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Polycyclic Aromatic Hydrocarbons in Marine Organisms from the Gulf of Naples, Tyrrhenian Sea

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Polycyclic aromatic hydrocarbons (PAHs) were determined by an HPLC method with fluorescence detection in bivalves (*Mitylus galloprovincialis*), cephalopods (*Todarodes sagittatus*), crustaceans (*Aristeus antennatus*), and fish (*Mullus surmeletus*, *Scomber scombrus*, *Micromesistius poutassou*, and *Merluccius merluccius*) caught in the Gulf of Naples (Tyrrhenian Sea, Italy). Anthracene, fluoranthene, pyrene, benz(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, and benzo(*k*)fluoranthene were detected, at different concentrations, in all of the examined marine organisms, whereas benzo(*a*)pyrene, dibenz(*a*,*h*)anthracene, benzo(*g*,*h*,*i*)perylene, and indeno(1,2,3-*cd*)pyrene were found only in Mediterranean mussels. Of mussels collected in winter 71.43% exceeded the maximum residual levels (MRL) fixed for the benzo(*a*)pyrene in European Regulation 208/2005/EC, whereas all samples collected in summer reported values lower than this limit. In comparison to the other marine organisms, the mussels showed the highest PAH concentrations ($p < 0.01$). Fish showed total PAH levels lower than those of cephalopods and, in particular, European hake showed the lowest values (6.06 ng/g of fresh weight).

KEYWORDS: Polycyclic aromatic hydrocarbons; fish; crustaceans; cephalopods; mussels; Tyrrhenian Sea

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are toxic compounds generally formed as the result of incomplete combustion of organic material (*1*). They are ubiquitous contaminants in the marine environment derived from uncontrolled petroleum spills, marine transport, discharges from ships, and urban runoff, all incomplete combustion at high temperature or pyrolytic processes involving fossil fuels and atmospheric deposition. However, the majority of PAH deposition is anthropogenic (*2*). They are known for their carcinogenic and mutagenic properties and for being responsible for background level contamination in environmental matrices (*3*). In the marine environment PAHs are bioavailable to fish via the food chain, as waterborne compounds and from contaminated sediments (*4*). As a result of their widespread presence PAHs were introduced in international monitoring programs, and recently a maximum residue limit (MRL) was fixed for benzo(*a*)pyrene in fresh fish (*5*). Due to their low water solubility PAHs show a high affinity for the organic fraction, and in water they are adsorbed on particulate matter, which can be deposited as sediments (*6*). As lipophilic compounds they can easily cross lipid membranes and have the potential to bioaccumulate in aquatic organisms (*7*). Their fate in marine organisms is considered to be species dependent. Generally, metabolic capacity in edible aquatic species appears to be best developed in fish, intermediate in crustaceans, and least in molluscs (*8*, *9*). Filtering organisms, such as mussels or oysters, accumulate PAHs and present elimination rates much less than those observed in vertebrates (*10*). PAHs in fish are subjected to an active metabolizing process, through the cytochrome P-450 oxidase, and these oxidation and conjugation reactions facilitate their excretion. During the metabolism of some PAH compounds, reactive intermediates can form that, upon binding to macromolecules such as DNA and RNA, produce covalently bonded adducts that are a necessary step toward the development of PAH-induced cancer (*11*). Fish may be victims of these carcinogenic metabolites, whereas many invertebrates, such as mussels, are protected against PAH-induced cancer by their metabolic incapacity (*12*). Furthermore, recent studies (*13*, *14*) suggest that some PAHs are capable of inducing embryotoxic effects of dioxin interacting with the aryl hydrocarbon receptor (AHr). The inevitable contamination from the accumulation of PAHs is a significant concern that involves both benthic and pelagic marine organisms and also the human population. Particular attention should be paid to the Gulf of Naples, off the coast of the Campania region of southern Italy,

