OCCURRENCE AND CONCENTRATION OF PAHS IN CLAMS AND SEDIMENTS OF THE MARINE COASTAL LAGOON OF GANZIRRI (ITALY). EXTRACTION, GC-MS ANALYSIS, DISTRIBUTION AND SOURCES.

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SUMMARY

The distribution of selected Polycyclic Aromatic Hydrocarbons (PAHs) in surface sediments and clams of marine coastal lagoons, called Ganzirri, located at the Sicilian coast of the Messina's strait, has been investigated by GC/MS analysis with Selected Ion Monitoring (SIM) mode.

The lagoon is characterized by abundant organic detritus, deriving from aquagenic and anthropogenic inputs. Anoxic/reduced conditions of sediments make them a preferential site for uptake and preservation of PAHs.

The investigations have been performed on the 16 PAHs recommended by US-EPA as priority pollutants to be monitored in the framework of environmental quality control.

The sediment PAH concentrations ranged from 135 to 1650 μg/kg dry matrix. The total concentrations of PAHs in clams ranged from 60 to 1427 μg/kg d.w. The relative standard deviation (RSD) for all samples of the concentration replicates of individual compounds ranged from 10% to 25%.

The resulting distributions and ratios of specific compounds have been discussed in terms of sampling location and origin of contaminants. The results obtained show that levels of contamination are not homogeneous throughout the stations.

In the Ganzirri Lagoon, the bioavailability of hydrophobic organic compounds, such as PAHs, seems to be mainly governed by chemical characteristics of the contaminants. Pyrolytic compounds (penta- and hexa-aromatics) are not readily available. In contrast, petroleum hydrocarbons (some tetra-aromatics) are accumulated by clams to a great extent.

From an eco-toxicological point of view, the aquatic ecosystem investigated appears to be moderately polluted.

KEYWORDS: PAHs, sediments, clams, bioaccumulation factor, lagoon, Ganzirri.

INTRODUCTION

Methods used to quantify contaminants in marine environments involve the study of the pollutant levels in seawater, sediments and biota. The low levels of pollutants in seawater create analytical sensibility and contamination problems during sample collection and preparation. Several quantification methods involving the study of certain marine organisms have already been developed [1-3]. It was found that some of these organisms can accumulate, and bioconcentrate seawater pollutants. The bivalves are well-known concentrators of persistent chemical pollutants and have been utilized in international programs for monitoring contaminants, such as the International Mussel Watch [4]. In particular, these organisms do not biotransform organic contaminants like PAHs, and for that reason the bivalves often are used as biomonitoring tools to assess PAH bioavailability [5]. When an organism is exposed to organic contaminants, the concentration of these compounds in its tissue varies until it reaches a steady-state level. This apparent constant concentration results from a balance between uptake (from the water, the sediment, the diet) and direct excretion [2]. In particular, the uptake of a contaminant is governed by its bioavailability, which, in the case of PAHs, is related to their water solubility.

