



Occurrence and toxicological assessment of polycyclic aromatic hydrocarbons (PAHs) in marine sediments under mussel farming influence

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Abstract

The use of marine resources for mussel culture has become increasingly important, particularly on the European Atlantic coast and notably in the Galician Rías in the northwest of Spain. Despite its importance, there is a lack of research and analysis in this area and of the potential problems that it could cause to the environment. This paper details the findings of a study that aimed to find the probable environmental impact of mussel culture activities and to evaluate the polycyclic aromatic hydrocarbon (PAH) content derived from this activity. The Ría de Arousa, where you can find over 70% of all installed rafts in Galicia, was selected for the present study, and nearly 40 marine sediment samples were collected there. The sediments were extracted by ASE (accelerated solvent extraction) procedure, and the quantification of PAHs was performed using gas chromatography coupled to mass spectrometry (GC-MS), with the aid of deuterated PAH internal standards. The total concentration of parental PAHs ranged from 11.66 to 30,272-ng g⁻¹ dry weight (d.w.), with a mean value of 3907-ng g⁻¹ d.w.; the concentration of alkyl PAHs varied from 3.72 to 1187-ng g⁻¹ d.w., with a mean value of 205.1-ng g⁻¹ d.w. Compositional patterns, principal component analysis (PCA) and hierarchical cluster association (HCA) yielded a sediment classification where the mussel raft impact is pointed out. PAH ratios indicated a predominance of combustion sources, except in two samples, located in small harbors. Only one station showed total potential carcinogenic PAH values in the range that would frequently cause negative biological effects, and the toxic equivalent concentrations based on BaP equivalents identified another ten positions where biological effects would occur occasionally.

Keywords PAH · Marine sediments · Mussel farming · Toxicology

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic pollutants with two or more fused aromatic rings. PAHs can be generated either from natural processes such as

diagenesis or from anthropogenic inputs such as oil spills, incomplete combustion, urban runoff, domestic and industrial wastewater discharges, atmospheric fallout of vehicle exhaust, and industrial emissions. Anthropogenic origins are generally the major sources of PAH pollution in the environment (Acquavita et al., 2014; C.-W. Chen & Chen, 2011) and can be classified into two main groups: petrogenic and pyrogenic. Petrogenic PAHs are derived from crude and refined petroleum products, while pyrogenic sources include incomplete combustion processes. Particularly in the Ría de Arousa, in addition to the possible industrial sources of PAHs, the presence of mussel farming must be considered. Among the negative impacts that arise from the culture of mussels, some are directly associated to the mussels' biology (Otero et al. 2006): (i) discharge of biodeposits (feces and pseudofaeces) with high TOC (total organic carbon) associated, (ii) release of drugs, (iii) increase in sedimentation, and (iv) increase in the

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concentration of nutrients in sediments beneath and near rafts. Other impacts are associated with farm maintenance (Wang et al. 2010; Crawford et al. 2003; Rosenthal et al. 1987): (i) tars or waterproof paints used to avoid raft corrosion, (ii) oil spillage due to vessel circulation around the raft, and (iii) organic wastes such as dead mussels and algae from the harvesting process. Some authors (Iribarrren Lorenzo, 2010; Sityo et al. 1983) and the local press have reported that the use of paints and tars intended to prevent wood corrosion and the analysis of this product composition (Ruiz, et al. 2014) indicate a high presence of PAHs, primarily with a pyrogenic profile. Moreover, in such situations, mussels, as filter feeders, will absorb the toxic substances present in the water and hence cause an increase in the concentration of the pollutants in their feces and in turn transfer them to the sediment.

In terms of human health, PAHs are thought to be carcinogenic through multiple routes of exposure and can affect the immune, reproductive, nervous, and endocrine systems. Due to this potential carcinogenic activity, the U.S. EPA (Environmental Protection Agency) has designated 16 PAH compounds as priority pollutants—naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene. This list of 16 EPA priority PAHs, issued by the U.S. EPA in 1976, is often used as a measurement in environmental samples; however, a recent publication discusses the appropriateness of this list and wonders if more compounds should be added as, for example, alkylated PAHs (Andersson & Achten, 2015). In this respect, OSPAR (Convention for the Protection of the Marine Environment of the North-East Atlantic) has also included alkylated PAHs in the pre-CEMP (Coordinated Environmental Monitoring Programme) list, and their monitoring may be required in the future once appropriate monitoring guidance, quality assurance procedures, and/or assessment tools are developed (OSPAR, 2010). Alkylated derivatives of PAHs are defined as PAH compounds with one to four carbon atoms replaced by alkyl groups.

In marine pollution studies, marine sediments represent an ideal matrix for gathering information regarding human activities in the coastal areas. Although no relevant regulation has been established, the monitoring of pollutants in sediments can provide information for the assessment of the potential toxic risk of these compounds, as well as supporting the decision-making of management authorities.

The studies on PAH concentrations in marine sediments are numerous and give an idea of the importance that the scientific community gives to the matter, while also providing concentration levels in different locations. He et al. (2014) present a comparison of the concentration determined in locations all over the world where concentrations from 21.72–319.61 ng/g

in a Bay in China to 30,000–1,670,000 ng/g in Marmara Sea can be found. The work of Viñas et al. (2010) carried out close to the current study area and gathered data from the Iberian coast where the concentration of PAHs varied from 0.7–136.5 ng/g on the Guipuzcoan Coast (Grimalt et al. 1992) to the values detected in the Bilbao Estuary (Prieto et al., 2008) that varied from 1493 to 47,480 ng/g.

This work, besides the inherent environmental study, is intended to provide evidence regarding the influence of rafts in the area. The study of an environment with very few polluting agents, other than the rafts, will be of great value in revealing the potential negative effects of the rafts on sediment pollution. Moreover, the mussels as a filter organism will suffer the effects from this increase in pollution.

