The PAH composition in limpets (Patella vulgata L.) from the coasts of Sicily (Italy)

2 authors:

Antonio Gianguzza
Università degli Studi di Palermo
166 PUBLICATIONS **2,644** CITATIONS

Santino Orecchio
Università degli Studi di Palermo
103 PUBLICATIONS **1,544** CITATIONS

Some of the authors of this publication are also working on these related projects:

Management of Sicily aquifers View project

THE PAH COMPOSITION IN LIMPETS
(PATELLA VULGATA L.) FROM THE COASTS
OF SICILY (ITALY)

Antonio Gianguzza
Santino Orecchio

QUERY SHEET

Q1: Au: Caption ok?
Q2: Au: Cite in text table 4.
Q3: Au: Au: City?
Q4: Au: Au: City?

Taylor & Francis
Taylor & Francis Group

THE PAH COMPOSITION IN LIMPETS (PATELLA VULGATA L.) FROM THE COASTS OF SICILY (ITALY)

Antonio Gianguzza
5   **Santino Orecchio**
Dipartimento di Chimica Inorganica e Analitica, Università di Palermo, Palermo, Italy

This article examines the presence, distribution, nature and sources of 19 Polycyclic Aromatic Hydrocarbons (PAH), in
10 *Patellae (patella vulgate L.) and seaweed (vulva) sampled in different stations of Sicilian coastal environments and analyzed for their polycyclic aromatic hydrocarbon (PAH) content.*

Analysis was performed by gas chromatography/mass
15 *spectrometry (GC/MS) with selected ion monitoring (SIM), after saponification of the sample and clean up of the extract. In the limpets the total concentration of polycyclic aromatic hydrocarbons ranged from 34 to 750 μg/Kg of dry matrix. The relative standard deviation (RSD) of the*
20 *replicas on the concentrations of individual compounds ranged from 4 to 20%. The accuracy of method was estimated by analyzing "blank" samples spiked with known quantities of analytes and the recover percentage was 82 ± 12%. The detection limit (LOD) of analytical procedure was*
25 *less than 0.2 μg/Kg d.w. for all analytes. The quantification limit (LOQ) of analytical procedure was less 0.7 μg/Kg d.w.*

The resulting distributions and weight ratios of specific compounds are discussed in terms of sampling location and origin. The results obtained show that levels of
30 *contamination vary from one sampling station to the next,*

2   *A. Gianguzza and S. Orecchio*

*while relative distributions of PAH is homogeneous in most
stations. Lipidic content and total PAH concentrations were
found not to be correlated and the compounds present in
limpets were shown to be mainly of petrogenic origin. There
is no evidence of coal-tar contamination.*   35

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are of special concern because of their widespread distribution throughout the environment and   40
their often toxic and carcinogenic properties (1, 2). In general, the two
main sources of PAH in the environment are fossil fuels, mainly crude
oil, and the incomplete combustion of organic materials such as wood,
coal and oil. In addition to the many domestic and industrial combustion
processes in use today, coal tar containing coating systems are also a   45
major source of PAH in aquatic systems. To these we may add offshore
activities, oil spills, offshore installations and shipping exhausts (3). Under anaerobic conditions some PAH can also be derived from biogenic
precursors such as pigments and steroids (4, 5).

As a consequence of their hydrophobic nature, PAH in aquatic environments rapidly tend to become associated with particulates. Sediments   50
therefore represent the most important reservoir of PAH in the marine
environment (6). Some PAH are persistent and toxic to aquatic organisms and can influence the development of liver tumors in several fish
species and may adversely affect the reproductive process in fish and   55
other aquatic species.

A practical problem in analyzing environmental contaminants (heavy
metals, organic compounds, etc.) in water is the very low concentrations
near or below the detectable analytical limits at which they often occur.
In water, concentrations vary widely over time, therefore the number of   60
analyses needed for statistical processing is high (7). Interpreting trace
contaminants concentrations in water and sediment and predicting the
threat they pose to aquatic life under variable physical-chemical conditions is very difficult. The question of bioavailability of PAHs is very
complicated and sediment concentrations are a poor guide to the potential toxicity of PAHs to species (8). Bioaccumulators are used to measure   65
contaminant concentrations in aquatic ecosystems through the accumulation of contaminants in their tissues. There are many advantages of

using organisms. They essentially provide information on the average
70 variation in time and space of the concentrations of contaminants in the
surrounding water. Also, they can concentrate the water contamination
making the analyses simpler and cheaper.

Generally, for aquatic organisms, the bioaccumulation of lipophilic
compounds depends on their concentration in ambient, water and food,
75 on the mechanisms and kinetics that control the distribution between
water and food, on the position of the organism in the food chain, on
metabolism, and so on (9, 10).

Animal and aquatic plants can accumulate hydrocarbons from the en-
vironment even when these pollutants are present at very low concentra-
80 tions; thus marine organism have been widely used as bioindicators and
integrators of organic pollution in marine ecosystems (11, 12). Oysters
and limpets have been recommended as indicator organisms. However,
recently, Cantillo (13) remarked that oysters and limpets are not equal in
their ability to concentrate xenobiotic compounds. In fact, their bioac-
85 cumulation response may be affected by various parameters pertaining
to the organism itself as well as those concerned with the environment
where the organism is sampled (water temperature, salinity, position in
the shoreline, etc.) (14, 15).

The Italian marine environment, where strategies for monitoring pol-
90 lution are required, is characterized by a great variety of species. How-
ever, the most useful bioindicator organisms (mussels and oysters) do
not occur along the Sicilian coast. Consequently, it is necessary to look
at other species present in these coastal areas as possible sentinel organ-
isms. The use of new species as possible bioindicators made it necessary
95 to develop research and monitoring strategies simultaneously. The aim
of this study is to assess the content and distribution of PAH in the *Patella
vulgata*.

Patella vulgata is a (univalve) gastropod, a soft-bodied invertebrate
that is protected by a very hard, flattened conical shell. Limpets cling
100 tightly to a rock (using the muscular foot). *Patella vulgata* feeds on a
wide range of seaweeds. Limpets eat using a radula, a rough tongue-like
organ that has thousands of tiny denticles. The biggest limpets are up
to 10 cm wide, but most are smaller. The limpet's shell is not coiled.
Limpets may be preyed upon by birds, crabs, starfish and whelks.
105 In this work, limpets (*Patella vulgata*) from several areas of the Si-
cilian coasts were sampled and analyzed for their PAH content. Some
samples of seaweeds were also analyzed because limpets live in or on
the benthos and are grazers feeding on algae.

Potential sources of pollution are well defined in Sicily, as petroleum
110 refinery, dock activities, and some transformation industries can be

4   *A. Gianguzza and S. Orecchio*

found. In fact, the main parts of human activities in the island are related
to agriculture, tourist resorts, and fishing activities localized on the coast.
This knowledge makes it easy to know what the main pollutant sources
are.

