

Article

Application of Positive Matrix Factorization for Source Apportionment of Polycyclic Aromatic Hydrocarbons (PAH) in the Adriatic Sea, and the Evaluation of PAH-Related Carcinogenic Risks

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Abstract: Concentrations of polycyclic aromatic hydrocarbons (PAH) were studied in 36 sediment samples collected from 29 sites in the central Adriatic Sea. The total concentration of PAH showed high variability, ranging from 42.85 $\mu\text{g kg}^{-1}$ in open sea sediments to 28,662 $\mu\text{g kg}^{-1}$ in the sediments of Šibenik Bay. Unsubstituted, parent PAH were dominant PAH compounds in the entire area of Šibenik Bay and most of the stations in the coastal area. In Kaštela Bay, methyl PAH concentrations were higher than the concentrations of unsubstituted PAH, whereas in the sediments along the mid-Adriatic transect, similar concentrations of both PAH fractions were determined. PAH sources were investigated by applying the Positive Matrix Factorization software developed by the U.S. Environmental Protection Agency (USEPA). The results indicate the presence of four sources, two of which can be considered petrogenic and two pyrogenic. The contribution of the sources to the total concentration PAH at each station indicates the dominance of pyrogenic sources in Šibenik Bay and at most stations in the coastal area. In the sediments along the mid-Adriatic transversal, as well as in Kaštela Bay, PAHs originate mainly from petrogenic sources. The obtained results show that PMF can be a suitable tool for control of PAH pollution and thus, for the management of various activities in the Adriatic region. Seasonal differences between the contributions of sources in the Kaštela and Šibenik bays indicate that the contribution of traffic to the total concentration of PAH was higher during the tourist season. The carcinogenic risk of PAH determined by toxicity equivalent (TEQ) calculations showed an increased carcinogenic risk in the whole area of Šibenik Bay, regardless of the number of PAH compounds considered for the calculations. A low PAH-related carcinogenic risk was found for the rest of the study area.

Keywords: PAH; sediments; Adriatic Sea; Positive Matrix Factorization; source apportionment; toxic equivalent quantity



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1. Introduction

Polycyclic aromatic hydrocarbons are a large class of organic compounds whose structure consists of fused aromatic rings [1]. They are emitted from numerous sources in the form of a complex mixture that includes a significant amount of heterocyclic aromatic compounds in addition to PAH [1]. Although PAH may originate from natural sources, most of these compounds are emitted into the environment from anthropogenic sources such as transportation, power generation, industrial furnaces, waste incineration, coke and carbon production, asphalt and petroleum cracking [2–4]. PAH of anthropogenic origin are usually divided into the class of petrogenic PAH, which are formed during the conversion of organic-rich materials at low temperatures, and pyrogenic PAH, which are formed during the high-temperature combustion of organic materials in industrial furnaces, vehicle engines, power plants, etc. [5–10]. PAH mixtures emitted from petrogenic sources

usually contain a greater amount of low molecular weight PAH and alkyl-substituted PAH, while PAH mixtures from pyrogenic sources are enriched in unsubstituted PAH compounds with higher molecular weight. PAH are introduced into the marine environment mainly through rivers, atmospheric deposition, municipal and industrial runoff, and accidental spills of fresh petroleum derivatives. Due to their high hydrophobicity, PAHs adsorb to particles in the water column and sink through the water column. Sediments can accumulate large amounts of PAH, which degrade over long periods of time and pose a threat to ecosystem health [2,3].

PAHs have been shown to have disruptive effects on the lymphatic system and hematopoiesis [11,12], while chronic exposure to PAH causes mutagenicity due to their tendency to interact with DNA [13]. While pyrogenic PAHs are associated with greater environmental and health risks, they are primarily associated with chronic toxicity [11,13]. In contrast, petrogenic PAH are more soluble and bioavailable to marine organisms, and as such considered to cause acute toxicity. Due to their adverse effects on human health and the health of marine organisms, the U.S. Environmental Protection Agency (USEPA) has included 16 PAHs in a list of priority pollutants to be monitored in the environment [14]. Various approaches have been developed to assess the environmental risk associated with PAH, of which the use of Sediment Quality Guidelines (SQGs) and bioassays have been the most widely used [15,16]. Laboratory experiments have been conducted on a variety of marine animals, including microorganisms, algae, invertebrates, fish and marine mammals [11,13,17], and the results served as the basis for the carcinogen classification system for PAH and mixtures of PAH, and for the development of the Toxic Equivalent (TEQ) method for PAH-related environmental risk assessment [13,17–20]. This method assumes that all carcinogenic PAH are equitoxic to benzo[*a*]pyrene and that the carcinogenic effect of a complex PAH mixture can be calculated as the sum of the effects of the individual PAH compounds [17,20]. Although this approach may lead to overestimation or underestimation of the effects of PAH due to the inhibitory/synergetic properties of PAH compounds when present as a complex mixture, the TEQ method has been used in many studies to assess the PAH-related environmental risk [18–20].

Since the toxicity of a PAH mixture is determined by its composition and, consequently, by the nature of the source from which the PAH mixture originated, knowledge of the PAH sources at a particular site is important for the assessment of PAH-related environmental risk. Many approaches have been applied to constrain PAH sources, of which diagnostic ratios, statistical tools and modelling have been most commonly used. The use of statistical multivariate analyses, such as principal component analyses (PCA), cluster analyses and alternating least squares (ALS) are a cost-effective method for attributing PAH sources, although these methods require the identification of a larger number of PAH compounds at a larger number of locations [19,21,22]. To provide a reliable tool for addressing PAH pollution, the USEPA has developed a specific model based on the factor analysis method: the Positive Matrix Factorizations (PMF) [23]. The PMF results provide the number of factors (sources), the contributions of the factors to the concentration of each PAH compound, and a contribution of the factors to the total pollution PAH for each case [4,19,21,24].

This study aims to obtain detailed information on the most important PAH sources and their contributions in the overall PAH pollution by application of PMF software. Another goal was to determine the PAH-related sediment toxicity by calculation of toxic equivalents at each station. The obtained results will be used for the management of various economic activities in the Adriatic region, as well as for the development of strategies to mitigate the PAH pollution and PAH-associated environmental risk.

2. Material and Methods

2.1. Study Area

Figure 1 shows PAH concentrations that were analyzed in surface sediments of 29 stations along the Adriatic coast and along the mid-Adriatic transect. The coastal area includes 11 stations located from the island of Pag in the north, to the city of Dubrovnik in the south.

Stations were selected to cover urbanized areas along the Croatian coast as well as areas that were heavily industrialized in the past. Six stations along the horizontal transect from Split to Mt. Gargano in Italy were selected to identify PAH concentrations and the main PAH sources in this area. Due to the greater anthropogenic pressures, Kaštela Bay and Šibenik Bay were selected for more in-depth study. In these areas, sediment samples were obtained at a greater number of stations during winter and early autumn, in order to assess the impact of touristic activities on trends in PAH pollution. Additional information for each station is given in Tables S1 and S2 of Supplementary Information (SI).