The objectives of the present work were as follows: (1) to investigate the pollution level and distribution of PAHs in the Ría de Arousa, (2) to determine the possible origin of PAHs found, and (3) to evaluate the influence of mussel farming by means of principal component analysis (PCA).

Thirty-five PAHs, including 13 methylated PAHs (Table S2), were chosen on the basis of priority pollutants defined by CEMP and pre-CEMP lists to be monitored in surface sediments. TOC analyses were also carried out as a complementary parameter that could help to explain the distribution and contamination level assessment of PAHs. To complete this study, PAH diagnostic ratios and statistical tools were applied to reveal more information about pollution provenance and to discuss the current state of the ría. Additionally, ecotoxicological sediment assessment was performed based on two sediment quality guidelines (SQGs) approaches: (a) the effect range low (ERL), effect range median (ERM) values, and (b) the total toxic BaP equivalent (TEQ^{carc}). The results obtained will add new data to the global database and provide valuable information to improve the environmental quality of the estuary.

Material and methods

Chemicals and reagents

SupraSolv-grade acetone, hexane, silica gel (0.063–0.2 mm), neutral alumina (70–230 mesh), and anhydrous sodium sulfate for analysis were obtained from Merck (Darmstadt, Germany). A standard mixture of deuterated PAHs containing naphthalene-d8, biphenyl-d10, anthracene-d10, dibenzophthiophene-d8, pyrene-d10, and benz[a]pyrene-d12 was obtained from CIL (MA, USA). A PAH mixture containing naphthalene, biphenyl, 2-methylnaphthalene, 1-methylnaphthalene, 2,3-dimethylnaphthalene, acenaphthylene, acenaphthene, 2,3,6-trimethylnaphthalene, fluorene, dibenzothiophene, phenanthrene, anthracene, 4-methyldibenzothiophene, 2-methylphenanthrene, 2,8-

dimethyldibenzothiophene, 1,6-dimethylphenanthrene, fluoranthene, 2,4,7-trimethyldibenzothiophene, pyrene, 1,2,8-trimethylphenanthrene, 1-methylpyrene, benzo(c)phenanthrene, benz(a)anthracene, triphenylene, chrysene, 2-methylchrysene, 7,12-dimethylb(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene, and benzo (g,h,i) perylene was prepared from mixtures from CPA Chem (Stara Zagora, Bulgaria) and Chiron (Trondheim, Norway).

Study area

The Ría de Arousa (Fig. 1) is located in northwest Spain between 42° 25′–42° 42′ N and 8° 40′–9° 07′ W with an area of 230 km² and a maximum water depth of 69 m (Rosón et al. 1991). It has numerous islands and islets, among which, Arousa Island, inside the estuary, and Sálvora, near its mouth, are the most important. Two rivers create the drainage system of the ría, the rivers Ulla and Umia. The river Ulla flows into

its headwaters and the river Umia flows into the cove that is formed by the El Grove peninsular and the Cambados coast.

The Ría de Arousa has the largest area among the Galician Rías and contains the highest number of mussel rafts (2292). In all, almost 70% of the total rafts in Galicia can be found in the Ría. As a result, this ría has been selected in order to evaluate whether or not the mussel rafts can be blamed for part of the PAH pollution.

Sampling was carried out during the 2011 spring period on board the oceanographic vessel “José María Navaz.” The water temperature varied from 15.9 to 18 °C, and the salinity conditions were in the range from 32.8 to 36‰. Sediments were collected with a box corer dredge, and the top 3 cm of sediment was placed in aluminum containers and frozen on board. They were then freeze-dried and homogenized in the laboratory. Coordinates of sampling locations, water depth, and principal impact factors identified only by means of proximity to possible sources are shown in Table S1.

In order to cover the whole estuary, the sediments were collected in an area from the coast to the open sea. All of them