Visual observations made by us have shown the presence of both tar   115
balls and oil slicks around the island and stranded on the beaches, which
could be related to the parent crude oils. The increasing power of tourist
resources and fishing activities around this island makes necessary the
establishment of pollution monitoring programs.

In the present study, investigations were carried out into some PAH   120
identified by the US-EPA as requiring priority monitoring action within
the framework of environmental quality control (16). Other non US-
EPA listed PAH, namely perylene and some methyl derivatives, were
also investigated in order to obtain further information on their origins
(17–20).   125

In this work, together with PAH, we analyze water and lipid content
because for other matrices it has been demonstrated that the concentra-
tions of PAH in organisms were affected by chemical composition of the
matrices such as organic matter and water content (21). Relationships
between PAH concentration and distribution, which are useful for evalu-   130
ating possible sources of contamination, were defined by comparing the
profiles of 19 PAH compounds recovered from samples.

EXPERIMENTAL PROCEDURE

Study Site

Patella vulgata samples were collected at 16 sampling sites (Figure 1),   135
characterized by different human impacts and different hydrodynamic
and natural conditions. Sites of sampling were chosen in the Gulfs of
Palermo, Catania, Siracusa (Table 1, Figure 1) because of their high con-
centration of contamination sources, including municipal developments,
important trading port and, in the area of Siracusa, some petroleum-plants 140
and oil loading terminals. The Marsala sampling site was taken as refer-
ence. Seaweed samples were collected at four sampling sites, referred to
as Augusta harbour (2a), Club Nautico (5a), Ortigia (9a), Palermo (12a)
(Table 1, Figure 1).

Sampling   145

The samples (approx. 30 limpets with a shell length of 3–5 cm) were
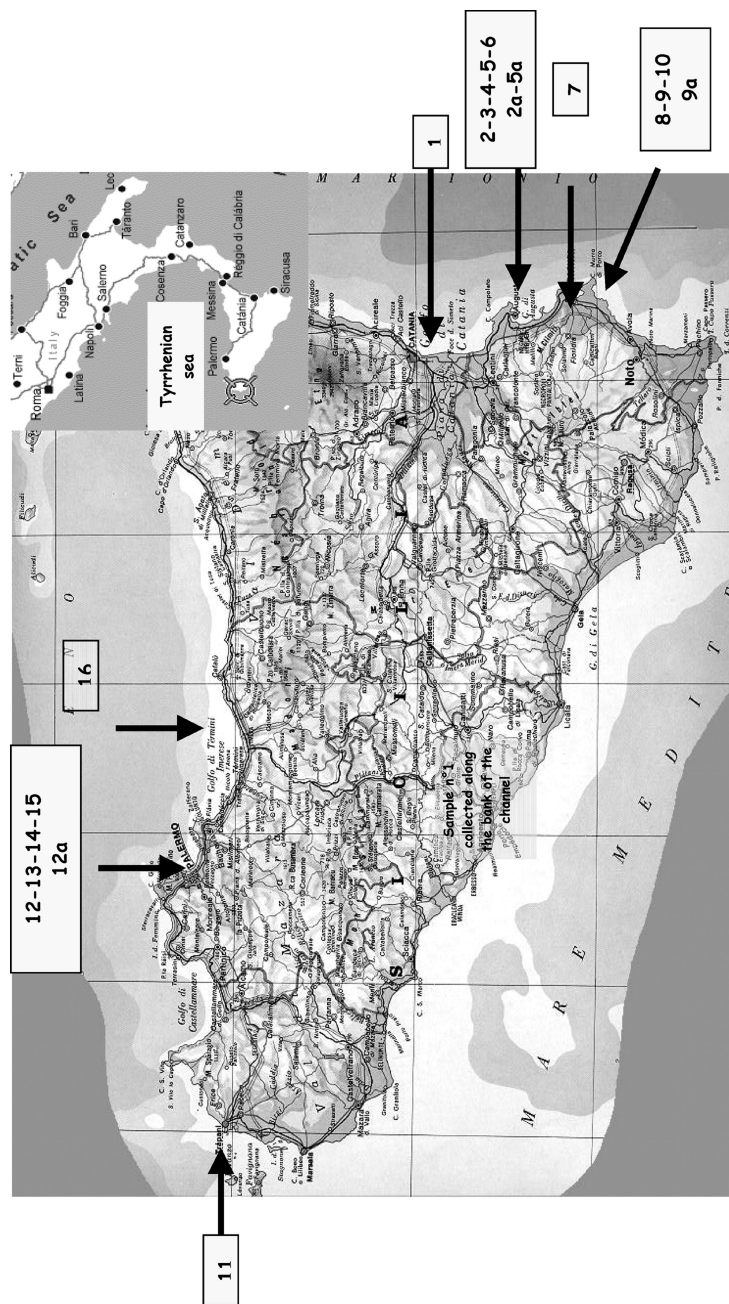taken along the shoreline at middle tide through 2003–2004. Efforts

FIGURE 1. Location of sampling sites.

6   *A. Gianguzza and S. Orecchio*

TABLE 1. Characteristics of Sampling Sites

Station N°	Denomination of station	Characteristics of sites
1	Catania harbor	On the inside of trading harbor. The main human influence is due to maritime traffic.
2-2a	Augusta harbor	On the inside of trading harbor. The main human influence is due to maritime traffic.
3	Augusta breakwater	On the inside of breakwater.
4	Augusta breakwater	On the outside of breakwater.
5-5a	Augusta nautical club est	The main human influence is due to maritime traffic.
6	Augusta nautical club ovest	The main human influence is due to maritime traffic.
7	Priolo	Is the closest to the industrial area where a number of chemical industries are running.
8	Siracusa Targia	Is assumed to be few contaminated site, because of the limited human influences and the strong water exchange with the sea.
9-9A	Siracusa Ortigia harbor	On the inside of trading harbor. The main human influence is due to maritime traffic.
10	Siracusa Ortigia harbor	On the inside of trading harbor. The main human influence is due to maritime traffic.
11	Marsala	Is assumed as reference, because of the limited human influences and the strong water exchange with the sea.
12-12A	Palermo F. Italico in	On the inside of breakwater. Represents an area characterized by domestic inputs and maritime traffic.
13	Palermo F.Italico out	On the inside of breakwater. Represents an area characterized by domestic inputs and maritime traffic.
14	Palermo Sperone right-hand	On the inside of breakwater. Represents an area characterized by domestic inputs and maritime traffic.
15	Palermo Sperone left-hand	On the inside of breakwater. Represents an area characterized by domestic inputs and maritime traffic.
16	Cefalù	Represents an area characterized by light maritime traffic.

were made to obtain specimens of homogeneous size at every station but size differences may occur among the stations. Seaweed was manually collected from four stations. The limpet and seaweed samples, wrapped in aluminium foil, were immediately refrigerated (4°C) on site, stored avoiding exposure to light, and then rapidly transported to the laboratory where they were frozen (-20°C) less than 6 hours later prior to analysis. Tissues were prepared for analysis by manually removing limpet tissue from their shell and homogenizing it.

