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## Distribution of alternant and non-alternant polycyclic aromatic hydrocarbons in sediments and clams of the Pialassa Baiona Lagoon (Ravenna, Italy)

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The distribution of polycyclic aromatic hydrocarbons (PAHs) in surface sediments and clams (*Tapes philippinarum*) in a Ramsar site (Pialassa Baiona Lagoon, Italy) was investigated by high-performance liquid chromatography (HPLC) with fluorescence detection and gas chromatography–mass spectrometry (GC–MS). Surface sediments exhibited a variable degree of PAH contamination, with high concentration values (>100 mg/kg) in the southern area, in the proximity of the industrial district, and much lower values (<1 mg/kg) in the northern area. The molecular distribution of PAHs was very similar throughout the Lagoon, confirming previous studies indicating a common source of contamination attributed to partial combustion of methane. With the exception of the northernmost site, the molecular distribution was characterized by high levels of non-alternant PAHs, among which the probable mutagenic cyclopenta[*cd*]pyrene was the predominant species. PAH levels in tissues of clams collected in the polluted area, where fishing is forbidden, and in the less-polluted permitted area were similar (~0.1 mg/kg dry weight). In both sites, the PAH distribution in clams was different from that observed in sediments. In particular, the levels of non-alternant PAHs with an external cyclopenta ring (CP-PAHs), acenaphthylene and cyclopenta[*cd*]pyrene, were remarkably lowered in clams with respect to sediments, probably as a result of their chemical/biological reactivity.

*Keywords:* Polycyclic aromatic hydrocarbons; Sediments; *Tapes philippinarum*; Lagoon

### 1. Introduction

Pialassa Baiona is a brackish intertidal lagoon connected to the Northern Adriatic Sea through the harbour of the historical city of Ravenna (Italy). This wetland hosts a variety of rare fauna species including protected waterfowls, which find in it ideal habitats for nesting, feeding and breeding. For its peculiar characteristics, the Lagoon is a national protected area belonging to the Nature Park 'Delta del Po' as well as a wetland of European (Natura 2000) and international importance (Ramsar Convention).

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The environmental status of the Lagoon has been strongly affected by anthropogenic activities. Besides the intense agriculture and the rapid urbanization of its neighbouring areas responsible for eutrophication, the petrochemical district of Ravenna caused severe chemical contamination due to past discharges from a tributary channel located in its southern end (figure 1). Remarkable levels of polycyclic aromatic hydrocarbons (PAHs), mercury and synthetic polymers occur in sediments of the southern area [1–4]. Although the spatial pattern of these contaminants may vary considerably within a small scale, due to the irregularity of the bottom and the complex hydrodynamic of the basin, they exhibit a similar distribution in the southern area. This correlation was attributed to their common source in the local chemical productions based on the pyrolysis of biogenic methane to produce acetylene (with undesired PAHs formation), which in turn was utilized for the synthesis of acetaldehyde and vinyl chloride by means of Hg catalysts [3].

The toxicity of Hg and PAHs and their ability to bioaccumulate in benthic organisms may raise health problems, as edible species, notably *Tapes philippinarum*, are harvested in the Lagoon. Studies on the distribution and speciation of mercury in sediments and benthic organisms have been carried out [4, 5], but data concerning the biota/sediment relationship are lacking for PAHs. Nonetheless, this latter aspect is worth of consideration also for the unusual molecular pattern of sedimentary PAHs, which is characterized by a consistent contribution of molecular species containing a five-membered ring in their structure (non-alternant PAHs) [3]. Besides non-alternant PAHs normally monitored in environmental studies being

