**An Acad Bras Cienc (2022) 94(Suppl. 2): e20211392 DOI 10.1590/0001-3765202220211392**
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CHEMICAL SCIENCES

# Community-based assessment of marine resources contamination after a large-scale oil spill

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**Abstract:** As of August 2019, several oil slicks reached the Brazilian coast, compromising local ecosystems and the economy of coastal communities. In this context, this study aimed to assess seafood quality at the Canavieiras Extractive Reserve (RESEX), located in the state of Bahia, Northeastern Brazil, by determining polycyclic aromatic hydrocarbons (PAHs) concentrations in local biota following the oil spill. It was designed and carried out in a participatory manner, involving RESEX fishers, shellfish and crab gatherers and managers, as well as university researchers. A total of 72 biota samples were analyzed, and the sum of the 16 priority USEPA PAHs ranged from less than the limit of detection to 30.81 ng $g^{-1}$ (w.w.). When also considering alkylated compounds, concentrations ranged from 3.53 to 360.75 ng $g^{-1}$ (w.w.). No sample contained PAHs concentrations suggestive of human consumption risks. No difference in PAHs concentrations between the six studied areas and regarding different biota feeding habits were observed. Higher PAHs concentrations were generally noted in molluscs, as these organisms do not have enzyme systems capable of metabolizing these compounds. This initiative demonstrated the feasibility of participatory construction resulting in a study concerning valued species to local communities, ensuring a contribution to local fishing and marketing activities.

**Key words:** Brazil, crustaceans, fish, molluscs, petroleum pollution, polycyclic aromatic hydrocarbons.

## INTRODUCTION

The initial event of what is now considered the greatest coastal environmental disaster in Brazil took place on August 30th, 2019, when the first orphan oil slick reached the Brazilian coast, namely the coast of the state of Paraíba. Oil slicks reached several points of the coast, in a continuous dispersion process according to the dominant current system. According to Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) estimates, about 1,000 km of the Brazilian coast were affected by these oil slicks (IBAMA 2020).

Although until September 2021 the exact source of the oil had not been confirmed, subsequent analyses indicated its origin as crude oil from Venezuela, either highly degraded due to its time at sea or comprising a product containing heavy oil produced in that country (Oliveira et al. 2020).

The leak was estimated at 5,000 to 12,500 $m^3$ of oil and postulated as originating from a continuous release from a vessel ranging from the size of a PANAMAX to a SUEZMAX, containing between 60,000-80,000 and 120,000-170,000 deadweight tonnage (Zacharias et al 2021). Following investigations, finally, in December 2021, the Brazilian Federal Police indicated the

Boubolina tanker as responsible for the oil spill (Official Press of the Brazilian Government 2021).

Several local coastal systems were affected, such as beaches, estuaries, reefs and mangroves, profoundly compromising not only important and sensitive ecosystems and biota, but also the local economies and coastal communities that inhabit the affected regions, especially concerning fishing activities, the tourism sector and commerce (Araújo et al. 2020, Magris & Giarrizzo 2020).

Although the IBAMA began collecting oil from the Brazilian coast on September 2nd, 2019, the National Contingency Plan was only activated on October 11th, 2019, and the first mention of the Monitoring and Action Group (Grupo de Acompanhamento e Ação - GAA) was only noted on October 14th (GAA 2020). This delay by the competent authorities has raised many criticisms, especially from fishers, NGOs and scientists.

As a reaction to the authorities' unresponsiveness in presenting effective actions to prevent and battle the continuously dispersing oil, several communities organized themselves and began cleaning and containment actions regarding the multiplying oil stains spreading along the Brazilian coast at the time (Pena et al. 2020, Brum et al. 2020). A large part of the battle against oil carried out by volunteers was performed without adequate protection, and several volunteers were directly exposed to the oil, putting their health at risk. Soonafter, alerts were projected by specialists and health institutions concerning these risks. In addition to exposure to the crude oil itself, the economic consequences caused by this disaster contributed to mental health disorders, especially in populations that suffer greater socioeconomic vulnerability. Nevertheless, the voluntary actions of coastal communities alongside the Civil Defense and IBAMA, strongly reduced the disaster effects in affected ecosystems, as highlighted by Craveiro et al. (2021).

