/g collected from sediment near an oil refinery in Bahrain (Tolosa et al. 2005). The determination of total aromatic hydrocarbons (AH) has sparked the most interest in research. In the present study, we showed that the AH level was at its highest at SMTB-1-C1 and its lowest at SMTB-3-C1. The variability of the AH values is in agreement with that reported in previous studies. Mille et al. (2007) reported that the aromatic fraction varied from 2.5 to 70.0 μg/g in the French coastal region. Nishigima et al. (2001) found that aromatic fractions varied from 0.08

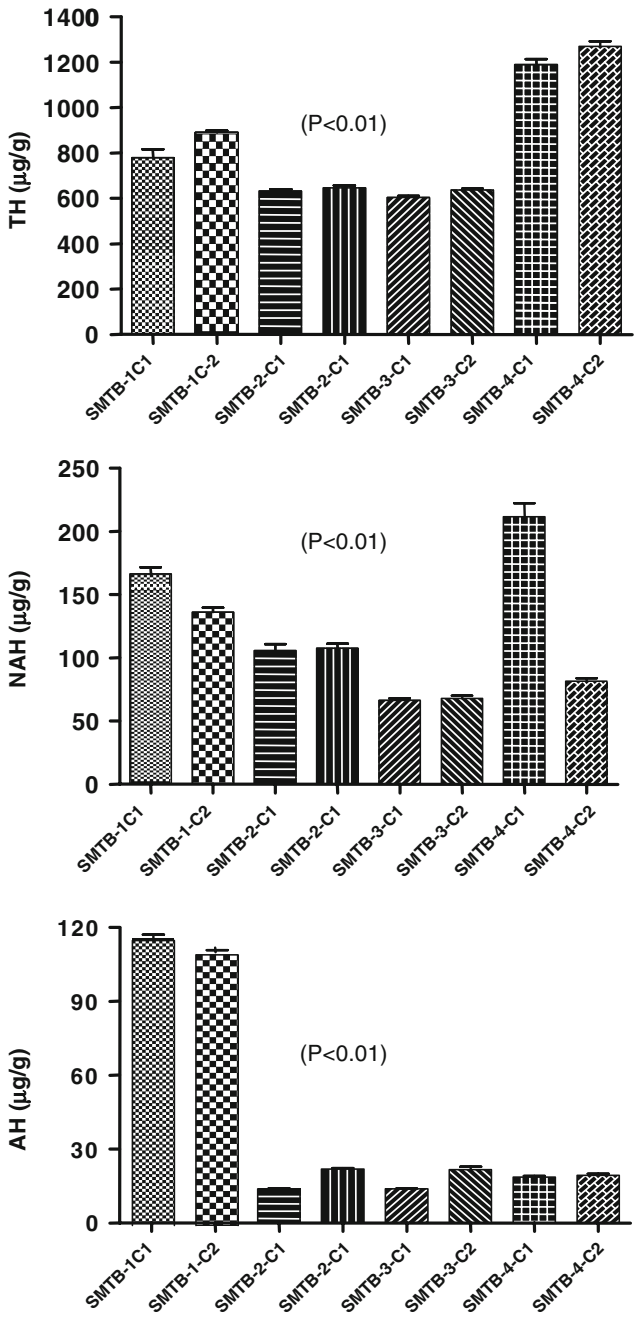

Fig. 2 Statistical variations of TH, NAH, and AH concentrations in the Jarzouna coastal region. A statistical study was made using a one-way ANOVA, followed by a Newman-Keuls Multiple Comparison Test. Histograms were created using the mean relative values ± SE for 8 separate groups. Differences in TH, NAH, and AH concentrations at each site and from the two layer depths were considered significant at $p < 0.05$ or less