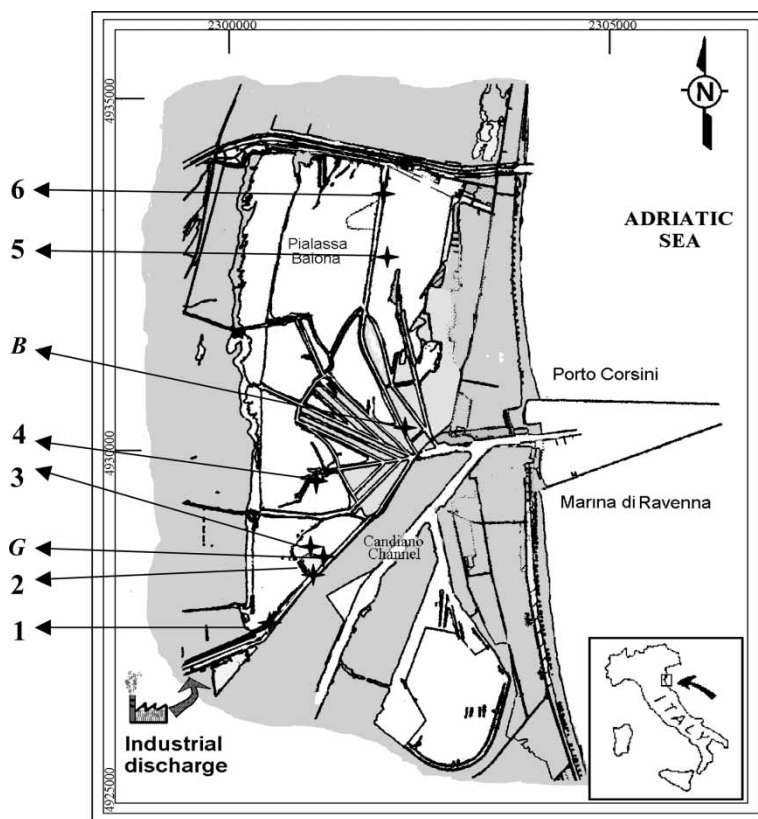


Figure 1. Map of Piasassa Baiona with sampling sites. Clams and corresponding sediments were collected in sites G and B.



attention was focussed on PAH species with an external five-membered ring in their structure (here briefly referred to as cyclopenta-PAHs, CP-PAHs) for their uncommon abundance in sediments and their possible biological significance.

## 2. Materials and methods

### 2.1 Sampling activity

Surface sediment samples and native clams (*T. philippinarum*) were collected in summer 2001 in the two sites labelled with a letter as reported in figure 1 (sites B and G). Six surface sediment samples were withdrawn from a previous campaign in 1999 in sites labelled with numbers (figure 1).

Sediment samples were collected with a box corer (~10 cm depth), gross vegetable material and animal rests or shell residues were eliminated and the samples were homogenized and stored at  $-25^{\circ}\text{C}$  prior to analysis.

Clams were manually collected, sorted by size (those with length  $>20$  cm were selected for analysis) and kept for 2–3 h in water coming from the sampling sites. Organisms were opened and placed on shell edges for 5 min to allow the intra-shell water to drain off. Tissues were dissected from the shells, and freeze-dried, homogenized under liquid nitrogen and stored at  $-25^{\circ}\text{C}$ . Mean loss of weight after freeze-drying was  $81 \pm 1\%$ .

### 2.2 Extraction of PAHs

**2.2.1 Sediments.** PAHs isolation and determination were performed according to a previous study [3]. Briefly, ~10 g (exactly weighed) of wet sediment was mixed with 10 g anhydrous sodium sulphate and Soxhlet-extracted with dichloromethane (DCM) for 16 h. The DCM solution was concentrated by rotary evaporation and added dropwise into excess *n*-hexane in order to precipitate synthetic polymers which were centrifuged off. This step was necessary for the most polluted samples containing synthetic polymers deriving from the past industrial activity, which could interfere in the clean-up procedure. The *n*-hexane solution containing PAHs was evaporated to 2 ml and liquid chromatographed on a glass column containing anhydrous sodium sulphate, copper powder and 10 g of activated silica gel (60 mesh). Three fractions were collected. The first fraction (25 ml) was eluted with 100% *n*-hexane; the second (35 ml) fraction was eluted with a mixture DCM/*n*-hexane (40/60 v/v) and the third fraction (25 ml) was eluted with 100% DCM. The second fraction containing PAHs was evaporated, and the residue was dissolved in 5 ml acetonitrile for HPLC and GC–MS analyses.

