**Environmental
Contamination
and Toxicology**

Polycyclic Aromatic Hydrocarbons and *n*-Alkanes in *Osilinus attratus* from the Coast of Tenerife (Canary Islands)

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Anthropogenic compounds, derived from terrestrial and atmospheric sources, are subjected to physical/chemical/biological processes during their residence time in seawater ecosystems These processes ultimately take part in the distribution and fate of contaminants in marine environments. In this way, sediment and particulate associated contaminants are available to aquatic plants and animals, which can accumulate petrogenic hydrocarbons from the marine environment in their lipid tissues (Farrington *et* al. 1982; Metcalf and Carlton 1990) even if pollutants are present at very low concentrations (Malins 1977).

Therefore, marine organisms can be widely used as bioindicators and integrators of water quality conditions in seawater ecosystems because they can provide useful information about the potential for biomagnification in the food webs (Mason 1987; Burns and Knapp 1989).

Because of their geographical situation, the Canary Islands are in an area of high tanker traffic. Moreover, the Canary Islands Stream and Trade Winds may carry the pollution which originates far away from the islands coasts. On 19 December 1989 the Iranian tanker Khark-5 was disabled 150 mi off the Moroccan coast and, by the beginning of January, had lost about 60,000 t of heavy Iranian crude oil. However, 220,000 t of oil remained inside the tanker, which was towed along the north of the Canary Islands and down to Cape Verde Islands to discharge the cargo. Next on 30-31 December 1989 the damaged Spanish tanker Aragón spilled 25,000 t of Maya crude oil off Madeira and was towed into the port of Santa Cruz de Tenerife (Canary Islands) to discharge the cargo.

The objective of this paper, which is a part of a global monitoring project of the coast of Tenerife, is to examine the content of n-alkanes and polycyclic aromatic hydrocarbons in specimens of the marine winkle *Osilinus attratus* Wood (Mollusca: Gastropoda: Prosobranchia: Archaeogastropoda: Trochidae) readily found in this coast and very appreciated as a food.

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MATERIALS AND METHODS

Analytical reagent grade chemicals were used throughout the study. n-Hexane was distilled, treated with sulfuric acid, and re-distilled under reflux. Milli-Q water was extracted with purified n-hexane. Samples of *O. attratus,* size 12.2±0.3 mm, were collected by hand at mid-tide throughout 1990 and 1991, at three locations in the south east coast of Tenerife (Figure 1). The samples were wrapped in aluminium foil and stored, not longer than 2 wk, at -20°C until analyzed.

All analyses were carried out on a composite sample for each sampling date and station, prepared from about 37 specimens, which were freeze-dried, freed from their shells and weighed (5-10 mg). Then the internal standard 9-methyl- phenanthrene (100% recovery) was added to each composite sample for the purposes of instrument calibration and percent recovery calculation. Subsequent analysis included alkaline hydrolisys (8% KOH in ethanol), addition of 40% aqueous sodium chloride to avoid emulsion formation, and extraction with 3x50 mL of n-hexane. The organic layer was first washed with 10% HCl (3x100 mL) and then with purified water until neutral, dried over Na_2SO_4 and concentrated to 1-2 mL in a rotary evaporator. The aliphatic and aromatic fractions were then separated by column chromatography on florisil-alumina-silica (deactivated at 3%, 5% and 5%, respectively, with purified water), which is a modification of the analytical schema proposed by Law *et al.* (1988), using n-hexane and n-hexane-dichloromethane (8+2) as the eluents. Both fractions were taken almost to dryness in the rotary evaporator and dissolved into n-hexane, aliphatic fraction, or toluene, aromatic fraction. Both fractions were analysed by gas chromatography with flame ionization detector (Varian 3300, Walnut Creek, CA) on a SPB-50.32 mm x 15m column (N_2 carrier 1.4 mL/min, N_2 make-up 30.12 mL/min, splitless/split injection, 90°C for 2 min, 8°C/min until 280°C, held for 30 min), calibration was carried out using Aldrich standards (catalog 30,027-6 and 29,850-6, Milwaukee, NY) for n-alkanes (Table 1) and PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, carbazol, 4H-cyclopenta-fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benz-[*b*]anthracene, benzo[*e*]-acephenanthrilene, perylene, benzo[*a*]pyrene, benzo[*e*]-pyrene, coronene). When analytical peaks overlap with peaks of impurities or the UCM (see later) is great enough, individual compounds were identified using GC-MS.

RESULTS AND DISCUSSION

Mean values for paraffinic hydrocarbons and PAHs concentration in *Osilinus attratus* at the three sampling stations are given in Table 1 along with various geo-geochemical correlation parameters such as the n-C_{17}/pristane, n-C_{18}/phytane and pristane/phytane ratios.