Laboratory Equipment

All glassware and sample containers were thoroughly washed with hot detergent solution followed by rinsing with distilled water and pure acetone respectively. These were finally baked in the oven at 150°C overnight. To avoid contaminations of samples, different glassware and syringes were used for standards and for solutions extracted from samples.

Chemicals

All chemicals used were of analytical grade with high purity. In particular, n-pentane and dichloromethane, from Fluka, were 99.8% pure, ethanol was analytical grade, from Scharlau Chemie S.A. (Barcelona, Spain). Acetone (Envisolv for analysis of dioxins, furans and PCB) from Fluka was $\geq$99.8% pure. KOH for trace analysis from Fluka, Standard PAH mixtures (EPA 610 PAH mix, lot LA-96245) and perdeuterated internal standards (fortification solution B Lot N° LA-92479) and benz(a)anthracene d_{12} were from Supelco.

Alumina (150 basic, type T particle size 0.063–0.2 mm) and silica (Silica gel, particle size 0.063–0.2 mm; Merck, Darmstadt, Germany) were washed with CH_2Cl_2 and activated for 14 h at 150°C.

Extraction and Clean Up of Limpets

Quality assurance study was carried out in terms of recovery of PAHs. Some techniques and solvents were tested in order to identify the most efficient extraction procedure (Table 2) because certified samples of patellae for PAHs analysis are not available. Together with the PAH analysis, performed on the samples, different recovery tests were carried out by using limpets not containing polycyclic aromatic compounds. These "blank" samples were obtained performing several extraction steps in 48 hours on three samples. After the complete PAH extraction was

A. Gianguzza and S. Orecchio

TABLE 2. Results of Recovery Tests Carried out on a Mixture of 19 Compounds

Extraction method	Recovery % (mean of three analysis)
Soxhlet	72 ± 11
Saponification	82 ± 12
Ultrasound (n-pentane: $CH_2Cl_2 = 1:1$)	61 ± 13
Ultrasound (CH_2Cl_2)	65 ± 14

obtained (checked by GC-MS analysis), a known amount of PAH (EPA) standard mixture was added to each purified "blank" sample. The test samples obtained in this way were extracted using three different methods. The extraction tests, together with the average recoveries, calculated for the compounds investigated, and the relative mean deviations are reported in Table 2. The results favor the saponification method because it allowed us to recover the highest percentage of PAH and are in good agreement with literature data (20).

The internal standard (benz(a)anthracene-d_{12}) was added to 1 g of limpet tissue and the sample was digested for 4 h under reflux after addition of 50 mL of an ethanolic solution of KOH (2 mol/L). After cooling, decantation and addition of 20 mL of water, the digest was liquid-liquid extracted three times with 15 mL of pentane. The extracts were reduced to a small volume using a rotary evaporator (T = $35 \pm 1°C$).

The purification of the extract was performed by liquid chromatography after dilution with dichloromethane/pentane (35:65 v/v) on an alumina micro-column (1.4 g of Al_2O_3, length ca 7 cm). The hydrocarbons were eluted with 8 mL of dichloromethane/pentane (35:65 v/v). The solution was taken up to dryness, diluted with 1 mL of pentane and then transferred to a silica micro-column (0.8 g of SiO_2). The stationary phase was saturated with pentane, the alkanes were eluted with 2 mL of pentane and the aromatic fraction was eluted with 5 mL of the $C_2H_2Cl_2/C_5$ mixture.

The last stage in the procedure involved drying the solution containing PAH under a weak nitrogen flow at room temperature. The dry residue was dissolved in 250 μL solution containing the following perdeuterated internal standards in cyclohexane (0.2 mg/L each): acenaphthene d_{10}; background. phenanthrene d_{10}, chrysene d_{12} and perylene d_{12}.

To increase the quality of results, every four-five analysis, a blank experiment using uncontaminated water and following all procedures of extraction and cleanup described above was carried out which gave a clean background.

Lipid Determination

A Soxhlet extraction procedure was used. About 5 g of dried limpet
tissue were extracted with 50 mL of Freon (Carlo Erba, Milano) for 4 h.
The obtained solution was filtered through a filter containing sodium
220   sulphate and the lipid extract was evaporated to dryness under vacuum.
The residue was weighted.

Extraction and Clean Up of Algae

About 5 g of sample was treated with precleaned (Soxhlet extracted
with dichloromethane for 24 h) anhydrous Na_2SO_4 (Carlo Erba). A so-
225   lution of a perdeuterated internal standard (benz(a)anthracene-d_{12}) was
added.

All samples were extracted in a Soxhlet extractor for 24 hours by
using the solvent mixture dichloromethane-pentane 1:1 (Carlo Erba,
Milano). The extracts were filtered through pre-cleaned Pasteur's pipette
230   filled with solvent-rinsed glass wool and pre-cleaned anhydrous Na_2SO_4,
rinsed and concentrated in a rotary evaporator with the thermostatic
bath at T = 35 $\pm$ 1°C. The final volume was around 5 mL. Finally,
the solution containing PAH was taken to dryness under a weak ni-
trogen flow. The dry residue was dissolved in 0.5 mL solution con-
235   taining the following perdeuterated internal standards in cyclohexane
(0.2 mg/L each): Acenaphthene d_{10}; Phenanthrene d_{10}, Chrysene d_{12} and
Perylene d_{12}.

PAH Analysis

PAH were analyzed with a Shimadzu gas chromatograph-mass spec-
240   trometer (GC-MS mod. GC-17A quadrupole detector GCMS-QP5000)
equipped with a capillary Equity-5 (30 m × 0.25 id, 0.5 μm) column
from Supelco (Milano, Italy) and a splitless injector (0.61 min). The gas
carrier was He at a flow rate of 1.4 ml min^{-1}. The injector was heated
at a temperature of 280°C; the column temperature was 40°C for 2 min,
245   then it was raised to 100°C at 40.0°C min^{-1}, to 200°C at 10°C min^{-1},
to 325°C at 30°C min^{-1} and kept for 8 min. The detector was seated at
250°C. The total run time was about 35 min. The spectrometer was used
in selected ion monitoring mode (SIM).

Identification of the components of the standard mixture was carried
250   out by comparing retention times for each component in the mixture
with those of pure components analyzed under the same experimental
conditions. Identification was confirmed by comparing the spectra of the

10   *A. Gianguzza and S. Orecchio*

single components with those stored in the acquisition system library. The identification of PAH in the solutions extracted from limpets and seaweeds was carried out on the basis of previously determined retention **255** times and confirmed using mass spectra.

The PAH content in the sample was quantified relatively to the perdeuterated PAH added to the dry residue. The response factors for different compounds were measured by injecting, every two days, a mixture containing standard compounds and having the same concentration of **260** perdeuterated PAH as that used for spiking the sample. The most abundant ion was used for quantification and two other ions were additionally used for confirmation. Selected ion chromatogram of a *patella vulgata* extract is given in Figure 2.