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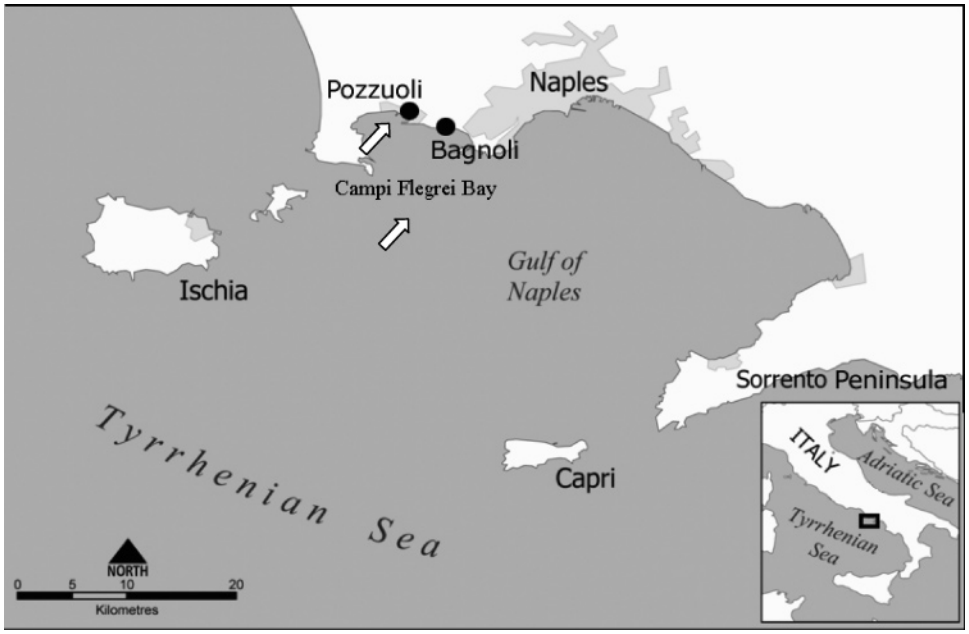

Figure 1. Sampling site in the Gulf of Naples (Italy).

that represents an area of vital environmental importance, where many resident marine species live, feed, and reproduce.

This area is subject to PAH pollution by the proximity to major coastal settlements, by the large industrial complexes clustered along the coast, and possibly by insufficient rates of diffusion due to its semienclosed nature. Furthermore, the Bagnoli industrial area, in western Naples, was dominated by the presence of a steel plant (ILVA) that was in operation from 1905 until 1990, when all activities of the plant terminated. In 1935, between the Island of Nisida and the mainland, a strip of land was built which altered the existing water circulation and sediment distribution. Between 1962 and 1964, part of the sea between the two piers was filled, utilizing contaminated soil from the plant area, to create new space for the widening and development of the industrial activity (*15*).

Despite this actual environmental condition, little is known about the PAH contamination levels in marine organisms living in the Gulf of Naples. The PAH contents in invertebrate organisms, such as bivalves, have been widely investigated, but poor information is available on PAH levels in marine vertebrates, such as fish, living in the Mediterranean Sea. This study investigated the levels of priority PAHs in benthic, demersal, and pelagic fish, crustaceans, and shellfish fished in the Gulf of Naples in order to understand their status of contamination. Mussels were chosen as good bioindicators of pollution due to their ability to filter the water and accumulate PAHs in the marine area. Crustaceans were chosen due to their necrophagous behavior and their benthic characteristics and for having a preferential uptake from sediments. Cephalopods and fish were chosen due to their different feeding behaviors and trophic levels. The influence of biological factors, such as season, feeding habitat, and metabolic capacity was also examined.

In addition, considering the current MRL for benzo(*a*)pyrene in muscle of meat of fish, crustaceans, cephalopods, and bivalves, its presence was evaluated to assess the potential risk for consumers.

MATERIALS AND METHODS

Reagents and Materials. PAH-mix9 (100 ng/μL in acetonitrile) was supplied by Dr. Ehrenstorfer, Reference Materials (Augsburg, Germany), stored at 4 °C, and used for the preparation of working standard solutions (5, 10, 25, 50, and 100 ng/mL in acetonitrile).

Ethanol (ACS, for analysis), cyclohexane (for the analysis of pesticide residues), acetonitrile for HPLC, water plus for HPLC, potassium hydroxide pellets, and anhydrous sodium sulfate crystals were provided by Carlo Erba (Milan, Italy), and Strata FL-PR Florisil (170 μm, 80 A) tubes, 1000 mg/6 mL, were supplied by Phenomenex Inc. (Torrance, CA).