The PAHs include molecules containing fused aromatic rings, and are of special concern because of their widespread distribution throughout the environment and their often toxic and carcinogenic properties [6, 7]. PAHs are hydrophobic compounds, and this property is represented by the octanol-water partition coefficient (K_{OW}). Their bioavailability decreases as K_{OW} and molecular weight increase [2]. As a consequence of their hydrophobic nature, PAHs in aquatic environments rapidly tend to become associated with particulates. Sediment, therefore, represents the most important reservoir of PAHs in the marine environment [8]. In this paper, we report results on concentration levels and the distribution of PAHs in sediments and various species of clams (*Tapes decussatus, Cardium edule* and *Chamelea gallina*) of a marine coastal lagoon, called Ganzirri, located at the Sicilian coast of Messina's strait. Due to its geographical position, and very nearness to the town, and to economic traffic around, the Ganzirri lagoon ecosystem is threatened by accidental and chronic releases of PAHs. The Ganzirri Lake is devoted to clam farming. Because of the environmental, tourist and economic interest, this lake was chosen for the biomonitoring with clams. In fact, the most useful bioindicator organisms (mussels and oysters) do not occur along the Sicilian coasts, so it has been necessary to look for other species present in the coastal areas as possible sentinel organisms. The determination of PAHs in bioaccumulator organisms and sediments is of great importance to precise the origin and fate of these widely spread compounds throughout the environment. Knowing the level of hydrocarbons, several indexes can be calculated [9, 10] and, therefore, the biogenic or anthropogenic origin of these hydrocarbons can be established. In general, the two main sources of PAHs in the environment are fossil fuels, mainly crude oil, and the incomplete combustion of organic materials, such as wood, coal and oil [11]. Under anaerobic conditions, some PAHs can also be derived from biogenic precursors [12]. They are also formed naturally in forest fires and volcanic eruptions. Anthropogenic activities are generally recognized to be the most important source of PAH release into the environment. In the present study, investigations were carried out concerning PAHs identified by the US-EPA as required priority monitoring action within the framework of environmental quality control [13]. Bioaccumulation factors (BAFs) were calculated for the individual PAHs, and discussed in terms of compound bioavailability to estimate the most probable route of contaminant uptake relative to the molecular weight of the compounds.

MATERIAL AND METHODS

Study site

Ganzirri Lake has an elongated form and its surface is 338.400 square meters with an average depth of about 6.50 m (Fig. 1). It is connected to the sea by means of two input/output canals, which are periodically open in order to allow the seawater re-circulation. A third canal connects it to the Faro Lake. The lagoon is characterized by abundant organic detritus, deriving from aquagenic and anthropogenic inputs. Anoxic/reduced conditions of sediments make them a preferential site for uptake and preservation of PAHs. Sediments were collected at 7 sampling sites (Fig. 1), and clams were collected at 3 stations.

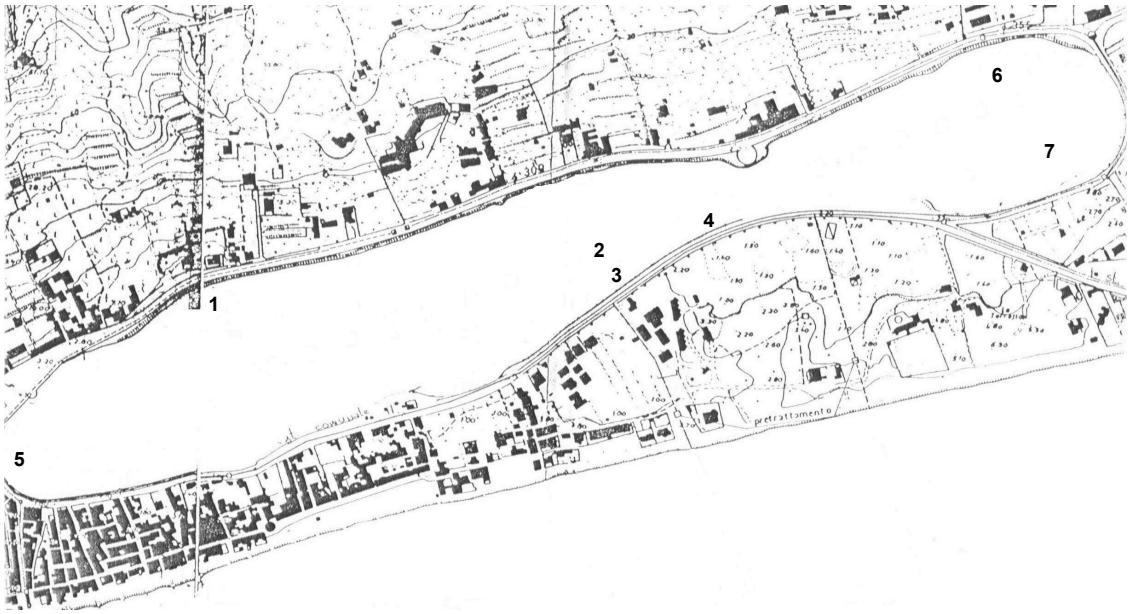


FIGURE 1 - Location of sampling sites.