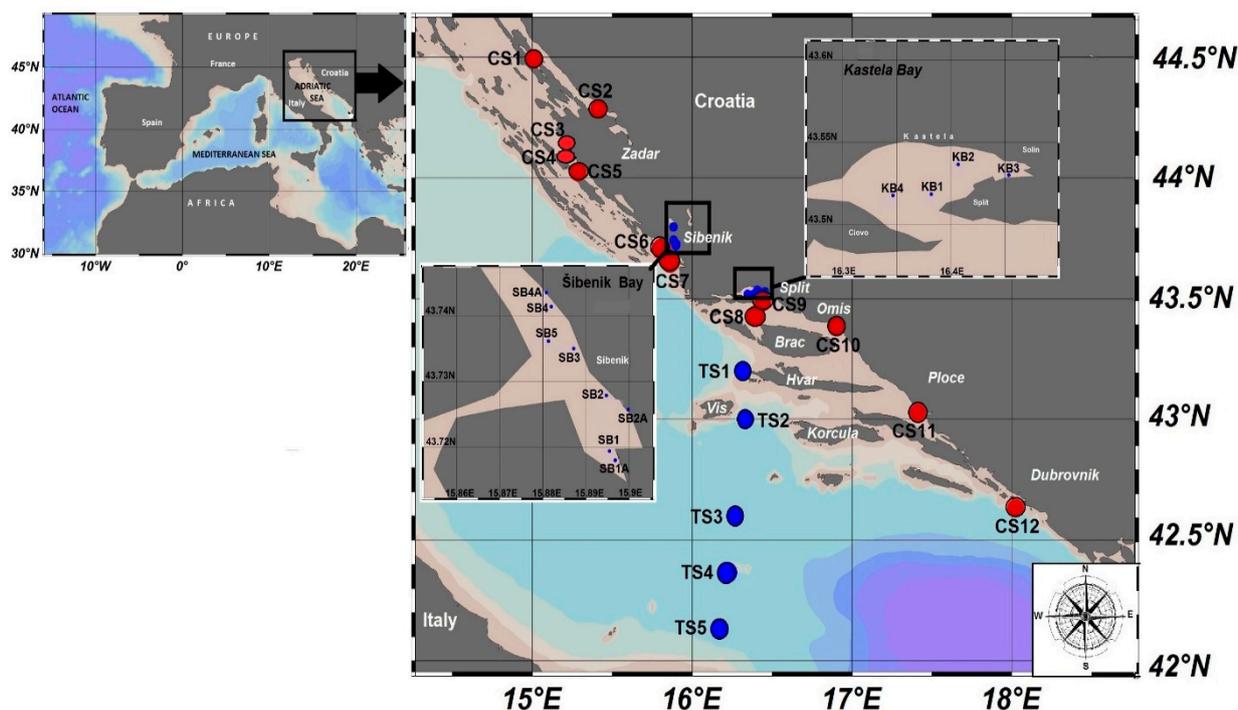


Figure 1. Sediment-sampling stations selected for source apportionment of polycyclic aromatic hydrocarbons and PAH-related toxicity evaluation in Adriatic Sea.

2.2. Determination of Polycyclic Aromatic Hydrocarbon in Sediment Samples

Polycyclic aromatic hydrocarbons were collected from the research vessels BIOS DVA using the Van Ween sampling device. Sediment samples were transferred in the laboratory for biogeochemistry of organic contaminants (*Biogéochimie des Contaminants Organique*) at the French institute for exploration of the sea: Ifremer (Institut Français de Recherche pour l'exploitation de la Mer) in Nantes, for further analyses. Analytical procedure and instrumental analyses followed the protocol developed by Ifremer. Extraction of the sediment samples was performed using accelerated solvent extraction (ASE), with dichloromethane as the solvent. The sediment extracts were purified and fractionated by column chromatography, using activated SiO₂ and Al₂O₃ as adsorbents. After fractionation, the solvent was reduced by rotary evaporation and further concentrated under gentle nitrogen steam. Activated copper was added to the samples prior to instrumental analysis to remove sulphur traces, which may interfere with the chromatography column. Internal standards (phenanthrene-d₁₀, benzo[*e*]pyrene-d₁₂, fully deuterium-labelled compounds) were added to each sample prior to extraction to control the quality of the analyses. Instrumental analysis of PAHs was performed by high-resolution gas chromatography, using a HP 6890 gas chromatograph equipped with a 5973-mass selective detector (MSD). PAHs were separated on a DB5-MS capillary column made of fused silica (coated with 5% phenylmethyl-silicone) with a length of 60 m and an inner diameter of 0.25 mm, using helium as the carrier gas. Injections were performed using a Combi-PAL CTC autosampler. Injection volume of 1–2 μL with an on-column port was used. The mass selective detector was

operated in an electron impact mode at 70 eV and for quantitative analysis of individual PAHs in selected ion monitoring mode (SIM) in conjunction with perdeuterated calibration standards added prior to injection with the autosampler (acenaphthene-d10, fluorene-d10, pyrene-d10, benzo[*a*]anthracene-d12 and Indeno [1,2,3-*cd*]pyrene-d10). The list of analyzed PAH compounds, along with target ions, internal standards used for quantification, and abbreviations used in the text are listed in Table S3 of SI.

Identification of each compound was based on the single mass fragment ion characteristic for the compound and its retention time. The concentrations of the compounds were determined in the extracts using an eight-point calibration curve (linear regression) prepared for each compound and laboratory-prepared calibration solutions. The calibration curves were in the range of the concentrations of the analytes in the samples. A separate calibration curve was used for very low PAH concentrations in the extracts. Calibration solutions were prepared in iso-octane by appropriate dilutions (by weight). Prior to injection, deuterated surrogates for quantification were automatically added to each sample extract and to external calibration solutions. Example of characteristic chromatogram of PAH mixture in sediment samples, obtained by GC-MS operating under described conditions, is shown in Figure S1 of SI. Sediment samples with recoveries greater than 110% and lower than 70% were reanalyzed until recoveries for both internal standards were within the desirable range. Standard reference materials (SRM 1491 and SRM 2977) from the National Institute of Standards and Technology (NIST) were incorporated into a batch of 10 sediment samples for additional control of the analytical procedure. Internal standard recovery factors were used as an uncertainty matrix for the PMF model.

A detailed description of PAH analyses in sediment samples from the Adriatic Sea has been described in previous publications [22,25].

2.3. PAH Source Apportionment by Positive Matrix Factorization

Positive Matrix Factorization is a statistical method that reduces the large number of variables in datasets to combinations of species, called source types, and source contributions with weighted least squares regression [4,19,24].

The PMF model assumes that the concentration of each PAH compound in each sediment sample can be described as the sum of the concentration of the PAH compound emitted by the source and the source contributions for each sample, as shown in the following equation:

$$X_{ij} = \sum_{k=1}^p g_{ik} f_{kj} + e_{ij}$$

where X_{ij} is the concentration of the i th PAH compound in the j th sample; g_{ik} is the concentration of the i th species in the k th source, f_{kj} is the contribution of the source k th to the sample j th and e_{ij} is the model error for the specie j determined in sample i .

The results are constrained to provide positive source contributions that make their interpretation physically meaningful. Dataset of PAH sediment concentrations at each station was used as model input, along with uncertainty data calculated using the following equation:

$$\text{Measurement uncertainty} - \text{M.U.} = |100 - \text{RF}\%|,$$

where RF is an average value of the recovery factor for two internal standards used for quality control of the analytical protocol.