**2.2.2 Clams.** The adopted procedure was similar to that reported by Baumard *et al.* [13]. About 1 g (exactly weighed) of dry-tissue was homogenized and saponified with 50 ml of methanolic KOH (2 M), for 4 h under reflux. After the addition of 20 ml deionized water, the digest was extracted three times with *n*-pentane, the organic layers were pooled, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and reduced to ~0.5 ml by rotary evaporation. The solution was fractionated over 1.8 g alumina, and PAHs were eluted with 8 ml of a DCM/*n*-pentane (35/65 v/v) solution. The collected fraction was subjected to column chromatography over 0.8 g silica gel. Two fractions were collected. The first fraction (2 ml) was eluted with 100% *n*-hexane and the second fraction (5 ml) was eluted with a mixture DCM/*n*-hexane (60/40 v/v). The second fraction, containing PAHs, was concentrated to 0.1 ml for GC–MS analysis (0.001 ml injected) and diluted with 0.5 ml of acetonitrile for HPLC analysis.

### 2.3 Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis was performed with a Varian 3400 GC coupled with an ion-trap Varian Saturn 2000. Samples were injected in splitless mode using a 1078 Varian injector on a Restek RTX-5 fused silica capillary column (30 m × 0.25 mm ID and 0.25 μm film thickness) programmed from 50 (held 5 min) to 300 °C at 10 °C/min. The injector was kept at 280 °C and the transfer line at 270 °C. The operating conditions of the ion trap were ion source at 200 °C, acquisition range  $m/z$  50–350, electron ionization at 70 eV and 1.2 scan/s.

### 2.4 High-performance liquid chromatography (HPLC)

Measurement solutions (20 μl) were injected into a Perkin Elmer 250 (HPLC), and PAHs were separated on a 25 cm × 4.6 mm internal diameter (ID) Supelcosil LC-18 reverse-phase column. The solvent programme consisted of operating isocratically for the first 0.2 min with a mixture of acetonitrile/water 65/35 (v/v), programming to acetonitrile/water 80/20 for the next 35 min using a linear gradient and finally programming to acetonitrile/water 100/1 for the last 10 min with a linear gradient. The flow rate was kept constant at 1.5 ml/min throughout the programme. Eluting PAHs were detected with an LC 240 spectrofluorimeter detector for quantitative analysis; the wavelength programme was as follows: first 6.5 min 260 nm excitation, 340 nm emission; next 8 min 255 nm excitation, 390 nm emission; next 9.5 min, 260 nm excitation, 420 nm emission and last 22 min 255 nm excitation, 390 nm emission.

### 2.5 PAH quantitation and molecular distribution

PAH concentrations were determined by HPLC with fluorescence detection using the external standard calibration procedure. Calibration solutions were prepared by serial dilutions from a standard PAH solution (0.500 mg/l) purchased from Supelco. Precision measured from three replicate analyses of a lagoon sediment sample as a percentage standard deviation of PAH concentrations ranged from 18% (fluoranthene) to 37% (anthracene), 25% on average. Three replicate analyses of a clam tissue sample produced percentage standard deviations of PAH concentrations ranging from 4% (fluoranthene) to 44% (benzo[ghi]perylene), 19% on average. Limit of detection (concentration of calibration solution giving a signal to noise ratio > 3) was in the 0.2–7 ng/g range. Procedural blank tests resulted in no significant contamination. All concentrations were expressed on a dry-weight basis.

The relative distribution of PAHs was determined by GC–MS (some non-alternant PAHs are not fluorescent). Peak areas were determined in the mass chromatogram mode using the molecular ion, and values were corrected for response factors relative to pyrene. Relative response factors were determined from the analysis of the standard solution of PAHs (average of three replicates). For non-priority PAHs, it was used as the response factor of the most structurally similar PAH. Relative standard deviation ranged from 8% (acenaphthylene) to 40% (benzo[a]pyrene), 20% on average.

## 3. Results and discussion

### 3.1 Sediments

Concentrations of 11 priority PAHs in surface sediments are reported in table 1. Total PAH concentrations in the southern sub-basin, directly affected by the discharge point of industrial effluents, are heavily contaminated sites confirming previous studies [3, 14]. Concentrations

tend to decrease with increasing distance from the discharge point, and the lowest concentrations are registered in the northernmost site. The concentrations of Hg, which is a marker of past discharges into the lagoon, are reported in table 1. Mercury distribution is rather similar to that of total PAHs, in accordance to results relative to core sediments collected in the southern area [3]. This finding suggests that these pollutants share a common source and a similar redistribution process through the basin.