Sampling

A total of 7 sediment samples were collected employing a metallic box corer. About 1 kg aliquots of sediment were placed in plastic bags.

The samples of clams (approx. 30 mussels with a shell length of 3-5 cm) were taken 2005 during spring. Efforts were made to obtain specimens of homogeneous size at every station, but size differences may occur among the stations.

The clam and sediment samples, wrapped in aluminum foils, were immediately refrigerated (4 °C), 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.

Preparation of sample

Tissues were prepared for analysis by manually removing limpet tissue from their shell. We homogenized the tissues by lyophilization. This process consists of two major steps: freezing of a protein solution, and drying of the frozen solid under vacuum. The drying step is further divided into two phases: primary and secondary drying. The primary drying removes the frozen water, and the secondary one removes the non-frozen 'bound' water [14].

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 of analytical grade, from Scharlau Chemie S.A. (Barcelona-Spain). Acetone (Envisolv for analysis of dioxins, furans and PCBs) from Fluka was ≥ 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 benzo(a)anthraxcene 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 clams

Various techniques were tested in order to identify the most efficient preparation and extraction procedure. Different recovery tests were carried out by using clam tissues 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 obtained (checked by GC-MS analysis), a known amount of PAH (EPA) standard mixture was added to each "blank" sample. The test-samples obtained in this way were extracted using two different methods. In particular, we have compared the extraction after saponification with 2 M ethanolic KOH, and the Soxhlet extraction with a 1:1 mixture of dichloromethane/n-pentane.

The extraction tests, together with the total PAH concentrations, were calculated for the compounds investigated, and the relative mean deviations reported in Table 1. The results showed that the Soxhlet method allowed us to extract the highest PAH total amounts with a smaller standard deviation.

TABLE 1 - Extraction tests carried out with a 16 compounds` mixture.

Extraction method	Total PAHs (µg/kg d.w.)
Soxhlet	153 ± 63
After saponification (KOH 2M)	52 ± 23

The sample was extracted in Soxhlet apparatus for 24 h with dichloromethane:pentane/1:1. The extract was reduced to a small volume using a rotary evaporator (T = 35±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 of the PAH solution under a weak nitrogen flow at room temperature. The dry residue was dissolved in 250 µL hexane containing the following perdeuterated internal standards (0.2 mg/L each): acenaphthene d_{10}; phenanthrene d_{10}, chrysene d_{12} and perylene d_{12}.

Treatment and extraction of sediment

About 5 g of sediment, after centrifugation for 10 min, was treated with pre-cleaned (Soxhlet-extracted with dichloromethane for 24 h) anhydrous Na_2SO_4 (Carlo Erba). Activated copper (200 mg) was added to the extraction vessel to remove elemental sulphur. The copper (40 mesh, 99.5% purity, from Aldrich) was activated with 1N HCl, and then washed with water, acetone and CH_2Cl_2. The PAH extraction was performed in a Soxhlet apparatus as described by Giacalone et al. [8].

GC/MS analyses

PAHs were detected with a Shimadzu gas chromatograph-mass spectrometer (GC–MS mod. GC-17A quadrupole detector GCMS-QP5000), equipped with a capillary Equity-5 (30 m x 0.25 i.d., 0.5 µm) column from Supelco (Milano, Italy) and a splitless injector (0.61 min). The carrier gas was He at a flow rate of 1.4 ml min^{-1}. The injector was heated to 280 °C; the column temperature was 60 °C for 2 min, 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 heated at 250°C. The

total runtime was about 35 min. The spectrometer was used in Selected Ion Monitoring (SIM) mode.

Identification of the components of the standard mixture was carried 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 single components with those stored in the acquisition system library. The identification of PAHs in the solutions extracted from clams and sediments was carried out on the basis of previously determined retention times and confirmed using mass spectra.