Since the average recovery values ranged from 75 to 105%, the measurement uncertainties were within 0.25–0.05, depending on the sample (SI Tables S4–S7). The model was run for 4 to 6 factors and initialized with different starting points, changing the seed value from 1 to 20. The result with 4 factors, for which the Q/Q theoretical was 1.32, was considered satisfactory and adopted as the result. A more detailed description of the receptor model for source apportionment, as well as the guidelines used for model application in this work, were described in EPA guidelines [23].

2.4. Calculation of PAH-Related Toxic Equivalent-TEQ

Carcinogenic potential related with sediment PAH concentrations were calculated as summed toxic equivalent quantity for 9 EPA PAH compounds that are classified as known carcinogens, as well as for all 16 EPA PAH enlisted by USEPA as priority pollutants in the environment [14,18–20]. Toxic equivalent factors used for calculation of BaP equivalent toxicity for 16 EPA PAHs are given in Table 1.

Table 1. Toxic equivalent factors of 16 EPA PAHs (USEPA, 2014). * PAH compounds enlisted by EPA as known carcinogenic compounds.

PAH Compound	Toxic Equivalent Factor—TEF
Naphtalene *	0.001
Acenaphtene *	0.001
Acenaphthylene	0.001
Fluorene	0.001
Phenanthrene *	0.001
Anthracene	0.001
Fluoranthene *	0.001
Pyrene	0.001
Benzo[a]anthracene *	0.1
Chrysene *	0.001
Benzo[b]fluoranthene	0.1
Benzo[k]fluoranthene *	0.1
Benzo[a]pyrene *	1
Indeno [1,2,3-cd]pyrene	0.1
Dibenzo[a,h]pyrene	1
Benzo[ghi]perylene *	0.01

The original sediment concentrations of selected PAH compounds were multiplied by their toxic equivalent factor relative to the BaP, as follows:

$$TEQ = \sum C(n) \times (TEF)n$$

where c is the concentration of the PAH compound and TEF is a toxic equivalent factor relative to the Benzo[a]Pyrene of a specific PAH compound.

3. Results and Discussion

3.1. PAH Concentrations in the Sediments

Concentrations of all PAH compounds determined in the sediments of the study area, along with detection limits and recovery factors for two internal standards, are given in Tables S4–S7 of SI.

Concentrations of T-PAH and the characteristic groups of PAHs in sediments from 11 stations in the coastal area are shown in Figure 2. T-PAH concentration ranged from 180.71 $\mu\text{g kg}^{-1}$ to 2793.18 $\mu\text{g kg}^{-1}$. The highest T-PAH concentration was measured in the sediment of the Brac-Split channel near the town of Omis (CS9), while the lowest PAH concentration was measured in the sediment near the island of Pag (CS1). High PAH concentrations in the coastal area are observed at the station near the town of Zadar (CS3). At this station, concentrations of parent-PAH were higher than those of C-PAH, indicating that PAH in this area originates mainly from pyrogenic sources, such as inland and marine traffic, residential heating, etc. C-PAH were the predominant PAH compounds only in the sediment at station CS2, whereas on the rest of stations, unsubstituted PAH were the predominant PAH compounds in the sediments. A high proportion of C-PAH fraction was also observed at stations CS4, CS9, CS10 and CS11. These stations are in the harbor (CS4) and in channel areas with dense marine traffic. Similar results with a dominance of pyrogenic PAH have been reported for sediments from coastal areas in the Mediterranean Sea, the Bay of Mexico and other coastal regions worldwide [2,26–31]. The dominance of

C-PAH in the sediment at station CS2 suggests that PAH in this area are mainly derived from unburned petroleum derivatives [10,32]. A petrogenic source of pollution at this station is also indicated by the relatively high amounts of S-PAH, which may be present in crude petroleum up to 5% [33,34].

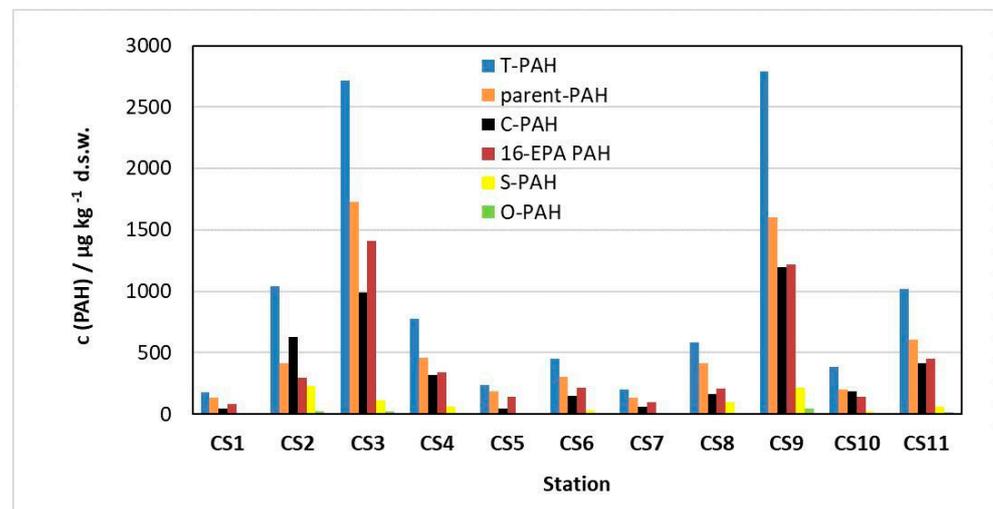


Figure 2. Concentration of total PAH (T-PAH), 16 EPA priority PAH (16 EPA PAH), unsubstituted PAH (parent-PAH), methyl-substituted PAH (C-PAH), sulphur-containing-PAH (S-PAH) and oxygen-containing-PAH (O-PAH) at stations in the coastal area.

PAH concentrations measured in the sediments of the stations along the mid-Adriatic transect are shown in Figure 3. Concentrations of T-PAH ranged from $42.859 \mu\text{g kg}^{-1}$ (TS2) to $963.517 \mu\text{g kg}^{-1}$ (TS6). PAH concentrations tend to decrease as the distance from the coast increases. Contributions of pyrogenic PAH were similar to the contribution of C-PAH at each station, except at TS1, where the concentration of parent-PAH was higher than the concentration of C-PAH fraction. Station TS1 is located near the island of Brač, where forest fires frequently occur in summer, which contributes to high levels of parent-PAH [35]. Similar results have been obtained for open sea regions around the world, where maritime traffic is the most pronounced anthropogenic pressure [2,27,29,36].