To estimate the possible toxicological significance of chemical concentrations in sediments, PAHs data were compared with sediment quality guidelines established by the National Oceanic and Atmospheric Administration (NOAA) [15, 16]: ER-L (effect range-low) is indicative of concentration level below which adverse effects on biota are rarely observed and ER-M (effect range-median) is taken as a warning level over which adverse effects frequently occur. Although the guidelines provide simply informal interpretative tools, they can be used to identify areas relatively uncontaminated and those with a high risk of toxicity. Data of table 1 show that sites in the southernmost area contain one or more PAH species with concentrations greater than ER-M values (sites 1, 2 and 3). Sediments from central and northern areas exhibit PAH concentrations lower than ER-L values, with a notable exception for site 4 which presents very high PAH levels. On the other side, Hg occurs at concentrations higher than its corresponding ER-M value in almost all the site, confirming this metal as the master pollutant of the entire Lagoon. However, previous studies performed on southern sediments have revealed the occurrence of non-priority PAHs for which ecotoxicological data are not available [3, 6].

The relative abundance of PAH species in the investigated sediments determined by GC-MS is reported in table 2. Pyrene is always the most abundant priority PAH. With the exception of the northernmost site (site 6), non-priority non-alternant PAHs contribute significantly to the total amount of PAHs in the whole lagoon. The most abundant non-priority non-alternant PAHs are cyclopenta[*cd*]pyrene, followed by benzo[*ghi*]fluoranthene and 4H-cyclopenta[*def*]phenanthrene. Notably, cyclopenta[*cd*]pyrene is the second most abundant PAH in all the sediments (excluding site 6).

Table 1. Concentrations of PAHs in surface sediments (mg/kg dry weight) determined by HPLC and comparison with numerical sediment quality guidelines.

PAHs	Sites <sup>†</sup>								Guidelines	
	1	2	3	G	4	B	5	6	ERL <sup>‡</sup>	ERM <sup>§</sup>
Phenanthrene	<b>4.7</b>	<b>7.5</b>	0.67	0.1	0.16	0.08	0.07	0.011	0.24	1.5
Anthracene	<b>1.4</b>	1.0	0.12	0.034	0.021	0.02	0.007	¶	0.09	1.1
Fluoranthene	<b>15</b>	<b>13.1</b>	0.96	0.16	<b>12</b>	0.12	0.13	0.005	0.60	5.1
Pyrene	<b>60</b>	<b>43.6</b>	<b>3.26</b>	0.37	<b>33</b>	0.28	0.33	0.02	0.66	2.6
Benzo[ <i>a</i> ]anthracene	1.7	0.8	0.11	0.016	0.03	0.015	0.02	0.005	0.26	1.6
Chrysene	0.15	1.4	0.09	0.016	0.02	0.015	0.016	0.005	0.38	2.8
Benzo[ <i>b</i> ]fluorantene	2.93	3.5	0.26	0.021	0.07	0.01	0.026	0.006		
Benzo[ <i>k</i> ]fluorantene	0.84	1.1	0.07	0.006	0.02	0.005	0.008	¶		
Benzo[ <i>a</i> ]pyrene	5.2	3.4	0.19	0.043	1.83	0.03	0.03	¶	0.43	1.6
Dibenzo[ <i>a,h</i> ]anthracene	17.5	3.2	0.08	0.13	0.03	0.07	0.012	¶	0.06	0.26
Benzo[ <i>ghi</i> ]perylene	24.8	16.9	1.53	0.14	4	0.09	0.14	0.008		
Sum	<b>135</b>	<b>96</b>	<b>7.3</b>	1.0	<b>52</b>	0.73	0.79	0.060		
Hg	<b>250<sup>  </sup></b>	<b>160<sup>  </sup></b>	<b>4.5<sup>  </sup></b>	<b>1.3<sup>  </sup></b>	<b>4.6<sup>  </sup></b>	<b>1.7<sup>  </sup></b>	<b>1.6<sup>  </sup></b>	0.47 <sup>  </sup>	0.15	0.71

<sup>‡</sup>ERL, effect range-low from Long *et al.* [15].