The PAH content in the sample was quantified relatively to the perdeuterated PAHs added to the dry residue. The response factors for different compounds were measured by injecting a mixture containing standard compounds at the same concentrations of perdeuterated PAHs used for spiking the sample. The most abundant ion was used for quantification, and two other ions were additionally used for confirmation. The list of groups of PAHs formed, the perdeuterated standards employed, the quantification ion, and the confirmation ion for each PAH are shown in Table 2.

The individual concentrations of PAHs in organisms and sediments are given on a dry weight basis in Table 3.

TABLE 2 – List of PAHs analyzed, the perdeuterated standards employed (underlined), the quantification ion and confirmation ion for SIM GC-MS mode.

Chemical	Quantification ion	Confirmation ion
Acenaphthylene	152	76, 151
Acenaphthene	154	152, 76
Fluorene	166	164, 165
Acenaphthene d_{10}	164	
Phenanthrene	178	188, 89
Anthracene	178	188, 89
Fluoranthene	202	101, 200
Pyrene	202	101, 200
Benzo(a)anthracene	228	114, 226
Phenanthrene d_{10}	188	
Chrysene	228	114, 226
Benzo(b)fluoranthene	252	126, 250
Benzo(k)fluoranthene	252	126, 250
Benzo(a)pyrene	252	126, 250
Chrysene d_{12}	240	
Perylene	252	126, 250
Indeno(1,2,3-cd)pyrene	276	138, 277
Dibenzo(a,h)anthracene	278	139, 279
Benzo(g,h,i)perylene	276	138, 277
Perylene d_{12}	264	

TABLE 3 – Organism and sediment concentrations in µg/kg of dry matrix.

Sediment →								*Chamelea →*				*Tapes→*				*Cardium→*		
Compounds	1	2	3	4	5	6	7	*Gallina*	1	2	3	*Decussatus*	1	2	3	*Edule*	1	2	3
Acenaphthylene	0.0	0.0	0.0	3.7	1.0	25.6	11.9		0.9	1.0	2.5		2.5	0.0	0.0		0.0	4.9	2.1
Acenaphthene	0.0	0.0	0.0	0.0	0.7	3.8	14.5		10.6	7.8	4.4		8.5	6.6	5.9		1.8	8.4	0.6
Fluorene	5.9	3.0	6.7	3.3	2.7	11.1	19.9		13.9	20.9	8.8		8.4	5.3	3.4		0.0	24.3	3.5
Phenanthrene	29.5	24.4	43.5	21.8	8.2	119.3	128.2		28.2	41.0	35.8		23.8	19.6	39.0		9.2	79.4	34.9
Anthracene	5.1	2.9	1.0	2.5	1.4	13.6	27.1		2.5	4.8	3.5		2.7	3.2	1.6		0.5	4.0	4.3
Fluoranthene	27.9	22.0	41.6	41.9	24.6	166.6	234.3		27.8	178.3	78.8		28.1	51.2	114.2		14.1	79.8	188.6
Pyrene	21.7	16.0	23.0	67.7	32.1	163.1	215.5		27.2	162.7	836.5		30.6	50.7	1220.6		19.9	392.0	921.9
Benzo(a)anthracene	10.3	0.0	7.8	57.4	13.6	61.1	427.9		14.8	16.8	2.8		10.2	3.7	12.0		3.1	12.5	63.5
Chrysene	10.3	0.0	11.3	16.7	11.6	120.7	196.1		13.5	18.1	12.2		13.0	6.7	17.1		7.1	13.9	62.8
Benzo(b)fluoranthene	9.0	0.0	0.0	29.6	1.6	129.7	74.6		0.0	0.0	0.0		0.0	0.0	0.0		0.0	0.0	0.0
Benzo(k)fluoranthene	0.5	0.0	0.0	16.0	0.5	45.3	21.0		0.0	0.0	0.0		0.0	0.0	0.0		0.0	0.0	0.0
Benzo(a)pyrene	17.1	35.9	8.3	15.3	7.6	97.3	154.7		3.1	4.8	0.0		0.0	7.9	7.4		1.7	0.0	24.8
Perylene	0.7	2.4	0.7	1.4	0.0	20.8	19.7		2.3	13.7	9.1		0.1	3.8	5.6		2.7	6.6	0.9
Indeno(1,2,3-cd)pyrene	10.5	32.5	6.6	5.5	9.3	116.1	64.6		1.8	0.0	0.0		0.0	0.0	0.0		0.0	0.0	10.4
Dibenzo(a,h)anthracene	7.3	85.1	3.5	3.4	0.0	28.8	4.4		0.5	0.0	0.0		0.0	0.0	0.0		0.0	0.0	5.6
Benzo(g,h,i)perylene	10.0	44.1	15.2	9.3	19.9	81.9	35.8		3.2	0.0	0.0		0.0	0.0	0.0		0.0	0.0	11.5
Total PAHs[a]	165.8	268.3	169.2	295.5	134.8	1204.8	1650.2		150.3	469.9	944.4		127.9	158.7	1426.8		60.1	625.8	1335.4