Sample Collection. Blue and red shrimp (*Aristeus antennatus*), red mullet (*Mullus surmeletus*), European flying squid (*Todarodes sagittatus*), Atlantic mackerel (*Scomber scombrus*), blue whiting (*Micromesistius poutassou*), and European hake (*Merluccius merluccius*) were collected by trawling to a depth of 30−60 m in the Gulf of Naples (**Figure 1**). Mediterranean mussel (*Mytilus galloprovincialis*) came from a breeding farm located in the Campi Flegrei Bay, off the coast of Pozzuoli (northern Gulf of Naples). All samples were collected on two occasions: 49 samples in July and the same number in December 2004. Fish, crustacean, and cephalopods, all of commercial size, were measured, weighed, and stored at −20 °C.

Sample Analysis. A number from 2 to10 specimens for each species of fish and 20−40 specimens for mussels and crustaceans were pooled. The shell and byssus of mussels were removed, and the soft tissues as well as the filet of each fish were homogenized before the analysis. PAH content was determined according to the method of Dafflon et al. (*16*). Two grams of homogenized sample was saponified, using dark glass vials, with 10 mL of 1 M KOH in an ethanol solution for 3 h at 80 °C in a water bath. Then 10 mL of water and 20 mL of cyclohexane were added, and samples were mixed by an orbital agitator for 5 min using dark glassware. The hexanic phase was recovered, and the polar mixture was washed once with cyclohexane and then discharged. The extracts were filtered, concentrated under a nitrogen gas stream to about 1 mL, and run on a column filled with Florisil. The eluates were dried under a flow of nitrogen and dissolved with 1 mL of acetonitrile before the analysis. Quantitative analysis of PAHs was carried out with a high-performance liquid chromatography (HPLC) apparatus equipped with a 20 μL loop and a fluorescence detector (Pro-Star 363, Varian, Palo Alto, CA) with variable excitation and emission wavelengths. The software used was Star Chromatography Workstation version 5.2 (Varian).

Table 1. Fluorescence Detection of PAHs at Different Wavelength Combinations ($\lambda_{ex/em}$), Recovery, Linearity, Limit of Detection, and Limit of Quantification

compd	wavelength ($\lambda_{ex/em}$, nm)	recovery[a] (%)	linearity	LOD (ng/g)	LOQ (ng/g)
AN	250–406	41.3 ± 17.9	0.9870	0.13	0.15
FA	280–450	58.8 ± 7.0	0.9992	0.17	0.21
PY	270–390	66.5 ± 12.3	0.9965	0.49	0.62
BaA	265–380	51.7 ± 3.2	0.9899	0.12	0.15
CHR	265–380	55.1 ± 6.0	0.9991	0.16	0.18
BbFA	290–430	54.6 ± 2.9	0.9852	0.05	0.06
BkFA	290–430	60.3 ± 5.8	0.9982	0.01	0.02
BaP	290–430	54.9 ± 5.0	0.9967	0.07	0.10
DBahA	290–410	62.1 ± 7.3	0.9968	0.06	0.08
BghiP	290–410	58.6 ± 9.9	0.9996	0.16	0.24
IP	300–500	51.4 ± 2.7	0.9990	0.21	0.26

[a] Average of five measurements at three concentrations (25, 50, and 100 ng/mL).

PAHs were separated at ambient temperature using a C18 Envirosep-pp column (Phenomenex; 12.5 cm × 4.60 mm, particle size = 3 μm) and a gradient elution program with a flow rate of 1.4 mL/min. The initial mobile phase was 65% acetonitrile and 35% HPLC water for 8 min, which was then gradiently changed to 100% acetonitrile over 1 min, held at 100% for 8 min, and then decreased to initial phase (65−35%). The investigated PAHs were anthracene (AN), fluoranthene (FA), pyrene (PY), benz(*a*)anthracene (BaA), chrysene (CHR), benzo(*b*)-fluoranthene (BbFA), benzo(*k*)fluoranthene (BkFA), benzo(*a*)pyrene (BaP), dibenz(*a*,*h*)anthracene (DBahA), benzo(*g*,*h*,*i*)perylene (BghiP), and indeno(1,2,3-*cd*)pyrene (IP). The different wavelengths for each compound are shown in **Table 1**. PAHs were identified on the basis of retention time, and quantification was performed by using an external standard method. Analyte recoveries were determined by using unpolluted fish tissue spiked with solutions containing 25, 50, or 100 ng/mL of the PAH standard. Detection limit (LOD) calculated as signal-to-noise 3:1 and quantification limit (LOQ) calculated as signal-to-noise 6:1 are reported in **Table 1**.

