

Polycyclic Aromatic Hydrocarbons in Bivalves From Mangrove Ecosystems Affected by the 2019 Brazilian Oil Spill

Carlos Henrique Cordeiro de Amaral

Federal University of Bahia: Universidade Federal da Bahia

Basílio Magno Tavares Sotão Neto

Federal University of Bahia: Universidade Federal da Bahia

Gabriel Izar

Federal University of Bahia: Universidade Federal da Bahia

Mariana dos Santos Figueiredo de Freitas

Federal University of Bahia: Universidade Federal da Bahia

Arthur Henrique Silva de Assis

Federal University of Bahia: Universidade Federal da Bahia

Yasmin Caroline Anunciação Gomes

Federal University of Bahia: Universidade Federal da Bahia

Tatiane Combi

Federal University of Parana: Universidade Federal do Parana

João Bosco Gusmão

Federal University of Parana: Universidade Federal do Parana

Ruy Kenji Papa de Kikuchi

Federal University of Bahia: Universidade Federal da Bahia

Carlos Alexandre Domingos Lentini

Federal University of Bahia: Universidade Federal da Bahia

Rita de Cássia Franco Rego

Federal University of Bahia: Universidade Federal da Bahia

Ana Cecília Rizzatti Albergaria-Barbosa

`cecilia.albergaria@unifesp.br`

Federal University of Sao Paulo: Universidade Federal de Sao Paulo <https://orcid.org/0000-0003-2121-9792>

Research Article

Keywords: Mangrove, PAHs, Biomonitoring, Marine Pollution, Health Risk Assessment

Posted Date: June 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9954200/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

POLYCYCLIC AROMATIC HYDROCARBONS IN BIVALVES FROM MANGROVE ECOSYSTEMS AFFECTED BY THE 2019 BRAZILIAN OIL SPILL

Carlos Henrique Cordeiro de Amaral^{a,b*}; Basílio Magno Tavares Sotão Neto^{a,b}; Gabriel Izar Mendes^c; Mariana dos Santos Figueiredo de Freitas^b; Arthur Henrique Silva de Assis^b; Yasmin Caroline Anunciação Gomes^b; Tatiane Combi^{a,d}; João Bosco Gusmão^{b,d}; Ruy Kenji Papa de Kikuchi^e; Carlos Alexandre Domingos Lentini^{a,f}; Rita de Cássia Franco Rego^g; Ana Cecília Rizzatti de Albergaria-Barbosa^{a,b*}

^a. Graduate Program in Geochemistry: Oil and Environment - Federal University of Bahia – Rua Barão de Jeremoabo, s/n, 40170-020 – Salvador – BA – Brazil

^b. Center of Excellence in Petrochemical Geochemistry, Geoscience Institute - Federal University of Bahia – Rua Barão de Jeremoabo, s/n, 40170-020 – Salvador – BA – Brazil

^c. Polytechnic School, Interdisciplinary Center of Energy and Environment - Federal University of Bahia (UFBA), Rua Barão de Jeremoabo, s/n, 40170-290 - Salvador – BA - Brazil

^d. Center for Marine Studies - Federal University of Paraná - Av. Beira-mar, s/n, 83255-976 - Pontal do Sul – PR - Brazil

^e. Graduate Program in Geology and Graduate Program in Ecology - Federal University of Bahia – Rua Barão de Jeremoabo, s/n, 40170-020 – Salvador – BA – Brazil

^f. Physics Institute - Federal University of Bahia – Rua Barão de Jeremoabo, s/n, 40210-340 – Salvador – BA - Brazil

^g. Graduate Program in Health, Environment and Work - Federal University of Bahia – Largo do Terreiro de Jesus, s/n, 40026-010 – Salvador – BA – Brazil

^h. Sea Institute - Federal University of São Paulo – Rua Carvalho de Mendonça, 144, 11070-100 - Santos – SP - Brazil

* Corresponding author: cecilia.albergaria@unifesp.br

ACKNOWLEDGMENTS

This study was developed within the Graduate Program in Geochemistry: Petroleum and Environment (POSPETRO), Federal University of Bahia, with financial support from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) (Finance Code 001; AUXPE No. 29/2020), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Grant Nos. 440829/2020-8 and 440784/2020-4), the Brazilian Ministry of Health (Decentralized Execution Agreement – TED No. 49/2021), the Ministério da Ciência, Tecnologia e Inovação (MCTI), through the INCT AmbTropic – Phase II (CNPq Grant No. 465634/2014-1), and the European Union's Horizon 2020 Research and Innovation Programme (Grant Agreement No. 862923). R.K.P. Kikuchi, T. Combi, and A.C.R. Albergaria-Barbosa acknowledge CNPq for their research grants (Nos. 314270/2023-0, 307484/2022-0, and 309648/2023-8, respectively).

ABSTRACT:

The 2019 oil spill along the Brazilian coast affected extensive mangrove ecosystems and raised concerns regarding hydrocarbon contamination in seafood resources. This study evaluated the accumulation of polycyclic aromatic hydrocarbons (PAHs) in marine bivalves collected from mangrove areas along the Bahia coast, including oil-spill-affected sites, a chronically contaminated estuary (São Paulo River), and a reference area (Itacaré). *Crassostrea rhizophorae*, *Mytella charruana*, and *Anomalocardia flexuosa* were sampled during two campaigns (August–September 2020 and February–April 2021), and PAHs were quantified by gas chromatography–mass spectrometry. Total PAH concentrations ranged from 25.9 to 537 ng g⁻¹ dry weight. Spatial differences were evident, with higher concentrations generally observed in Belmonte and the São Paulo River and lower levels at the reference site. Significant differences among species were detected (one-way ANOVA, $p = 0.009$), with *M. charruana* exhibiting the highest concentrations, which may be related to its closer association with fine, organic-rich sediments that favor PAHs retention. No significant temporal variation was observed between sampling campaigns (two-way ANOVA, $p = 0.765$), suggesting continued exposure to PAHs after the spill period. Multivariate analysis revealed distinct compositional patterns among sites and indicated the influence of multiple contamination sources. The results support a multifactorial contamination scenario involving residual oil-derived inputs, pre-existing contamination, and ongoing diffuse anthropogenic sources. Incremental Lifetime Cancer Risk (ILCR) assessment indicated negligible to low carcinogenic risk for most samples under short- and medium-term exposure scenarios, whereas potential risk was restricted to a small number of samples from Belmonte and the São Paulo River under long-term exposure conditions. Non-carcinogenic risk remained negligible for all evaluated compounds. These findings demonstrate that contamination patterns in tropical estuaries affected by oil spills may be strongly influenced by pre-existing chronic pollution, complicating the assessment of environmental recovery and seafood safety

Keywords: Mangrove; PAHs; Biomonitoring; Marine Pollution, Health Risk Assessment.

1 INTRODUCTION

In August 2019, an oil spill occurred off the coast of Brazil with international repercussions. Bahia state, which has the longest coastline in Brazil (over 1,100 km), received a substantial portion of the oil residue. More than 130 coastal locations in Bahia were affected, and approximately 1,700 tons of oily waste were collected along the shorelines (IBAMA 2020). Several affected areas contain highly sensitive and ecologically valuable habitats such as coral reefs and mangrove forests. Mangroves rank among the most productive tropical coastal areas and provide key ecosystem services, functioning as nurseries for marine organisms, carbon sinks, and natural barriers against coastal erosion (Barbier et al. 2011). They are also highly vulnerable to oil contamination. Hydrophobic compounds tend to be retained in fine organic-rich sediments, which make oil removal from mangrove sediments particularly difficult (Duke et al. 2016). In addition, this prolongs contaminant persistence and increases the exposure of benthic and other marine organisms, making mangroves a priority area for environmental protection and monitoring during oil spill events (Matos-Barbosa et al. 2025; Garcia and Martins 2021).

Petroleum is a complex mixture of hundreds of hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs). These are organic substances that contain more than one condensed benzene ring and can be of major concern in areas affected by chronic or acute oil contamination. They are known to be toxic, environmentally persistent, bioaccumulative in organisms, and possess carcinogenic and mutagenic properties (Ohiozebau et al. 2017; Oliva et al. 2017). Natural sources of PAHs include volcanic eruptions and forest or coastal fires. However, they more commonly originate from anthropogenic sources such as oil spills, incomplete combustion of organic matter, and industrial processes (Abdel-Shafy and Mansour 2016). Once released, they can be transported from their original source to marine systems, including estuarine and coastal habitats, via riverine discharge, surface runoff, sediment resuspension, and atmospheric deposition (Ko et al. 2014; Cheng et al. 2018).

One of the main challenges in evaluating PAHs contamination after large oil spill events is distinguishing recently introduced petroleum-derived compounds from hydrocarbons associated with long-term contamination. Chronically contaminated estuaries may maintain elevated background levels of PAHs for extended periods, complicating the interpretation of post-spill contamination patterns and ecological recovery processes (Patel et al. 2020). Therefore, understanding the combined influence of residual oil contamination and pre-existing anthropogenic sources is essential for accurately assessing environmental quality and potential risks to marine organisms and human populations.

Marine bivalves are considered global indicator species for assessing PAHs contamination. Their sedentary behavior, high filtration rates, and limited metabolic capacity for organic contaminants make them effective bioindicators of PAHs contamination (Martins et al. 2020; Eça et al. 2021). As bivalves filter suspended particles from the surrounding waters, PAHs accumulation in their tissues generally reflects local contamination levels, although species-specific biological factors may influence accumulation patterns (Wade et al. 1988; Baumard et al. 1999; Sericano et al. 1995, Sericano 2000). Besides, bivalves are a common food source (Costa et al. 2023). Therefore, they are key organisms for assessing human exposure to these pollutants through diet and the associated health risks. The use of bivalves as biomonitors for long-term monitoring has proven effective in monitoring programs such as Mussel Watch, which has been operating since the 1970s (O'Connor 1998; Wade et al. 1998).