Chromatograms of both paraffinic and PAHs fractions show a deformed baseline, the well-known Unresolved Complex Mixture (UCM), which is considered to be a consequence of the exposure of organisms to weathered or biodegraded crude petro-leum and its derivatives, along with peaks corresponding to the acyclic isoprenoid
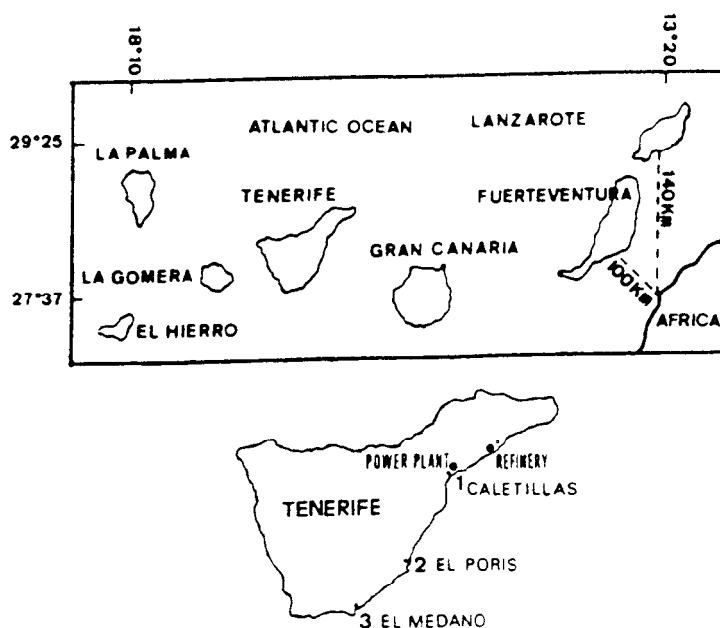
Figure 1. Sampling stations in the south east coast of Tenerife

hydrocarbons pristane and phytane.

One can see in Figure 2 for Caletillas station, that the individual concentrations of the paraffinic hydrocarbons with n<20 are higher for the n-C_{15} and n-C_{17} which suggests a greater tendency for the accumulation of these odd hydrocarbons. However, the fact that many n-alkanes, especially the odd numbered carbon chain alkanes, are biosynthesized in the marine environment, fluctuations in this source of input to the winkles or their own biosynthesis of such compounds can influence n-alkane concentration (Farrington and Meyers 1975). The n-alkanes in the range C_{21}-C_{30} seem to show no odd-to-even predominance which is an indication of a petrogenic input characteristic of crude petroleum.

Nevertheless, the Carbon Preference Indexes (CPI = Σn-C_{odd}/-Σn-C_{even}) presented in Table 2 are more relevant. In this sense, one can see that while CPI_{21-30} remains more or less constant near 0.50 at Caletillas, El Porís and El Medano stations, the temporal variation for CPI_{12-20} is much greater and shows values much higher than unity at the three sampling stations. During the first 6 mon of 1991, the CPI_{12-20} values were very high, from 18 to 28, which seems an abnormal accumulation of odd carbon chain n<20 alkanes along this period. Taking into account the geographical situations of these beaches, and the presence of a constant source of pollution due to the power station near to Caletillas, one can think of these beaches as having an almost constant petrogenic pollution level and that a new petrogenic input came from outside during 1991, as this increase is not a seasonal one.

Table 1. Mean concentration (ng/g±SD, dry weight) and range for hydrocarbons and geochemical correlation parameters in *Osilinus attratus* from the three sampling stations*.