The external standard multipoint calibration technique was used to determine the linear response interval of the detector, and in all cases, regression coefficients were higher than 0.9852 for all of the analytes.

Statistical Analysis. For all samples with concentrations below the limit of quantification, zero was used in the calculation. Normality of the data of PAHs, calculated on a fresh basis, was assessed by means of the Kolmogorov−Smirnov test. In some compounds data were not normally distributed; therefore, they were log transformed and normality was assessed again. Then, analysis of variance (ANOVA) was performed to detect significant differences among groups (according to species and season) with the statistical package SPSS 14.0.2 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The PAH concentrations in samples collected in July are reported in **Table 2**, whereas **Table 3** shows the results for samples collected in December.

Mussels were the most polluted marine organisms, showing the highest PAH concentrations ($p < 0.01$). These values range from 44.67 to 207.12 ng/g of fresh weight and appear particularly high if compared with some other data reported for Mediterranean areas (*17*, *18*). This pattern of contamination could depend on the proximity of the sampling site to the filled-in area and the presence of particularly contaminated sediments and materials dumped during landfill. Furthermore, it is important to consider that Pozzuoli Bay belongs to the Campi Flegrei volcano-tectonic system and represents the northern margin of a volcanic caldera. Its geology is very dynamic and related to an intense and relatively recent volcanic activity, displayed by underwater gas emissions and bradyseismic movements (*15*).

With regard to BaP, which represents the most potentially carcinogenic PAH (*19*), European Regulation 208/2005/EC fixed at 10 μg/kg of wet weight the MRL in bivalve molluscs. This compound was detected in all samples of mussels. Thirty-five percent of these marine invertebrates showed values higher than the cited MRL. In particular, a significant difference was found between seasons ($p < 0.01$). All samples collected in summer showed BaP values lower than the MRL, whereas 71.43% of mussels collected in winter exceeded this limit. Total PAH levels found in mussels collected in December were higher than those found in July with a particular congener distribution (**Figure**

Table 2. Range, Mean, and Standard Error Values of PAHs and ΣPAHs (Nanograms per Gram of Wet Weight) in Selected Marine Species Collected in Summer in the Gulf of Naples