Despite the extensive geographic extent of the 2019 Brazilian oil spill, information on long-term PAHs accumulation in commercially important bivalves from impacted mangroves remains limited, particularly regarding the distinction between spill-derived contamination and pre-existing chronic sources. Given the ecological sensitivity of mangroves, their role as natural reservoirs of hydrophobic contaminants, and their socioeconomic value, it is essential to quantify PAHs accumulation in native bivalves to assess the environmental impacts of the 2019 oil spill and any potential effects on human health. This study aimed to evaluate the accumulation and distribution patterns of PAHs in marine bivalves collected from mangrove ecosystems along the Bahia coast, including areas affected by the 2019 Brazilian oil spill, a chronically contaminated estuary, and a relatively preserved reference site. By comparing different species, sampling locations, and collection periods, this study investigated the relative influence of residual oil contamination and long-term anthropogenic inputs on PAHs bioaccumulation, as well as the potential implications for environmental quality and human health risk.

2 MATERIALS AND METHODS

2.1 STUDY AREA SELECTION

The study areas were selected based on the occurrence and spatial distribution of the 2019 oil spill. Socioeconomic relevance was also considered, including the presence of tourism, artisanal fisheries, shellfish harvesting, and aquaculture (Machado et al. 2025). Many of these territories are inhabited by traditional communities, such as artisanal fishers, *marisqueiras* (traditional shellfish gatherers), *quilombola* groups (Afro-Brazilian communities descended from enslaved people who escaped), and Indigenous populations, whose livelihoods directly depend on healthy marine and estuarine ecosystems (Rêgo et al. 2026; Machado et al. 2025). Furthermore, many impacted areas were previously considered relatively well-preserved, with limited anthropogenic pressure (Soares et al. 2022), reinforcing the need for environmental quality assessments to better understand the ecological impacts of the spill (Soares et al. 2020).

Sampling sites included Belmonte (Jequitinhonha River estuary), Canavieiras (Salsa River estuary), Siribinha (Itapicuru River estuary), Boipeba (Bainema Point), and Cumuruxatiba (Queimado River estuary) (Figure 1). The degree of contamination in these areas varies markedly, with some areas showing heavy accumulation of oil and others exhibiting more diffuse or intermittent impacts (Soares et al. 2022) (Table 1). Additional samples were collected from a relatively non-contaminated reference site (Itacaré, Contas River estuary) and a chronically impacted estuary (Candeias, São Paulo River estuary) (Figure 1).

2.2 SAMPLE COLLECTION

Crassostrea rhizophorae (mangrove oysters), *Mytella charruana* (charru mussel), and *Anomalocardia flexuosa* (wedge shell clam) were sampled in August-September 2020 and February-April 2021 at the mangroves of the Bahia coast (Figure 1, Table 1). The three bivalve species were selected based on their recognized role as sentinel organisms (Eça et al. 2021) and their high consumption by local populations (Lucena-Frédou et al. 2021).

Sampling was performed manually. For each species and site, soft tissues from at least ten individuals were pooled to obtain a composite sample representative of each sampling campaign (Table 1).

Not all bivalve species were present at all sampling sites or during both sampling campaigns, reflecting the natural differences in species distribution and local ecological conditions along the Bahia coast. Therefore, species collection depended on their natural availability in each mangrove ecosystem and during the sampling period. Owing to the limited number of individuals, only one sample was analyzed per species, site, and sampling period.

Fig. 1. Geographical location of the Bahia coast and sampling areas of the present study indicating the species found in each region.

Table 1. Geographic location (coordinates), sampling dates, and environmental classification of the studied areas.

Study area	Municipality	Oil impact*	Anthropogenic pressures**	Latitude	Longitude	Campaign 1	Campaign 2
Belmonte	Belmonte, BA	Highly impacted by the oil spill	Paper industry, untreated sewage, agricultural runoff, and upstream mining activities	15.850° S	38.868° W	August 2020	February 2021
Rio São Paulo	Candeias, BA	Not directly impacted by the oil spill/Chronically impacted	Oil refinery, sewage discharge, and high population density	12.721° S	38.538° W	September 2020	April 2021
Canavieiras	Canavieiras, BA	Moderately impacted by the oil spill	Artisanal fisheries and shrimp farming	15.763° S	38.899° W	–	February 2021
Siribinha	Conde, BA	Highly impacted by the oil spill	Artisanal fisheries and coconut plantation	11.751° S	37.522° W	September 2020	April 2021
Cumuruxatiba	Prado, BA	Moderately impacted by the oil spill	Tourism and artisanal fisheries	17.011° S	39.173° W	–	February 2021
Itacaré	Itacaré, BA	Not directly impacted by the oil spill/Relatively preserved	Tourism	14.278° S	39.004° W	September 2020	February 2021
Boipeba	Cairu, BA	Moderately impacted	Tourism and offshore oil extraction	13.635° S	38.896° W	September 2020	February 2021

* Oil impact categories (highly, moderately, and not directly impacted) were defined qualitatively based on documented shoreline oiling, reported environmental impacts, and official records for the 2019/2020 oil spill along the northeastern Brazilian coast (Soares et al. 2022; IBAMA 2023).

** Anthropogenic pressures were summarized from published descriptions of coastal uses and environmental impacts along the northeastern Brazilian coast, including industrial activities (oil refining, paper industry), shrimp farming, tourism, and artisanal fisheries (Soares et al. 2022; Mussi et al. 2022).

2.3 LABORATORY ANALYSIS

For PAHs analysis, the procedures were based on Pinheiro et al. (2021) with minor modifications. Briefly, p-terphenyl-D14 ($200 \mu\text{g L}^{-1}$) was added to approximately 1 g of dry tissue as a surrogate standard. Pressurized liquid extraction was performed using an accelerated solvent extractor system (ASE 300) with an in-cell purification step during three cycles with a dichloromethane (DCM):methanol mixture (4:1 v:v). The resulting extracts were concentrated to 1000 μL . Purification was performed using a chromatographic adsorption column containing 10 g of silica (5% water deactivated) and 7 g of alumina (2% water deactivated) and eluted with a 75 mL mixture of DCM:n-hexane mixture (1:1; v:v). The final eluate was concentrated and adjusted to a final volume of 1000 μL .

A gas chromatograph (Agilent 7890B) coupled to a mass spectrometer (Agilent 5977A) was operated in the single ion monitoring (SIM) mode to analyze the PAHs. An HP-5MS silica capillary column (30m $\times$ 250 μm ; film thickness 0.25 μm) was used. The oven was programmed from an initial temperature of 50 $^{\circ}\text{C}$, increased at the rate of 20 $^{\circ}\text{C min}^{-1}$ up to 200 $^{\circ}\text{C}$, and held for 1.5 min before further increasing to 300 $^{\circ}\text{C}$ at a rate of 25 $^{\circ}\text{C min}^{-1}$. The temperature was maintained at 250 $^{\circ}\text{C}$ for 2 min, and the final ramp was increased at a rate of 5 $^{\circ}\text{C min}^{-1}$ until it reached 300 $^{\circ}\text{C}$. The ion source and quadrupole temperatures were set at 230 $^{\circ}\text{C}$ and 150 $^{\circ}\text{C}$, respectively. High purity helium was used as the carrier gas at a constant flow rate of 1.5 mL min^{-1} . A calibration curve was constructed by injecting five standards at concentrations ranging from 10.0 to 200 $\mu\text{g L}^{-1}$. The accepted linear correlation coefficient of the analytical curve was higher than 0.995.

Target analytes included the 16 priority polycyclic aromatic hydrocarbons (PAHs) defined by the United States Environmental Protection Agency (USEPA): Naphthalene (Nap), Acenaphthylene (Acy), Acenaphthene (Ace), Fluorene (Flu), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Flt), Pyrene (Pyr), Benz[a]anthracene (BaA), Chrysene (Chr), Benzo[b]fluoranthene (BbF), Benzo[k]fluoranthene (BkF), Benzo[a]pyrene (BaP), Indeno[1,2,3-cd]pyrene (IP), Dibenz[a,h]anthracene (DBaA), and Benzo[ghi]perylene (BghiP). The final results were expressed as individual concentrations in dry weight (dw).

The recovery of the surrogate standard (p-terphenyl-D14) ranged from 59% to 127% in all samples (Online resource 1), indicating acceptable performance of the extraction and clean-up procedures for most analyses. Concentrations were not corrected for recovery. The limit of detection (LOD) was estimated from seven replicate measurements of the lowest-concentration fortified procedural blank and defined as three times the standard deviation of these measurements. LOD values ranged from 0.95 to 1.87 $\text{ng g}^{-1} \text{ dw}$ (dry weight) (Online resource 1). All analytical data were checked using procedural blanks and matrix-spike samples. PAH concentrations in all blanks were below the respective LODs, indicating low contamination throughout the analytical procedures. Matrix-spike samples prepared from three pooled estuarine matrices (fish – *Mugil curema*, fish – *Rachycentron canadum* and crab – *Ucides cordatus*) were used to evaluate accuracy. Mean recoveries ranged from 32.7% to 133.9%, with approximately 94% of compound–matrix combinations falling within the 50–120% range considered acceptable for complex biological matrices (Wade and Cantillo 1994). PAH analysis was also performed on replicate spiked samples to evaluate method precision. The relative standard deviation was < 20% for all compounds, except for naphthalene,

which showed values of 38.8% (Group 2) and 144.1% (Group 3) in two compound–matrix combinations, reflecting the higher volatility and instability of this analyte in complex biological matrices.

2.4 HEALTH RISK ASSESSMENT

Health risk calculations were performed using concentrations expressed on a wet weight basis, which is more directly comparable to food safety guidelines and dietary exposure assessments. So results were converted, accomplished by obtaining average moisture content values for all species from publications and regional environmental monitoring reports, which can be found in Table 2.