In parallel with the attempts to contain the oil and clean up affected systems, several concerns and uncertainties regarding the contamination and quality of marine resources (fish, crustaceans and molluscs) exploited for local subsistence and commercialization were noted. The manifestations of authorities, scientists and institutions on the quality and suitability of fish from different areas of the Northeast for consumption multiplied in 2019 and 2020. However, many of these manifestations were either not based on chemical analyses on the products themselves or involved a reduced number of samples obtained from different sampling points, such as the monitoring carried out by Bahia Pesca (State Fisheries Agency of Bahia) and the Ministry of Agriculture, Livestock and Supply (Bahia Pesca 2019a, b). This scenario resulted in the mistrust, insecurity and uncertainty of the local populations, especially fishers and shellfish and crab gatherers who depend on the sale and consumption of their products for survival and who were already suffering hardships, due to consumers fears regarding product purchasing.

The nature of the spill, with no identified responsible party, and its spatial and temporal scope (mainly from September 2019 to January 2020 with circa 3000 km of coastline affected in different magnitudes), made the battle against pollution even more complex. The economic issues arising from the oil pollution were aggravated by the arrival of the COVID pandemic which, although of a different nature, affected the same activities as the oil spill, such as local tourism, which was still recovering from the ecological disaster (Câmara et al. 2021), while also decreasing available funds for the restoration of oil-affected ecosystems (Magalhães et al. 2021).

In this context, this study emerged from a citizen science perspective, aimed at evaluating the quality of seafood caught, consumed and marketed by the Canavieiras Extractive Reserve (RESEX) fishing and extractive communities, in the state of Bahia, Northeastern Brazil. The study was developed and carried out in a participatory manner, involving fisher and extractive communities, RESEX managers, environmental analysts, and researchers, who jointly decided on sites and species to be collected. Canavieiras seafood contamination was, therefore, evaluated by determining polycyclic aromatic hydrocarbons (PAHs) concentrations, the most toxic fraction of oil and derivatives.

## MATERIALS AND METHODS

### Study Area

This study was carried out at the Extractive Reserve (RESEX) located in Canavieiras (Bahia, Northeastern Brazil) (Figure 1). The local climate according to the Koppen classification comprises the Af type, hot and humid rainy, with an average temperature of 24 °C and an average annual rainfall of 1,648 mm, with the rainy period