compd	Mediterranean mussels	blue/red shrimp	red mullet	European flying squid	Atlantic mackerel	blue whiting	European hake
AN	9.39–16.62 (13.60 ± 0.94)	1.31–2.83 (1.90 ± 0.20)	2.42–6.23 (3.91 ± 0.48)	2.75–4.35 (3.67 ± 0.22)	1.80–4.03 (2.82 ± 0.30)	0.42–5.47 (3.36 ± 0.64)	ND[a]–0.24 (0.12 ± 0.04)
FA	9.47–14.73 (12.45 ± 0.68)	0.83–1.62 (1.14 ± 0.10)	0.23–1.30 (0.60 ± 0.13)	1.27–3.03 (2.24 ± 0.25)	ND–2.57 (0.95 ± 0.33)	0.74–1.20 (1.07 ± 0.07)	ND
PY	16.44–29.95 (23.23 ± 1.99)	1.81–5.78 (3.62 ± 0.61)	2.85–7.52 (4.76 ± 0.63)	3.26–7.23 (4.92 ± 0.55)	1.31–5.36 (3.14 ± 0.56)	1.02–4.26 (3.00 ± 0.42)	ND
BaA	0.44–1.96 (1.46 ± 0.19)	3.25–5.40 (3.94 ± 0.29)	1.71–4.31 (3.10 ± 0.31)	1.46–3.47 (2.52 ± 0.25)	0.90–2.10 (1.45 ± 0.16)	2.77–4.53 (3.57 ± 0.20)	1.61–4.08 (3.12 ± 0.32)
CHR	0.60–8.22 (4.74 ± 1.14)	0.22–0.63 (0.45 ± 0.05)	ND	ND	ND–0.54 (0.37 ± 0.07)	0.34–1.11 (0.76 ± 0.10)	0.28–0.91 (0.56 ± 0.09)
BbFA	2.58–14.71 (9.52 ± 1.93)	ND	2.04–6.51 (4.70 ± 0.56)	0.77–1.89 (1.21 ± 0.16)	1.54–13.39 (5.78 ± 1.61)	2.66–4.11 (3.24 ± 0.22)	ND
BkFA	0.68–3.80 (2.41 ± 0.51)	1.42–3.03 (2.22 ± 0.19)	ND–1.48 (0.79 ± 0.19)	1.56–2.24 (1.82 ± 0.10)	ND–1.26 (0.76 ± 0.22)	0.70–1.38 (1.11 ± 0.08)	1.36–2.15 (1.65 ± 0.10)
BaP	0.31–2.67 (1.61 ± 0.44)	ND	ND	ND	ND	ND	ND
DBahA	ND–0.96 (0.45 ± 0.16)	ND	ND	ND	ND	ND	ND
BghiP	ND–7.37 (4.60 ± 1.05)	ND	ND	ND	ND	ND	ND
IP	1.57–5.95 (3.53 ± 0.67)	ND	ND	ND	ND	ND	ND
Σ PAHs	77.69 ± 9.18	13.26 ± 1.00	17.85 ± 0.81	16.39 ± 0.71	15.26 ± 1.02	16.11 ± 1.28	5.45 ± 0.36

[a] Not detected.

Table 3. Range, Mean, and Standard Error Values of PAHs and ΣPAHs (Nanograms per Gram of Wet Weight) in Selected Marine Species Collected in Winter in the Gulf of Naples

compd	Mediterranean mussels	blue-red shrimp	red mullet	European flying squid	Atlantic mackerel	blue whiting	European hake
AN	11.97–16.34 (13.63 ± 0.55)	1.73–2.99 (2.35 ± 0.19)	1.73–6.38 (2.71 ± 0.62)	2.77–4.94 (3.66 ± 0.31)	1.20–3.93 (2.18 ± 0.34)	3.53–6.95 (5.50 ± 0.46)	0.20–4.20 (1.40 ± 0.70)
FA	33.98–49.10 (40.57 ± 1.91)	ND[a]–1.18 (0.74 ± 0.19)	ND–1.08 (0.42 ± 0.13)	1.30–2.47 (1.89 ± 0.13)	ND–1.27 (0.47 ± 0.23)	1.28–2.40 (1.79 ± 0.16)	ND–0.98 (0.35 ± 0.18)
PY	34.00–47.35 (40.47 ± 1.59)	1.32–3.63 (2.21 ± 0.36)	2.40–4.91 (3.19 ± 0.35)	4.54–7.96 (5.65 ± 0.45)	0.66–2.91 (1.74 ± 0.26)	2.76–5.34 (4.18 ± 0.33)	ND
BaA	4.73–7.38 (6.05 ± 0.37)	2.63–4.93 (3.53 ± 0.30)	1.78–3.39 (2.65 ± 0.23)	3.01–18.19 (9.56 ± 2.45)	1.19–2.81 (2.07 ± 0.21)	2.23–4.31 (3.38 ± 0.32)	1.48–4.04 (2.84 ± 0.39)
CHR	12.04–20.23 (16.54 ± 1.16)	0.19–1.25 (0.61 ± 0.13)	ND	20.33–88.12 (46.01 ± 10.09)	0.37–2.26 (1.21 ± 0.26)	0.36–1.09 (0.81 ± 0.11)	0.34–0.88 (0.64 ± 0.06)
BbFA	15.70–22.74 (19.10 ± 0.89)	ND	4.19–5.65 (4.60 ± 0.19)	0.59–2.70 (1.77 ± 0.28)	2.36–16.81 (6.72 ± 2.17)	3.12–7.27 (4.68 ± 0.58)	ND
BkFA	5.02–7.23 (5.82 ± 0.35)	0.11–2.69 (1.21 ± 0.47)	0.18–1.34 (0.86 ± 0.18)	2.37–3.60 (2.68 ± 0.19)	1.08–2.65 (1.74 ± 0.18)	0.14–0.77 (0.43 ± 0.09)	0.98–1.93 (1.45 ± 0.15)
BaP	8.42–13.51 (11.62 ± 0.71)	ND	ND	ND	ND	ND	ND
DBahA	0.63–1.44 (1.18 ± 0.10)	ND	ND	ND	ND	ND	ND
BghiP	9.61–16.06 (10.93 ± 0.88)	ND	ND	ND	ND	ND	ND
IP	6.99–10.95 (9.18 ± 0.45)	ND	ND	ND	ND	ND	ND
Σ PAHs	175.10 ± 6.79	10.66 ± 0.84	14.44 ± 0.80	71.23 ± 10.30	16.13 ± 3.00	20.77 ± 0.62	6.67 ± 0.61