Table 2. Average moisture content values of bivalve species used to convert PAHs concentrations from dry weight to wet weigh

Species	% Average Moisture	Reference Source
<i>Crassostrea rhizophorae</i>	82%	Martino and Cruz (2004)
<i>Mytella charruana</i>	82%	Lira et al. (2004)
<i>Anomalocardia flexuosa</i>	80%	Lira et al. (2004)

The PAHs concentrations were converted to a wet weight basis using Eq. (1) (Rakib et al. 2021):

$$C_{ww} = C_{dw} \times \left(\frac{100 - \%ws}{100} \right) \quad (1)$$

where: C_{ww} = PAHs concentration on a wet weight basis (ng g⁻¹ in wet weight), ws = water content of the sample (%), C_{dw} = PAHs concentration on a dry weight basis (ng g⁻¹ in dry weight).

The benzo[a]pyrene-equivalent toxicity concentrations (TEQ_{BaP}) in bivalve tissues were calculated using the following equation (Yu et al. 2016):

$$TEQ_{BaP} = \sum(C_i \times TEF_i)$$

where C_i (ng g⁻¹) is the concentration of an individual PAHs in the seafood and TEF_i is the toxicity equivalency factor for that compound. These values were obtained from Nisbet and Lagoy (1992).

The daily dietary intake (DDI) of PAHs in adults due to seafood consumption was calculated as proposed by Xia et al. (2010) using the following equation:

$$DDI = TEQ_{BaP} \times IR$$

where IR is the daily seafood intake (g day⁻¹). We assumed an IR of 60 g day⁻¹ for shellfish, as proposed by ANVISA (2019).

The incremental lifetime cancer risk (ILCR) in adults caused by shellfish ingestion by adults was calculated as described by Xia et al. (2010):

$$ILCR = \frac{DDI \times EF \times ED \times SF \times CF}{BW \times AT}$$

where *EF* is the Exposure Frequency (assuming seafood consumption occurs 1/3 of the year for the fisheries population, *EF* = 122 days year⁻¹), *ED* is the Exposure Duration (it was calculated for 5, 10 and 30 years), *SF* is the oral cancer slope factor (7.3 mg kg⁻¹ day⁻¹) (Xia et al. 2010), *CF* is the conversion factor (10⁻⁶ mg ng⁻¹) (Xia et al. 2010), *BW* is body weight (60 kg for adults) (ANVISA 2019), *AT* is the average lifetime for carcinogens (25,550 days), as proposed by Xia et al. (2010).

Non-carcinogenic risks were preliminarily assessed for PAHs with robust chronic oral reference doses (RfDs) available in regulatory databases. For each sample and compound, the the average daily dose by ingestion (*ADD*, mg kg⁻¹ day⁻¹) was calculated as:

$$ADD = \frac{C \times IR \times EF \times ED \times CF}{BW \times AT}$$

where *C* is the PAH concentration in seafood on a wet weight basis (ng g⁻¹), *IR* is the ingestion rate (g day⁻¹), *EF* is the exposure frequency (days year⁻¹), *ED* is the exposure duration (years), *CF* is the unit conversion factor (10⁻⁶ mg ng⁻¹), *BW* is the body weight (kg), and *AT* is the averaging time (days). For non-carcinogenic effects, *AT* = *ED* × 365 was adopted, following the usual approach for chronic non-carcinogenic exposure assessment. Hazard quotients for each compound were then calculated as:

$$HQ_i = \frac{ADD_i}{RfD_i}$$

where *RfD_i* is the chronic oral reference dose of compound *i*, expressed in mg kg⁻¹ day⁻¹. RfD values from the EPA IRIS database were used for naphthalene, fluorene, fluoranthene, and pyrene.

The non-carcinogenic hazard index (HI) was calculated as the sum of the individual hazard quotients:

$$HI = \sum HQ_i$$

Because RfDs are not consistently available for all 16 analyzed PAHs, the resulting HI should be interpreted as a partial indicator, potentially underestimating the overall non-carcinogenic risk associated with the complete PAH mixture.

2.5 STATISTICAL ANALYSIS

All statistical analyses were performed using PAHs concentrations expressed on a dry weight basis (dw). Statistical tests were conducted using Jamovi (v. 2.3), an open-source software based on the R language (Jamovi Project, 2022), and Microsoft Excel were used for data organization and preliminary

checks. Normality was evaluated using the Shapiro–Wilk test, inspection of histograms, and assessment of residual normality using Q–Q plots. Homogeneity of variances was assessed using Levene’s test. For all analyses, a significance level of 95% ($p \leq 0.05$) was adopted.

To investigate spatial differences in total PAHs concentrations, a two-way analysis of variance (ANOVA) was applied, with campaign as factor 1 (fixed, two levels) and site as factor 2 (fixed, seven levels). When appropriate, one-way ANOVA was used to compare sites within each campaign. Tukey’s test was used for post-hoc comparisons.

Taxonomic differences in PAHs accumulation were evaluated using a separate one-way ANOVA on total PAHs concentrations (dw), with species as a fixed factor (three levels: *Crassostrea rhizophorae*, *Mytella charruana*, and *Anomalocardia flexuosa*). To avoid the confounding effects of spatial differences in contamination among estuaries, this analysis was restricted to data from Belmonte, the only site where the three species were sampled in both campaigns. When significant differences were detected, Tukey’s test was used to identify the species that differed from each other.

Temporal variations in PAHs concentrations were assessed using only *C. rhizophorae* and *A. flexuosa*, as these species showed the most consistent occurrence across sampling campaigns. For this analysis, only sites sampled in both campaigns were included, allowing a direct comparison between Campaign 1 (August–September 2020) and Campaign 2 (February–April 2021). For each individual sample, the total PAHs, low-molecular-weight PAHs (LMW; 2–3 rings), and high-molecular-weight PAHs (HMW; 4–6 rings) were calculated on a dry weight basis. Owing to the small and unequal sample sizes among sites and campaigns, temporal patterns were primarily explored graphically using individual data points and site-specific campaign medians rather than boxplots, without the application of paired or longitudinal statistical tests. Accordingly, the temporal analysis was interpreted descriptively, and any statistical results were considered with caution.

To explore the patterns of PAHs composition and potential source influences in the bivalve samples, Principal Component Analysis (PCA) was performed. PCA was applied to log-transformed and standardized concentrations of the 16 target PAHs (dw basis) and computed from the correlation matrix. The analysis was conducted using Python (v. 3.13) using the scikit-learn library for PCA, with data preprocessing carried out in pandas and graphical outputs generated using matplotlib. This multivariate approach was used as an exploratory tool to summarize the main compositional gradients among species, sampling locations and campaigns recognizing that interspecific differences, selective bioaccumulation and limited biotransformation capacity may modify the original contaminant signatures in bivalve tissues.

3. RESULTS AND DISCUSSION

3.1 OCCURRENCE, SPATIAL DISTRIBUTION AND PAHs COMPOSITION

PAHs were detected in all bivalve samples (Online resource 2 and 3), with total concentrations (Total PAHs) ranging from 25.9 to 537 ng g⁻¹ dw across all sites and sampling periods (Figure 2). These values are comparable to those reported for bivalves collected along the coast of Bahia by Eça et al. (2021), who found Total PAHs concentrations between 66 and 505 ng g⁻¹ dw in bivalve tissues. They also fall within the range observed in other marine organisms affected by the 2019 oil spill, with concentrations ranging from 8.71 to 418 ng g⁻¹ ww (Magalhães et al. 2022). Consistently, Matos-Barbosa et al. (2025)

detected PAHs in all mangrove sediment samples collected at the same sites as those in the present study, reinforcing the evidence of regional contamination. Although differences in analytical scope (number of compounds) and reporting basis (dry vs. wet weight) limit direct comparisons, these results indicate that PAH levels reported here are broadly consistent with previously described contamination patterns in the region.

Fig. 2. Total concentration of the 16 priority PAHs (dry weight, ng g⁻¹) in bivalve samples collected from seven different areas and two periods evaluated in the present study.

Extending this comparison to other major oil spills worldwide provides additional context for the magnitude of the contamination observed along the Bahia coast. In the context of large tanker accidents, the PAH concentrations measured in the present study are lower than the highest levels reported for heavily impacted coasts, but still fall within the range documented for contaminated bivalve populations. Following the *Prestige* tanker accident on the Galician coast (north-western Spain), Soriano et al. (2006) reported ΣPAH concentrations in wild mussels (*Mytilus galloprovincialis*) ranging from 17 to 7782 µg kg⁻¹ dry weight, with the upper end of this range reflecting intense local oiling. After the *Sea Prince* spill in South Korea, Yim et al. (2002) found ΣPAH concentrations of 144–664 ng g⁻¹ dry weight in oysters (*Crassostrea* spp.) and 172–1364 ng g⁻¹ dry weight in mussels (*Mytilus* spp.) five years after the accident, indicating persistent contamination of coastal food webs. By comparison, the 25.9–537 ng g⁻¹ dry weight range measured in bivalves along the Bahia coast lies within the lower to intermediate portion of the ranges reported for bivalves in other oil-impacted coasts, remaining well below the most extreme values documented for the *Prestige* spill and supporting the interpretation of a mixed scenario involving spill-derived inputs superimposed on pre-existing and ongoing anthropogenic sources.

At the opposite end of the contamination gradient, Itacaré, located on the southern coast of Bahia within the Itacaré/Serra Grande Environmental Conservation Area (ECA), was used as a reference site. In this area, the Total PAHs concentrations in bivalves were the lowest among all sites, ranging from 25.9 ng g⁻¹ dw in *Mytella charruana* to 120 ng g⁻¹ dw in *Crassostrea rhizophorae*. Except for this single *C. rhizophorae* sample collected in February 2021, most concentrations were lower than or close to 50 ng g⁻¹ dw (Figure 2), a range typically associated with environments with little or no PAH input (Soriano et al. 2006). Itacaré was only lightly affected by the 2019 oil spill, with oil residues reported on beaches but not in the estuaries or mangroves (IBAMA 2020). Overall, the Total PAHs levels in bivalves from this site were consistent with background pollution and indicated minimal hydrocarbon inputs in this preserved region.