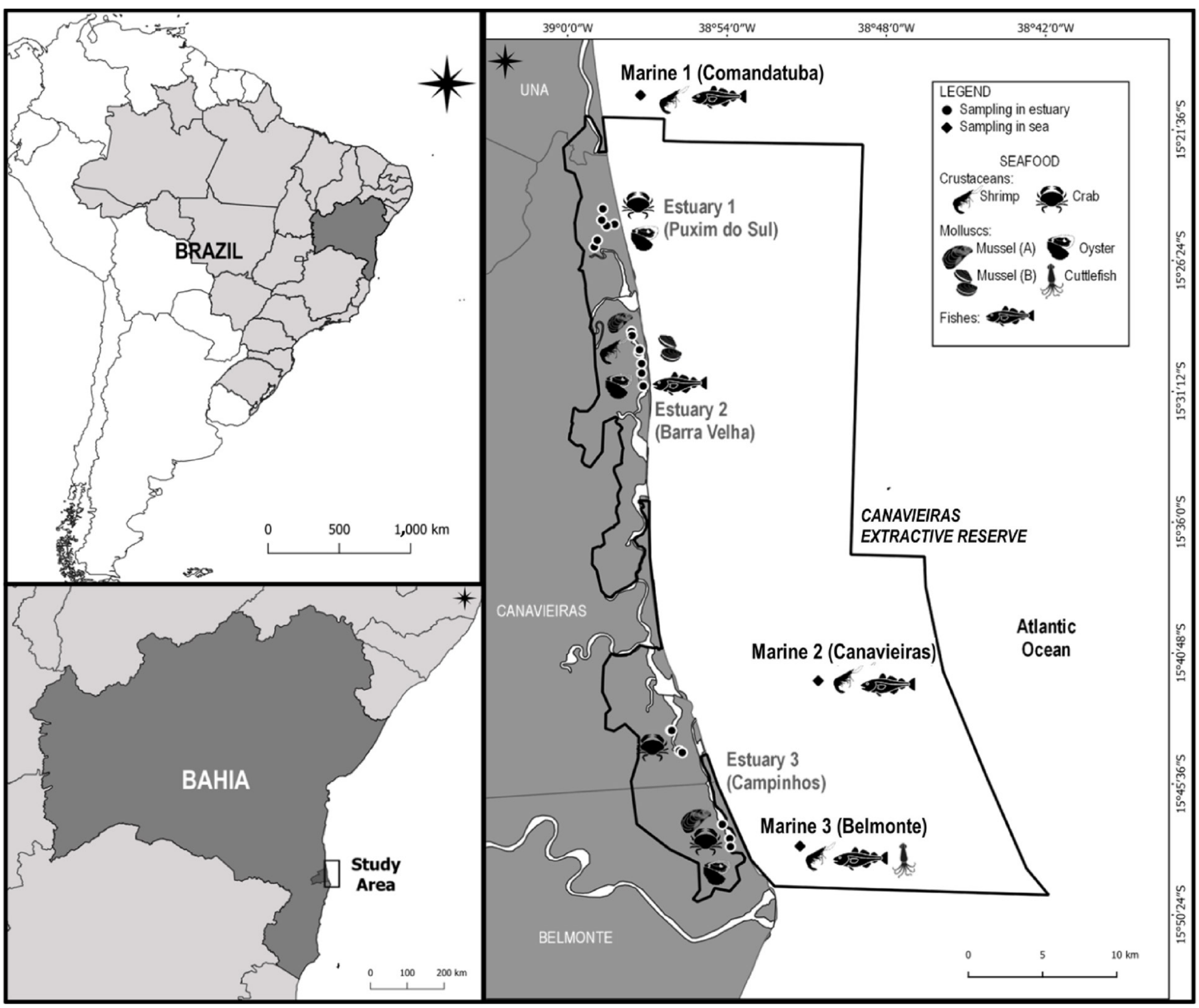


**Figure 1. Location of sampling stations in estuaries (3 sites) and sea (3 sites), as well as the sampling biota in the Canavieiras region. Crustaceans: shrimps and crabs; molluscs: mussels, shellfishes, oysters and squid; and fishes.**

occurring between January and February and the dry period, between August and September (PNMA Canavieiras 2016). The area is subject to a mesotidal regime, with spring tides presenting a maximum amplitude of 2.4 m (Nascimento 2006).

The Canavieiras RESEX, created in 2006, is located mostly in the Canavieiras municipality, and partially in the municipalities of Belmonte to the South and Una to the North. It occupies approximately 100,000 hectares, distributed throughout marine environments (82.33%), rivers and mangroves (12.01%), *restingas* (4.82%) and beaches (0.38%) (PNMA Canavieiras 2016, Nascimento & Dominguez 2010). The Canavieiras human population numbered 32,336 inhabitants in 2010, with a population density of 24.37 hab/km² (IBGE 2010). It is one of the most fish dense areas in Bahia, and one of the main sources of income and survival of artisanal marine and estuarine extractive fishers, with fish and shrimp most noteworthy in the coastal strip and crab and molluscs in estuaries, mainly in mangroves protected by the Canavieiras RESEX (Curado 2014, Dumith 2018).

The fishing and extractive communities distributed in the North-South direction, namely Oiticica, Puxim de Fora, Puxim do Sul, Barra Velha, Atalaia and Campinhos, form an important economic sector in this municipality, which exploit Canavieiras RESEX resources (Aguiar 2011, PNMA Canavieiras 2016). According to ICMBio (Chico Mendes Institute for Biodiversity Conservation - Ministry of the Environment) registration data, 1,866 user families were located within the Canavieiras RESEX between 2013 and 2014, totaling 5,819 people registered as direct RESEX beneficiaries. Of this total, about 1,550 families (83%) reside in the Canavieiras municipality, while 15% reside in Belmonte and 1.7%, in Una (Dumith 2018).