to 157.90 µg/g in the Santos area of Brazil, yet no aromatic compound was detected in the Cananéia region of the same country. In coastal sediment from the Rio de la Plata area, Argentina, Colombo et al. (2006) reported that the aromatics ranged from 0.01 to 78.00 µg/g. Commendatore and Esteves (2007) showed that aromatic concentrations varied from non-detected to 737 µg/g in sediment found in Patagonia, Argentina. In a study of the continental shelf near Tabasco, Mexico (Botello et al. 1991), the aromatics ranged from 501 to 3120 ng/g. This comparison therefore does not enable us to confirm any chronic contamination by aromatic compounds in the region examined in our study. In fact, these compounds are known to be less degraded by indigenous bacteria and, compared to NAH, are more resistant. Regarding the aromatics compound, most recent studies (Trabelsi and Driss 2005; Wang et al. 2006; Rao et al. 2007; Unlu 2007) have focused on individual aromatics and particularly PAHs (polycyclic aromatic hydrocarbons) which are known to display mutagenic and carcinogenic properties (Menzie et al. 1992; Baird et al. 2005). The NAH concentrations in the Jarzouna coastal region were relatively similar to those found in sediments from the other Mediterranean areas. In fact, NAH concentrations registered an average of 76–124 µg/g in Algeria's Gulf of Arzew, in the southwest Mediterranean area (Buscail et al. 1999), while an average of 3.75–221.00 µg/g was recorded near Monaco (Raoux et al. 1999). Compared to our findings in the Jarzouna area (Table 1), national comparisons (Louati et al. 2001) revealed elevated NAH concentrations (349–1983 µg/g) off the northern coast of Sfax, Tunisia. Higher average concentrations were also found in other Mediterranean coastal regions, such as Alexandria's eastern harbour (Egypt), with an NAH concentration registering at between 60.7 and 1356.3 µg/g (Aboul-Kassim and Simoneit 1995) and the French Mediterranean coast at Berre Lake (Marseille), with an average between 112 and 5458 µg/g (Jacquot et al. 1999). The aliphatic compounds are represented as the sum of n-alkanes and UCM following GC determination. Our results show that the TA levels varied from 12.4 µg/g at SMTB-3 to 55.7 µg/g at SMTB-1; these findings are moderate compared to those of other studies. In fact, Commendatore and Esteves (2007) showed the total aliphatic hydrocarbons to be approximately 1304 µg/g off the Argentinian coast. The Egyptian coastal area (Abu Quir Bay) sediment showed the aliphatic hydrocarbon concentration to be between 6.0 and 565.2 ng/g (El Deeb et al. 2007). Botello et al. (1991) found sedimentary aliphatic concentrations ranging from 0 to 255 ng/g in Mexico City's Lagoon Estuary. In our study, the n-alkane concentration varied from 11.5 µg/g at SMTB-3 to 53.7 µg/g at SMTB-1. These levels are therefore less significant than those found at various sites around the world (Nishigima et al. 2001). Inter-station comparisons of the AH, aliphatics, and n-alkanes showed an accumulation of these chemicals at the SMTB-1 site, located closest to the refinery rejection point, although SMTB-4 contained the highest concentration of NAH. These data suggest that the region under study was only moderately contaminated with petroleum

compounds, which may signify that the STIR refinery at Jarzouna houses an efficient effluent treatment system.

Petroleum compounds are generally readily identified by their gas chromatogram traces. NAH analysis was carried out using gas chromatography for aliphatic biomarkers which enabled us to determine the origin of the hydrocarbons present in the sediments. Table 2 presents the diagnostic criteria used to determine the origin identification characteristics. The chromatograms (Fig. 3) obtained from layer-1 and layer-2 sediments recorded a similar NAH distribution in the two layers of each sediment sample (SMTB-1, SMTB-2, and SMTB-3). Figure 3 also shows a regular NAH distribution at each site. The NAH fraction gas chromatograms of the sediments from these stations showed that the predominant resolved compounds were n-alkanes (n–C_{15} and n–C_{21}), with no predominance of odd-over-even carbon chains. The chromatograms also revealed a gradual decrease in n-alkanes in SMTB-1, SMTB-2, and SMTB-3, according to the number of carbons, with no marked disparity in the number of carbons between n-C_{23} and n-C_{31}. The carbon preference index (CPI) values were calculated according to the method of Leal-Granadillo et al. (2000). The CPI values expressed in Table 2 indicate that an n-alkane input from the petroleum in each sediment sample collected from SMTB-1, SMTB-2, and SMTB-3 were close to the unit ($\leq$1). These values are in accordance with those previously reported (Zheng et al. 2000; Kalaitzoglou et al. 2004). In addition, the chromatograms of all of the samples collected from SMTB-1, SMTB-2, and SMTB-3 showed evidence of developed UCM, generally considered to be indicative of petroleum contamination (Volkman et al. 1992; Wang and Fingas 2003). Our organic analysis also showed the presence of high levels of pristane and phytane in the samples from these three collection sites. Their abundance and relative importance, compared to that of the n-alkanes, also suggest petroleum contamination (Volkman and Maxwell 1986). Table 2 also presents the different ratios of Pr/Ph, C_{18}/Pr, and C_{17}/Ph, which were used to identify the anthropogenic origin when $\leq$1. The overall results therefore indicate that the four sites under investigation underwent moderate petroleum contamination. The gas chromatograms of the sediments from SMTB-4 revealed a different n-alkane status, characterized by an irregular NAH distribution with dominant odd carbon-numbered compounds ranging from n–C_{27} to n–C_{34}. In most of the SMTB–4 samples, n–C_{29} and the three terrigenous compounds (n–C_{27}, n–C_{29} and n–C_{31}) displayed major alkanes, indicating an input from higher terrestrial plants, as previously reported (Volkman et al. 1992). Figure 3 shows the