In contrast, higher PAHs concentrations were observed in Belmonte, São Paulo River, and Canavieiras, where several samples reached Total PAHs values around or above 200 ng g⁻¹ dw. Belmonte, located within the Canavieiras Marine Extractive Reserve (MER), showed the highest concentrations, with *M. charruana* samples reaching 537 ng g⁻¹ dw in August 2020 and 515 ng g⁻¹ dw in February 2021. Concentrations close to or greater than 500 ng g⁻¹ dw are usually associated with areas under active PAHs input and are often described as contamination “hotspots” (Soriano et al. 2006). Belmonte was one of the regions most affected by the 2019 oil spill and receives hydrocarbon inputs from the Jequitinhonha River.

Additional pressures, including untreated sewage, agricultural runoff, upstream mining activities, and the local paper industry, may help explain the persistent enrichment of PAHs in local bivalves, despite the protected status of this coastal zone.

The São Paulo River represents a contrasting scenario of chronic contamination in Brazil. This estuary is located in a region that has been historically affected by industrial and petroleum-related activities (Miranda et al. 2016). In this area, the Total PAHs concentrations reached 363 ng g⁻¹ dw in *M. charruana* and 355 ng g⁻¹ dw in *C. rhizophorae*. These elevated values, combined with the known history of contamination, suggest that long-standing anthropogenic inputs are the dominant factor controlling PAHs accumulation in the local biota. Thus, in the São Paulo River, contamination cannot be attributed solely to the 2019 oil spill but reflects a combination of recent and legacy sources.

Intermediate Total PAHs levels were detected in the mangroves of Canavieiras, Siribinha, Cumuruxatiba, and Boipeba, all of which are located within environmentally protected areas. Canavieiras lies within the Canavieiras MER, Siribinha within the Northern Coast of the State of Bahia Environmental Conservation Area (ECA), Boipeba within the Boipeba–Tinaré ECA, and Cumuruxatiba within the Cumuruxatiba MER. In these regions, Total PAHs concentrations generally ranged between 114 and 315 ng g⁻¹ dw. The maximum values were 315 ng g⁻¹ dw in Canavieiras, 195 ng g⁻¹ dw in Cumuruxatiba, and 144–218 ng g⁻¹ dw in Siribinha, whereas Boipeba exhibited comparatively lower maximum concentrations of 114 ng g⁻¹ dw. Under baseline conditions, PAHs levels in protected areas are expected to remain low. However, these mangroves were severely impacted by the 2019 oil spill and may also be influenced by local and regional inputs, such as riverine discharge and urban effluents, which likely contributed to the intermediate levels observed.

Taken together, PAH concentrations in bivalves differed markedly among sites, with the lowest values at the reference area (Itacaré), higher concentrations at Belmonte and at the chronically impacted São Paulo River, and intermediate levels at the remaining mangrove areas (Canavieiras, Siribinha, Cumuruxatiba, and Boipeba). These contrasts are consistent with the varying intensity of local anthropogenic pressures and with the documented oil and industrial contamination histories of these sites.

To better understand the compositional patterns underlying these concentration differences, Principal Component Analysis (PCA) was applied to the PAHs dataset (Figure 3).

Fig. 3. Principal Component Analysis (PCA) biplot of PAHs concentrations in bivalve samples, expressed on a dry weight basis. The points represent individual samples, colors indicate sampling locations, and symbols indicate sampling campaigns. The arrows represent the PAHs loading vectors, and only the most influential loadings are labeled to improve visual clarity.

The first principal component (PC1), which accounted for 37.1% of the variance, was primarily associated with medium- to high-molecular-weight PAHs, including fluoranthene (Flt), pyrene (Pyr), benzo[b]fluoranthene (BbF), and benzo[ghi]perylene (BghiP). Similar sets of compounds have been reported as dominant contributors to compositional variability in coastal and estuarine environments, particularly in multivariate analyses of biota and sediments (Soclo et al. 2000; Baumard et al. 1998). In this

context, PC1 represents a compositional gradient in which samples with higher scores show increased contributions from these higher molecular weight compounds.

PC2, which accounted for 17.5% of the variance, captured additional differences in PAH composition that did not reflect a simple contrast between low- and high-molecular-weight compounds. Therefore, this axis likely represents more complex variability among sites that is not straightforward to attribute to a single environmental or biological driver. Similar patterns have been reported in multivariate analyses of PAHs in marine organisms, in which secondary components are often interpreted cautiously as reflecting mixed influences of environmental availability and biological processes affecting PAH bioaccumulation (Baumard et al. 1999).

Together, PC1 and PC2 explained 54.6% of the total variance in PAHs composition, leaving approximately 45% of the variability unaccounted for by the first two components. This indicates that additional factors, such as local hydrodynamics, sediment characteristics, and species-specific physiology, also play important roles in shaping the PAHs patterns in bivalves. Consequently, the PCA results should be interpreted as an exploratory summary of the main compositional gradients, rather than as a complete description of all processes controlling PAHs accumulation in these organisms.

Spatially, the PCA revealed distinct, yet partially overlapping, compositional patterns among the sampling areas. Samples from Belmonte were broadly distributed across the ordination space, with a tendency to associate with phenanthrene (Phe) and fluorene (Flu). These compounds are often more readily accumulated in bivalve tissues and have been linked to relatively recent or less weathered hydrocarbon inputs in marine environments, although such interpretations must be made cautiously when based solely on multivariate patterns. The mixed position of the Belmonte samples in the ordination supports a scenario in which multiple contamination pathways coexist, including contributions from the 2019 oil spill, river-borne hydrocarbons, and local anthropogenic activities.

In contrast, samples from the São Paulo River and, to a lesser extent, Siribinha were more frequently associated with vectors representing higher molecular weight PAHs, such as Pyr, Flt, and BghiP. These compounds are typically more persistent and have been widely reported in environments influenced by chronic anthropogenic inputs, particularly combustion-derived sources (Soclo et al. 2000; Baumard et al. 1998). The association of these sites with high-molecular-weight PAHs supports the interpretation that long-term, combustion-related and industrial inputs play a major role in controlling PAHs composition in these estuaries.

Intermediate compositional patterns were observed in Canavieiras, Boipeba, and Cumuruxatiba, where samples occupied positions between the gradients defined by the Belmonte and São Paulo River/Siribinha. This distribution suggests mixed PAHs profiles and the contribution of multiple sources, combining oil-related inputs with more diffuse and chronic contamination. Finally, samples from Itacaré, the reference site, showed a more dispersed distribution and generally weaker association with the dominant loading vectors, consistent with lower contamination levels and reduced anthropogenic influence on PAHs composition.

Overall, the multivariate analysis indicated that the PAHs composition in bivalve tissues reflects the influence of multiple contamination sources and environmental processes rather than a single dominant origin. Source inputs related to the 2019 oil spill, legacy industrial contamination, combustion-derived

hydrocarbons, and diffuse anthropogenic discharges interact with hydrodynamic conditions, sediment dynamics, and species-specific bioaccumulation capacities to shape the observed patterns. These results support the hypothesis that, along the Bahia coastline, PAHs accumulation in bivalves arises from a complex, multifactorial contamination scenario in which both acute and chronic inputs play important roles.

3.2 DIFFERENCES BETWEEN SPECIES (TAXONOMIC VARIATION)

Statistically significant differences in PAHs concentrations were found among the species ($p = 0.009$). Post-hoc comparisons indicated that *M. charruana* (the most contaminated species) differed significantly from both *C. rhizophorae* ($p_{Tukey} = 0.01$) and *A. flexuosa* ($p_{Tukey} = 0.03$). These species have different modes of exposure to the contaminants.

C. rhizophorae is found attached to the roots and stems of *Rhizophora mangle* and is therefore primarily exposed to contaminants present in the tidally influenced water column (Nishida et al. 2006). In contrast, *A. flexuosa* and *M. charruana* are infaunal species that inhabit the surface sediments. While *A. flexuosa* occurs on intertidal sand–clay banks, which are generally more dynamic and oxygenated, *M. charruana* buries itself in muddier sediments (Leonel et al. 1982; Narchi 1972). Finer sediments, such as mud, generally exhibit higher contaminant concentrations because of their larger surface area and higher organic matter content, which favor the adsorption of hydrophobic compounds such as PAHs (Guimarães et al. 2020). Furthermore, these finer sediments tend to present lower oxygen concentrations (anoxia or hypoxia) due to reduced water circulation, which can decrease PAHs degradation rates by microorganisms and increase their residence time within the sediment matrix. These factors may have increased the bioavailability of PAHs to *M. charruana*.

The infaunal habit of *M. charruana*, which lives buried in these muddy sediments, likely increases its exposure to sediment-bound contaminants compared with epifaunal species such as *C. rhizophorae*. Similar patterns have been reported in other studies, in which infaunal bivalves tend to accumulate higher PAH concentrations because of their closer interaction with contaminated sediments and their bioturbation activity, which redistributes particles and associated contaminants within the sedimentary matrix (Bendell et al. 2020).

Biological factors may also contribute to the observed differences. Although it was not possible to analyze the lipid content in the samples from the present study, differences in lipid content among species can influence the accumulation of hydrophobic compounds such as PAHs, given their affinity for adipose tissues (Arnot and Gobas 2006). Therefore, species-specific physiological traits, such as lipid content, metabolic rates, and detoxification capacity, may also modulate PAH accumulation and should be considered when interpreting these patterns. Overall, the higher PAH concentrations observed in *M. charruana* are most plausibly explained by the combined influence of sediment characteristics and species-specific ecological traits, particularly its closer association with fine, organic-rich, and poorly oxygenated sediments.