Water Content Analysis   **265**

About 2 g of homogenized sample (limpets or seaweeds) was dried at 105°C for one night. The water content was determined by weight loss and was utilized to correlate all the results with dry weight.

(a)

(b)
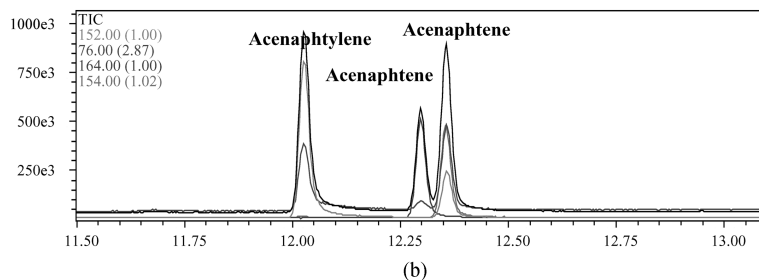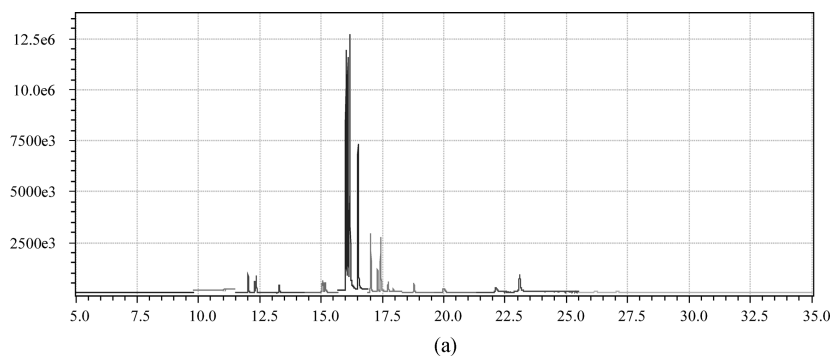
FIGURE 2. (a) Select ion monitoring chromatogram of a limpet extracted. (b) Chromatogram of three components of a limpet extracted.

Q1

RESULTS AND CONCLUSIONS

270   Extraction yields, utilizing perdeuterated internal standard (benz(a)-anthracene-d_{12}) was never less than 70% and in most cases almost 100%.

In Table 3 are reported the average of three independent measurements for individual and total concentrations of PAH, water and lipid content detected in limpet and seaweed samples.   Q2

275   To evaluate the precision of the analysis, three replicates of field samples (limpets and seaweeds) were analyzed. For the limpets, the relative standard deviation (RSD) of the replicas on the concentrations of individual compounds ranged from 4 to 20%, while for the seaweed ranged from 7 to 18%.

280   The detection limit (LOD), estimated as 3σ (three times the background noise) (IUPAC criterion), was similar for all analyzed compounds and results less than 0.2 μg/Kg d.w. for all analytes. The blank values of analytical procedure remained always below the quantification limit (LOQ): 0.7 μg/Kg d.w., estimated as 10 times σ.

285   For the limpets, the total concentration of the 22 compounds investigated, expressed as the sum of concentrations, ΣPAH, varies from 34 to 750 μg/Kg of dry matrix. The wide range of PAH concentrations found in the limpets indicates heterogeneous levels of contamination. This can be explained by considering the different level of contamination of the

290   sites.

The highest PAH concentrations have been measured in stations n° 2, 1 (Augusta Harbor, Catania Harbor) and n° 4 (Augusta breakwater). The high concentrations of PAH recorded at stations corresponding to harbors are due to the petroleum contamination (in some cases roughly

295   manifest) in the considered sites. Station n° 4, located at Augusta, represents an area characterized by domestic inputs and maritime traffic. These stations probably represent localized sites of high contamination and do not reflect the concentrations found in the remainders of areas.

300   The concentrations of PAH in limpets close to pollution sources (n° 2, 1, 4) had high concentrations compared to limpets sampled away from point sources and in reference station (n° 11) located at Marsala. This evidence is in good agreement with results of Baumard (23) who suggested that away from pollution sources there was a threshold below which or-

305   ganisms balanced the uptake and depuration of PAH thus maintaining a relatively constant concentration.

The concentration of carcinogen PAH in the samples of *patella vulgata* ranged from 0.8 to 240 μg/Kg d.w. expressed as benzo(a)pyrene (24, 25) (Figure 3).

12

TABLE 3. Results of Analysis (Individual, Total PAH Concentration (μg/Kg dry weight) Content Carried out in Mytilus Galloprovincialis by Baumard et al. (30)

Station	P	A	Fluo	Py	BaA	Ch	BF	HaF	ReP	BaP	Per	IP	BPer	DA	ΣPAH
Mytilus galloprovincialis															
Le Planier	17.5	2.28	10.2	18.5	3.25	8.86	4.2	0.32	4.50	1.90	0.44	2.29	4.49	0.93	79.6
Carteau	12.5	1.27	8.23	5.59	1.87	9.02	2.83	0.25	3.17	0.54	0.57	0.79	1.24	0.20	48.1
Fort Brescou	10.4	1.14	5.66	4.69	1.79	5.43	2.79	0.25	2.17	0.66	0.96	1.18	1.78	0.15	39.0
Leucate harbor	16.3	1.20	4.68	4.08	2.60	5.36	5.44	0.37	3.70	1.32	1.95	1.40	1.62	0.27	50.3
Vendres harbor(o)	12.8	1.14	5.19	4.12	1.20	6.50	3.38	0.17	2.83	0.38	0.22	0.87	1.45	0.12	40.3
Vendres harbor (i)	60.8	19.1	29.5	47.5	10.0	71.0	38.2	1.24	29.9	3.61	1.97	9.16	12.9	1.10	337
Cola Manjoy	15.3	1.29	4.06	2.83	1.13	3.96	2.66	0.20	1.48	0.34	0.19	1.54	1.89	0.09	37.5
Calleta	13.6	4.35	9.00	10.2	4.79	8.88	6.75	0.56	5.08	1.72	0.34	6.26	9.78	0.35	82.2
Barcelona harbor	33.9	5.05	41.0	64.2	17.6	73.0	26.4	1.74	30.3	5.31	21.2	5.39	9.37	1.37	336
Barcelona harbor	7.11	1.64	6.55	8.21	4.11	10.33	7.05	0.54	6.17	1.24	2.51	1.76	2.35	0.46	60.0
Cap de Salou	13.5	1.12	4.91	4.69	2.22	6.65	4.92	0.39	3.9	1.31	0.46	2.57	4.14	0.25	51.0
Cap Roig	5.62	0.74	3.65	1.75	1.35	3.74	2.38	0.17	2.09	0.40	1.88	0.41	0.90	0.05	25.1

TABLE 4. Results of Analysis (Individual, Total and Carcinogenic PAH Concentration (μg/Kg dry weight) a), Lipid Content Carried out in Limpets and Seaweeds