Extractive fishing production in the municipality of Canavieiras is represented by sea bass, mullet, among other fish, as well as shrimp, crabs, prawns, and lobsters (Aguiar 2011). Local fishing activities are characterized as artisanal and organized by local fishing communities, with catches mostly destined for commercialization. Corporate fishing is also present and takes place at sea, according to reports of communities and fishermen associated with the Canavieiras Extractive Reserve Association (AMEX) (Aguiar 2011).

**Sampling strategy**

The sampling design was developed alongside RESEX fishers and shellfish and crab gatherers, UERJ researchers and ICMBio managers to assess the main seafood species obtained for local consumption and marketing in the Canavieiras region.

Biota samples were collected between November 16 and 20, 2019. Sampling was carried out jointly by shellfish and crab gatherers and fishers from the RESEX and the LAGOM/UERJ team at the main collection and fishing sites indicated by local fishers, totaling six sampling areas (Figure 1), three located in estuarine areas (Campinhos, Barra Velha and Puxim do Sul) and three in marine areas (Comandatuba, Canavieiras, Belmonte). Whenever possible, samples were collected in triplicate, to prepare three composite samples per species and per sampling location (Table I).

*Mytella* sp., *Ucides cordatus*, *Callinectes exasperatus*, *Goniopsis cruentata*, *Crassostrea* sp., *Anomalocardia brasiliana*, *Mugil curema*, *Callinectes danae*, *Litopenaeus schmitti* and *Lucina pectinata* were obtained at the three estuaries, totaling 49 analyzed samples. Of these, *Callinectes exasperatus*, *Crassostrea* sp. and *Goniopsis cruentata* were obtained from all three estuaries. *Xiphopenaeus kroyeri*,

**Table I.** **PAH concentrations, in ng $g^{-1}$, feeding habit and size (length) of organisms collected in the Canavieiras region after the 2019 oil spill in the Brazilian coast.**