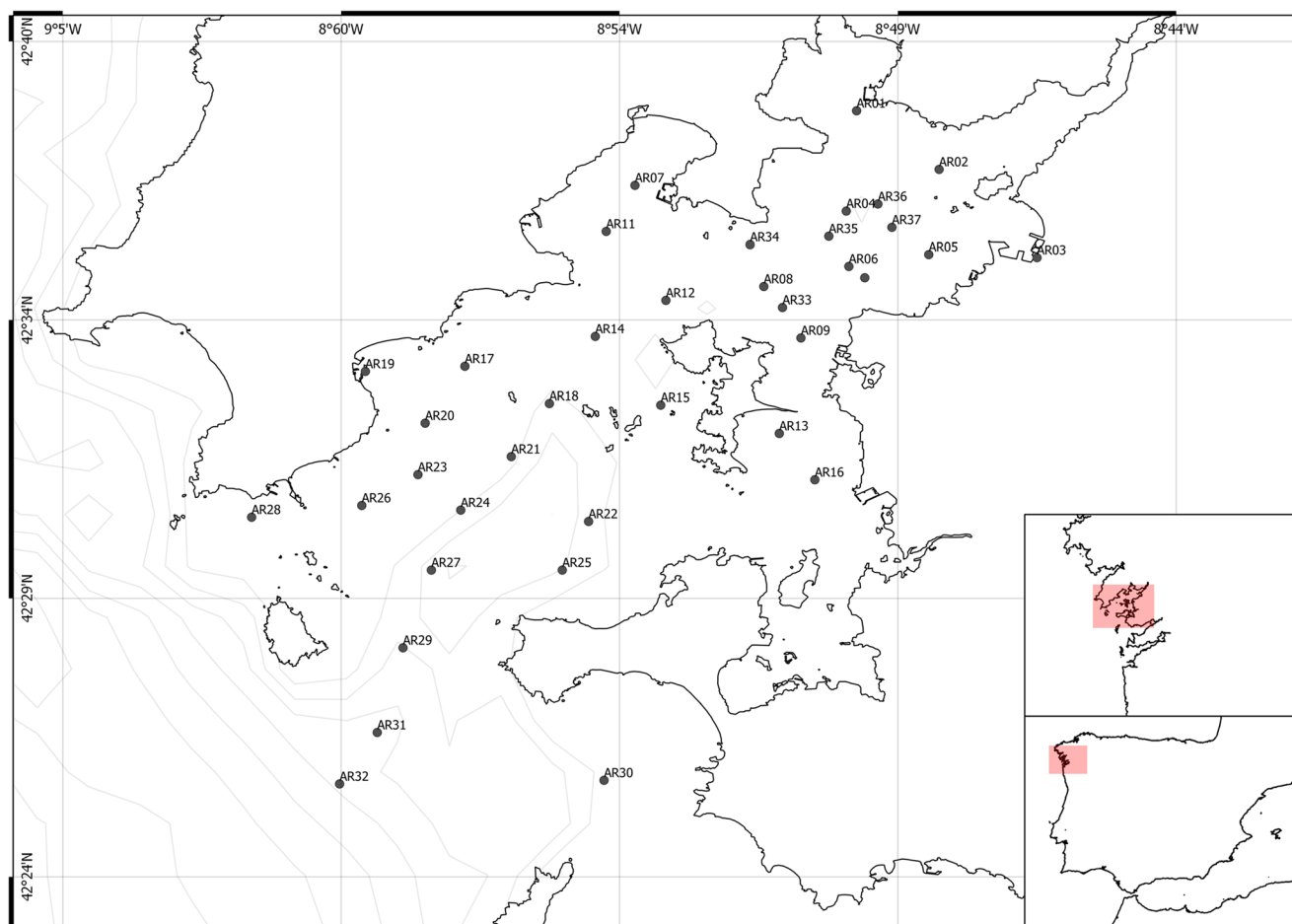


Fig. 1 Map of Ría de Arousa showing principal cities and sampling positions

show a sea character (salinity around 35‰) and so correspond to the marine part of the estuary.

The idea to cover the whole estuary, without taking into account the raft positions, would allow for the comparison between both sets of samples and also enable a complete characterization of the estuary. As a result, this wide sampling distribution provides samples from the less impacted positions in the entrance of the estuary, to the more polluted ones, placed near rafts, and/or exposed to human activities like harbors or villages. A previous paper (Pérez-Fernández et al. 2015) described the state of the bay and some of the samples are also cited here. The use of the complete set of samples presented in this paper allows for a better comparison of samples with different degrees of impact and can help reach new conclusions.

As an initial classification, we can identify AR07, AR15, AR16, AR25, AR33, AR34, AR35, AR36, AR37, and AR38 as being located close to mussel rafts; AR04, AR06, and AR09 as being situated between rafts; AR01, AR03, AR07, AR11, and AR19 as being found in or near harbors; and finally, among the cleanest positions, AR13 is located in a natural park and AR27, AR28, AR29, AR30, AR31, and AR32 can be found in the outer area of the ría.

Sample extraction and analysis methods

The analytical method was thoroughly described in Pérez-Fernández et al. (2015). In brief, PLE (pressurized liquid extraction) (ASE™ 350, Thermo Scientific™ Dionex™, Sunnyvale, CA, USA) was used to prepare the samples by means of the following procedure: ~3 g of the portion and <2 mm of the freeze-dried sediment together with diatomaceous earth were loaded in ASE cells and spiked with a mixture of six deuterated PAHs (naphthalene-d8, biphenyl-d10, anthracene-d10, pyrene-d10, pyrene-d10 and benzo[a]pyrene-d12). The solvent used was a 1:1 mixture of hexane and acetone. Activated copper was added to the extract for desulfurization. The extract was concentrated to 1 ml using a gentle stream of nitrogen and cleaning through an alumina column to obtain a suitable extract to be analyzed.

The analytical characterization was performed by a GC/MS Thermo model DSQ II (Thermo Electron Corporation, Austin, TX, USA) equipped with a TriPlus automatic sampler. The sample was injected in on-column mode into a 0.25-mm i.d. 60-m fused silica capillary column with a 5% phenyl-substituted methylpolysiloxane phase (0.25-μm film thickness) (TR-5 MS, Thermo Fischer Scientific, USA) using helium as carrier gas. The GC temperature program was initially set at 50 °C for 3 min, ramped to 200 °C at 6 °C/min, and finally to 315 °C at 4 °C/min holding that temperature for 15 min. Transfer line and ion source temperatures were held at 280 and 220 °C, respectively. The MS was operated in the electron impact (EI) mode at 70 eV and the spectrum divided

into eight SIM windows, each scanned for up to seven molecular masses previously optimized for the corresponding analytes and the deuterated internal standards eluting in each window.

Quality assurance and quality control (QA/QC) of analysis

Reagent blanks and recovery procedures were performed simultaneously with every batch of samples. The methodology has a detection limit in the range of 0.25 (for triphenylene + chrysene) to 2.62-ng g⁻¹ d.w. (for perylene) and a mean recovery rate of 83%.

Quantification of parent and alkylated PAHs reported in Table S2 was corrected for every sample with the corresponding deuterated compound with Thermo Scientific™ Xcalibur™ software package.

A minimum of five PAH mixture standards was run with every batch of samples to build the linear regression curves by plotting the peak area ratios versus each PAH concentration; the obtained correlation coefficient has to be at least ≥ 0.99 to be acceptable.

Four different sediments, named QMM64, QMM73, QMM74, and QMM78, analyzed in the lab as part of intercalibration exercises organized by the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) were used as reference internal materials obtaining satisfactory results.

Sediment characterization

Collected sediments were characterized in terms of grain size and TOC content. The sediment fraction was determined by wet sieving on a 63-μm sieve to obtain two grain-sized fractions, < 63 and > 63 μm.