Hydrocarbon	Caletillas	El Porís	El Medano
n-C_{15}	675.7±362.0 (123-1,368)	619.1±425.7 (24-1,095)	289.9±241.2 (66-847)
n-C_{16}	95.7±53.3 (37-179)	145.4±112.3 (50-348)	99.5±113.7 (32-413)
n-C_{17}	370.2±227.5 (67-784)	319.5±260.0 (ND-623)	182.1±76.3 (53-272)
n-C_{18}	150.4±68.4 (60-247)	211.0±92.2 (58-417)	55.8±41.5 (ND-72)
n-C_{19}	144.1±93.0 (74-279)	188.8±156.7 (52-417)	27.7±9.1 (ND-72)
n-C_{20}	234.6±175.1 (113-435)	232.1±198.1 (ND-467)	54.2±99.0 (ND-272)
n-C_{21}	147.0±142.0 (8-382)	306.7±160.0 (122-471)	27.1±32.5 (ND-282)
n-C_{22}	167.8±124.0 (65-473)	296.2±204.4 (112-548)	70.1±92.8 (ND-282)
n-C_{23}	199.5±176.0 (62-618)	328.0±295.4 (25-799)	92.0±85.5 (ND-277)
n-C_{24}	264.0±211.6 (99-779)	524.7±564.0 (46-1,426)	92.8±84.2 (ND-266)
n-C_{25}	242.5±166.7 (97-658)	363.0±354.0 (ND-1,021)	93.8±80.0 (ND-276)
n-C_{26}	320.3±196.0 (193-665)	631.2±632.0 (10-1,410)	88.0±86.1 (ND-271)
n-C_{28}	235,4±116.4 (151-441)	437.4±385.3 (ND-933)	72.0±80.0 (ND-218)
n-C_{30}	274.0±119.2 (156-550)	368.2±279.0 (ND-842)	169.0±191.0 (ND-647)
Total Aliphatics	2,998±1.540 (1,558-6,662)	4,366±3,276 (674-9,908)	1,351±769 (563-2,641)
Pr/Ph	4.6±6.3 (0.4-16)	1.1±2.0 (ND-5)	0.6±0.5 (ND-1)
C_{17}/Pr	3.8±4.0 (ND-11)	4.4±4.7 (ND-11)	3.5±4.1 (ND-10)
C_{18}/Ph	1.4±1.7 (0.5-5)	4.4±4.7 (0.2-13)	3.5±4.1 (0.6-3)

*Pr = pristane, Ph = phytane, ND = not detected.

Table 2 Carbon preference index (CPI = $\Sigma n\text{-}C_{odd}/\Sigma n\text{-}C_{even}$) in the sampling stations

Collection date	CPI_{total}	CPI_{12-20}	CPI_{21-30}
	Caletillas		
19-01-90	--	1.50	--
22-03-90	1.10	2.31	0.25
12-06-90	0.55	0.86	0.34
20-07-90	1.05	1.69	0.51
07-08-90	0.78	6.05	0.09
21-08-90	1.40	1.60	0.77
22-01-91	1.06	1.73	1.99
20-04-91	0.77	0.71	0.88
17-05-91	0.76	1.06	0.43
26-06-91	0.77	4.51	0.45
26-08-91	2.24	17.9	0.49
	El Porís		
07-04-90	1.14	3.41	0.51
19-05-90	0.90	1.45	0.61
15-06-90	0.86	1.73	0.52
18-07-90	0.70	1.12	0.57
14-08-90	0.49	0.79	0.34
29-01-91	3.20	28.2	0.49
24-02-91	3.44	28.1	0.49
12-05-91	3.27	28.1	0.49
30-06-91	3.27	28.1	0.49
	El Medano		
26-04-90	0.63	1.18	0.47
27-06-90	0.32	0.85	0.18
27-07-90	0.29	1.41	0.42
24-08-90	0.30	0.30	0.28
09-01-91	1.11	1.11	1.11
30-04-91	3.27	28.1	0.49
21-05-91	3.27	28.1	0.49
21-06-91	2.16	18.0	0.48

Another possibility to explain the higher CPI_{12-20} and lower CPI_{21-30} values as compared with the corresponding values for Iranian and Arabian crude oils, the most commonly transported oils near the Canaries, 0.79-0.85 and 0.77-0.98, respectively,
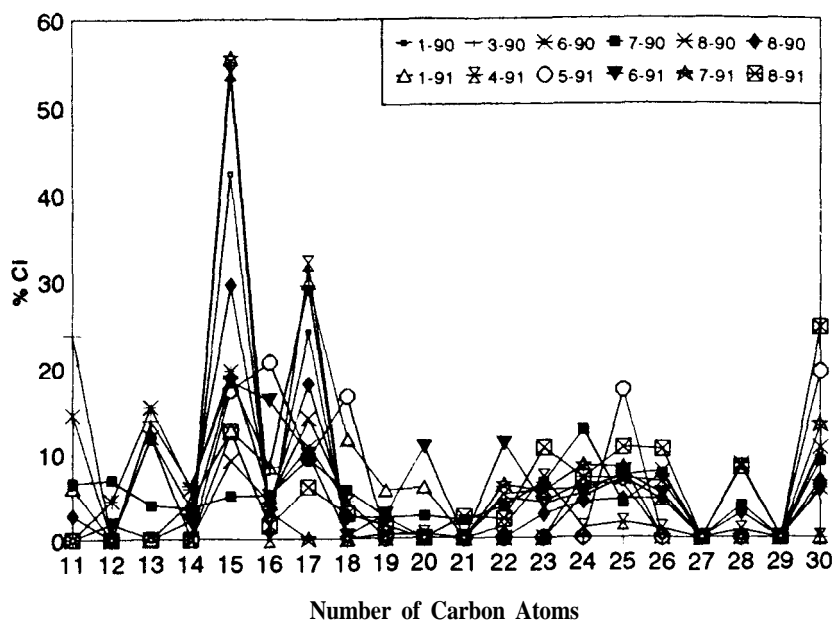
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Figure 2. Variation of the relative abundance of the n-C$_i$ hydrocarbons along the sampling period in Caletillas station samples. Legend for dates in box.