| Sampling Station | Organism | TYPE[1] | 16 HPAs | Total HPAs | Feeding habit[2] | Length (cm) |
|---|---|---|---|---|---|---|
| E1 | Goniopsis cruentata | C | 4,.98 ±3.49 | 28.09 ± 14.34 | omnivorous/sediment | 2.9 - 4.9 |
| E1 | Ucides cordatus | C | 15.56 ± 2.66 | 101.86 ± 47.60 | omnivorous/sediment | 6.2 - 8.5 |
| E1 | *Crassostrea* sp. | M | 11.62 ± 16.63 | 24.86 ± 22.95 | omnivorous/filter or pelagic | 6.0 - 9.1 |
| E1 | *Mytella* sp. | M | 5.65 ± 2.14 | 43.14 ± 28.17 | omnivorous/filter or pelagic | 5.4 - 7.0 |
| E1 | Callinectes exasperatus | C | 5.90 ± 5.43 | 25.14 ± 10.96 | omnivorous/sediment | 10.1 - 12.8 |
| E2 | Goniopsis cruentata | C | 4.83 ± 3.11 | 27.04 ± 16.42 | omnivorous/sediment | 4.1 - 5.4 |
| E2 | Anomalocardia brasiliiana | M | 6.38 ± 5.01 | 38.36 ± 14.48 | omnivorous/filter or pelagic | 3.0 - 3.9 |
| E2 | *Crassostrea* sp. | M | 6.04 ± 4.22 | 35.14 ± 19.90 | omnivorous/filter or pelagic | 7.9 - 11.6 |
| E2 | Callinectes exasperatus | C | 1.83 ± 1.16 | 12.30 ± 3.20 | omnivorous/sediment | 10.1 - 12.1 |
| E2 | Mugil curema | F | 2.53 ± 1.42 | 15.90 ± 4.44 | omnivorous/filter or pelagic | 19.0 - 28.0 |
| E2 | Callinectes danae | C | 6.99 ± 5.06 | 31.69 ± 25.12 | omnivorous/sediment | 7.8 - 11.2 |
| E2 | *Mytella* sp. | M | 7.96 ± 3.15 | 54.13 ± 23.38 | omnivorous/filter or pelagic | 5.0 - 6.8 |
| E2 | Litopenaeus schmitti | C | 4.10 | 33.45 | omnivorous/sediment | 2.3 - 5.4[3] |
| E3 | Lucina pectinata | M | 5.53 ± 1.04 | 43.34 ± 7.47 | omnivorous/filter or pelagic | 3.1 - 5.4 |
| E3 | Callinectes exasperatus | C | 4.06 ± 3.84 | 20.15 ± 21.58 | omnivorous/sediment | 11.2 - 12.5 |
| E3 | Goniopsis cruentata | C | 6.53 ± 9.98 | 23.66 ± 25.34 | omnivorous/sediment | 3.6 - 4.5 |
| E3 | *Crassostrea* sp. | M | 13.10 ± 5.33 | 35.02 ± 12.43 | omnivorous/filter or pelagic | 3.5- 10.0 |
| M1 | *Cynoscion* sp. | F | 13.22 | 41.70 | carnivore | 19.0 - 24.0 |
| M1 | Xiphopenaeus kroyeri | C | 4.36 ± 2.81 | 24.11 ± 4.41 | omnivorous/sediment | 2.6 - 13.9[3] |
| M1 | Litopenaeus schmitti | C | 7.27 | 21.45 | omnivorous/sediment | 7.2 - 39.3[3] |
| M1 | Rajidae | F | 1.91 ± 1.43 | 17.75 ± 9.27 | carnivore | n.d.[4] |
| M2 | Lutjanus synagris | F | 3.83 ± 0.55 | 17.87 ± 15.95 | carnivore | 27.0 - 43.0 |
| M2 | Larimus breviceps | F | 4.45 | 18.38 | carnivore | 7.0 - 10.0 |
| M2 | Litopenaeus schmitti | C | 4.02 | 31.14 | omnivorous/sediment | 8.1 - 35.6[3] |
| M2 | *Cynoscion* sp. | F | 4.60 | 18.68 | carnivore | 9.0 - 11.0 |
| M3 | Litopenaeus schmitti | C | 1.49 | 12.27 | omnivorous/sediment | 14.1 - 35.1[3] |
| M3 | Paralonchurus brasiliensis | F | 2.33 | 13.39 | omnivorous/sediment | 5.0 - 13.0 |
| M3 | Lolliguncula brevis | M | 6.50 | 20.36 | carnivore | n.d.[4] |
| M3 | *Anchoa* sp. | F | 17.24 | 36.34 | omnivorous/filter or pelagic | 9.4 - 12.9 |
| M3 | *Cynoscion* sp. | F | 4.39 | 14.00 | carnivore | 12.0 - 13.0 |
| M3 | Odonthognathus mucronatus | F | 21.60 | 52.82 | carnivore | 11.7 - 14.4 |
| M3 | Xiphopenaeus kroyeri | C | 12.52 ± 15.85 | 136.56 ± 194.24 | omnivorous/sediment | 3.9 - 11.6 |

*Litopenaeus schmitti*, *Cynoscion* sp., *Rajidae*, *Lutjanus synagris*, *Paralonchurus brasiliensis*, *Odonthognathus mucronatus*, *Lolliguncula brevis*, *Anchoa* sp. and *Larimus breviceps* were obtained from the marine areas, totaling 23 samples. *Litopenaeus schmitti* and *Cynoscion* sp. were obtained from all three sea sampling areas. In all, 72 biota samples from the Canavieiras region were analyzed, comprising fish (eight species, one estuarine and seven marine), molluscs (five species, four estuarine and one marine) and crustaceans (seven species, five estuarine and two marine). All samples were wrapped in aluminum foil, identified, frozen in plastic bags, placed in coolers and transported to the UERJ laboratory in Rio de Janeiro.