3.3 TEMPORAL VARIATION OF PAHs CONCENTRATION

Only the total PAH concentrations measured in *Crassostrea rhizophorae* and *Anomalocardia flexuosa* were used in the temporal analysis, as these species provided the largest number of observations across sites and campaigns. For this reduced dataset, total PAH concentrations ranged from 31.0 to 355.6 ng g⁻¹ dw in the first sampling campaign and from 91.1 to 315.8 ng g⁻¹ dw in the second campaign, with median values of approximately 146.6 and 176.2 ng g⁻¹ dw, respectively. No significant temporal differences in total PAH concentrations were detected between the sampling periods (two-way ANOVA on ΣPAHs, time effect: $F = 0.12$, $p = 0.765$), and the interaction between site and period was also non-significant ($F = 1.27$, $p = 0.485$), indicating that short-term temporal trends were weak relative to the pronounced spatial variability among mangroves.

This result must be interpreted in the context of the sampling design. Collections began several months after the spill because of restrictions imposed by the COVID-19 pandemic and therefore did not capture the immediate post-impact phase. Consequently, the available data do not allow a direct reconstruction of the initial contamination trajectory.

The lack of a clear temporal trend suggests that PAHs exposure in these estuarine organisms may reflect the combined influence of residual oil-derived contamination and pre-existing or ongoing chronic inputs. This interpretation is consistent with evidence from northeastern Brazil, indicating that sediments affected by the 2019–2020 oil spill may record overlapping acute and chronic PAHs sources rather than a single contamination signal (Bertoldi et al. 2024). This is also consistent with previous studies of petroleum-influenced estuarine systems in Bahia, such as the São Paulo River estuary in Todos os Santos Bay, where PAHs were already present in surface sediments prior to this event, with spatial and seasonal variability linked to local environmental conditions and historical anthropogenic pressure (Araújo et al. 2018; Martins et al. 2020).

Overall, the persistence of PAHs in filter-feeding bivalves across the sampling periods indicates continuous exposure after the initial contamination event. However, the available data are insufficient to precisely separate the relative contribution of the 2019 spill from that of chronic local or regional sources. Long-term monitoring integrating biota, sediments, and the hydrodynamic context would be necessary to clarify whether the observed contamination reflects gradual post-spill attenuation, recurrent remobilization of previously deposited residues, or sustained diffuse anthropogenic inputs.

3.4 FOOD SAFETY / ASSOCIATED RISKS

The incremental lifetime cancer risk (ILCR) associated with bivalve consumption was estimated based on the benzo[a]pyrene toxic equivalency (TEQ-BaP), using concentrations expressed on a wet weight basis. The calculated ILCR values ranged from 4.8×10^{-8} to 4.9×10^{-6} for the 5-year exposure scenario, from 9.7×10^{-8} to 9.8×10^{-6} for 10 years, and from 2.9×10^{-7} to 2.9×10^{-5} for 30 years of exposure (Table 3). According to widely adopted risk classification criteria, ILCR values below 10^{-6} are considered negligible, values between 10^{-6} and 10^{-4} indicate potential carcinogenic risk, and values above 10^{-4} represent an unacceptable or high risk to human health (Usepa 1989, 2005; Xia et al. 2010; Tiwari et al. 2017; Gereslassie et al. 2018). Based on these thresholds, most samples analyzed in this study fell within the negligible-to-low risk categories, particularly under the 5- and 10-year exposure scenarios.

Under the 30-year exposure scenario, a limited number of samples exceeded the threshold of 10^{-5} , indicating a non-negligible carcinogenic risk under prolonged consumption. Specifically, three out of 25 samples (12%) showed ILCR values above this level: *Mytella charruana* from the São Paulo River (Sep/2020; 1.5×10^{-5}) and Belmonte (Aug/2020; 2.9×10^{-5}), and *Anomalocardia flexuosa* from Belmonte (Aug/2020; 1.3×10^{-5}). The remaining samples presented ILCR values between 2.6×10^{-7} and 9.7×10^{-6} , remaining below, but in some cases approaching, the intermediate risk threshold.

Table 3. The incremental lifetime cancer risk (ILCR) was calculated for adults due to the consumption of contaminated bivalves for 5, 10, and 30 years. Values in bold represent non-negligible carcinogenic risks. *Crassostrea rhizophorae*: CR, *Mytella charruana*: MC, *Anomalocardia flexuosa*: AF.

Local	Bivalve	Period	ILCR adult	ILCR adult	ILCR adult
			5 exposure	10 exposure	30 exposure
			Year	Year	Year
Belmonte	MC	Aug/2020	4.9×10^{-6}	9.8×10^{-6}	2.9×10^{-5}
		Feb/2021	2.3×10^{-7}	4.6×10^{-7}	1.4×10^{-6}
	AF	Aug/2020	2.2×10^{-6}	4.4×10^{-6}	1.3×10^{-5}
		Feb/2021	1.6×10^{-6}	3.2×10^{-6}	9.7×10^{-6}
	CR	Aug/2020	6.3×10^{-7}	1.3×10^{-6}	3.8×10^{-6}
		Feb/2021	4.3×10^{-8}	8.6×10^{-8}	2.6×10^{-7}
Siribinha	MC	Sep/2020	7.8×10^{-8}	1.6×10^{-7}	4.7×10^{-7}
		Apr/2021	5.1×10^{-7}	1.0×10^{-6}	3.1×10^{-6}
	AF	Sep/2020	4.7×10^{-7}	9.3×10^{-7}	2.8×10^{-6}
		Apr/2021	6.0×10^{-7}	1.2×10^{-6}	3.6×10^{-6}
Itacaré	MC	Sep/2020	4.8×10^{-8}	9.7×10^{-8}	2.9×10^{-7}
		Apr/2021	2.6×10^{-7}	5.1×10^{-7}	1.5×10^{-6}
	AF	Sep/2020	4.0×10^{-7}	8.1×10^{-7}	2.4×10^{-6}
		Apr/2021	6.0×10^{-8}	1.2×10^{-7}	3.6×10^{-7}
Boipeba	CR	Sep/2020	5.4×10^{-8}	1.1×10^{-7}	3.2×10^{-7}
		Apr/2021	8.7×10^{-8}	1.7×10^{-7}	5.2×10^{-7}
São Paulo River	MC	Sep/2020	2.6×10^{-6}	5.1×10^{-6}	1.5×10^{-5}
		Apr/2021	2.5×10^{-7}	5.0×10^{-7}	1.5×10^{-6}
	AF	Sep/2020	9.2×10^{-8}	1.8×10^{-7}	5.5×10^{-7}
		Apr/2021	2.0×10^{-7}	4.0×10^{-7}	1.2×10^{-6}
	CR	Apr/2021	1.5×10^{-7}	2.9×10^{-7}	9.3×10^{-7}
Canavieiras	CR	Feb/2021	1.2×10^{-6}	2.4×10^{-6}	7.1×10^{-6}
Cumuruxatiba	CR	Feb/2021	4.8×10^{-8}	9.7×10^{-8}	2.9×10^{-7}

Under realistic short- and medium-term exposure scenarios, the consumption of bivalves from the studied areas is associated with a low carcinogenic risk. However, under long-term exposure conditions, localized increases in risk may occur, particularly in areas such as Belmonte and the São Paulo River, where higher PAHs concentrations were observed. The spatial distribution of ILCR values was consistent with the contamination patterns discussed in the previous sections, with higher risks associated with sites influenced by either chronic contamination (São Paulo River) or combined inputs from multiple sources (Belmonte). This reinforces the interpretation that long-term exposure risks are more closely related to persistent and multifactorial contamination processes than to a single contamination event.

It is important to emphasize that these results represent a conservative and preliminary assessment of the potential health risks. The ILCR estimates are based on a limited number of samples, generalized exposure parameters, and toxicity equivalency factors derived from the literature rather than site-specific consumption data. In addition, the temporal scope of this study may not fully capture the variability in PAHs concentrations over time.

Therefore, the non-negligible, but relatively low ILCR values observed in a small subset of samples should not be generalized to entire populations or locations. Nevertheless, given the dependence of local communities on bivalve consumption, the identification of non-negligible risk under prolonged exposure conditions represents an important signal that warrants attention.

Overall, these findings highlight the need for continued monitoring and more refined risk assessments that incorporate larger datasets, site-specific consumption patterns, and long-term environmental data to better evaluate the potential health impacts associated with seafood consumption in contaminated coastal regions.

Regarding non-carcinogenic risk, all HQ values calculated for the individual PAHs with available oral reference doses (naphthalene, fluorene, fluoranthene, and pyrene) remained below 1 in all exposure scenarios, and the HI values also remained below 1 for all samples (Table 4).

These results indicate negligible non-carcinogenic risk for the assessed compounds under the assumed consumption conditions. However, because oral RfDs are not consistently available for several other PAHs detected in the samples, the overall non-carcinogenic risk associated with the full PAH mixture may be underestimated and should therefore be interpreted with caution.

Table 4. Hazard quotients (HQ) and hazard indices (HI) for Naphthalene (Nap), Fluorene (Flu), Fluoranthene (Flt), and Pyrene (Pyr) in the muscle of *Crassostrea rhizophorae*, *Mytella charruana*, and *Anomalocardia flexuosa* by sampling site and period.