Organic carbon content was determined using an elemental analyzer (model 2400 series II, CHNS/O, Perkin Elmer, Inc., Shelton, USA). In order to prepare the samples for this elemental analysis, sediment samples were ground in an agate mortar and then treated with hydrochloric acid 25% to remove inorganic carbon as described in Boitsov et al. (2013).

PAHs sources

The main sources of PAHs in the environment are classified into three groups: pyrogenic, petrogenic, and diagenetic. By means of three crossplots of the following ratios IP/(IP + BghiP), Ant/(Ant + Phe), and BaA/(BaA + Chr) versus Fla/(Fla + Pyr), different sources can be inferred in the environmental samples (Tobiszewski & Namiesnik, 2012; Yunker et al., 2002). For the diagnostic ratio, Fla/(Fla + Pyr) and IP/(IP + BghiP) ratios below 0.4 and 0.2, respectively, indicate petrogenic origins, while ratios at 0.5 and above indicate

biomass and coal combustion pollution. It is accepted for the relationship $\text{Ant}/(\text{Ant} + \text{Phe})$ that values < 0.1 are related to petroleum origins and values above this figure indicate a dominance of combustion sources. $\text{BaA}/(\text{BaA} + \text{Chry})$ ratios lower than 0.2 indicate a large amount of spilled oil. Values from 0.2 to 0.35 characterize the combustion of petroleum, and values larger than 0.5 indicate other combustion sources.

Statistical tools

The statistical treatment of the data (PCA and hierarchical cluster analysis (HCA)) was performed using R statistical software (<https://www.r-project.org>).

The PCA and HCA can help to recognize different sources of PAHs and to identify and classify those sediments subjected to raft pressure against other polluted sediments. For statistical analysis, the values were firstly transformed by scaling them, thus minimizing the different scales.

Toxicity assessment in sediments

SQGs, defined as numerical chemical concentrations intended to be protective of biological resources, predictive of adverse effects to those resources, or both, are important tools for the assessment of contamination in marine and estuarine sediments. In 1995, Long et al. (1995) developed two guideline values: ERL and ERM, for a number of trace metals and organic compounds as the 10th and 50th percentile of a series of studies that provide concentrations of contaminants in sediments and observed biological effects. The defined values establish three ranges at concentrations $< \text{ERL}$ where biological effects rarely occur; at concentrations $\geq \text{ERL} \leq \text{ERM}$ where biological effects occasionally occur; and at concentrations $> \text{ERM}$ where negative biological effects frequently occur. In the present paper, the sediment was evaluated based on the SQG defined for the seven PAHs: BaA, Chry, BbF, BkF, BaP, IP, and DBaH classified as group B2, probable human carcinogenic by the EPA (Peters et al., 1999). An ERL concentration of 1373 and an ERM concentration of 8410 ng g^{-1} were established in the cited article for the sum of the seven carcinogenic PAHs listed.

Another SQG, frequently used in the evaluation of sediment affected by PAH pollution, is the total toxic BaP equivalent (TEQ_{carc}). As different PAH congeners evoke different toxicity, the potential toxicity of sediment is better evaluated as the toxic equivalency (TEQ) of the compounds, based on the equivalency factors (TEF) of each individual hydrocarbon, determined with respect to the carcinogenicity of BaP (C.-W. Chen & Chen, 2011; US EPA, 1993). According to the US EPA, TEFs for BaA, Chry, BbF, BkF, BaP, IP, and DBaH are 0.1, 0.001, 0.1, 0.01, 1, 0.1, and 1, respectively.

Results and discussions

Sediment analysis and PAH concentrations

Sediment characterization in terms of TOC (C), hydrogen (H), and nitrogen (N) can be seen in Fig. 2; the samples closer to rafts—AR33, AR34, AR35, AR36, AR37, and AR38—are easily recognizable. They are characterized by the highest values of carbon, low levels of hydrogen, and not as high nitrogen levels, all of them yield a C/N of around 7, identified by Rullkotter, as related to biodeposits (Rullkotter, 2006). Samples located in protected areas or outside the ría—AR13 and AR28 to AR32—are characterized by low concentrations of carbon (C) and hydrogen (H). Sample AR12 shows the highest level of nitrogen and a high level of hydrogen, to be expected given its location in a bifurcation of marine currents where upwelling processes have been identified (Álvarez-Salgado et al. 2000; Otto, 1975; Rosón et al. 1997).

Among the samples, those situated in the proximity of rafts show the highest %C, around a fourfold increase. The existence of a correlation between PAHs and organic matter is generally accepted (Chen et al. 2013; Liu et al., 2008; Viguri et al. 2002); nevertheless, some exceptions can be found in the literature and were indeed observed in our samples. In Fig. 3, neither grain size nor organic matter shows a good correlation with the PAH content in the studied samples. This absence of correlation indicates that the increase in %C is not the only responsible for the high PAH concentrations found in these samples and consequently, a first evidence that some other input (not related with the PAHs associated to detritus) should be liable for the PAHs present in the areas near rafts.

PAHs were determined in ca. 40 samples reaching values that ranged from 15.38 to 31,460 ng g^{-1} , with a mean value of 3830 ng g^{-1} ; the sampling points with the lowest PAH concentrations are those found in the outer part of the estuary (AR28–AR32) and one sample (AR13) in a protected area under the shelter of Arousa Island; the most polluted sites are located near harbors, rafts, or in the mouth of the river Ulla.

An overall study of the obtained results (Fig. 4) identified the Ría de Arousa as a Ría with high PAH concentrations. Other nearby rías, even the more populated ones, show values that do not exceed 5000 ng/g (Franco et al. 2006; Viñas et al., 2009), and this study has identified nine sampling sites that exceed this value and even two that are twice as high as the referred value. This situation is totally unexpected as the Ría de Arousa has very low levels of industrial activity and does not suffer from the influence of a high population. It was even characterized in previous studies as a typically low contaminated area regarding other pollutants such as heavy metals (Besada et al., 2014; Prego et al., 2006). This is the main reason for this research into the probable causes of these unforeseen PAH concentrations and trying to elucidate a possible correlation with the raft positions.