Compounds/Samples N°	1	2	2a	3	4	5	5a	6	7	8	9	10	11	12	12a	13	14	15	16
Acenaphthene	0,1	7,1	0,2	3,1	18,5	0,0	0,3	0,0	0,0	0,4	0,5	0,5	0,5	1,1	1,1	2,8	0,9	0,5	0,3
Acenaphthylene	3,1	15,1	1,4	2,6	9,3	1,2	0,4	1,2	2,2	1,3	0,9	1,6	2,6	3,7	0,2	3,9	2,3	2,5	1,7
Fluorene	20,8	65,1	2,0	8,5	14,4	4,7	1,9	8,3	9,8	6,4	6,3	6,2	2,3	14,1	3,4	16,8	9,0	8,4	8,8
Phenanthrene	123,6	175,0	7,9	14,0	30,0	15,8	12,2	43,7	45,0	19,6	55,0	26,8	22,9	73,1	17,2	60,5	37,7	29,2	19,9
Anthracene	4,4	6,4	2,4	1,1	2,6	0,7	1,3	0,9	4,0	1,8	5,1	2,7	0,7	5,3	2,1	2,5	2,2	2,5	0,8
9-methylanthracene	0,1	17,3	0,2	1,0	4,9	1,2	0,4	0,0	3,2	1,1	0,0	1,0	0,2	7,3	0,2	7,8	4,9	3,9	3,4
3-methylanthracene	0,0	0,2	0,1	0,5	2,6	0,2	0,2	0,0	0,0	0,2	0,0	0,0	0,1	0,4	0,1	0,1	1,1	0,4	1,9
Fluoranthene	38,5	7,1	0,1	5,7	16,0	2,9	5,7	14,8	13,2	2,7	37,2	16,2	2,3	13,8	0,1	5,7	7,8	13,3	4,3
Pyrene	252,6	6,5	0,1	5,0	15,6	3,1	3,6	9,8	21,3	4,6	30,3	15,5	3,0	47,3	0,1	7,2	16,9	12,5	4,4
1 methyl pyrene	8,3	10,8	0,3	1,2	10,8	0,5	0,5	0,4	1,0	0,4	1,0	1,0	0,5	1,8	0,2	0,5	2,6	2,0	0,1
Benz(a)anthracene	10,7	1,9	0,2	4,4	16,9	0,2	0,4	0,9	9,1	0,4	16,1	16,5	0,2	3,2	0,2	0,4	1,9	10,7	0,2
Crysene	18,9	10,9	1,0	4,2	19,0	0,2	1,0	1,3	11,6	0,3	17,5	19,7	0,2	4,7	2,7	0,2	1,9	7,9	0,5
Benzo(b)fluoranthene	14,3	2,3	2,1	9,5	44,1	0,2	1,6	1,9	4,6	0,2	16,3	22,4	0,3	0,2	0,3	0,3	1,2	10,3	3,3
Benzo(k)fluoranthene	4,7	4,1	0,7	5,2	25,1	0,2	0,9	1,0	1,6	0,2	18,4	20,2	0,4	0,2	0,3	0,3	1,2	8,5	1,5
Benzo(a)pyrene	5,7	3,3	0,6	5,4	25,5	0,4	0,8	0,1	11,2	0,4	25,4	34,7	0,5	0,4	0,5	0,5	0,8	8,4	0,3
Perilene	13,9	33,2	0,7	2,3	20,2	0,4	0,9	1,9	2,4	0,4	12,6	11,4	0,7	0,4	0,7	0,5	1,5	0,9	3,0
Indeno(1,2,3-cd) pyrene	0,2	87,9	0,6	9,9	28,5	0,4	0,9	0,1	2,3	0,4	20,9	30,1	0,9	0,4	0,7	0,5	0,8	4,0	26,2
Dibenz(a,h,)anthracene	0,3	226,0	1,3	12,0	53,1	0,8	1,7	0,1	4,5	0,7	11,5	22,3	1,6	0,8	1,4	1,0	1,3	6,4	11,3
Benzo(g,h,i)perilene	0,1	69,3	0,9	7,7	48,1	0,5	1,2	0,0	10,5	0,5	17,8	41,5	1,0	0,6	1,0	0,7	0,9	7,3	19,1
Cancerogenic PAH (microg/Kg d.w.)	10,9	240,0	2,4	20,6	92,0	1,3	3,1	0,8	18,6	1,3	45,8	67,9	2,3	2,1	2,3	1,7	2,8	19,0	14,8
Total PAH (microg/Kg d.w.)	520	750	23	103	405	34	36	87	157	42	293	290	41	179	32	112	97	139	111
Fatty substance (%)	1,5	1,80	—	1,50	1,10	1,20	—	1,40	1,56	1,60	1,75	nd	0,85	1,60		1,35	2,20	2,22	

(Continued on next page)

13

TABLE 4. Results of analysis (individual, total and carcinogenic PAH concentration (μg/Kg dry weight) a), lipid content carried out in limpets and seaweeds (*Continued*)

COMPOUNDS/SAMPLES N°	9a	10	11	12	12a	13	14	15	16
Acenaphthene	1,4	0,5	0,5	1,1	1,1	2,8	0,9	0,5	0,3
Acenaphthylene	1,9	1,6	2,6	3,7	0,2	3,9	2,3	2,5	1,7
Fluorene	0,7	6,2	2,3	14,1	3,4	16,8	9,0	8,4	8,8
Phenanthrene	6,3	26,8	22,9	73,1	17,2	60,5	37,7	29,2	19,9
Anthracene	0,7	2,7	0,7	5,3	2,1	2,5	2,2	2,5	0,8
9-methylanthracene	0,3	1,0	0,2	7,3	0,2	7,8	4,9	3,9	3,4
3-methylanthracene	0,2	0,0	0,1	0,4	0,1	0,1	1,1	0,4	1,9
Fluoranthene	3,2	16,2	2,3	13,8	0,1	5,7	7,8	13,3	4,3
Pyrene	2,9	15,5	3,0	47,3	0,1	7,2	16,9	12,5	4,4
1 methylpyrene	0,4	1,0	0,5	1,8	0,2	0,5	2,6	2,0	0,1
Benz(a)anthracene	0,3	16,5	0,2	3,2	0,2	0,4	1,9	10,7	0,2
Crysene	0,2	19,7	0,2	4,7	2,7	0,2	1,9	7,9	0,5
Benzo(b)fluoranthene	0,3	22,4	0,3	0,2	0,3	0,3	1,2	10,3	3,3
Benzo(k)fluoranthene	0,3	20,2	0,4	0,2	0,3	0,3	1,2	8,5	1,5
Benz(a)pyrene	0,6	34,7	0,5	0,4	0,5	0,5	0,8	8,4	0,3
Perilene	0,7	11,4	0,7	0,4	0,7	0,5	1,5	0,9	3,0
Indeno(1,2,3-cd) pyrene	0,7	30,1	0,9	0,4	0,7	0,5	0,8	4,0	26,2
Dibenz(a,h,)anthracene	1,4	22,3	1,6	0,8	1,4	1,0	1,3	6,4	11,3
Benzo(g,h,i)perilene	1,0	41,5	1,0	0,6	1,0	0,7	0,9	7,3	19,1
Cancerogenic PAH (microg/Kg d.w.)[a]	2,2	67,9	2,3	2,1	2,3	1,7	2,8	19,0	14,8
Total PAH (microg/Kg d.w.)[a]	24	290	41	179	32	112	97	139	111
Fatty Substance (%)[b]	—	1.35	0,85	1,60	—	1,35	2,20	2,22	1.40