[a] **Relative standard deviations range from 10% to 25%.**

Water content analysis

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

Organic Matter

To characterize chemical nature of sediment, total organic matter was determined by ignition at 550 °C for 6 hours. The organic content was determined by weight loss.

Carbonate content

The carbonate content in the sediment was determined by weight loss at 1000 °C for 6 hours.

RESULTS AND DISCUSSION

For the clams, the total concentration of the 16 compounds investigated, expressed as the sum of concentrations, ∑PAH, varies from 60 to 1427 µg/kg of dry sample. The wide range of PAH concentrations found in the clams

indicates heterogeneous levels of contamination. This can be explained by considering the different characteristics of the sampling sites.

The highest PAH concentrations have been measured in station n° 3. The high concentrations of PAH recorded at stations corresponding to sewer trunk line with water containing domestic organic matter, but also washing-out water from road, and petroleum products. The concentration of carcinogenic PAHs in the samples of clams ranged from 1.5 to 44 μg/kg d.w., expressed as benzo(a)pyrene [15].

For the sediments, the total levels of the 16 PAHs investigated varied from 135 to 1650 μg/kg of dry weight and, practically, showed spatial trends. This high variability is correlated to the different physical-chemical characteristics (Table 4) of collected sediments.

On the basis of literature data [16], two types of sediments can be characterized by water content (about 20% for sands, and more than 40% for muds). Muddy sediments are known to accumulate hydrophobic compounds to a much greater extent than sandy ones. In our samples, water content ranged from 6.2 to 59%. The overall PAH concentration is lower in sand (samples n° 1-5), ranging from 135 to 295 μg/kg, than in mud (samples n°6, 7) with 1205-1650 μg/kg. The lower absorption of PAHs by sandy sediments has been already well-recognized, and the present results are in good agreement with literature data [8].

TABLE 4 – Physical-chemical characteristics of sediments.

Sediment	Organic matter (%)	Carbonate (%)	H_2O (%)
1	1.1	1.4	21.7
2	0.1	0.5	22.4
3	0.8	1.4	23.5
4	0.2	1.2	22.8
5	2.5	2.1	6.2
6	28.1	0.4	56
7	23.7	3.4	59

The different nature of the bottom of the lake is justified by letting in of terrigenous matter for clams` farming. In fact, in the sampling sites, identical PAH distribution (expressed as weight percentage) was not observed.