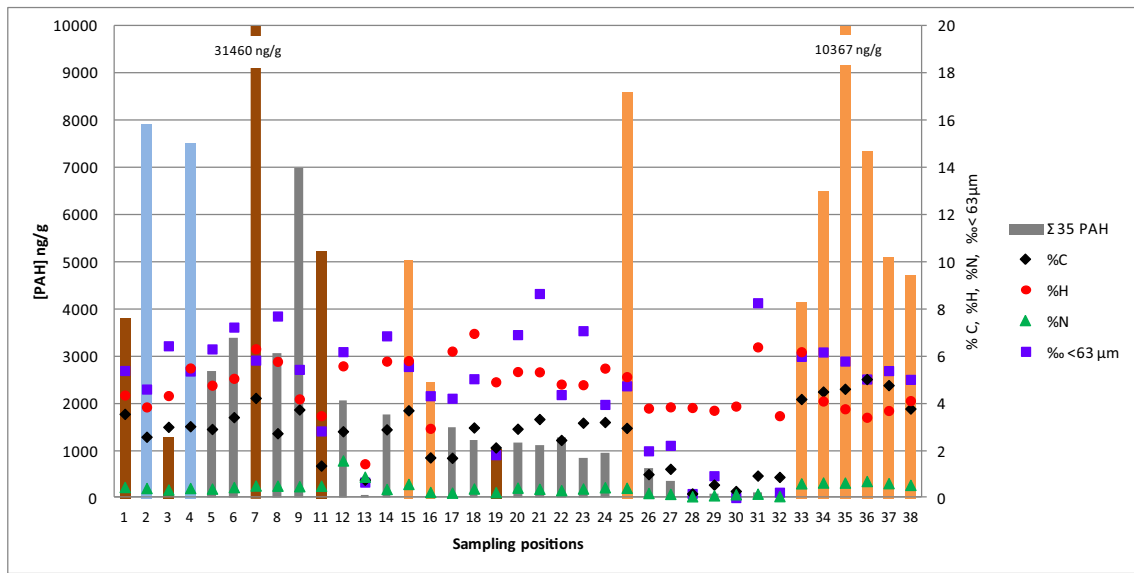


Fig. 2 PAH concentration expressed as sum of 35 PAHs and sediment characteristics (secondary axis) in terms of %C, %H, %N, and fine fraction (% particles less than 63 μm). The relation C/N for all the

samples is also presented. Color bars indicate potential impact: mussels' rafts in orange, harbors in brown, and rivers in blue

The detritus material associated with rafts and the presence of cities and harbors in the area can be pointed out as the possible pollution actor; nevertheless, two facts indicate that other activities should be participating in the pollution present in the area. One is the idea that other areas with similar TOC values, such as Ría de Vigo and Ría de Pontevedra (Vilas et al. 2005; Viñas et al. 2009), do not reveal so high PAH concentrations indicating that the detritus material is not a unique factor. The other fact is the presence of high PAH values far from the coast and an absence of a PAH depletion as we move away from harbors or populated areas, indicating a located pollution in the raft surroundings.

In this sense, the human activities associated to the rafts: paints used in the maintenance activities, boats, winches, and other activities associated with the harvesting are pointed out as pollution sources in this area.

In terms of compound distribution, the sample profiles are quite homogenous along the sampling sites with Fla, Pyr, BbF, and Phe being generally the most abundant compounds (Fig. S1). The average representation of the sediments with the high PAH concentration determined—A—shows this tendency and reveals a predominant presence of Fla, Pyr, Phe, and BbF. The average representation of those sampling points located close to rafts—B—shows a similar distribution to the previous samples located close to harbors or cities. This fact would imply the existence of pollution sources possessing similar characteristics, and indeed, this would match the idea of the pollution coming as a result of the tar paints used in raft and other harvesting and maintenance activities, given that, the same as industries, they present a markedly pyrogenic profile. The less polluted areas—C—show a slightly different profile where, despite the predominance of 4- to 5-ring PAH,

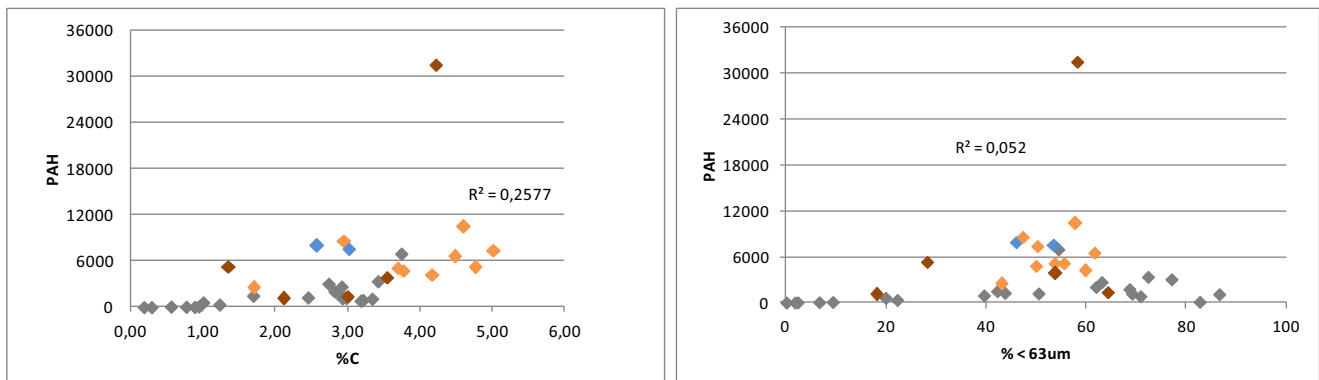


Fig. 3 Correlation plots of %COT and grain size distribution against PAH concentration