a) Relative standard deviations for limpets range from 4 to 20% while for seaweed from 7 to 18%.
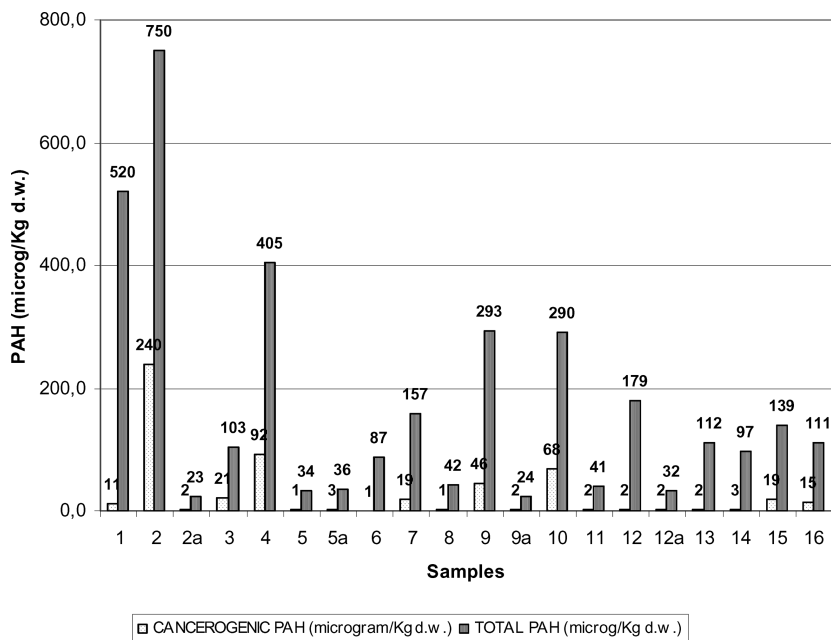
b) Relative standard deviations less than 5%.

14

FIGURE 3. Total and carcinogenic PAH concentration (μg/Kg dry weight).

310   For the seaweeds, the total concentration of the 19 compounds investigated varies from 23 to 36 μg/Kg of dry sample and, practically, shows no distinct spatial trends. These low and nearly constant concentrations constitute an apparent baseline level, which is through the result from equilibrium between uptake and depuration of the absorbed PAH.

315   In most of the sampling sites, the same distribution of PAH (expressed as weight percentage) is observed. Phenanthrene, Fluoranthene and Pyrene are the three most abundant components in nearly all the samples.

     In Table 3, as comparison, are shown the PAHs concentrations

320 recorded on bivalves (*Mytilus galloprovincialis*) sampled on European coasts and analysed by Baumard et al. (23). In this case PAH concentrations ranged from 25 to 337 μg/Kg d.w. The mussels, filter-feeding organisms, were mainly exposed to the soluble fraction of PAHs. Therefore, they accumulate the bio available fraction of the contaminants present in

325 the water column (dissolved and particulate PAHs and the compounds present in the plankton and the PAH content of their tissues reflect the profile of the compounds they have been exposed to).

     The accumulation of contaminants in the limpets may be either indirectly caused by stimulation of the animal's feeding and metabolic

activities, or by an increased uptake of contaminated food particles. **330**
Limpets are a food source for other species; in fact, directly or indirectly the presence of the pollutants in their tissues may be a risk for potential bioaccumulation in the food.

For the limpets, the lipid content is in the range from 0.85 to 2.2%. With the aim to find a relationship between the lipid content and the **335** concentration of total PAHs determined in samples investigated, we carried out a linear regression analysis. The results show that there is no correlation of considered parameters.

Some processes can generate polycyclic aromatic compounds. What makes it difficult to accurately identify PAH origins is the fact that there **340** exist a number of possible sources and processes that analytes can undergo prior to absorption in seaweeds and limpets.

The molecular patterns generated by each source, however, are like fingerprints, which make it possible to hypothesize which processes generate PAH by studying their distribution in samples. Pyrolytic sources **345** are characterized by the presence of PAH over a wide range of molecular weights, while petrogenic sources are dominated by the lowest molecular weight PAH.

The presence of low molecular weight compounds is typically from spill-associated hydrocarbons (24), while pyrogenic polycyclic aromatic **350** hydrocarbons are generally characterized by the dominance of high molecular mass PAH over those with low molecular mass (25).

If we group polycyclic aromatic compounds into different classes depending on the number of aromatic rings present in their structure, it can be observed (Figure 4) that the PAH with 2,3 and 4-rings found **355** in Patella vulgata samples at the sites under investigation constitute from 44 to 98% of the total, while for the seaweed ranged from 82 to 88%.

However a preferential accumulation of the lower molecular weight and more water soluble PAH was observed. Thus, limpets accumulated **360** preferentially phenanthrene, the most soluble PAH measured, but also the tetra-aromatic PAH, compounds that are present in great abundance in petroleum. These evidences suggest that PAH contamination in the limpets and in the seaweeds might originate mainly from the pollution caused by the petrogenic sources. **365**

In our stations, limpets were located on piers of jetty with high water depth, far from the sediment and the seaweeds and are exposed to the petroleum contamination present in the upper part of the water column and/or deposited on surface of rocks. The occurrence of petroleum on the surface of the water may often be observed by the diffraction of the **370** sun light.
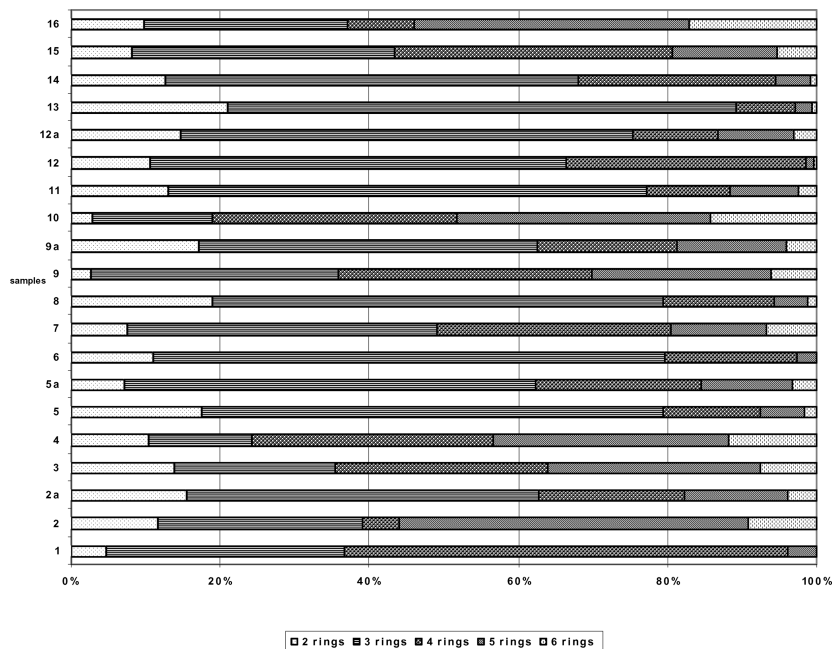
FIGURE 4. Contribution of 2,3,4,5 and 6 rings to total PAH.