can be the result of the selective activity of the membrane surfaces more permeable to lower than to higher molecular weight n-alkanes (Stegeman and Teal 1973).

The pristane-to-phytane (Pr/Ph) ratio has been proposed as a measure of the redox potential of sediments (Didyk *et al.* 1978) as well as a way to identity oil slicks and tar balls (Balkas *et al.* 1982; Glover and Bullin, 1989) even though Ten Haven *et al.* (1987, 1988) and Powell (1988) state that this ratio is of restricted utility as a paleoenvironmental indicator. The higher Pr/Ph ratios observed in *O. attratus* may suggest that the phytyl side chain of chlorophyl is degraded to pristane in the digestive system of *O. attratus* or comes from petroleum hydrocarbons as it is not found in most biota that have not been exposed to petroleum hydrocarbons (Anderlini *et al.* 1981; Farrington *et al.* 1982).

Furthermore, as one can see in Table 3, only eight out of the nineteen quantified PAHs could be detected in the samples analyzed and only naphthalene is present in almost all the samples (22, 78.6%); besides naphthalene is the only PAHs detected in the samples coming from El Médano station with higher concentrations than in the samples from the other two stations.

In the samples coming from Caletillas station, the presence of PAHs other than naphthalene is sporadic while samples coming from El Porís station show the pres-

Table 3. Mean concentration (ng/g±SD, dry weight) and range of PAHs in *Osilinus attratus* from the three sampling stations (ND= not detected)

PAH	Caletillas	El Porís	El Médano
Naphthalene	19.8±7.7 (ND-86.8)	13.2±8.4 (2.28-26.6)	19.8±27.7 (2.10-86.8)
Fluoranthene	2.04±0.82 (ND-3.00)	4.27±2.66 (2.00-8.10)	2.04±0.83 (1.20-3.00)
Pyrene	1.68±1.29 (ND-3.12)	3.91±2.80 (1.84-7.10)	1.68±1.29 (ND-3.12)
Benz[a]anthracene	1.00±1.41 (ND-2.00)	2.50±0.50 (2.00-3.00)	1.00±1.40 (ND-2.00)
Chrysene	ND	5.88±2.38 (3.10-8.80)	ND
Benz[b]anthracene	5.90±5.00 (3.10-15.5)	33.6±34.9 (2.53-88.3)	5.90±5.00 (3.10-15.5)
Perylene	13.7±22.0 (ND-29.1)	42.2±44.2 (1.50-89.2)	13.7±22.0 (ND-39.1)
Benzo[a]pyrene	4.05±3.54 (1.40-9.00)	11.0±12.2 (3.00-25.0)	4.05±3.54 1.40-9.00)

Table 4. Concentration (ng/g±SD, dry weight) and range of PAHs in *Osilinus attratus* from El Porís station.

	Collection date								
PAH	4-90	5-90	6-90	7-90	8-90	1-91	2-91	5-91	6-91
1	4.6	19.3	13.0	10.3	ND	16.1	ND	26.7	2.3
2	ND	2.7	8.1	2.0	6.0	2.5	ND	ND	ND
3	ND	2.8	ND	ND	ND	ND	ND	7,9	1,9
4	ND	3.0	2.5	2.0	ND	ND	ND	ND	ND
5	ND	ND	3.1	5.2	8.8	6.4	ND	ND	ND
6	ND	88.3	19.0	18.0	65.4	8.2	ND	2.6	ND
7	1.5	ND	ND	ND	ND	89.3	ND	35.8	ND
8	ND	ND	25.0	3.0	5.0	ND	ND	ND	ND

1: naphthalene, 2: fluoranthene, 3:pyrene, 4: benzo[a]anthracene, 5: chrysene, 6: benzo[b]anthracene, 7: perylene, 8: benzo[a]pyrene

ence of most of the PAHs quantified and with high concentrations, Table 4. This fact may be related to the higher contamination of this beach, which has many areas covered with very thick layers of weathered petroleum residues.

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