Local	Bivalve	Period	HQ _{NAP}	HQ _{FLU}	HQ _{FLT}	HQ _{PYR}	HI
Belmonte	<i>Anomalocardia flexuosa</i>	1	4.1×10^{-5}	1.3×10^{-6}	3.8×10^{-6}	8.0×10^{-6}	5.4×10^{-5}
	<i>Crassostrea rhizophorae</i>	1	2.2×10^{-5}	1.2×10^{-6}	2.4×10^{-6}	7.1×10^{-6}	3.2×10^{-5}
	<i>Mytella charruana</i>	1	2.3×10^{-5}	2.3×10^{-6}	4.6×10^{-6}	1.5×10^{-5}	4.4×10^{-5}
	<i>Anomalocardia flexuosa</i>	2	2.8×10^{-5}	1.7×10^{-6}	4.6×10^{-6}	1.3×10^{-5}	4.7×10^{-5}
	<i>Crassostrea rhizophorae</i>	2	3.2×10^{-5}	8.3×10^{-7}	3.0×10^{-6}	5.8×10^{-6}	4.2×10^{-5}
	<i>Mytella charruana</i>	2	2.4×10^{-5}	4.0×10^{-6}	3.9×10^{-7}	2.5×10^{-7}	2.9×10^{-5}
Boipeba	<i>Crassostrea rhizophorae</i>	2	5.6×10^{-6}	1.9×10^{-6}	6.7×10^{-7}	3.6×10^{-6}	1.2×10^{-5}
	<i>Crassostrea rhizophorae</i>	2	2.1×10^{-5}	6.8×10^{-7}	5.9×10^{-6}	2.1×10^{-5}	4.8×10^{-5}
Canavieiras	<i>Crassostrea rhizophorae</i>	2	3.3×10^{-5}	3.6×10^{-6}	1.7×10^{-5}	6.7×10^{-5}	1.2×10^{-4}
Cumuruxatiba	<i>Crassostrea rhizophorae</i>	2	2.8×10^{-5}	1.6×10^{-6}	3.7×10^{-6}	7.7×10^{-6}	4.1×10^{-5}
Itacaré	<i>Crassostrea rhizophorae</i>	2	1.2×10^{-5}	7.7×10^{-7}	7.9×10^{-6}	2.9×10^{-5}	5.0×10^{-5}
	<i>Anomalocardia flexuosa</i>	1	3.3×10^{-6}	3.6×10^{-7}	1.3×10^{-6}	4.0×10^{-6}	9.0×10^{-6}
	<i>Crassostrea rhizophorae</i>	1	8.3×10^{-6}	1.3×10^{-6}	1.9×10^{-6}	6.4×10^{-6}	1.8×10^{-5}
	<i>Mytella charruana</i>	1	4.6×10^{-6}	1.7×10^{-7}	1.3×10^{-6}	5.0×10^{-6}	1.1×10^{-5}
São Paulo River	<i>Crassostrea rhizophorae</i>	2	1.3×10^{-5}	1.2×10^{-6}	5.1×10^{-6}	1.2×10^{-5}	3.1×10^{-5}
	<i>Mytella charruana</i>	2	1.2×10^{-5}	9.7×10^{-7}	4.8×10^{-6}	1.1×10^{-5}	2.9×10^{-5}
	<i>Anomalocardia flexuosa</i>	1	1.5×10^{-5}	2.7×10^{-7}	3.7×10^{-6}	1.3×10^{-5}	3.2×10^{-5}
	<i>Crassostrea rhizophorae</i>	1	1.2×10^{-5}	5.8×10^{-7}	3.4×10^{-6}	1.0×10^{-5}	2.6×10^{-5}
	<i>Mytella charruana</i>	1	9.4×10^{-6}	2.4×10^{-7}	6.4×10^{-6}	2.1×10^{-5}	3.7×10^{-5}
Siribinha	<i>Crassostrea rhizophorae</i>	2	4.4×10^{-6}	7.5×10^{-7}	4.5×10^{-6}	3.2×10^{-5}	4.1×10^{-5}
	<i>Anomalocardia flexuosa</i>	1	9.8×10^{-6}	2.0×10^{-6}	8.0×10^{-6}	4.1×10^{-5}	6.1×10^{-5}
	<i>Crassostrea rhizophorae</i>	1	9.0×10^{-6}	9.1×10^{-7}	3.3×10^{-6}	1.4×10^{-5}	2.7×10^{-5}
	<i>Mytella charruana</i>	1	4.2×10^{-6}	6.6×10^{-7}	9.2×10^{-7}	4.0×10^{-6}	9.8×10^{-6}

4. CONCLUSIONS

This study provides an integrated assessment of PAH accumulation in marine bivalves from multiple coastal environments along the Bahia coast, including areas affected by the 2019 oil spill, a chronically contaminated estuary (São Paulo River), and a reference site (Itacaré). PAH concentrations varied markedly among locations and species, reflecting the combined influence of site-specific contamination patterns and species-specific ecological traits on contaminant accumulation.

Among the species analyzed, *Mytella charruana* consistently showed higher PAH concentrations, primarily attributed to its stronger association with fine, organic-rich sediments that favour the retention and persistence of hydrophobic contaminants. In contrast, *Crassostrea rhizophorae* and *Anomalocardia flexuosa* exhibited lower levels, reflecting differences in habitat and exposure pathways.

No consistent temporal trends in PAH concentrations were observed between the sampling campaigns. This absence of a clear decline indicates that short-term monitoring is insufficient to capture the environmental evolution of contamination following oil-spill events. Instead, the results show that PAH exposure in these tropical estuarine systems arises from the superposition of residual oil-derived inputs, long-standing industrial contamination, and diffuse anthropogenic sources. The contrasting behaviour of sites such as Belmonte and the São Paulo River reinforces the importance of local environmental conditions and chronic contamination in shaping current PAH patterns.

The ILCR assessment indicated that, under short- and medium-term exposure scenarios, the consumption of bivalves from the studied areas is associated with a negligible to low carcinogenic risk. Under long-term exposure conditions, however, a limited number of samples, primarily from Belmonte and the São Paulo River, reached values indicative of non-negligible risk, suggesting that potential health concerns are spatially localized and dependent on prolonged exposure rather than being widespread across the study area.

In parallel, non-carcinogenic risk assessment based on hazard quotients (HQ) and hazard indices (HI) for naphthalene, fluorene, fluoranthene, and pyrene showed that all values remained below 1 in all samples and exposure scenarios, indicating negligible non-carcinogenic risk under the consumption assumptions adopted in this study. Nonetheless, this evaluation should be regarded as preliminary and partial, because chronic oral reference doses are not available for several other PAHs detected in the samples, and the overall non-carcinogenic hazard associated with the complete PAH mixture may therefore be underestimated.

Taken together, these findings demonstrate that post-spill contamination in tropical estuarine systems cannot be interpreted solely as the consequence of a single acute event, but rather as the outcome of interactions between residual oil residues and pre-existing chronic contamination. This insight is particularly relevant for tropical estuaries worldwide, where historical industrial activities and diffuse coastal pressures are likely to modulate the ecological and human-health consequences of future oil-spill events. Consequently, our results highlight the need for integrated and long-term biomonitoring strategies, combining multiple bioindicator species and environmental compartments, to distinguish chronic and episodic contamination sources and to support evidence-based spill-response planning and coastal management in vulnerable tropical regions.

REFERENCES

- Abdel-Shafy HI, Mansour MSM (2016) A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egypt J Pet* 2(1):107–123.
- Araújo LS, Mafalda PO, Moreira, I (2018) Avaliação de HPAs nos manguezais dos estuários dos rios São Paulo e Jaguaripe, Bahia de Todos os Santos, Bahia. *Rev Bras Gestão Ambiental e Sustentabilidade*. 5(10): 599-606
- Annot JA, Gobas FAPC (2006) A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environ Rev*. 14: 257–297.
- Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011). The value of estuarine and coastal ecosystem services. *Ecolol Monogr*. 81(2):169–193.
- Baumard P, Budzinski H, Garrigues P, Sorbe JC, Burgeot T, Bellocq J (1998) Concentrations of PAHs (polycyclic aromatic hydrocarbons) in various marine organisms in relation to those in sediments and to trophic level. *Mar Pollut Bull* 36:951-960.
- Baumard P, Budzinski H, Garrigues P, Dizer H, Hansen PD (1999) Polycyclic aromatic hydrocarbons in recent sediments and mussels (*Mytilus edulis*) from the Western Baltic Sea: occurrence, bioavailability and seasonal variations. *Mar Environ Res* 47:17-47.
- Bendell LI, Lecadre E, Zhou W (2020) Use of sediment dwelling bivalves to biomonitor plastic particle pollution in intertidal regions: a review and study. *PLoS One* 15:e0232879.
- Bertoldi CF, Dolatto RG, Oliveira LFL, Reis LF, Canela MCC, Melo JTB, Rodrigues AS, Paim AP, Grassi MT (2024) Vestígios do derrame de petróleo de 2019: avaliação da presença de HPA e BTEX em produtos de pesca. *Quim Nova* 47:20240063.
- Brasil Agência Nacional de Vigilância Sanitária (ANVISA) (2019) Instrução Normativa No. 2 of 13 November 2019. Procedures and criteria for human health risk assessment associated with chemical contaminants in food. *Diário Oficial da União*, Brasília.
- Costa MB, Otegui MBP, Zamprogno GC, Caniçali FB, Cozer CR, Pelletier E, Graceli JB (2023) Abundance, composition, and distribution of microplastics in intertidal sediment and soft tissues of four species of Bivalvia from Southeast Brazilian urban beaches. *Sci Total Environ* 857:159352.
- Cheng C, Bi C, Wang D, Yu Z, Chen Z (2018) Atmospheric deposition of polycyclic aromatic hydrocarbons (PAHs) in Shanghai: the spatio-temporal variation and source identification. *Front Earth Sci* 12:63-71.
- Duke N (2016) Oil spill impacts on mangroves: recommendations for operational planning and action. *Mar Pollut Bull* 109(2):700-715.
- Eça GF, Albergaria-Barbosa ACR, Souza MM, Costa PG, Leita, AS, Fillmann G, Hatje V (2021) Polycyclic aromatic hydrocarbons in sediments and shellfish from Todos os Santos Bay, Brazil. *Mar Pollut Bull* 173:113071.
- Garcia MR, Martins CC (2021) A systematic evaluation of polycyclic aromatic hydrocarbons in South Atlantic subtropical mangrove wetlands under a coastal zone development scenario. *J Environ Manage* 287:112421.