## Analytical procedures

At the laboratory, samples were thawed and individuals belonging to the same species were dissected to remove usually consumed tissues, for fishes, muscle, and for crustaceans and molluscs, all soft tissues. The number of dissected individuals varied as a function of the mass of each species. Thus, composite samples were prepared containing from 3 to 20 individuals. Each composite sample was then homogenized in a tissuemeizer-type cell disruptor equipment and transferred to glass vials where they were maintained frozen until analysis.

For the analyses, about 5 g of each wet homogenized sample were weighed and mixed with anhydrous sodium sulfate and 100 ng of a surrogate p-terphenyl-$D_{14}$ standard. PAHs extraction was based on the EPA 3540C method (EPA 1986a), employing Soxhlet and a dichloromethane:acetone (9:1, v/v) solution for 24 hours. Purification was carried out by eluting the extract through a glass column filled with 2% deactivated alumina. The aromatic fraction was then obtained by liquid chromatography employing an open silica/alumina column, according to the EPA 3630C method (EPA 1986b). The extracts were concentrated, made-up to 1 mL and internal quantification standards (naphthalene-D8, acenaphthene-D10, phenanthrene-D10, chrysene-D12 and perylene-D12) were added at a final concentration of 100 ng $mL^{-1}$.

All 16 priority USEPA PAHs, namely naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k) fluoranthene, benzo(a)pyrene, indene (1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene; in addition to benzo(e) pyrene, dibenzothiophene and perylene, were determined, as well as alkylated PAHs C1 to C4-naphthalenes, C1 to C3-fluorenes, C1 to C4-phenanthrenes, C1 to C3-dibenzothiophenes, C1 and C2-pyrenes, C1 and C2 – chrysenes.

The PAHs analysis methodology followed the EPA-8270D (EPA 1986c) method with modifications. Quantification was performed by internal standardization and calibration curves containing the authentic standards of non-alkylated PAHs from 2 to 1000 ng $L^{-1}$ on a GCMS ITQ Thermo equipment in the selective ion monitoring (SIM) mode. A VF-5MS column (30m, 0.174 mm in diameter and 0.25 mm of film) was used, and the following programming was set: 50°C/5.0 min; 6°C/min up to 280°C holding time 8 min; 12.0 °C/min up to 305°C holding time 7 min. The average recovery of the p-terphenyl-D14 surrogate standard in the analyzed samples was 83.6%. Analytical blanks were also analyzed to ensure the quality of the PAHs determinations. The limit of quantification for each compound was 0.34 ng $g^{-1}$ (w.w.) and the limit of detection for each compound ranged from 0.02 to 0.10 ng $g^{-1}$ (w.w.).

### Data analysis

All statistical analyses were performed using the Statistic 7.0 software (Star Soft. Inc., Tulsa, OK, USA). Data normality was verified by the Shapiro-Wilk test. Differences in PAHs concentrations according to taxonomic group, sampling site and feeding habit were assessed by the Kruskal-Wallis tests followed by a pos-test for multiple mean rank comparisons for all groups. The significance level was set at p = 0.05 for all cases.

## RESULTS AND DISCUSSION

The sum of the 16 USEPA PAHs ranged from less than the limit of detection (0.34 ng $g^{-1}$) for *Lutjanus synagris*, sampling station M2 to 30.81 ng $g^{-1}$ (w.w.) for *Xiphopenaeus kroyeri*, sampling station M3. When also considering alkylated compounds, dibenzothiophene and its alkylated homologues and benzo(e)pyrene (Total PAHs), PAHs concentrations ranged from 3.53 ng $g^{-1}$ (w.w.) (*Crassostrea* sp., sampling station E1) to 360.75 ng $g^{-1}$ (w.w.) (*Xiphopenaeus kroyeri*, sampling station M3).