<sup>§</sup>ERM, effect range-median from Long *et al.* [15].

<sup>||</sup>From Trombini *et al.* [4].

<sup>¶</sup>Below the detection limit.

<sup>†</sup>Concentrations exceeding ERM value are in bold.

Table 2. Percentage distribution of PAHs determined by GC-MS normalized to pyrene in surface sediments.

Site	1	2	3	G	4	B	5	6
Naphthalene	2.7	–	–	0.7	0.2	2.9	–	57
Acenaphthene	1.2	–	0.4	0.6	0.5	1.2	–	6.4
Acenaphthylene	21	43	6.5	10	11	15	4.0	17
Fluorene	2.8	6.0	1.5	2.2	2.1	3.4	1.4	–
Phenanthrene	12	20	11	14	13	19	16	53
4H-cyclopenta[def]phenanthrene	6.0	7.6	6.0	7.3	7.2	8.8	7.2	6.9
Anthracene	1.6	2.9	1.6	1.7	1.7	2.2	2.4	54
Fluoranthene	24	29	28	36	34	44	39	63
Pyrene	100	100	100	100	100	100	100	100
Benzo[ghi]fluoranthene	18	21	26	25	31	24	35	19
Cyclopenta[cd]pyrene	71	68	90	63	98	62	102	28
Benzo[a]anthracene	1.1	1.6	1.3	4.6	5.5	6.4	1.8	12
Chrysene	0.9	0.8	0.0	0.7	1.0	0.7	1.4	1.3
Benzo[b]fluorantene	5.0	5.1	7.2	10	27	16	22	–
Benzo[k]fluorantene	3.2	3.1	3.8	3.5	7.1	2.9	7.3	2.9
Benzo[a]pyrene	7.8	7.6	9.9	6.2	27	7.1	20	–
Benzo[ghi]perylene	46	17	49	26	112	26	50	–

Note: (–) Less than 0.1%.

This finding supports the view that PAHs in most areas of the lagoon originated from a predominant source, probably the pyrosynthesis of methane in a past industrial process for producing acetylene [3, 6]. In accordance, the site located furthest from the discharge point (site 6) exhibits a somewhat different distribution, with the lowest levels of cyclopenta[cd]pyrene and other CP-PAHs.

### 3.2 Clams

Considering the unusual molecular pattern of PAHs in surface sediments of the Lagoon, it is worth considering their potential of bioaccumulation in dwelling organisms. Among them, *T. philippinarum* is widely distributed in the lagoon, harvested and commercialized by professional fishing. Studies on *T. philippinarum* transplanted in a contaminated lagoon have indicated the bioaccumulation activity of these bivalves for PAHs [17].

Clams were collected in two sampling sites, in the permitted area for fishing (site B) and in the forbidden area (site G). Although sediments collected in sites G and B exhibit low levels of contamination (PAH concentrations below ER-L levels, see table 1) due to the sandy characteristics of the sediments, site G is located in the highly polluted southern area, whereas site B is located in the less-polluted central area, thus they could be taken as representative of two environments with different contamination degree.

Concentrations of some priority PAHs in clam tissues are reported in table 3. Both in permitted and forbidden areas, PAH concentrations in clams are one order of magnitude lower than in sediments sampled at the same site. Bioaccumulation is related to bioavailability (*e.g.* PAH occurring in the interstitial water which is dependent on PAH solubility) and physiological processes of uptake and elimination [18]. Therefore, bioaccumulation is affected by several geochemical parameters (grain size, total organic carbon in dissolved and particulate phase, salinity and water turbidity) and biological factors (lipid content, diet and metabolizing activity). Desorption of PAHs from sediment to water is related to the structural and physicochemical properties (*e.g.* molecular mass, surface area and octanol–water partitioning coefficient  $K_{ow}$ ) [19].



Table 3. Concentrations of PAHs in clams (mg/kg dry weight).