- Gereslassie T, Workineh A, Liu X, Yan X, Wang J (2018) Occurrence and ecological and human health risk assessment of polycyclic aromatic hydrocarbons in soils from Wuhan, Central China. *Int J Environ Res Public Health* 15:2751.
- Guimarães LM, França EJ, Arruda GN, Albergaria-Barbosa ACR (2020) Historical inputs of polycyclic aromatic hydrocarbons in the preserved tropical estuary of the Itapicuru River, Bahia, Brazil. *Mar Pollut Bull* 156:111218
- Brasil Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) (2020) Localidades atingidas: manchas de óleo no litoral brasileiro, version 3. IBAMA, Brasília.
- Brasil Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) (2023) Incidente de poluição por óleo na costa brasileira: relatório técnico. IBAMA, Brasília.
- Jamovi Project (2022) jamovi (Version 2.3) [Computer software]. The jamovi project, Sydney.
- Ko FC, Chang CW, Cheng JO (2014) Comparative study of polycyclic aromatic hydrocarbons in coral tissues and the ambient sediments from Kenting National Park, Taiwan. *Environ Pollut* 185:35-43.
- Leonel RMV, Magalhães ARM, Lunetta JE (1982) Survival rate of *Anomalocardia brasiliiana* (Gmelin, 1791) (Mollusca: Bivalvia) at different levels of salinity. *Braz J Med Biol Res* 15:231-248.
- Lira MCB, et al (2004) Análise bromatológica de moluscos bivalves do litoral de Alagoas. In: 44th Congresso Brasileiro de Química, Fortaleza, Brazil. Associação Brasileira de Química, Fortaleza.
- Lucena-Frédou F, Eduardo LN, Lira AS, Pelage L, Passarone R, Frédou T (2021) Atividade pesqueira artesanal no Nordeste do Brasil. In: Viana DL, Oliveira JEL, Hazin FHV, Souza MAC (eds) *Ciências do mar: dos oceanos do mundo ao Nordeste do Brasil*, vol 2: Bioecologia, pesca e aquicultura. Via Design Publicações, Recife, pp 374–405.
- Machado LOR, Rêgo RCF, Northcross AL, Meyer A, Mota LSR, Silva C, Lino PGJ, Costa LCC, Kikuchi RKP, Lima VMC (2025) Exposure to oil spill among fishers on the Brazilian coast in 2019/2020. *J Agromedicine* 30:1-13.
- Magalhães KM, Carreira RS, Rosa Filho JS, et al (2022) Polycyclic aromatic hydrocarbons (PAHs) in fishery resources affected by the 2019 oil spill in Brazil: Short-term environmental health and seafood safety. *Mar Pollut Bull* 175:113334.
- Martino RC, Cruz GM (2004) Proximate composition and fatty acid content of the mangrove oyster *Crassostrea rhizophorae* along the year seasons. *Braz Arch Biol Technol* 47:955-962.
- Martins AB, Assis AHS, Filho MSC, et al (2020) Concentration and distribution of polycyclic aromatic hydrocarbons in oysters from Todos os Santos Bay (Bahia, Brazil). *Mar Pollut Bull* 151:110781.
- Matos-Barbosa T, Lourenço RA, Gusmão JB, Nossol ABS, Combi T (2025) Acute and chronic sources of polycyclic aromatic hydrocarbons (PAHs) in coastal sediments impacted by the 2019–2020 oil spill in NE Brazil. *Reg Stud Mar Sci* 89:104330.
- Miranda LS, Moreira ÍTA, Oliveira OMC, et al (2016) Oil-suspended particulate material aggregates as a tool in preventing potential ecotoxicological impacts in the São Paulo River, Todos os Santos Bay, Bahia, Brazil: Influence of salinity and suspended particulate material. *Mar Pollut Bull* 112:91-97.
- Mussi CS, Zago NI, Marenzi RC, Polette M (2022) Environmental sensitivity analysis of environments affected by the 2019 oil spill on the Brazilian coast. *Rev Bras Cienc Ambient* 57:644-653.

- Narchi W (1972) Comparative study of the functional morphology of *Anomalocardia brasiliensis* (Gmelin, 1791) and *Tivela mactroides* (Born, 1778) (Bivalvia, Veneridae). *Bull Mar Sci* 22:643-670.
- Nishida AK, Nordi N, Alves RRN (2006) Mollusc gathering in northeast Brazil: An ethnoecological approach. *Hum Ecol* 34:133-145.
- Nisbet ICT, LaGoy PK (1992) Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). *Regul Toxicol Pharmacol* 16:290-300.
- O'Connor TP (1998) Mussel Watch results from 1986 to 1996. *Mar Pollut Bull* 37:14-19.
- Ohiozebau E, Tendler B, Codling G, et al (2017) Potential health risks posed by polycyclic aromatic hydrocarbons in muscle tissues of fishes from the Athabasca and Slave Rivers, Canada. *Environ Geochem Health* 39:139-160.
- Oliva AL, La Colla NS, Arias AH, et al (2017) Distribution and human health risk assessment of PAHs in four fish species from a SW Atlantic estuary. *Environ Sci Pollut Res* 24:18979-18990.
- Patel AB, Shaikh SS, Jain KR, Desai C, Madamwar D (2020) Polycyclic aromatic hydrocarbons: Sources, toxicity, and remediation approaches. *Front Microbiol* 11:562813.
- Pinheiro CVG, Carreira RS, Massone CG (2021) Polycyclic aromatic hydrocarbon (PAHs) analyses in marine tissues using accelerated solvent extraction (ASE) in tandem with in-cell purification and GC-MS. *J Braz Chem Soc* 32:2153-2159.
- Rakib MRJ, Jolly YN, Ebiweni OJ, Akter S, Khandaker MU, Bradley DA, Kamal MA (2021) Assessment of trace element contamination in dried fish from the Bay of Bengal: A potential health hazard. *Mar Pollut Bull* 170:112613.
- Rêgo RF, Machado LOR, Sentilles F, Mota LSR, Lima VMC, Meyer A, Northcross AL (2026) Developing epidemiological research in response to an oil spill: Study design and questionnaire validation. *Cien Saude Colet* 31:1-12.
- Sericano JL, Wade TL, Jackson TJ, et al (1995) Trace organic contamination in the Americas: An overview of the US National Status and Trends and the International Mussel Watch programmes. *Mar Pollut Bull* 31:214-225.
- Sericano JL (2000) The Mussel Watch approach and its applicability to global chemical contamination monitoring programmes. *Int J Environ Pollut* 13:340-350.
- Soares MO, Teixeira CEP, Bezerra LEA, et al (2020) Oil spill in South Atlantic (Brazil): Environmental and governmental disaster. *Mar Policy* 115:103879.
- Soares MO, Teixeira CEP, Bezerra LEA, Paiva SV, Tavares TCL, Garcia TM, Araújo JTC, Campos CCG, Ferreira SMA, Matthews-Cascon H, Frota A, Mont'Alverne TCB, Silva ST, Rabelo EF, Barroso CX, Freitas JEP, Melo Júnior M, Campelo RPS, Santana CS, Carneiro PBM, Santos BA, Oliveira Filho EC, Thompson CC, Thompson FL (2022) The most extensive oil spill registered in tropical oceans (Brazil): The balance sheet of a disaster. *Environ Sci Pollut Res* 29:19869-19877.
- Soclo HH, Garrigues P, Ewald M (2000) Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: Case studies in Cotonou (Benin) and Aquitaine (France) areas. *Mar Pollut Bull* 40:387-396.

- Soriano JA, Viñas L, Franco MA, et al (2006) Spatial and temporal trends of petroleum hydrocarbons in wild mussels from the Galician coast (NW Spain) affected by the Prestige oil spill. *Sci Total Environ* 370:80-90.
- Tiwari M, Sahu SK, Pandit GG (2017) Distribution of PAHs in different compartment of creek ecosystem: Ecotoxicological concern and human health risk. *Environ Toxicol Pharmacol* 50:58-66.
- United States Environmental Protection Agency (USEPA) (1989) Risk Assessment Guidance for Superfund (RAGS), Volume I: Human Health Evaluation Manual (Part A). EPA/540/1-89/002. United States Environmental Protection Agency, Washington, DC
- United States Environmental Protection Agency (USEPA) (2005) Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001F. United States Environmental Protection Agency, Washington, DC
- Viñas L, Franco MA, Soriano JA, González JJ, Pon J, Albaigés J (2010) Sources and distribution of polycyclic aromatic hydrocarbons in sediments from the Spanish northern continental shelf: assessment of spatial and temporal trends. *Environ Pollut* 158:1551–1560
- Wade TL, Atlas EL, Brooks JM, Kennicutt MC II, Fox RG, Sericano J, Garcia-Romero B, DeFreitas D (1988) NOAA Gulf of Mexico status and trends program: trace organic contaminant distribution in sediments and oysters. *Estuaries* 11:171–179
- Wade TL, Cantillo AY (1994) Use of standards and reference materials in the measurement of chlorinated hydrocarbon residues. National Oceanic and Atmospheric Administration (NOAA), Silver Spring, MD
- Wade TL, Sericano JL, Gardinali PR, Wolff G, Chambers L (1998) NOAA's Mussel Watch Project: current-use organic compounds in bivalves. *Mar Pollut Bull* 37:20–26
- Xia Z, Duan X, Qiu W, Liu D, Wang B, Tao S, Jiang Q, Lu B, Song Y, Hu X (2010) Health risk assessment on dietary exposure to polycyclic aromatic hydrocarbons (PAHs) in Taiyuan, China. *Sci Total Environ* 408:5331–5337
- Yim UH, Kim NS, Ha SY, An JG, Kim SH, Shim WJ (2002) Identification of PAH sources in bivalves and sediments 5 years after the Sea Prince oil spill in Korea. *Environ Forensics* 3:143–154
- Yu ZL, Lin Q, Gu YG, Ke CL, Sun RX (2016) Spatial-temporal trend and health implications of polycyclic aromatic hydrocarbons (PAHs) in resident oysters, South China Sea: a case study of the eastern Guangdong coast. *Mar Pollut Bull* 110:203–211