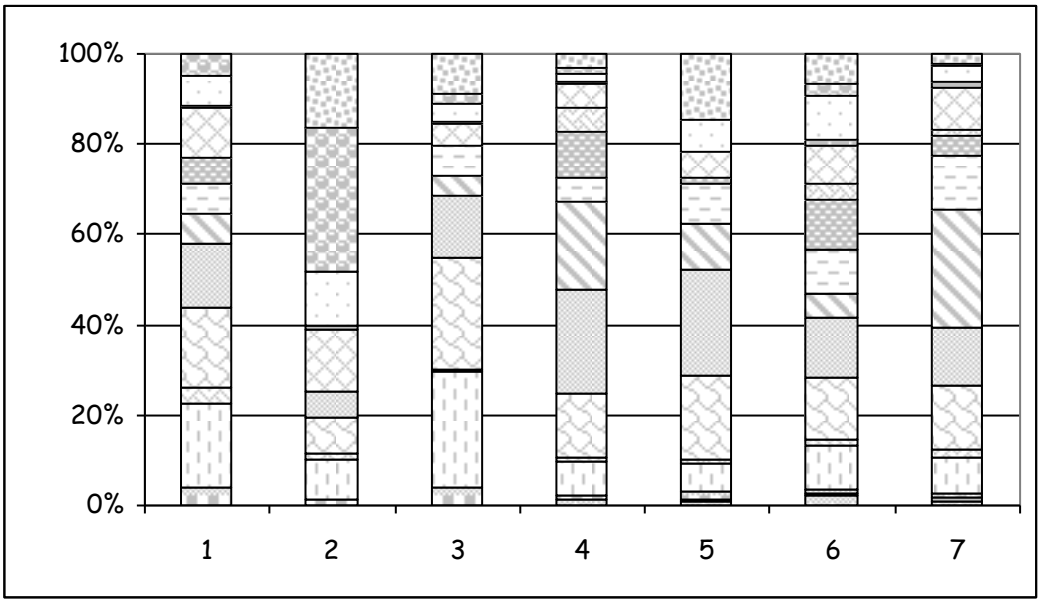


FIGURE 2 – Percentage distribution of Polycyclic Aromatic Hydrocarbons in sediments.

Clam bioaccumulation

Clams and sediments collected in different sites of Ganzirri lake were analyzed for the 16 PAHs, and their bioaccumulation factors (BAFs) in the sediments determined. The BAF factor is calculated as ratio of the organism concentration of a compound versus its sediment concentration, and this factor allows to estimate contaminant accumulation by organisms from the sediment. To allow comparison between the different stations, relative BAFs (BAF against the sum of all BAFs × 100) were then calculated for each station. The correlation between relative BAFs and log K_{OW} was studied. The BAFs were calculated for the three clam species and showed analogous behavior. They were found to be significantly negatively correlated with log K_{OW}, however, with a higher slope for station 3. As example, Table 5 shows the correlation log K_{OW} vs. BAFs only for a clam species (*Chamalea gallina*).

TABLE 5 – Linear correlation between relative bioaccumulation factors (BAFs) and log K_{OW} values.

Chamelea gallina	1	2	3
Slope	-5.90	-9.52	-2.18
Correlation coefficient (r^2)	0.27	0.38	0.41

As reported [2], this negative correlation between the bioaccumulation factor values and the log K_{OW} values indicates that the clams accumulate the bioavailable fraction of the contaminants present in the water column, and the PAH content of their tissue reflects the profile of pollution they have been exposed to. In fact, the clams are filter-feeding organisms and mainly exposed to the soluble fraction of PAHs, the most water-soluble one. The higher molecular weight compounds seem to be accumulated in the clams of all stations, particularly at station 3. There, the tetra-aromatic PAHs represent 90%. These compounds are present in great abundance in petroleum [17], and near S 3 there is a fuel and oil-containing sewer trunk line flowing into the lake water.