	Site G	Site B
Phenanthrene	0.022	0.022
Anthracene	nd	nd
Fluoranthene	0.038	0.033
Pyrene	0.054	0.039
Benzo[ <i>a</i> ]anthracene	0.009	0.003
Chrysene	0.004	0.002
Benzo[ <i>b</i> ]fluorantene	nd	nd
Benzo[ <i>k</i> ]fluorantene	nd	nd
Benzo[ <i>a</i> ]pyrene	0.001	0.001
Dibenzo[ <i>a,h</i> ]anthracene	nd	nd
Benzo[ <i>ghi</i> ]perylene	0.004	0.001
Sum	0.13	0.099

Note: nd, below the detection limit.

The molecular patterns of PAHs in sediments and clams are compared in figures 3 and 4. Although for both sediments and clams, pyrene is the most abundant PAH species, there are important differences. The levels of naphthalene are higher and those of benzo[*ghi*]perylene are lower in clams with respect to sediments. The effect of PAH water solubility (the highest for naphthalene and the lowest for benzo[*ghi*]perylene) in governing bioavailability probably explains the observed behaviour. However, in turbid waters, the effect of water solubility on bioaccumulation becomes less important, as filter-feeding organisms may absorb PAHs associated to fine particles by direct ingestion [20]. The high levels of pyrene and fluoranthene in clams with respect to phenanthrene would indicate that a fraction of assimilated PAHs could be associated with particles. In this context, the behaviour of CP-PAHs, cyclopenta[*cd*]pyrene and acenaphthylene, is quite anomalous, as their relative content is strongly reduced in clams with respect to sediments (figures 3 and 4). Water solubility could be hardly invoked to explain the much lower levels of cyclopenta[*cd*]pyrene in clams with respect to sediments. In fact, the non-alternant benzo[*ghi*]fluoranthene with the same molecular mass (226 Da) of cyclopenta[*cd*]pyrene exhibits similar relative abundance in sediments and clams. Moreover,

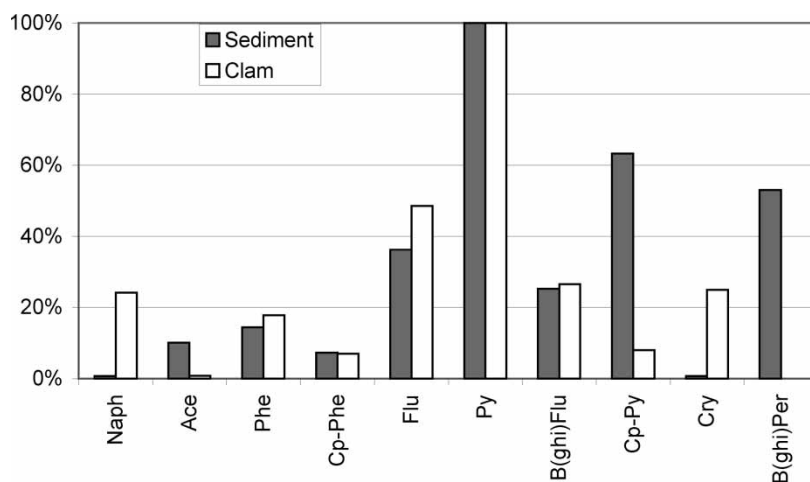


Figure 3. Percent molecular distribution of important PAH species (normalized to pyrene) in sediment and clams from site G.