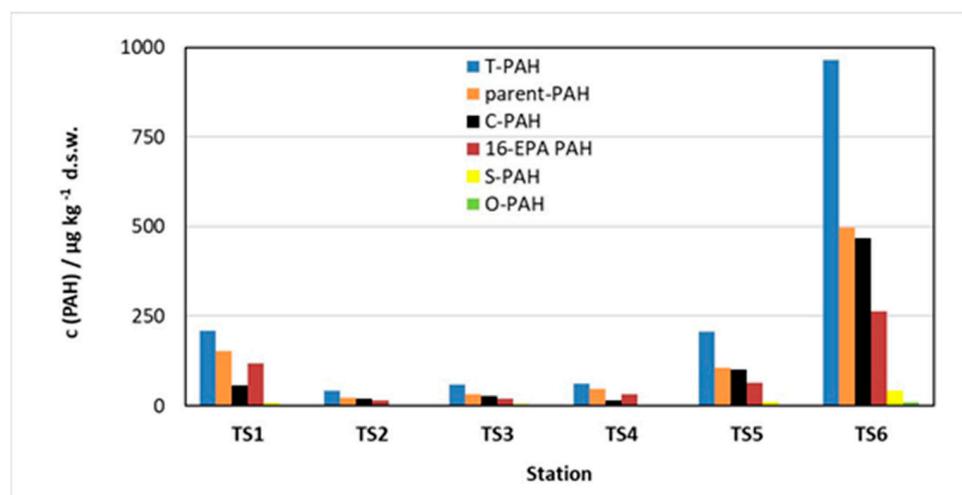


Figure 3. Concentration of total PAH (T-PAH), 16 EPA priority PAH (16 EPA PAH), unsubstituted PAH (parent-PAH), methyl-substituted PAH (C-PAH), sulphur-containing-PAH (S-PAH) and oxygen-containing-PAH (O-PAH) at stations along the mid-Adriatic transect.

PAH concentrations determined in the sediments of Kaštela Bay are shown in Figure 4. T-PAH concentration ranged from 278.64 $\mu\text{g kg}^{-1}$ to 2888.9 $\mu\text{g kg}^{-1}$. The highest T-PAH concentrations during the two campaigns were measured at station KB3, which is located near the industrial port of Split and the shipyard area. C-PAH were the dominant PAH compounds, with concentrations ranging from 151.62 $\mu\text{g kg}^{-1}$ to 1798.8 $\mu\text{g kg}^{-1}$. Predominance of C-PAH fraction at each station of Kaštela Bay indicate that PAH pollution in this area is mostly contributed by petrogenic sources [2]. PAH concentrations in Kaštela Bay are similar to the concentrations reported for the urban, coastal areas of the Mediterranean Sea, although they are lower than the PAH concentrations measured in the sediments of big harbour cities in the Mediterranean Sea, such as Toulon and Barcelona [8,26,27,30].

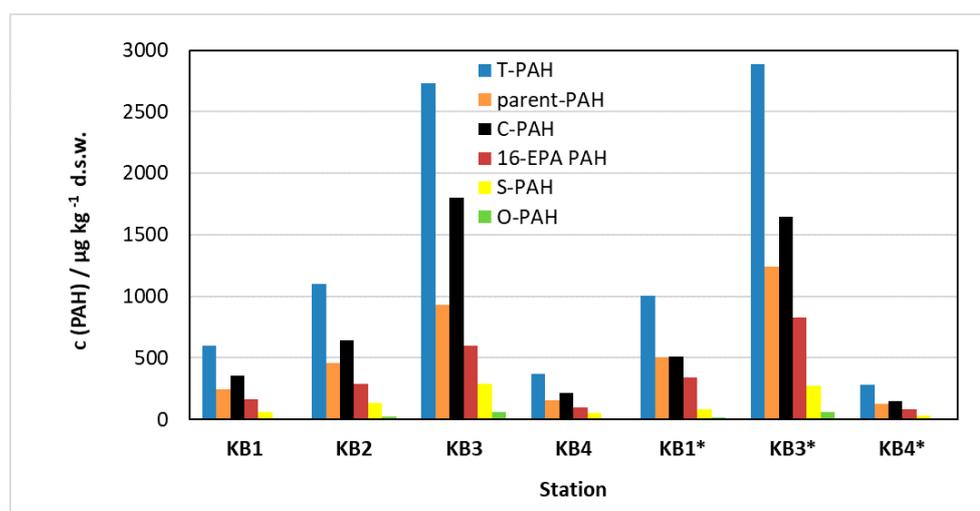


Figure 4. Concentration of total PAH (T-PAH), 16 EPA priority PAH (16 EPA PAH), unsubstituted PAH (parent-PAH), methyl-substituted PAH (C-PAH), sulphur-containing-PAH (S-PAH) and oxygen-containing-PAH (O-PAH) at stations in Kaštela Bay.

Concentrations of specific groups of PAH, determined in the sediment of Šibenik Bay, are shown in Figure 5. T-PAH concentrations ranged from 8280.8 $\mu\text{g kg}^{-1}$ to 28,662 $\mu\text{g kg}^{-1}$. The concentrations of the unsubstituted parent compounds were higher than the concentrations of C-PAH at every station. The highest concentrations of PAH were measured at station SB4A, which is closest to the area where a ferroalloy plant operated from 1880 to 1990. The high concentrations of PAH observed at station SB4 during both sampling periods can also be attributed to past industrial activities, as this station is only 100 m away from SB4A. A high proportion of the C-PAH fraction in the total concentrations of PAH was observed at station SB1, in February. Since this station is located near a tourist marina, the elevated concentrations of C-PAH can be explained by accidental leakage of petroleum derivatives during ship maintenance, which usually occurs during the winter season. The lowest concentration of T-PAH was observed at station SB5, which is farthest from shore. No seasonal trend in the concentration of PAH was observed. The very high PAH concentrations measured at each station in Šibenik Bay are similar to concentrations measured in the sediments of highly industrialized areas, such as Venice, Toulon, Barcelona, Mexico Bay and other coastal areas worldwide [8,19,28,30,31].

3.2. PAH Sources in the Adriatic Sea

Factor profiles obtained by the PMF modelling are shown in Figure 6. The application of the PMF revealed four main factors contributing to the pollution of PAH in the studied area. Each factor is considered as one of the specific sources of polycyclic aromatic hydrocarbons and is further discussed as such.

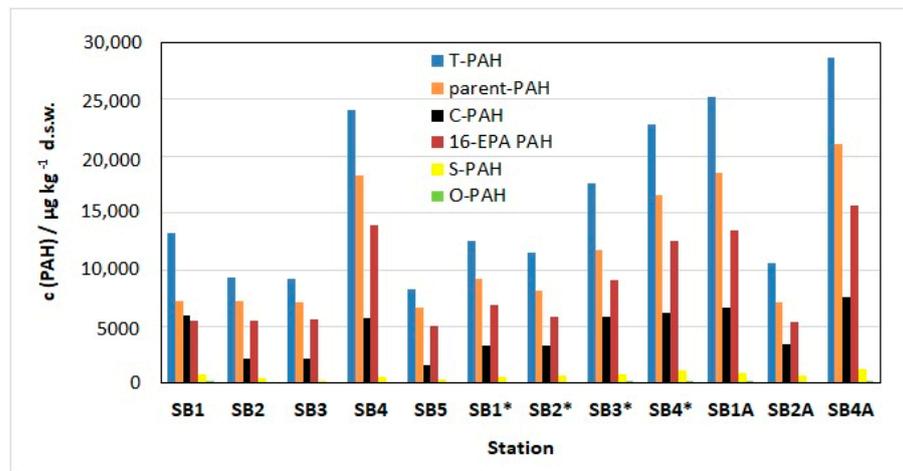


Figure 5. Concentration of total PAH (T-PAH), 16 EPA priority PAH (16 EPA PAH), unsubstituted PAH (parent-PAH), methyl-substituted PAH (C-PAH), sulphur-containing-PAH (S-PAH) and oxygen-containing-PAH (O-PAH) at stations in Šibenik Bay.