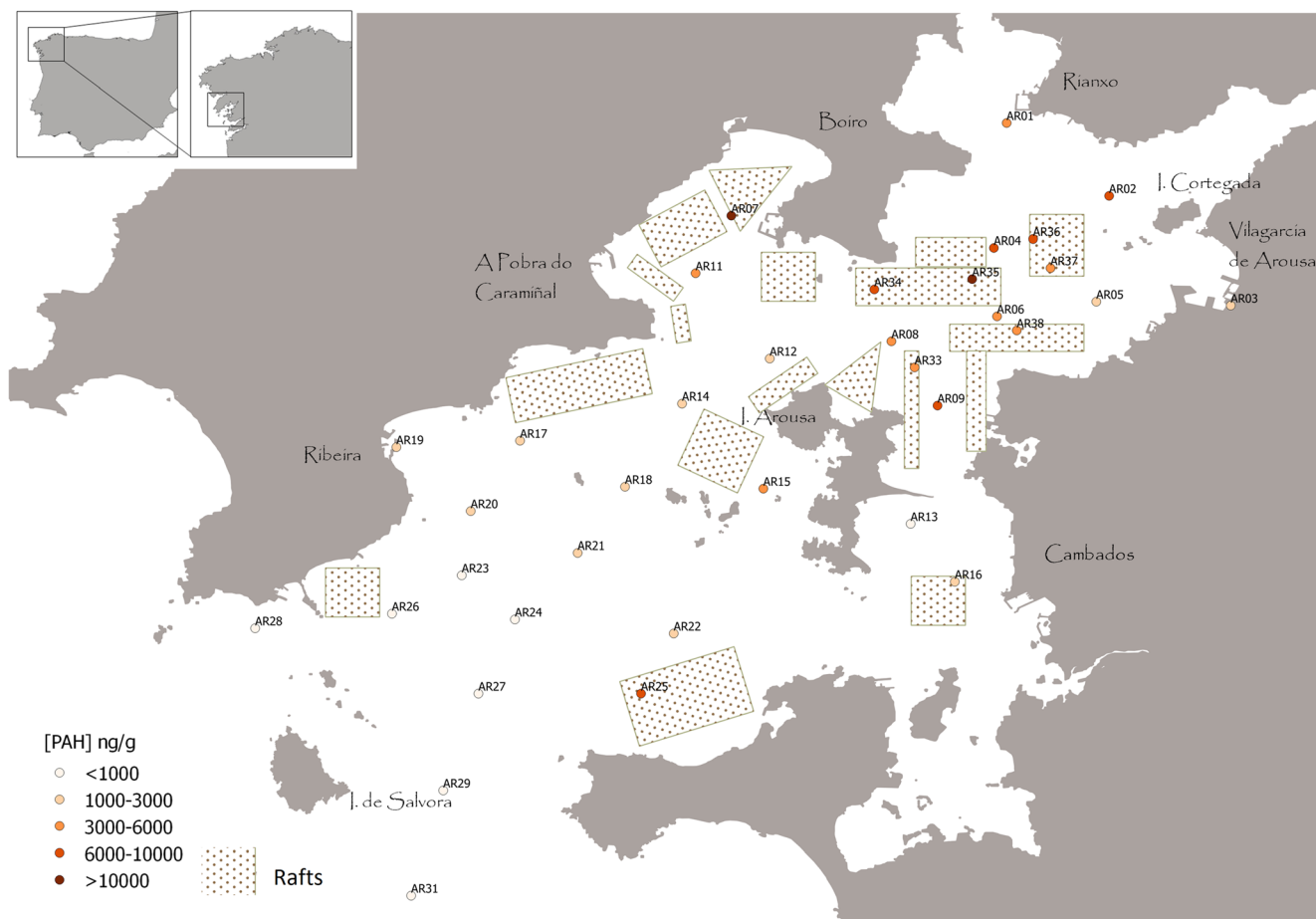


Fig. 4 Map of Ría de Arousa showing the determined PAH concentrations by GC/MS in each sample point, classified in five ranges. Rectangles with black dots indicate the locations of rafts. In order to focus

the map in the inner part of the ría, samples in the outer area of the Ría AR30 and AR32 are not represented; coordinates can be checked in Table S1

low molecular weight PAHs (3–4 PAH) also accounted for an important percentage of the total concentration.

PCA and cluster results

The purpose of PCA is to represent the total variation of the PAH data within a minimum number of factors (PC). Figure S2a–b represents the results of individual PAH and the sample data after the scaling processes. The scaling data of every individual PAH normalized to the sum of PAH was used in the PCA analysis. As shown in Table S3, PCA analysis classified the dataset of PAHs into four factors, accounting for 97.4% of the total variability in the data. Factor 1 (PC1) explained 87.40% of the total variance in the data. This factor is mainly weighted by Pyr, BcPhe, Triph/Chry, BkF, and BaP, although the loadings are in a small range and hence no firm influences can be determined.

The second factor (PC2) explained a total variance of 6.09% in the data and is predominately composed of 1,2,8-MPhe, 2,4,7-MDBT, 2,8-MDBT, 4-MDBT, 2,3,6-MNaph, all of them methylated compounds and mainly DBT derivatives; in some

papers (Ahad et al., 2015; Yang et al., 2011), these compounds were related to the presence of bitumen and therefore pointers of petrogenic sources. The third factor (PC3) is responsible for 2.31% of the total variance in the data and is again strongly related to 1-MNaph, 2,3,6-MNaph, Ant, 2,8-MDBT, 1,2,8-MPhe and thus appears as a new petrogenic indicator.

As can be seen in Table S3, there are only two principal components needed to describe more than 90% of the data variability among variables. Among the 37 samples, the PCA and the ulterior cluster association (Fig. 5) taking into account the two first PCs show a single-member cluster, where only station AR07 appears and another group of various, although not well-defined, clusters. A better representation of the associations among samples can be obtained by the representation of a dendrogram, where all cluster associations can be clearly checked.