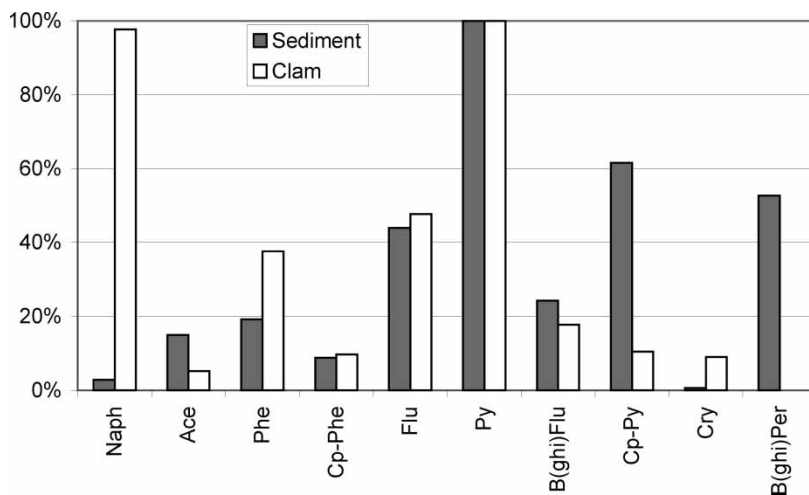


Figure 4. Percent molecular distribution of important PAH species (normalized to pyrene) in sediment and clams from site B.

chrysene is more abundant in clams than in sediments, despite its similar molecular mass (228 Da).

The different behaviour of cyclopenta[*cd*]pyrene with respect to similar PAHs could be related to the reactivity of the cyclopenteno double bond which is prone to metabolic oxidation. The corresponding metabolites can react with DNA to form DNA adduct, possibly responsible for its mutagenicity [21].

#### 4. Conclusions

The occurrence of the non-priority non-alternant PAHs, cyclopenta[*cd*]pyrene, 4H-cyclopenta[*def*]phenanthrene, benzo[*ghi*]fluoranthene, is a distinctive signature of PAH contamination in Pialassa Baiona Lagoon. Given the relatively high abundance of these characteristic PAHs throughout the entire basin, it is worth considering their presence in biota, especially for those species subjected to fishing activity. The obtained results show that non-alternant PAHs featuring sediment contamination are bioaccumulated by native clams. However, the body burden of CP-PAHs is lower in comparison with the levels observed in sediment for other non-alternant PAHs.

Recent studies on transplanted mussels have confirmed different bioaccumulated levels of cyclopenta[*cd*]pyrene and benzo[*ghi*]fluoranthene with respect to the levels found in sediments [22].

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#### References

- [1] D. Fabbri, O. Felisatti, M. Lombardo, C. Trombini, I. Vassura. The Lagoon of Ravenna (Italy): characterisation of mercury-contaminated sediments. *Sci. Total Environ.*, **213**, 121–128 (1998).