[a] Not detected.

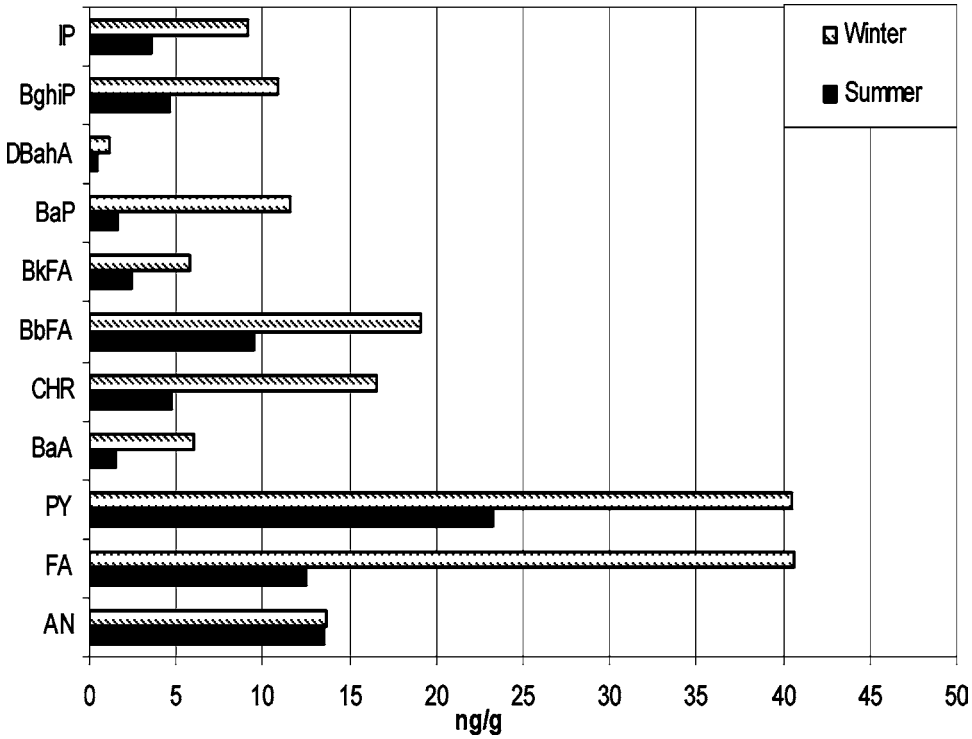

Figure 2. Distribution of individual PAHs in mussels collected during the winter sampling and summer sampling.

2). It seemed that during the winter sampling the mussels accumulated the higher weight compounds rather than the lower molecular weight PAHs. This fingerprint could be due to the presence in this area of groundwater and marine flow that during winter months, with a clockwise rotating circulation in the inner part of the gulf and a northward current offshore, characterize the hydrodynamics in the Gulf of Naples. On the contrary, during the summer, the inner circulation has an counterclockwise direction and a southward offshore current (*20*). Because of the direction of these currents and the sea storms occurring in the winter, the smaller particles were resuspended and may have constituted the main source of pollution to the mussels, due to the fact that the increasing turbidity of the water causes filter-feeding organisms to accumulate compounds adsorbed on sediment grains to a greater extent (*21*).