Origins of contaminants

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

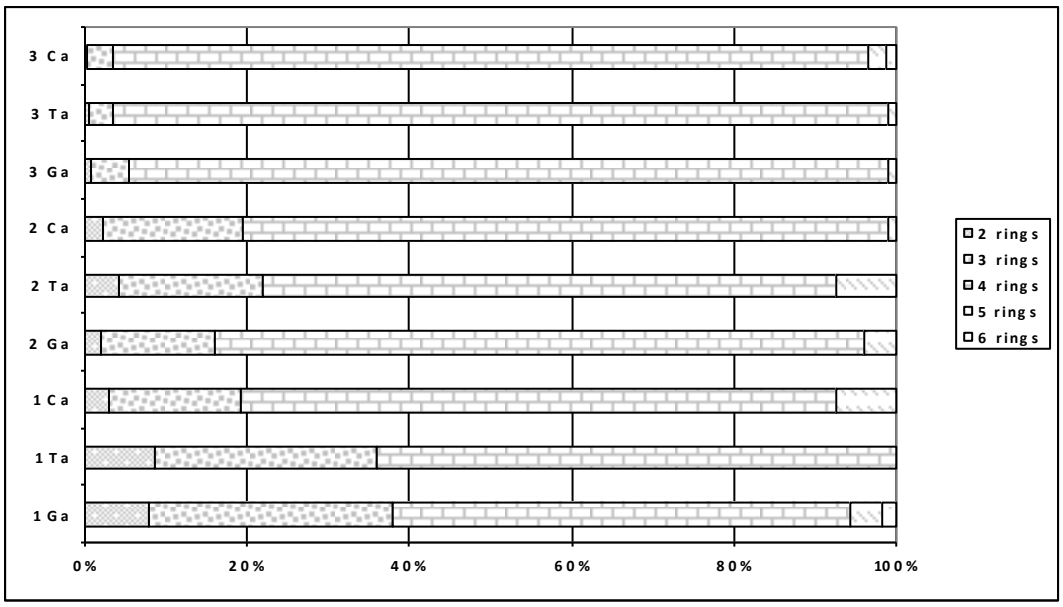
The molecular patterns generated by each source, however, are like *fingerprints*, which make it possible to hypothesize which processes generate PAHs by studying their distribution in samples. Pyrolytic sources are characterized by the presence of PAHs over a wide range of molecular weights, while petrogenic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

The presence of low-molecular weight compounds is typically from spill-associated hydrocarbons, while pyrogenic polycyclic aromatic hydrocarbons are generally characterized by the dominance of high-molecular PAHs [15].

If we group polycyclic aromatic compounds into different classes depending on the number of aromatic rings present in their structure (Fig. 3), we observed that PAHs with 4 rings, found in clam samples at the sites under investigation, constitute 60-90% of total PAHs.

However, a preferential accumulation of the more water-soluble PAHs was observed. Thus, clams accumulated preferentially pyrene that is present in great abundance in petroleum. These evidences suggest that PAH contamination in the clams might originate mainly from pollution by petrogenic sources.

Furthermore, 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 clams, likewise mussels, are characterized by low biotransformation capacities compared to vertebrates. PAH ratios in marine sediments for marine invertebrates (clams), commonly phenanthrene/anthracene and fluoranthene/ pyrene ratios, have commonly been used as means of the main PAH origins [9, 10].



Ca: ***Chamelea gallina*****; Ta:** ***Tapes decussatus*****; Ca:** ***Cardiume edule***

FIGURE 3 – Contribution of 2,3,4,5 and 6 ring-compounds to total PAH content in clams.

Phenanthrene and anthracene are both structural isomers. In particular, phenanthrene is more thermodynamically stable than anthracene. Therefore, in petrogenic PAH pollution the Phe/Ant ratio is very high, while high temperatures during the combustion process favor the formation of anthracene and a lowering of the Phe/Ant 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 PAHs from their origins, through environmental transport, to their uptake in marine organisms. In particular, a ratio of Phe/Ant <10 and Flu/Pyr >1 indicate a contamination due to pyrolytic origin. The data (Table 6) suggest that PAHs in the clams are mainly of petrogenic origin, while those in the sediments are of pyrolytic origin (Fig. 4).

TABLE 6 - Summary of Phe/Ant and Flu/Pyr ratios for clams.