Further, sources of PAH pollution in the aquatic ecosystems under investigation were estimated by comparing the distribution indexes of some polycyclic aromatic compounds with their concentration ratios. Supposing that patellae, likewise mussels, are characterized by low biotransformation capacities (23) compared to vertebrates, PAH ratios used in marine sediments we use for marine invertebrates (patellae). Phenanthrene/anthracene and fluoranthene/pyrene ratios have commonly been used as a means of determining the main origins of PAH (19, 28, 29).

Phenanthrene and anthracene are both structural isomers. In particular, phenanthrene is more thermodynamically stable than anthracene; therefore, in petrogenic PAH pollution the Ph/An ratio is very high, while high temperatures during the combustion process favor the formation of anthracene and a lowering of the Ph/An ratio. Because of the differences in reactivity and solubility of these two pairs of isomers, their respective ratios are not expected to remain constant and cannot, therefore, provide a picture of the progress of PAH from their origins, through environmental transport, to uptaking in marine organisms. In particular, a ratio of Phe/Ant <10 and Flu/Py >1 indicated a contamination due to pyrolytic
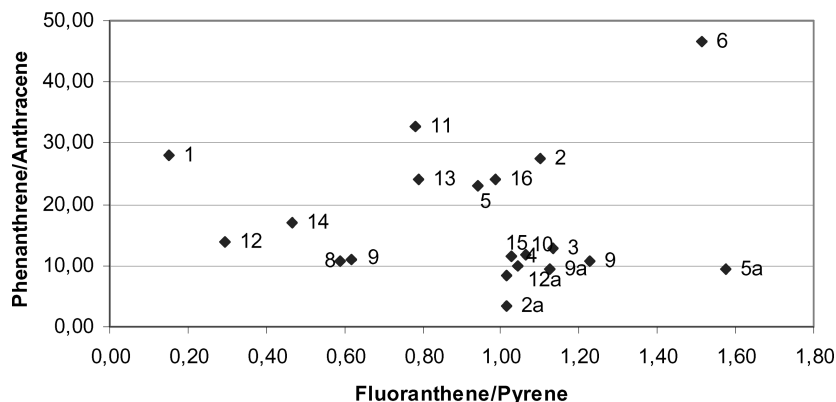
FIGURE 5. Plot of ratio Phenanthrene/Anthracene *vs*. ratio Fluoranthene/Pyrene.

origin (30). Figure 5 shows values of these ratios calculated for limpets and seaweeds samples.

The plot suggests that PAH in the limpets are mainly of petrogenic origin while in the seaweeds there is a small contribute of pyrolytic origin. Additionally, it is reported in literature (29) that a phenanthrene/-anthracene ratio of about four is typical of urban areas, while larger ratios were more common for remote locations. The average ratio for all the sites investigated in this work was 19.7 for limpets and 7.6 for seaweed, which are consistent with the relative long distance of urban site from the sampling stations.

Total methylated PAH ranged from 0.4 to 8.9% of total PAH. The highest methylated PAH concentrations have been measured in samples of limpets of stations n° 14, 13, 12 (Palermo Sperone, Palermo Foro Italico, Augusta Nautical Club). The higher concentrations of substituted PAH are recorded at stations corresponding to areas devoted to maritime activities and are due to the petroleum contamination (in some cases roughly manifest). Some authors stated that different sources will give mixtures with quite different relative amounts of unsubtituted PAH compared to their alkyl homologues and La-Flamme and Hites (30) report that high temperatures ($\approx 2200°$K) will yield soot devoid of alkyl PAH. Lower temperatures ($\approx 1100°$K) will yield quite abundant substituted compounds, while extended periods of time (10^6 years) at low temperatures ($\approx 430°$K) will yield PAH mixtures in which alkyl PAH dominate (as in petroleum).

Our main remarks are as follows:

1. The present study made it possible to optimize extraction and analytical conditions for the determination of PAH in patella. Under these

conditions, recoveries are very good: never less than 70% and in most cases almost 100%. Reproducibility is also satisfactory (relative standard deviation ranged from 4 to 20%).

2. The use of an Equity-5 column allowed complete separation in a shorter space of time (about 30 min) than proved possible in previous studies on PAH (6, 32). The results reported here represent the first quantitative investigations of PAH in *patella vulgate* of Mediterranean area.

3. The greater presence of PAH with low molecular weights (2–4 rings) in all samples and the Ph/An and Fl/Py ratio values used as PAH distribution indexes demonstrate that most samples owe their PAH to a predominant single origin, i.e., petroleum product.

4. Total PAH content in limpet samples were not correlate with PAH concentrations of seaweeds collected in the same place.

5. There is no evidence of coal-tar contamination; for coal-tar the Fl/Py ratio is ≥ 1.5 and this ratio is lower for limpets collected.

6. Total PAH concentrations in the limpets were not correlated with lipid content.

REFERENCES

1. E. J. Hoffman, G. L. Mills, J. S. Latimer, and J. G. Quinn, Urban Runoff as a Source of Polycyclic Aromatic Hydrocarbons to Coastal Waters, *Environmental Science and Technology* 18 (1984):580–587.

2. R. J. Pruell and J. G. Quinn, Geochemistry of Organic Contaminants in Narragansett Bay Sediments, *Estuarine Coastal and Shelf Science* 21 (1985):295–312.

3. M. Howsam and K. C. Jones, Sources of PAH in the Environment, in *The Handbook of Environmental Chemistry*—PAH and Related Compounds, vol. 3–I (1998):137–174, (Springer).   Q3

4. S. G. Wakeham, C. Schaffner, and W. Giger, Perylene in Sediments from the Namibian Shelf, *Geochimica et Cosmochimica Acta* 43 (1979):1141–1144.

5. S. G. Wakeham, C. Schaffner, and W. Giger, Polycyclic Aromatic Hydrocarbons in Recent Lake Sediment—II. Compounds Derived from Biogenic Precursors during Early Diagenesis, *Geochimica et Cosmochimica Acta* 44 (1980):415–429.

6. A. Giacalone, A. Gianguzza, M. R. Mannino, S. Orecchio, and D. Piazzese, Polycyclic Hydrocarbons in Sediments of Marine Coastal Lagoons in Messina, Italy: Extraction and GC/MS Distribution and Sources, *Polycyclic Aromatic Compounds*, 24(2), (2004):135–149.