Figure 5 graphically illustrates the obtained dendrogram as a result of the statistical analysis. A comprehensive reading of the dendrogram matches the previous assessments. From the center of the circle, it is possible to identify three large-scale groups identified in blue, red, and green. The blue samples are

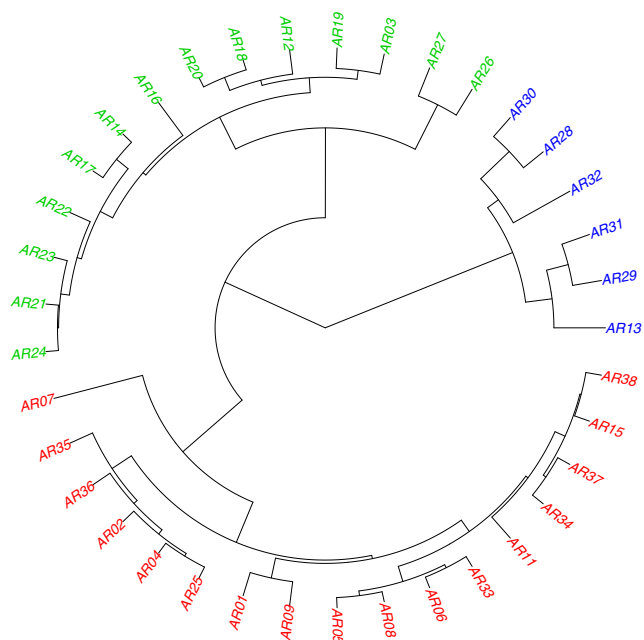


Fig. 5 Dendrogram resulting from cluster analysis of the composition of PAHs in sediments

the ones with the lowest detected levels of PAHs, i.e., the outer stations and stations in protected areas (AR13). The second group, in green, brings together those stations with a medium level of contamination, placed in the medium area of the Ría, and without other nearby pollution sources. The last cluster, in red, contains the stations with the highest pollution levels. All the samples positioned in the proximity of rafts, even those placed away from classical pollution sources (cities, harbors ...), are enclosed in this cluster, thereby revealing the impact of the rafts. Further divisions of this cluster identify more specific correlations; in this sense, AR07 is identified as a station with particular characteristics, previously identified by very high levels of PAHs.

Source identification by isomer ratios

As described in the “Material and methods” section, different sources can be identified by the distribution of a set of PAHs as detailed in Fig. S3.

The plots displayed in Fig. S3 confirm the idea that pyrolytic pollution is distributed along the ría, meaning that the same kind of source (pyrolysis) is present in the sites showing the highest pollution levels, both in the proximity to harbors and closeness to the mussel rafts.

In addition, different isomer ratios, as shown in Fig. 6, allow the identification of sources for all the sediment samples analyzed. It can be seen that only AR19 and AR28 exhibited evidence of petroleum-derived pollution, while the origin of the PAHs in the other samples may come primarily from biomass and coal combustion. It is worth noticing that both stations are located in small harbors where minor oil spills or

illegal disposal of uncombusted petrol could take place. The identification of pyrolytic sources, not only in the positions close to harbor but also in others in the middle of the ría, will again confirm the idea of a secondary pollution source. On this occasion, this could be clearly attributed to the rafts, given that the maintenance activities employ tar and tar-derived products as protective paints.

Toxicity assessment in sediments

An important toxicity assessment in sediment is determined by the bioavailability of a pollutant and thus their capability to enter into the food chain. As described in the “Material and methods” section, the total concentration of CPAHs can be used to identify a potential problem. The values of CPAHs found in the studied sediments ranged from 2.68 ng g⁻¹ at AR30 to 13,117 ng g⁻¹ at AR07 (with a mean value of 1600 ng g⁻¹) (Fig. S4). According to the obtained data, the samples contained in the clusters 1 and 2 yield figures below ERL indicating that biological effects are rare. On the other hand, the concentration found in AR07 is above the ERM, indicating that a negative biological effect would frequently occur in this area and it would be necessary to implement measures to improve the situation. The rest of the samples present values higher than the ERL but lower than the ERM, indicating that biological effects would occur occasionally.

The other approach described made use of the TEQ^{carc}. For the studied samples, the calculated TEQ^{carc}, plotted in Fig. S4, is in the range 0.58–3510 ng g⁻¹. To evaluate the relative toxicity of sediments, we applied the ERL and ERM modified in terms of TEQ^{carc} described in the literature (Sun et al. 2012); using these indicators, a similar identification of environmental disturbance is described. All samples contained in C1 and C2 and AR05, AR06, and AR08 yield figures below the ERL, indicating that biological effects are rare. AR07 is identified as sediment that may have adverse effects on ecological and human health. The remainder has concentrations that are higher than the ERL but below the ERM, indicating that a biological effect would occur occasionally.

A comparison of the results with other coastal areas is shown in Table S4. The ΣCPAHs found in sediment AR07 from Ría de Arousa is higher than other areas listed in the table. The rest of the values found in sediments from this study ranged from 2.68 to 4229 and are less polluted than the sediments from Naples Harbor, Kaohsiung Harbor (Taiwan), and Bahía Blanca Estuary (Argentina) but present higher values than those reported for the Barent Sea, Meiliang Bay (China), and Langkawi Island (Malaysia).