Isomeric Ratio	1	2	3
Flu/Pyr	0.88	0.77	0.13
Phe/Ant	12.4	11.4	14.3

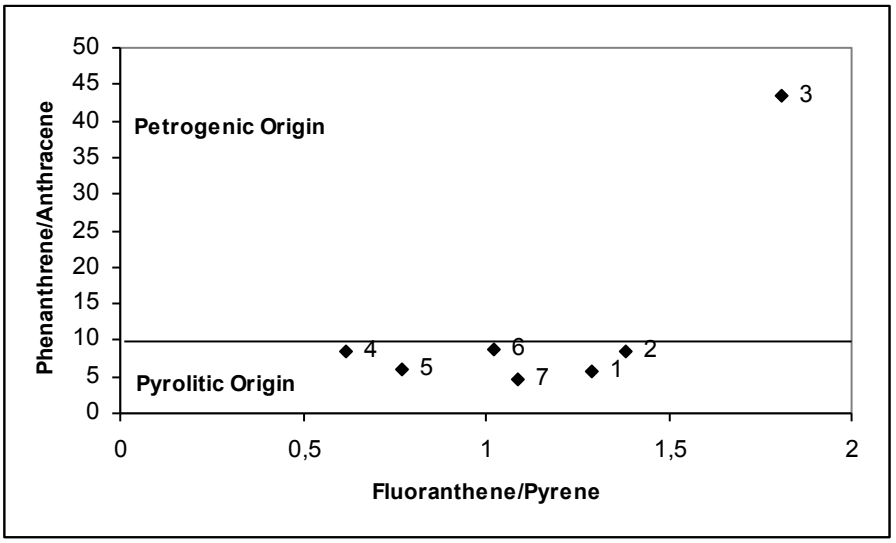


FIGURE 4 - Plot of ratio Phenanthrene/Anthracene *vs.* ratio Fluoranthene/Pyrene

However, there are some limitations in the use of only a few ratios. The simultaneous consideration of various molecular indices is thus necessary.

Additionally, it is reported in literature [10] that a chrysene/ benzo(a)anthracene ratio lower 1 indicates pyrolytic sources, while larger ratios indicate petrogenic origins. The ratios for all the sites investigated in this work ranged between 0.29 and 1.97.

The ratios reported in Table 7 confirm the pyrolytic origins for all stations of sediments, except for the station n° 3.

TABLE 7 - Summary of origins` ratios for sediments.

Isomeric Ratio	1	2	3	4	5	6	7
Flu/Pyr	1.29	1.38	1.81	0.62	0.77	1.02	1.09
Phe/Ant	5.9	8.5	43.5	8.6	6.0	8.8	4.7
Chr/B(a)ant	1.00	-	1.45	0.29	0.86	1.97	0.46

CONCLUSIONS

Our main remarks are as follows:

a) The analyses show that the characteristics of the Ganzirri lake bottom vary meaningfully, also for near points. This is the consequence of anthropic activities for clams` farming.

b) The present study made it possible to optimize extraction and analytical conditions for the determination of PAHs in clams. Under these conditions, reproducibility is satisfactory (relative standard deviation = 10-25%). The organism`s lyophilisation is indispensable to have a good reproducibility.

c) The clams are good bioaccumulators for PAHs in aquatic ecosystem. In fact, the clams are filter-feeding organisms and they concentrate in their tissues the compounds present in water, and, generally, the water contaminants` detection by direct analytical methods is difficult.

d) The PAHs bioaccumulation is not influenced by the different clam species.

e) The use of an Equity-5 column allowed complete separation in a shorter space of time (about 30 min), proven already in previous studies on PAHs [8, 18].

f) The results reported here represent the first quantitative investigation of PAHs in *clams* of the Mediterranean area.

g) The greater presence of low-molecular weight PAHs (4 rings) in all samples, and the Phe/Ant and Flu/Pyr ratio values used as PAH distribution indexes demonstrate that most of the clam samples contain PAHs predominantly from a single origin, i.e. a petroleum product. Instead, the sediments are characterized by pyrolitic origin.

h) Total PAH content in clam samples is not correlated with that in sediments from the sampling place.

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