Replica number 3 of the *Xiphopenaeus kroyeri* samples from the M3 sampling station is noteworthy, as it contained not only the highest PAHs concentration, but also due to its PAHs distribution profile (Figure 2), typical of petroleum or derivative product contamination, with a predominance of alkylated compounds, especially from the naphthalene, fluorene and phenanthrene series. Therefore, the organisms that make up this sub-sample seem to have had contact with a product of this nature. However, the other *Xiphopenaeus kroyeri* replicas collected at the same sampling station, M3, presented much lower Total PAHs concentrations (18.76 and

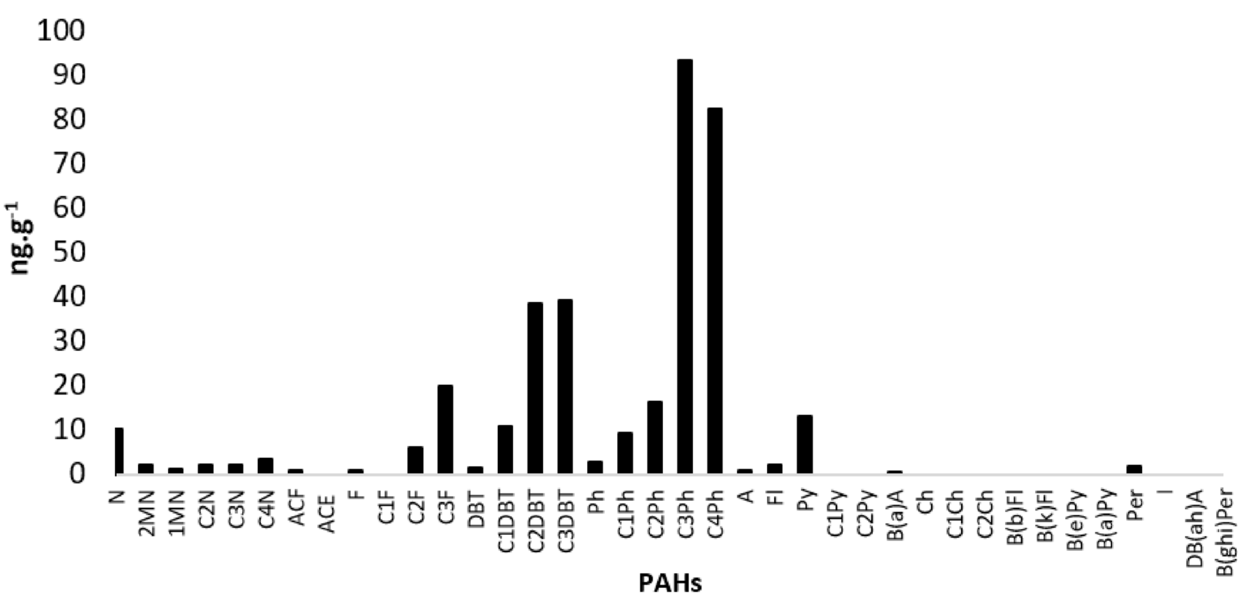


**Figure 2. N:** Naphthalene; **2MN: 2Methyl Naphthalene; 1MN: 1Methyl Naphthalene; C2N: C2 Naphthalene; C3N: C3 Naphthalene; C4N: C4 Naphthalene; ACF: Acenaphthylene; ACE: Acenaphthene; F: Fluorene; C1F: C1 Fluorene; C2F: C2 Fluorene; C3F: C3 Fluorene; DBT:** Dibenzothiophene; **C1DBT: C1 Dibenzothiophene; C2DBT: C2 Dibenzothiophene; C3DBT: C3 Dibenzothiophene; Ph:** Phenanthrene; **C1Ph: C1 Phenanthrene; C2Ph: C2 Phenanthrene; C3Ph: C3 Phenanthrene; C4Ph: C4 Phenanthrene; A:** Anthracene; **Fl:** Fluoranthene; **Py: Pyrene; C1Py: C1 Pyrene; C2Py: C2 Pyrene; B(a)A: Benzo(a)anthracene; Ch: Chrysene; C1Ch: C1 Chrysene; C2Ch: C2 Chrysene; B(b)Fl: Benzo(b) fluoranthene; B(k)fl: Benzo(k)fluoranthene; B(e)Py: Benzo(e)pyrene; B(a)Py: Benzo(a)pyrene; Per:** Perylene; **I: Indeno(1,2,3-cd)pyrene; DB(ah)A: Dibenzo(a, h)anthracene; B(ghi)Per: Benzo(g,h,i)perylene.**

30.17 ng $g^{-1}$ w.w.). Therefore, the contamination detected in the aforementioned replica seems to be unique and does not reflect the local environmental contamination. This, alongside the relatively high standard deviations of the replicate concentration values determined in the analyzed samples (Table I), highlights the importance of working with replicates and composite samples when evaluating biota contamination, especially concerning risks associated to their consumption as foodstuffs.