- [2] D. Fabbri, D. Tartari, C. Trombini. Analysis of poly(vinyl chloride) and other polymers in sediments and suspended matter of a coastal lagoon by pyrolysis-gas chromatography-mass spectrometry. *Anal. Chim. Acta*, **413**, 3–11 (2000).
- [3] D. Fabbri, I. Vassura, S. Cheng-Gong, C.E. Snape, C. McRae, A.E. Fallick. Source apportionment of polycyclic aromatic hydrocarbons in a coastal lagoon by molecular and isotopic characterisation. *Mar. Chem.*, **84**, 123–135 (2003).
- [4] C. Trombini, D. Fabbri, M. Lombardo, I. Vassura, E. Zavoli, M. Horvat. Mercury and methylmercury contamination in surficial sediments and clams of a coastal lagoon Pialassa Baiona, (Ravenna, Italy). *Cont. Shelf Res.*, **23**, 1821–1831 (2003).
- [5] M. Virgilio, N. Baroncini, C. Trombini, M. Abbiati. Relationships between sediments and tissue contamination and allozymic pattern in *Hediste diversicolor* (Polychaeta Nereididae) in the Pialassa lagoons (north Adriatic Sea). *Oceanol. Acta*, **26**, 85–92 (2003).
- [6] C. McRae, C.E. Snape, C.-G. Sun, D. Fabbri, D. Tartari, C. Trombini, A.E. Fallick. Use of compound-specific stable isotope analysis to source anthropogenic natural gas-derived polycyclic aromatic hydrocarbons in a lagoon sediment. *Environ. Sci. Technol.*, **34**, 4684–4686 (2000).
- [7] J.L. Durant, A.L. Lafleur, E.F. Plummer, K. Taghizadeh, Jr. W.F. Busby, W.G. Thilly. Human lymphoblast mutagens in urban airborne particles. *Environ. Sci. Technol.*, **32**, 1894–1906 (1998).
- [8] J.B. Howard, J.P. Longwell, J.A. Marr, C.J. Pope, W.F. Busby, Jr. A.L. Lafleur, K. Taghizadeh. Effect of PAH isomerization on mutagenicity of combustion products. *Combust. Flame*, **101**, 262–270 (1995).
- [9] Jr. B.A. Benner, G.E. Gordon, S.A. Wise. Mobile sources of atmospheric polycyclic aromatic hydrocarbons: a roadway tunnel study. *Environ. Sci. Technol.*, **23**, 1269–1278 (1989).
- [10] J.O. Allen, N.M. Dookeran, K.A. Smith, A. Saforim, K. Taghizadeh, A.L. Lafleur. Measurement of polycyclic aromatic hydrocarbons associated with size-segregated atmospheric aerosols in Massachusetts. *Environ. Sci. Technol.*, **30**, 1023–1031 (1996).
- [11] A. Gogou, I. Bouloubassi, E.G. Stephanou. Marine organic geochemistry of the Eastern Mediterranean: aliphatic and polyaromatic hydrocarbons in Cretan Sea surficial sediments. *Mar. Chem.*, **68**, 265–282 (2000).
- [12] J. Axelman, K. Naes, C. Näf, D. Broman. Accumulation of polycyclic aromatic hydrocarbons in semipermeable membrane devices and caged mussels (*Mytilus edulis* L.) in relation to water column phase distribution. *Environ. Toxicol. Chem.*, **18**, 2454–2461 (1999).
- [13] P. Baumard, H. Budzinsky, P. Garrigues. Analytical procedure for the analysis of PAHs in biological tissue by gas chromatography coupled to mass spectrometry: application to mussels. *Fresen. J. Anal. Chem.*, **359**, 502–509 (1997).
- [14] G. Matteucci, P. Rossini, A. Arcangeli, G. De Falco, P. Fonti, S. Guerzoni. Organic pollution in a Ramsar site (Pialassa Baiona, Northern Adriatic Sea). *Ann. Chim. (Rome)*, **91**, 445–457 (2001).
- [15] E.R. Long, D.D. MacDonald, S.L. Smith, F.D. Calder. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environ. Manag.*, **19**, 81–97 (1995).
- [16] E.R. Long, L.J. Field, D.D. MacDonald. Predicting toxicity in marine sediments with numerical sediment guidelines. *Environ. Toxicol. Chem.*, **17**, 714–727 (1998).
- [17] C. Nasci, L. Da Ros, N. Nesto, L. Sperti, L. Passarini, B. Pavoni. Biochemical and histochemical responses to environmental contaminants in clam, *Tapes philippinarum*, transplanted to different polluted areas of Venice Lagoon, Italy. *Mar. Environ. Res.*, **50**, 425–430 (2000).
- [18] J.P. Meador, J.E. Stein, W.L. Reichert, U. Varanasi. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Rev. Environ. Contam. Toxicol.*, **143**, 79–165 (1995).
- [19] J.F. Narbonne, J.E. Djomo, D. Ribera, V. Ferrier, P. Garrigues. Accumulation kinetics of polycyclic aromatic hydrocarbons adsorbed to sediment by the Mollusk *Curbicula fluminea*. *Eco Toxicol. Environ. Saf.*, **42**, 1–8 (1999).
- [20] P. Baumard, H. Budzinsky, P. Garrigues, J.F. Narbonne, T. Burgeot, X. Michel, J. Bellocq. Polycyclic aromatic hydrocarbon (PAH) burden of mussels (*Mytilus* sp.) in different marine environments in relation with sediment PAH contamination and bioavailability. *Mar. Environ. Res.*, **47**, 415–439 (1999).
- [21] C.H. Hsu, P.L. Skipper, S.R. Tannenbaum. DANN adduct formation by secondary metabolites of cyclopenta[cd]pyrene in vitro. *Cancer Lett.*, **136**, 167–141 (1999).
- [22] D. Fabbri, V. Baravelli, K. Giannotti, F. Donnini, E. Fabbri. Bioaccumulation of cyclopenta[cd]pyrene and benzo[ghi]fluoranthene by mussels transplanted in a coastal lagoon. *Chemosphere*. In press.