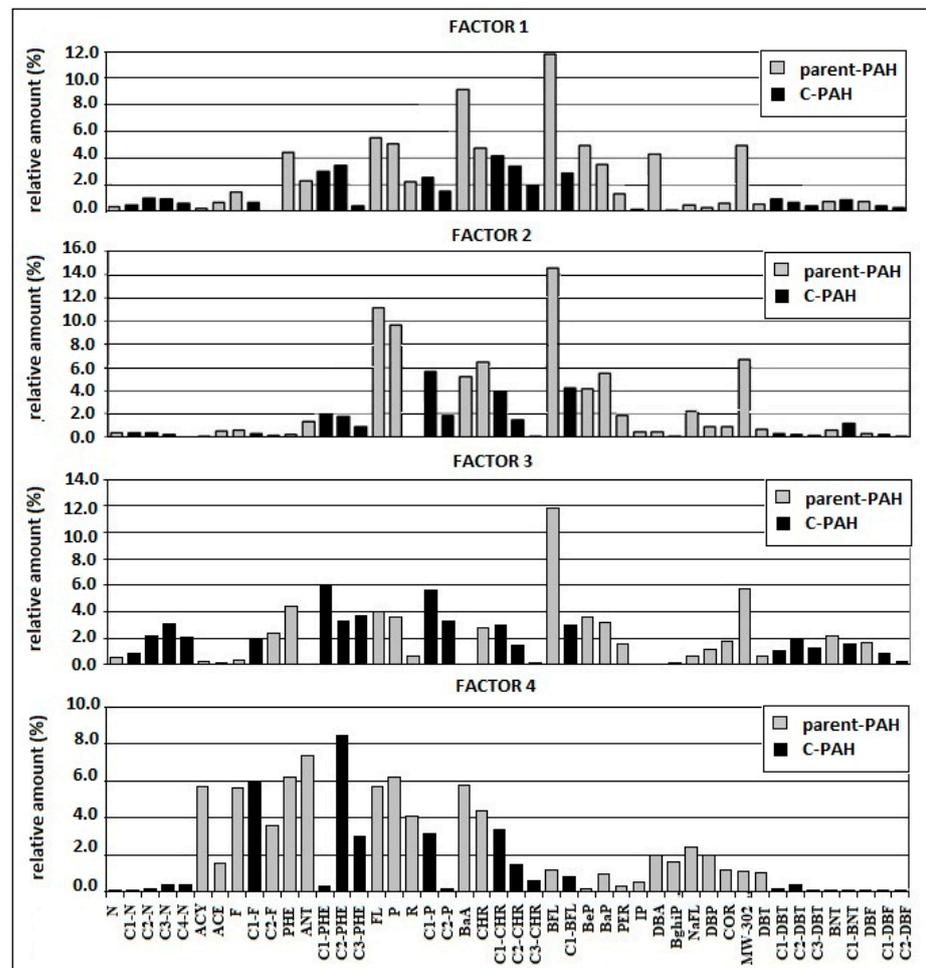


Figure 6. Factor (Source) profiles obtained by application of Positive Matrix Factorization.

Source 1 emits PAH mixtures enriched in unsubstituted PAH compounds. Dominant PAH compounds in the mixture are BFLs with a relative amount of 11.81% and BaA with a relative amount of 9.13%. Among the methyl-substituted compounds, C1-CHR (4.17%), C2-CHR (3.36%), C1-PHE (2.97%) and C2-PHE (3.43%) were the most abundant. The

relative amounts of S-PAHs and O-PAH in the PAH mixture derived from this source were less than 1%.

Unsubstituted parent-PAH compounds were the most abundant fraction of the PAH mixture emitted from Source 2. The relative amounts of BFLs, FL and Py were 14.53%, 11.61% and 9.65%, respectively. The predominant C-PAH compounds in the mixture were C1-P (5.66%) and C1-BFLs (4.19%), while the predominant heterocyclic aromatic compounds were C1-BNTs, DBTs and DBFs, with relative amounts of 1.18%, 0.65% and 0.30%.

The unsubstituted parent-PAH compounds with more than three aromatic rings in the structure were the predominant compounds in the PAH mixtures emitted from both Source 1 and Source 2, indicating that both sources refer to high-temperature combustion [33,34]. However, the mixture emitted from Source 1 contained lower amounts of unsubstituted PAH and higher amount of phenanthrene than the mixture emitted from Source 2. BFLs, BaP and BaA are compounds typically formed during gasoline combustion, while mixtures formed during diesel combustion are enriched in phenanthrene, fluoranthene, pyrene and chrysene. Comparing the obtained results with the literature data, it is observed that the PAH mixture emitted by Source 1 is similar to the composition of the PAH mixtures produced by the combustion of liquid fossil fuels in vehicle engines. In contrast, Source 2 emits PAH mixtures in which the amount of low-molecular weight PAH is significantly lower than the amount of high-molecular-weight PAH. PAH mixtures enriched in unsubstituted parent-PAH compounds, such as BFLs, BaA, FL, P and CHR resemble PAH mixtures typically formed during high-temperature combustion of crude fossil fuels in industrial furnaces or forest fires [2,10,33,34]. The above-mentioned differences in the composition of the PAH mixtures emitted from these two sources indicate that Source 1 can be ascribed to inland and marine transport, while Source 2 represents high-temperature combustion of crude fossil fuels and biomass.

PAH mixture emitted from Source 3 also contained a high proportion, but the contribution of the C-PAH fraction to the total concentration of PAH was higher than the contribution of the parent-PAH compounds. The PAH mixture contained higher relative amounts of methyl phenanthrenes, naphthalenes and pyrenes, while the dominant heterocyclic compounds were C1-BNTs with a contribution of 1.18%. Comparison with the literature data suggests that Source 3 is a petrogenic PAH source, mostly associated with recent accidental leakage of unburned fossil fuels and other petroleum derivatives [2,32,37].

Source 4 emitted a PAH mixture enriched in C2-FEN (8.41%), A (7.33%) and PHE (6.17%). Compared to the other three sources, the mixture emitted from Source 4 was enriched in PHE, ANT, ACE and F, which is similar to the composition of PAH mixtures in the heavier petroleum fractions but may also indicate weathered petroleum contamination. Thus, Source 4 may also be considered as a petrogenic source, but more related to accidental leakage of heavier petroleum fractions during vessel maintenance and weathered petroleum pollution [37].

Figure 7 shows the contribution of sources (factors) to the total concentration of PAH in the sediment of each studied station. According to the model results, the two most important sources in the coastal area were transportation, both inland and marine (Source 1), and weathered petroleum products (Source 4). Transport activities were the predominant PAH sources at the stations near the island of Pag (CS1), near Biograd (CS5), in the channel of Šibenik (CS6 and CS7) and in the port of Split (CS8), where Source 1 contributed with more than 50% to the total PAH pollution.

At the other stations in the coastal zone, the main source of PAH pollution was Source 4, which can be attributed to accidental spills of fresh motor oil and other heavier petroleum distillates during vehicle maintenance. Source 4 was present at every site in the coastal zone, with contributions ranging from 27.02% to 96.06%. Source 3 was also present at stations CS2, CS9 and CS10, indicating recent petroleum pollution in these areas. These stations are in the canal areas (CS2 and CS9) and in the port area (CS10), which explains the higher contribution of recently spilled petroleum. The contribution of coke and biomass burning

(Source 2) was not observed at any station in the coastal area, which can be explained by the absence of industrial activities in these areas.