Table 2 Characteristics of hydrocarbons in surface sediments from the Jarzouna coastal region

Site	UCM(μg/g)	CPI	Dominant peaks	Pristane (μg/g)	Phytane (μg/g)	nC_{17}/Pr	nC_{18}/Ph	Pr/Ph	TR (μg/g)	TA (μg/g)
SMTB-1C1	0.91	1.5	C_{19}–C_{22}	0.0431	0.0520	0.553	0.238	0.828	42.293	43.203
SMTB-1C2	1.87	0.97	C_{18}–C_{22}	0.0235	0.0199	0.224	0.380	1.180	53.705	55.775
SMTB-2C1	1.23	1.4	C_{17}–C_{21}	0.0651	0.0647	0.349	0.491	1.006	32.95	34.18
SMTB-2C2	1.43	1.2	C_{17}–C_{21}	0.0199	0.202	0.317	0.417	0.985	32.698	34.12
SMTB-3C1	0.98	0.98	C_{19}–C_{21}	0.0165	0.0172	0.241	0.455	0.959	11.516	12.496
SMTB-3C2	0.77	1.1	C_{17}–C_{22}	0.0231	0.0244	0.519	0.532	0.946	21.7808	22.5508
SMTB-4C1	Ab	2.3	C_{27}–C_{34}	0.2007	0.0329	2.012	4.123	6.1	30.987	30.987
SMTB-4C2	Ab	2.1	C_{26}–C_{32}	0.1095	0.0154	5.234	4.547	7.11	13.208	13.208

Ab, absent; UCM, unresolved complex mixture; CPI, carbon preference index; Pr, pristine; Ph, phytane; TR, total resolved; TA, total aliphatic. UCM, Pr, Ph, TR and TA (μg/g dw) were evaluated by gas chromatography (GC/FID)

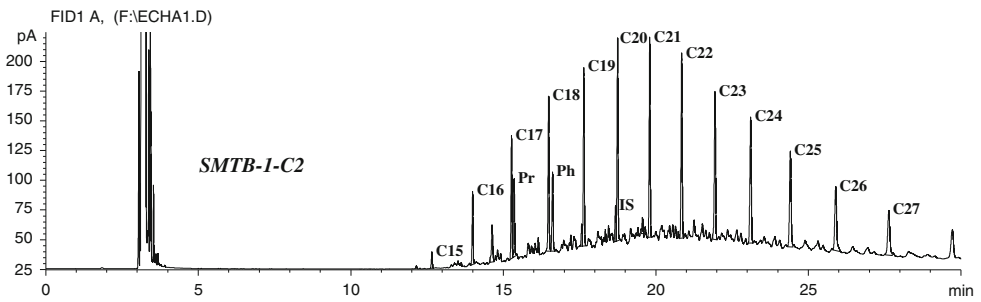

Fig. 3 Gas chromatograms of aliphatic hydrocarbons in representative polluted sediments. GC traces were obtained following NAH fractionation in a capillary column. Individual hydrocarbons are shown with their carbon number (C_{15}–C_{29}). The distribution of peaks with retention time demonstrates the hydrocarbon to be of petroleum origin. Is: standard (n-eicosene); Pr: pristane; Ph: phytane

three terrigenous compounds (n–C_{27}, n–C_{29}, and n–C_{31}). The CPI values of the sediment collected from SMTB-4 were greater than 2, confirming the exclusive vegetable/animal origin of the hydrocarbons, as previously reported (Louati et al. 2001). Finally, our study shows that the SMTB-4 samples contained no UCM and that the ratios of Pr/Ph, C_{18}/Pr, and C_{17}/Ph were >2, thus confirming the contribution of biogenic sources at this particular site (Rosa and Triguis 2007).

In summary, our findings indicate the predominance of petroleum hydrocarbon in the sediments collected near the STIR refinery effluents. Although moderate, these hydrocarbon concentrations should ideally be reduced.

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