TEQ^{carc} values calculated in samples—discarding AR07—range from 0.58 to 1027 ng g⁻¹ and are similar to those obtained in Meiliang Bay, China (94–856 ng g⁻¹), whose sediments were described as only rare causing acute toxic effects to benthic organisms while manifesting carcinogenic potential

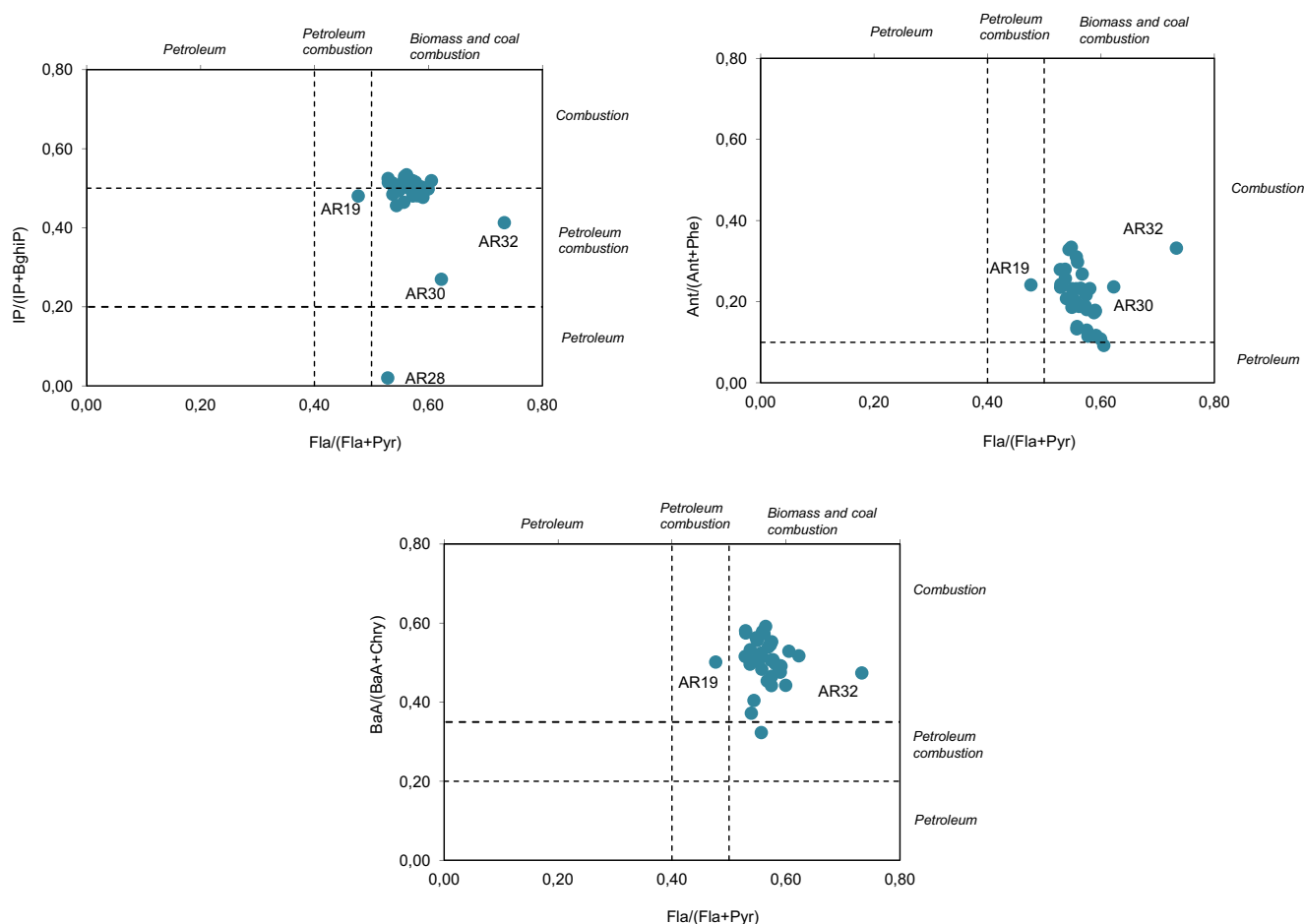


Fig. 6 Crossplots of PAH diagnostic ratios $IP/(IP + BghiP)$, $Ant/(Ant + Phe)$, and $BaA/(BaA + Chr)$ versus $Fla/(Fla + Pyr)$ identifying sources of PAHs in sediments

(Qiao et al., 2006), lower than those from Naples Harbor (Sprovieri et al., 2007) and greater than those from Bahia Blanca Estuary, Argentina (Oliva et al. 2015).

Conclusions

The present paper represents an extensive pollution study in terms of PAH contamination levels and their sources, allowing for the identification of a pollution source and its effects.

The analytical determination of 35 PAHs in more than 35 sampling points has been carried out and the crossplot representations: $IP/(IP + BghiP)$, $Ant/(Ant + Phe)$, and $BaA/(BaA + Chr)$ versus $Fla/(Fla + Pyr)$ identified pyrogenic origin as the predominant source of contamination. Moreover, the statistical treatment of the data resulted in the identification of three groups (clusters) of samples and describes the main actors responsible for pollution.

Based on the data analysis, a clear influence of mussel rafts has been detected, and a particular pollution pattern identified. Evidence of this raft influence can be seen in the high levels of

PAHs that have been detected in an area, not under the influences of industrial activity, or of a high urban population.

The spatial distribution of these high concentration levels of hydrocarbons is not only found in the ports (cities) but also in the middle of the ría, in areas close to the rafts, where no other identifiable sources can be found. Studies on the origins of the contamination tend to identify pyrolytic sources, which is expected given the consequences of industrial and urban activity. However, the identification of pyrolytic sources in most of the sampling sites, including those in the center of the ría, suggests the influence of tarring in the raft maintenance. All these point to the fact that the process of raft maintenance is causing a negative impact on the ría.

As a corollary study, the risks associated with the observed pollution have been identified by two indicators: the total concentration of carcinogenic PAHs and the toxic BaP equivalent. Both indicate the need for strict monitoring of water and sediment quality in the Galician Rías, with special attention being paid to contamination due to raft maintenance activities in those positions where toxicology indicators call for the implementation of urgent and necessary measures.

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