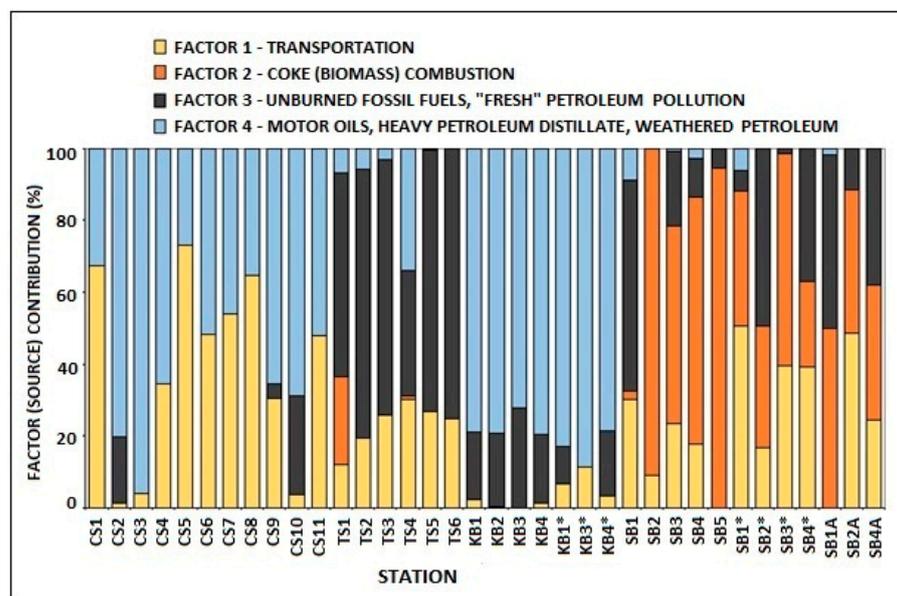


Figure 7. Factor (source) contributions (%) in the total PAH concentrations in the sediment of the investigated stations. * Refers to the sediments obtained in October of 2013.

The contribution of fresh petroleum pollution (Source 3) to the total PAH concentration was the highest in the sediments of the stations along the mid-Adriatic transect, ranging from 34.85%, as determined at station TS4, to 75.07%, as determined at station TS6. In the transect area, a high contribution of Source 1 was also observed, especially at station TS4, which is located along the high-density traffic route from the Strait of Otranto to the northern Adriatic Sea. These results indicate that accidental leakage of unburned fossil fuels and traffic are the main PAH sources in the transect area. This agrees well with the literature data for the open sea areas, where the main PAH sources are associated with maritime traffic. Elevated contributions of coke and biomass burning (Source 2) were determined in the sediment from station TS1 (24.67%), which is located near the island of Brač. Since this area is not heavily industrialized, these results can be explained by the frequent forest fires on the island of Brač, which occur frequently during the summer season [35].

In Kaštela Bay, the highest contribution to the sediment PAH concentration was determined for Source 4, ranging from 72.17% at KB3 in October, to 88.48% as observed at the same station in February. Seasonal differences in the contributions of each PAH source were observed only at station KB3, where the contribution of recently spilled petroleum was higher in February than in October. The highest contribution of Source 3 to the total concentrations of PAH in the sediment of Kaštela Bay was observed at station KB3 in February. This station is located near the industrial port of Split and the shipyard area, suggesting that the high contribution of Source 2 may be due to accidental spillage of unburned petroleum during ship maintenance [32,37]. The absence of Source 2, or minimal contributions from this source in the coastal area, Kaštela Bay and most of the open-sea stations are likely due to the absence of industrial activities in these areas.

In Šibenik Bay, a high contribution from Source 1 is observed at station SB1 in October. The contribution of Source 2 to the total PAH concentration was highest at each station in Šibenik Bay, especially at station SB5, where 94.57% of the PAH sediment concentration came from Source 2. High contributions from Source 2 were also observed in October at stations SB2 (91.07%) and SB4 (68.71%). These results can be explained by the influence of coke-burning in the ferroalloy factory that operated on the shores of the bay from 1880

to 1990. The high concentrations of PAH in the surface sediment are probably due to the diffusion of PAH from the deeper sediment layers that are oversaturated with PAH. The highest contributions from Source 3 were detected at station SB1 in February, where model results indicate that 58.72% of PAH originated from Source 3. Station SB1 is located near the tourist marina where most of the vessel maintenance is performed during the winter, suggesting that the high Source 3 contribution detected at this station in February is caused by accidental leaks of petroleum derivatives during vessel maintenance. An elevated contribution from Source 4 was also detected at this station, confirming the assumption that this station is greatly affected by vessel maintenance activities.

3.3. Benzo[a]pyrene Equivalent Toxicity of PAH Mixtures in the Adriatic Sea

Toxic equivalent quantity (TEQ) for 9 known carcinogenic PAH compounds and for 16 EPA compounds, calculated for the studied sediment samples are shown on Figure 8. It can be seen that summed TEQ for 9 and 16 PAHs exceeded $600 \mu\text{g kg}^{-1}$ only at the stations in Šibenik Bay. At the remaining stations, the TEQ values were below $200 \mu\text{g kg}^{-1}$, regardless of the number of compounds considered. In contrast, TEQ values in Šibenik Bay were significantly higher, especially at the stations near the former ferroalloy factory and the tourist marina. When all 16 PAH compounds were considered, TEQ values at most stations exceeded $600 \mu\text{g kg}^{-1}$, indicating a high carcinogenic risk associated with sediment PAH concentration in Šibenik Bay. Results obtained by calculation of TEQ are in good agreement with the results of PAH-related sediment toxicity obtained by applying the sediment quality guidelines, where a high environmental risk was observed for the total area of Šibenik Bay [38]. However, in the same study, the results of the bioassay application indicated poor environmental status associated with the PAH sediment concentration at stations outside Šibenik Bay. This suggests that assessing the toxicity of PAH solely on sediment concentration may significantly underestimate the environmental risk [38].

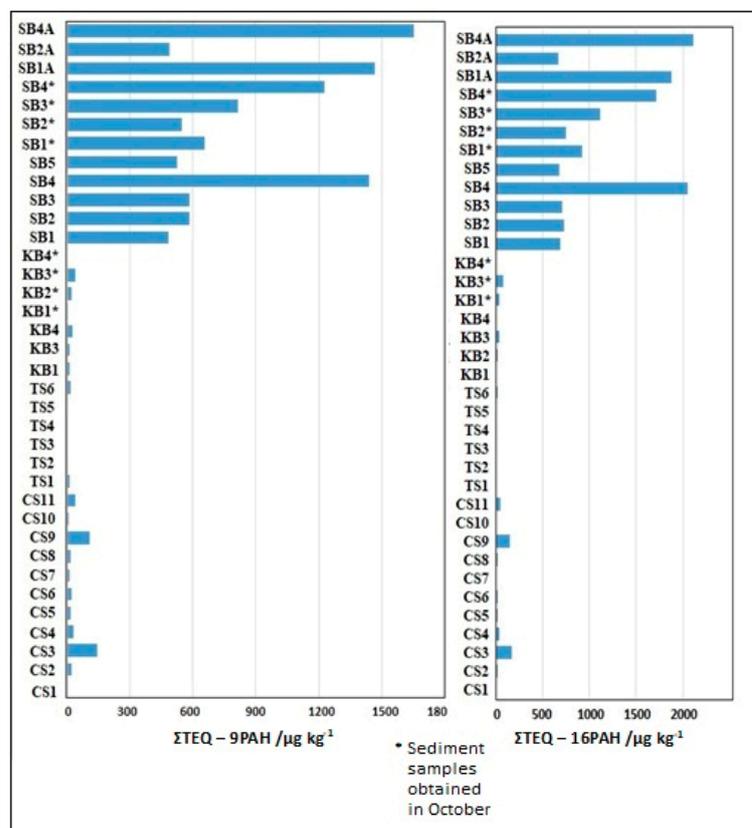


Figure 8. Toxic equivalent quantities for 9 known carcinogenic PAH and 16 EPA PAH in the sediments of investigated stations in the Adriatic Sea.