7. J. Koistinen, Sirpa S. Herve, M. Lahtiperä, and J. Paasivirta, Chloroaromatic Pollutants in Mussels Incubated in Two Finnish Watercourses Polluted by Industry, *Chemosphera* 34(12) (1997):2553–2569.

8. P. Somerfield, M. Gee, and R. M. Warwick, Benthic Community Structure in Relation to an Instantaneous Discharge of Wastewater from a Tin Mine, *Mar. Pollut. Bull.* 28(6) (1994):363–369.

20   *A. Gianguzza and S. Orecchio*

9. N. Kannan, T. B. H. Reusch, D. E. Shulz-Bull, G. Petrick, and J. C. Duinker, Chlorobiphenyls: Model Compounds for Metabolism in Food Chain Organisms and Their Potential use as Ecotoxicological Stress Indicators by Application of the Metabolic Slope Concept, *Environ. Sci. Technol* 29 (1995):1851–1859.

10. M. I. Dassenakis, M. A. Kloukiniotu, and A. S. Pavlidon, The Influence of Long Existing Pollution on Trace Metal Levels in a Small Tidal Mediterranean Bay, *Mar. Poll. Bull*. 22 (1996):275–282. **465**

11. P. W. Kwan and R. C. Clark, Assessment of Oil Contamination in Marine Environment by Pattern Recognition Analysis of Parafinic Hydrocarbon Content of Limpets, *Anal. Chim. Acta* 133 (1981):151–168. **470**

12. P. Baumard, P. Garrigues Budzinski, J. F. Narbonne, T. Burgeot, X. Michel, and J. Bellocq, Polycyclic Aromatic Hydrocarbon (PAH) Burden of Limpets (Mytilus Sp.) in Different Marine Environments in Relation with Sediment PAH Contamination, and Bioavailability, *Marine Environmental Research* 47 (1999):415–439.

13. A. Y. Cantillo, Comparison of Results of Mussels Watch Programs of the United States and Frame with Worldwide Mussel Watch studies, *Mar. Poll. Bull*. 36 (1998): 712–717. **475**

14. D. J. H. Phillips, *Quantitative Aquatic Biological Indicators*, Applied Pu. Ltd, London: (1980).

15. E. Mendez, M. S. Astorga Espana, and F. J. Garcia Montelongo, Polycyclic Aromatic Hydrocarbons and n-alkanes in the Intertidal Limpet Patella Crenata from the Coast of Tenerife (Canary Islands), *Bull. Environ. Contam. Toxicol*. 63 (1999):665–672. **480**

16. B. R. T. Simoneit, Biomarker PAH in the Environment in The *Handbook of Environmental Chemistry—PAH and Related M Compounds*, vol. 3–I, (1998):175–222, (Springer) **485**

17. S. Sportsol, N. Gros, R. G. Lichtenthaler, K. O. Gustavsen, K. Urdel, F. Ordel, and J. Skel, Source Identification of Aromatic Hydrocarbons in Sediments Using GC/MS, *Environmental Science and Technology*, 17 (1983):282–286.

18. J. C. Colombo, E. Pelletin, C. Brochn, M. Khall, and A. Catoggio, Determination of Hydrocarbon Distribution Indexes—Case Study: Rio de la Plata estuary, Argentina, *Environmental Science and Technology*, 23 (1989):888–894. **490**

19. H. Budzinski, I. Jones, J. Bellocq, C. Piérard, and P. Garrigues, Evaluation of Sediment Contamination by Polycyclic Aromatic Hydrocarbon in the Gironde Estuary, *Marine Chemistry*, 58 (1997):85–97. **495**

20. Irwin J. Roy, Environmental Contaminants Encyclopedia, www.nature.nps.gov/hazardssafety/toxic//, (1998).

21. G. B. Kim, K. A. Maruja, R. F. Lee, J. H. Lee, C. H. Koh, and S. S. Tanabe, Distribution and Sources of Polycyclic Aromatic Hydrocarbons in Sediments from Kyeonggi Bay, Korea, *Marine pollution Bulletin* 28 (1999):166–169. **500**

22. D. R. Oros and J. R. M. Ross, Polycyclic Aromatic Hydrocarbons in San Francisco Estuary Sediment, *Marine Chemistry* 86 (2004):169–184.

23. P. Baumard, H. Budzinski, P. Garrigues, J. C. Sorbe, T. Burgeot, and J. Bellocq, Concentrations of PAHs in Various Marine Organisms in Relation to Those in Sediments and to Trophic Level, *Marine Pollution Bulletin* 36 (1998):951–960.

Q4

505   24. P. R. Mc Clure, Evaluation of a Component-based Relative Potency Approach
          to Cancer Risk Assessment for Exposure PAHs. Poster presented at the Annual
          Meeting of the Society of Toxicology, Anaheim California, March 11, 1996, on
          line http//esc.syrres.com/≈esc1/poster1/poster.htm.
      25. C. Minoia and L. Perbellini, Monitoraggio Ambientale e Biologico dell'Esposizione
510   Professionale a Xenobiotici, Vol. 2 (Como): Morgan (2000).
      26. J. Conde, E. PenÄ a, and GarcõÂ a F. Montelongo, Sources of Tar Balls and
          Oil Slicks on the Coasts of the Canary Islands, *Inter. J. Environ. Anal. Chem.* 62
          (1996):77–84.
      27. G. Witt, Polycyclic Aromatic Hydrocarbons in Water and Sediments of the Baltic
515   Sea, *Marine Pollution Bulletin* 36 (1998):577–586.
      28. P. M. Gschwend and R. A. Hites, Fluxes of the Polycyclic Aromatic Hydrocarbons
          to Marine and Lacustrine Sediments in the Northeastern United States *Geochimica
          et Cosmochimica Acta* 45 (1981):2359–2367.
      29. N. Ohkouchi, K. Kawamura, and H. Kawahata, Distributions of Three- and Seven
520   Rings Polynuclear Aromatic Hydrocarbons on the Deep Sea Floor in Central Pacific,
          Environ. Sci. Technol. 33 (1999):3086–3090.
      30. R. E. LaFlamme and R. A. Hites, The Global Distribution of Polycyclic Aromatic
          Hydrocarbons in Recent Sediment, *Geochim. Cosmochim. Acta*, 42 (1978):289–
          303.
525   31. C. Y. Raoux and P. Garrigues, Mechanism Model of Polycyclic Aromatic Hydrocar-
          bons Contamination of Marine Coastal Sediments from the Mediterranean Sea. P.
          In Garrigues, & M. Lamotte (Eds.), Proceedings of the 13th international syposium
          on polynuclear aromatic hydrocarbons; Bordeaux, France 1–4 October, 1991 (pp.
          443–450). (Langhorn, PA: Gordon & Breach publishers).
530   32. L. Culotta, M. R. Melati, and S. Orecchio, The use of Leaves of *Rosmarinus of-
          ficinalis L.* as Samplers for Polycyclic Aromatic Hydrocarbons, Assessment of Air
          Quality in the Area of Palermo, *Ann. Chim. (Rome)* 92 (2002):837–845.