Funding

This study was developed within the Graduate Program in Geochemistry: Petroleum and Environment (POSPETRO), Federal University of Bahia, with financial support from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) (Finance Code 001; AUXPE No. 29/2020), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Grant Nos. 440829/2020-8 and 440784/2020-4), the Brazilian Ministry of Health (Decentralized Execution Agreement – TED No. 49/2021), the Ministério da Ciência, Tecnologia e Inovação (MCTI), through the INCT AmbTropic – Phase II (CNPq Grant No. 465634/2014-1), and the European Union's Horizon 2020 Research and Innovation Programme (Grant Agreement No. 862923). R.K.P. Kikuchi, T. Combi, and

A.C.R. Albergaria-Barbosa have received research support from CNPq (314270/2023-0, 307484/2022-0, and 309648/2023-8, respectively).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by Tatiane Combi and João Bosco Gusmão. Resources were provided by Tatiane Combi, Ana Cecília Rizzatti de Albergaria-Barbosa, Carlos Alexandre Domingos Lentini, Ruy Kenji Papa de Kikuchi, and Rita de Cássia Franco Rego. Statistical analyses were performed by Gabriel Izar Mendes. Validation and methodological analyses were performed by Carlos Henrique Cordeiro de Amaral, Basílio Magno Tavares Sotão Neto, Mariana dos Santos Figueiredo de Freitas, Arthur Henrique Silva de Assis, and Yasmin Caroline Anunciação Gomes. Investigation was performed by Carlos Henrique Cordeiro de Amaral, Gabriel Izar Mendes, and Basílio Magno Tavares Sotão Neto. The first draft of the manuscript was written by Ana Cecília Rizzatti de Albergaria-Barbosa and Carlos Henrique Cordeiro de Amaral, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Figures

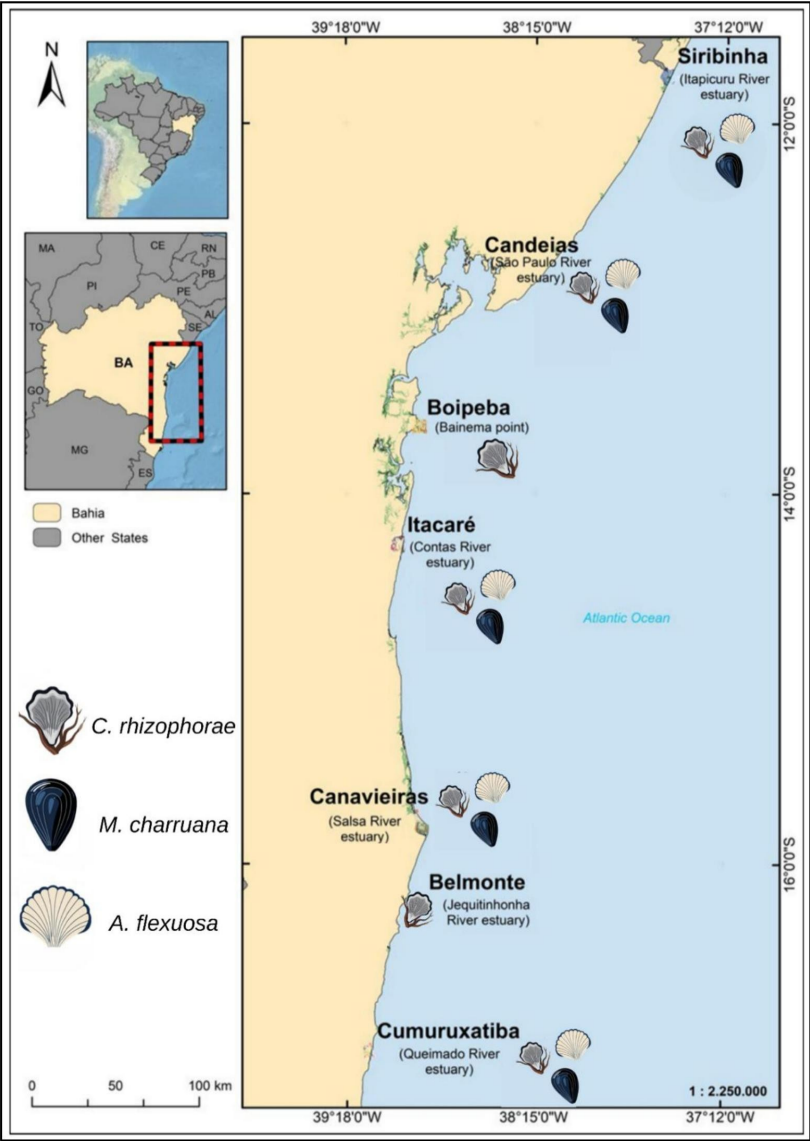


Fig. 1. Geographical location of the Bahia coast and sampling areas of the present study indicating the species found in each region.

Figure 1

See image above for figure legend

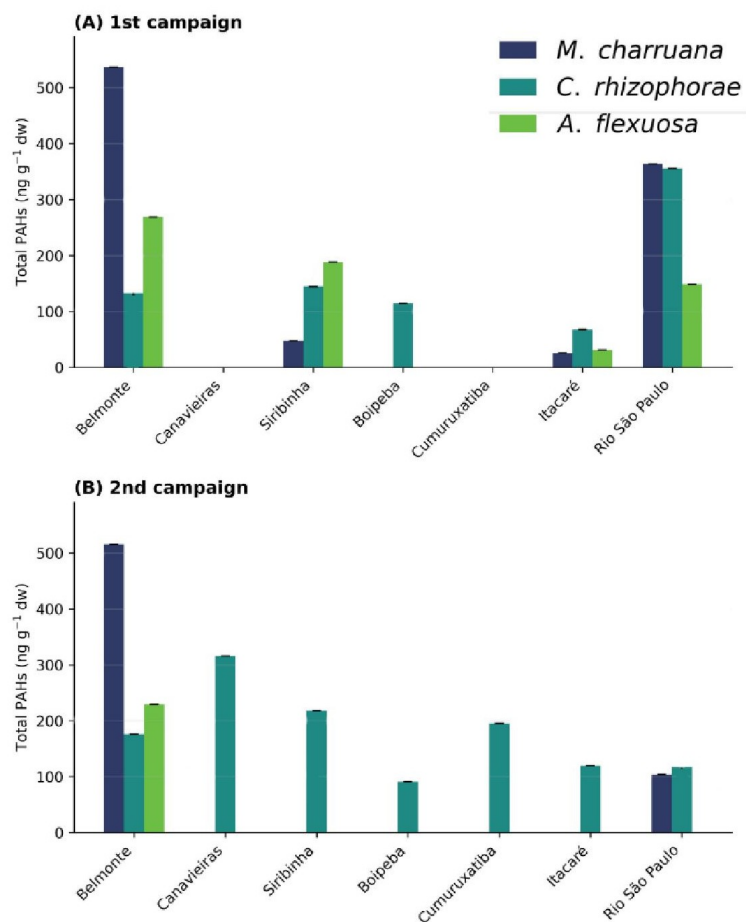


Fig. 2. Total concentration of the 16 priority PAHs (dry weight, ng g⁻¹) in bivalve samples collected from seven different areas and two periods evaluated in the present study.

Figure 2

See image above for figure legend

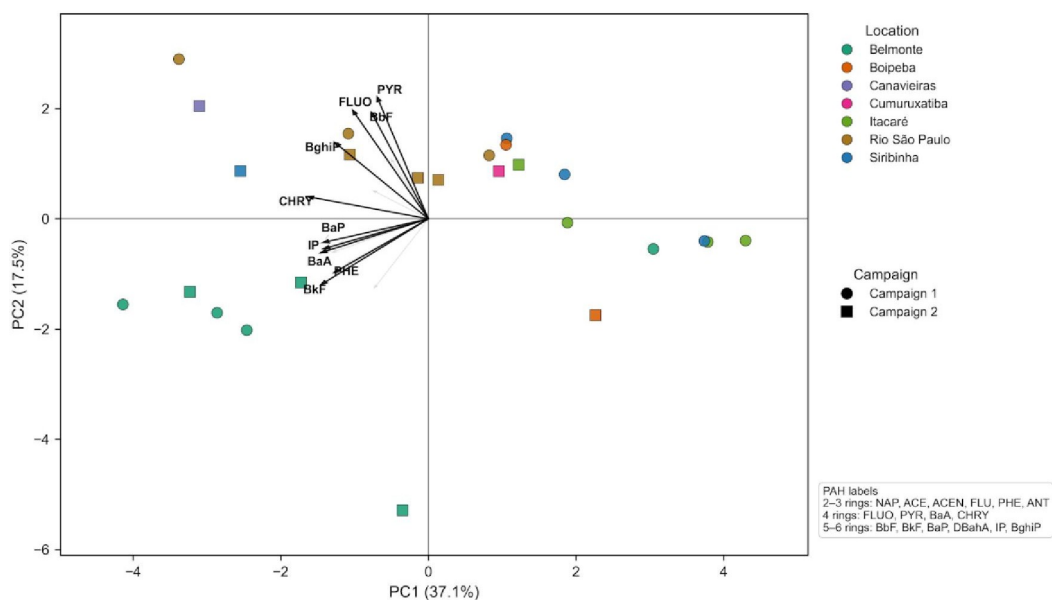


Fig. 3. Principal Component Analysis (PCA) biplot of PAHs concentrations in bivalve samples, expressed on a dry weight basis. The points represent individual samples, colors indicate sampling locations, and symbols indicate sampling campaigns. The arrows represent the PAHs loading vectors, and only the most influential loadings are labeled to improve visual clarity.

Figure 3

See image above for figure legend

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Amaral et al. Online resources.pdf](#)