4. Conclusions

PAH concentrations in sediments of the mid-Adriatic Sea show great variability, ranging from very low concentrations in the open sea area to very high concentrations in urbanized, coastal areas. The application of the PMF model for PAH source apportionment revealed the presence of four main sources of PAH pollution in the investigated area. Among them, Source 1 and Source 4 can be considered as the most important ones since their contributions to the total PAH pollution were observed in each studied area. Positive Matrix Factorization provided detailed information about PAH sources and the contribution of each source to the total PAH in each location, which makes it a reliable tool for the management of various activities in the Adriatic region. To obtain more accurate information on pollution sources, the dataset used as model input should include a greater number of stations as well as other pollutants, such as trace metals, pesticides, dioxins, antifouling chemicals, etc. The PAH-toxicity assessment revealed a high cancer risk associated with PAH concentration in the sediments of Šibenik Bay, while in the rest of the study area the cancer risk associated with the sediment concentrations of PAH was low. While these results are in good agreement with the results obtained by applying the sediment quality guidelines, they differ greatly from the results of the bioassay used to test the acute toxicity of the sediments. It can be concluded that additional bioassays conducted on a larger number of different marine species are needed to accurately assess the environmental risk associated with PAH.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app13126992/s1>, Figure S1: Chromatogram of the PAH mixture in sediment sample separated by DB5-MS column and obtained by GC-MS operating in the SIM mode. Table S1. Sampling locations and descriptions of the sites in the Coastal zone and along the Mid Adriatic transect selected for the Source apportionment and toxicity assessment. Table S2. Sampling locations and descriptions of the sites in Kaštela and Šibenik Bay selected for the Source apportionment and toxicity assessment. Table S3. List of parent and alkyl-substituted PAH, sulphur heterocyclic aromatic compounds and oxygen heterocyclic aromatic compounds determined in sediment samples; abbreviation, molecular weight and internal standard used as a reference. Table S4. PAH concentration ($\mu\text{g kg}^{-1}$ dry sediment weight) with limits of detection (LOD) determined in the sediments of Coastal area and measurements uncertainties for each sample used as input data matrices for PMF. Table S5. PAH concentration ($\mu\text{g kg}^{-1}$ dry sediment weight) with limits of detection (LOD) determined in the sediments of transect area and measurements uncertainties (M.U.) for each sample used as input data matrices for PMF. Table S6. PAH concentration ($\mu\text{g kg}^{-1}$ dry sediment weight) with limits of detection (LOD) determined in the sediments of Kastela Bay are and measurements uncertainties (M.U.) for each sample used as input data matrices for PMF. Table S7. PAH concentration ($\mu\text{g kg}^{-1}$ dry sediment weight) with limits of detection (LOD) determined in the sediments of Šibenik bay and measurements uncertainties (M.U.) for each sample used as input data matrices for PMF.

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References

1. Vollhardt, K.P.C.; Schore, N.E. *Organic Chemistry*, 3rd ed.; W. H. Freeman and Company: New York, NY, USA, 1999.
2. Neff, J.M. *Polycyclic Aromatic Hydrocarbons in the Aquatic Environment, Sources, Fates and Biological Effect*; Applied Science Publishers Ltd.: Essex, UK, 1979.
3. Tsapakis, M.; Stephanou, E.G.; Karakassis, I. Evaluation of atmospheric transport as a nonpoint source of polycyclic aromatic hydrocarbons in marine sediments of the Eastern Mediterranean. *Mar. Chem.* **2003**, *80*, 283–298. [[CrossRef](#)]
4. Duodu, G.O.; Ogogo, N.K.; Nanayakkara, M.; Sandya, W.; Harden, F.; Goonetilleke, A.; Ayoko, G. Source apportionment and risk assessment of PAHs in Brisbane River sediment, Australia. *Ecol. Indic.* **2017**, *73*, 784–799. [[CrossRef](#)]
5. Guo, J.; Wu, F.; Luo, X.; Liang, Z.; Liao, H.; Zhang, R.; Mai, B. Anthropogenic input of polycyclic aromatic hydrocarbons into five lakes in Western China. *Environ. Pollut.* **2010**, *158*, 2175–2180. [[CrossRef](#)] [[PubMed](#)]
6. Gschwend, P.M.; Hites, R.A. Fluxes of polycyclic aromatic hydrocarbons to marine and lacustrine sediments in the northeastern United States. *Geochim. Cosmochim. Acta* **1981**, *45*, 2359–2367. [[CrossRef](#)]
7. Sicre, M.A.; Marty, J.C.; Saliot, A.; Aparicio, X.; Grimalt, J.; Albaiges, J. Aliphatic and aromatic hydrocarbons in different sized aerosols over the Mediterranean Sea: Occurrence and origin. *Atmos. Environ.* **1987**, *21*, 2247–2259. [[CrossRef](#)]
8. Budzinski, H.; Jones, I.; Bellocq, J.; Pierard, C.; Garrigues, P. Evaluation of sediment contamination by polycyclic aromatic hydrocarbons in the Gironde estuary. *Mar. Chem.* **1997**, *58*, 85–97. [[CrossRef](#)]
9. Yunker, M.; MacDonald, R.; Vingarzan, R.; Mitchell, R.; Goyette, D.; Sylvestre, S. PAHs in the Fraser Rive Basin: A critical appraisal of PAH ratios as indicators of PAH source and composition. *Org. Geochem.* **2002**, *33*, 489–515. [[CrossRef](#)]
10. Tobiszewski, M.; Namiesnik, J. PAH diagnostic ratios for identification of pollution emission sources. *Environ. Pollut.* **2012**, *162*, 110–119. [[CrossRef](#)]
11. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs)*; Department of Health and Human Services, Public Health Service: Atlanta, GA, USA, 1995.
12. Santodonato, J.; Howard, P.; Basu, D. Health and Ecological Assessment of Polynuclear Aromatic Hydrocarbons. *J. Environ. Pathol. Toxicol.* **1981**, *5*, 100–368.
13. IARC (International Agency for Research on Cancer). *Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*; IARC Monographs on the Evaluation of Carcinogenic Risk to Humans Suppl. 7; IARC: Lyon, France, 1987.
14. USEPA (U.S. Environmental Protection Agency). *Ambient Water Quality Criteria for Polynuclear Aromatic Hydrocarbons*; EPA 440/5-80-069. US NTIS PB81-117806; USEPA: Washington, DC, USA, 1980.
15. McGrath, J.A.; Joshua, N.; Bess, A.S.; Parkerton, T.F. Review of Polycyclic Aromatic Hydrocarbons (PAHs) Sediment Quality Guidelines for the Protection of Benthic Life. *Integr. Environ. Assess. Manag.* **2019**, *15*, 505–518. [[CrossRef](#)]
16. Bihari, N.; Najdek, M.; Floris, R.; Batel, R.; Zahn, R.K. Sediment toxicity assessment using bacterial bioluminescence: Effect of an unusual phytoplankton bloom. *Mar. Ecol. Prog. Ser.* **1989**, *57*, 307–310. [[CrossRef](#)]
17. USEPA. *Human Health Evaluation Manual, Supplemental Guidance: Update of Standard Default Exposure Factors*; OSWER Directive 9200; USEPA: Washington, DC, USA, 2014; pp. 1–120. Available online: https://www.epa.gov/sites/default/files/2015-11/documents/oswer_directive_9200.1120_exposurefactors_corrected2.pdf (accessed on 8 March 2023).
18. Deelaman, W.; Choochuay, C.; Pongpiachan, S.; Han, Y. Ecological and health risks of polycyclic aromatic hydrocarbons in the sediment core of Phayao Lake, Thailand. *J. Environ. Expo. Assess.* **2023**, *2*, 3. [[CrossRef](#)]
19. Cao, Y.; Lin, C.; Zhang, X.; Liu, X.; He, M.; Ouyang, W. Distribution, source, and ecological risks of polycyclic aromatic hydrocarbons in Lake Qinghai, China. *Environ. Pollut.* **2020**, *266*, 115401. [[CrossRef](#)] [[PubMed](#)]
20. Delistraty, D. Toxic equivalency factor approach for risk assessment of polycyclic aromatic hydrocarbons. *Toxicol. Environ. Chem.* **1997**, *64*, 81–108. [[CrossRef](#)]
21. Lin, T.; Qin, Y.; Zhen, B.; Li, Y.; Chen, Y.; Guo, Z. Source apportionment of polycyclic aromatic hydrocarbons in the Dahuofang Reservoir, Northeast China. *Environ. Monit. Assess.* **2013**, *185*, 945–953. [[CrossRef](#)] [[PubMed](#)]
22. Mandić, J.; Tronczynski, J.; Kušpilić, G. Polycyclic aromatic hydrocarbons in surface sediments of the mid- Adriatic and along the Croatian coast: Levels, distributions and Sources. *Environ. Pollut.* **2018**, *242*, 519–527. [[CrossRef](#)]
23. USEPA. *Positive Matrix Factorization (PMF) 5.0 Fundamentals & User Guide*; USEPA: Washington, DC, USA, 2014. Available online: <http://www.epa.gov/heads/research/pmf.html> (accessed on 14 February 2023).
24. Joint Research Centre, Institute for Environment and Sustainability; Comero, S.; Gawlik, B.; Capitani, L. *Positive Matrix Factorisation (PMF)—An Introduction to the Chemometric Evaluation of Environmental Monitoring Data Using PMF*; Publications Office: Tokyo, Japan, 2011; Available online: <https://data.europa.eu/doi/10.2788/2497> (accessed on 8 March 2023).
25. Tronczyński, J.; Munschy, C.; Moisan, K.; Guiot, N.; Truquet, I.; Olivier, N.; Men, S.; Faurat, A. Contamination of the Bay of Biscay by polycyclic aromatic hydrocarbons (PAHs) following the T/V “Erika” oil spill. *Aquat. Living Resour.* **2004**, *17*, 243–259. [[CrossRef](#)]