Independent from the season, the PAH composition pattern in bivalves was dominated by the presence of PAHs with four rings (58%) followed by those with five rings (20%), six rings (11%), and three rings (10%). This pattern is not confirmed in fish, in which the higher molecular weight PAHs such as BaP, DBahA, BghiP, and IP were never detected. Normally, mussels are filter-feeding organisms that retain particles larger than 4

μm (*22*) and are exposed to both dissolved and particulate forms of PAHs present in the water column, which concentrate in their lipid tissue (*23*). Mussels adsorb lower molecular weight PAHs through interstitial filtered water, whereas heavier weight PAHs adsorb through the digestive system. Their metabolic activity is low, so an equilibrium is reached between the PAH levels accumulated and those present in the marine environment. In contrast, fish take up PAHs mainly in the dissolved form, and they can rapidly convert up to 99% of the PAHs to metabolites within 24 h of uptake, changing the pattern and the concentrations in the several tissues (*24*, *25*).

The pollutants presence depends on the geographical origin, too, and crustaceans, cephalopods, and fish that showed lower PAH concentrations and no presence of BaP were less exposed to petroleum because they came from an area not closer to the Campi Flegrei Bay.

The European hake showed the lowest total PAHs mean values, 6.06 ng/g of fresh weight ($p < 0.01$). Atlantic mackerel, blue whiting, and red mullet showed mean concentrations of 15.70, 18.44, and 16.15 ng/g of wet weight, respectively. European flying squid with PAH values of 43.81 ng/g of fresh weight was more contaminated than fish. The crustaceans showed a mean concentration of 11.96 ng/g of fresh weight. The pattern and relative PAH concentrations in fish are quite irregular, but this variability was confirmed by different authors (*26*, *27*). Depending on the season, no significant differences were found for crustaceans, cephalopods, and fish ($p > 0.05$).

The total PAH content observed in mussels in this study is slightly lower (126 ng/g of fresh weight) than that obtained by Amodio-Cocchieri et al. (*28*) in the same area. In the two-year periods 1998−1999 and 2000−2001 they reported for *M. galloprovincialis* total PAH concentrations of 334 and 241 ng/g of wet weight, respectively. This decreasing of hydrocarbon levels could be due to the environmental protection actions engaged during recent years in the Gulf of Naples with the dismantling of the industrial plant of ILVA. Because the releases of chemical contaminants, including PAHs, are already under regulatory control, further reductions in the environment will depend on the use of best management practices and possibly new regulatory improvements. Special attention should be paid to the PAH input to Campi Flegrei Bay from local sources, because these waters are extensively used for mariculture purposes.

Aside from carcinogenicity, PAHs have also been reported to cause hemato, cardio, renal, neuro, immuno, reproductive, and developmental toxicities in humans and laboratory animals (*29*). Studies on the bioavailability of these contaminants from various entry routes are important not only from the standpoint of scientific knowledge but also from the public health perspective. PAHs differ in carcinogenic potency. The U.S. Environmental Protection Agency classified BaA, BaP, BbFA, BkFA, CHR, DBahA, and IP as group B2 (probable human carcinogens). It was demonstrated that these compounds caused tumors in laboratory animals when they breathed them in the air, when they ate them, or when they had long periods of skin contact with them. Studies of people show that individuals exposed by breathing or skin contact for long periods to mixtures that contain PAHs and other compounds can also develop cancer (*30*). European Regulation 208/2005/EC established maximum admissible levels only for BaP. According to the Scientific Committee on Food, benzo(*a*)pyrene can be used as a marker for the occurrence and effect of carcinogenic PAHs in food, including also benz(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*j*)fluoranthene, benzo(*k*)fluoranthene, benzo(*g*,*h*,*i*)perylene, chrysene, cyclopenta(*c*,*d*)pyrene, dibenz(*a*,*h*)anthracene, dibenzo(*a*,*e*)pyrene, dibenzo(*a*,*h*)pyrene, dibenzo(*a*,*i*)pyrene, dibenzo(*a*,*l*)pyrene, indeno(1,2,3-*cd*)pyrene, and 5-methylchrysene. Further analyses of the relative proportions of these PAHs in foods would be necessary to inform a future review of the suitability of maintaining benzo(*a*)pyrene as a marker.

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