26. Lipiatou, E.; Tolosa, I.; Simo, R.; Bouloubassi, I.; Dachs, J.; Marti, S.; Sicre, M.A.; Bayona, J.M.; Grimalt, J.O.; Salliot, A.; et al. Mass budget and dynamics of polycyclic aromatic hydrocarbons in the Mediterranean sea. *Deep-Sea Res.* **1997**, *44*, 881–905. [[CrossRef](#)]
27. Wakeham, S.G. Aliphatic and polycyclic aromatic hydrocarbons in Black Sea sediments. *Mar. Chem.* **1996**, *53*, 187–205. [[CrossRef](#)]
28. Soclo, H.H.; Garrigues, P.H.; Ewald, M. Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal and marine sediments: Case studies in Cotonou (Benin) and Aquitaine (France) Areas. *Mar. Pollut. Bull.* **2000**, *40*, 387–396. [[CrossRef](#)]
29. Witt, G. Occurrence and transport of polycyclic aromatic hydrocarbons in the water bodies of the Baltic Sea. *Mar. Chem.* **2002**, *79*, 49–66. [[CrossRef](#)]
30. De Luca, G.; Furesi, A.; Leardi, R.; Micera, G.; Panzanelli, A.; Piu, P.C.; Sanna, G. Polycyclic aromatic hydrocarbons assessment in the sediments of the Porto Torres Harbor (Northern Sardinia, Italy). *Mar. Chem.* **2004**, *86*, 15–32. [[CrossRef](#)]
31. Ponce-Vélez, G.; Botello, A.V.; Díaz-González, G. Organic and inorganic pollutants in marine sediments from northern and southern continental shelf of the Gulf of Mexico. *Inter. J. Environ. Pollut.* **2006**, *26*, 295–311. [[CrossRef](#)]
32. Bjørseth, A.; Ramdahl, T. *Handbook of Polycyclic Aromatic Hydrocarbons*; Bjørseth, A., Ed.; Marcel Dekker: New York, NY, USA, 1985; Volume 2, p. 416.
33. Lima, A.L.; Farrington, W.J.; Reddy, C.M. Combustion-derived polycyclic aromatic hydrocarbons in the environment—A review. *Environ. Forensics* **2005**, *6*, 109–131. [[CrossRef](#)]
34. Khalili, N.R.; Scheff, P.A.; Holsen, T.M. PAH source fingerprints for coke ovens, diesel and gasoline engines, highway tunnels and wood combustion emissions. *Atmos. Environ.* **1995**, *29*, 533–542. [[CrossRef](#)]
35. Mifka, B.; Vučetić, V. Weather analysis during extreme forest fire on island of Brač from 14 to 17 July 2011. *Vatrog. I Upravlj. Požarima* **2012**, *1*, 13–25. Available online: <https://hrcak.srce.hr/103617> (accessed on 22 May 2023).
36. Stout, S.A.; Uhler, A.D.; Emsbo-Mattingly, S.D. Comparative evaluation of background anthropogenic hydrocarbons in surficial sediments from Nine urban waterways. *Environ. Sci. Technol.* **2004**, *38*, 2987–2994. [[CrossRef](#)] [[PubMed](#)]
37. Wang, Z.; Fingas, M.; Blenkinsopp, S.; Sergy, G.; Landriault, M.; Sigouin, L. Comparison of oil composition changes due to biodegradation and physical weathering in different oils. *J. Chromatogr. A* **1998**, *809*, 89–107. [[CrossRef](#)]
38. Mandić, J.; Veža, J.; Kušpilić, G. Assessment of environmental risk related to the polycyclic aromatic hydrocarbons (PAH) in the sediments along the eastern Adriatic coast. *Acta Adriat.* **2022**, *63*, 135–150. [[CrossRef](#)]

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