Although the predominance of the alkylates PAHs in relation of their parental compounds determined in replica 3 of the *Xiphopenaeus kroyeri* from the M3 sampling station (Figure 2) can be associated with weathering effect on spilled oil, considering the biota mechanism of absorption and metabolization of oil, some unpredictable compounds assemblages may occur, different from the original product spilled. So, it is difficult to ensure, even in this more contaminated sample, that there is weathered spill product signature.

The statistical analyses revealed no difference between the PAHs concentrations among the three estuarine areas or the three marine sampling sites (Kruskal-Wallis test, $p < 0.5$), indicating that all areas are subjected to the same conditions regarding PAHs contamination. A predominance of 2- to 3-aromatic ringed compounds was noted (Figure 3), which, alongside the presence of alkylated compounds, is a typical distribution resulting from contamination by oil and derivative products (Pampanin 2017).

When comparing the PAHs concentrations detected in fish, crustacean and molluscs tissues (Figure 4), higher concentrations were observed in the latter, which are filtering organisms and do not have enzymatic systems capable of metabolizing PAHs, corroborating that the metabolic capacity for PAHs follows the order fish > crustaceans > molluscs (Stegeman & Lech 1991, Livingstone, 1994).

The sampled animals were classified according to their feeding habits as: (i) omnivorous associated to the sediment; (ii) omnivorous filters or pelagic; (iii) carnivores (Table I). Considering these 3 feeding habits the Kruskall-Wallis test applied ($p < 0.05$)

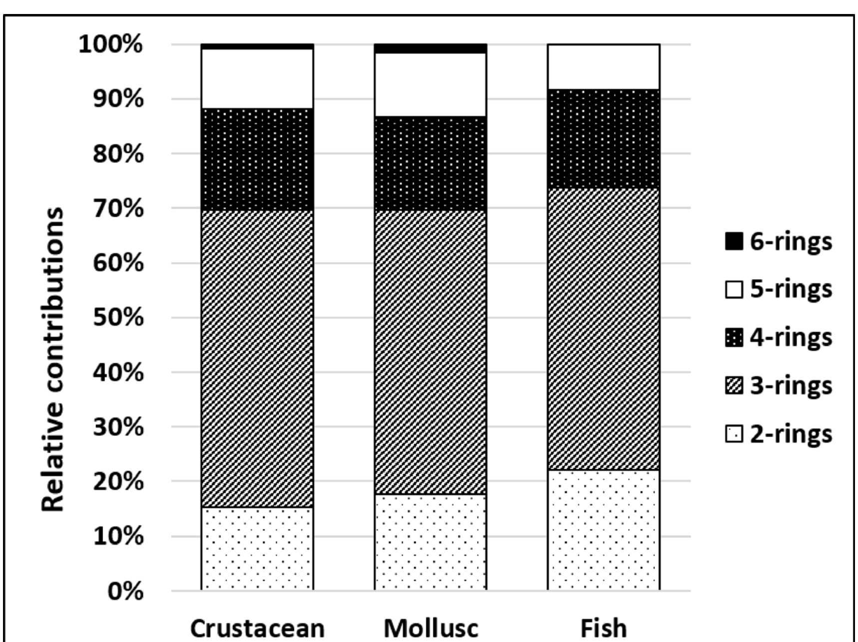


**Figure 3. Relative contribution of PAHs from 2 to 6 aromatic rings in samples of crustaceans, molluscs and fish collected in the Canavieiras region after the 2019 oil spill in the Brazilian coast.**

revealed no difference between the PAHs values determined in these organisms, highlighting the absence of feeding habit influence in the final PAHs concentrations in the analyzed tissues.

The mean concentrations of the 16 USEPA HPAs in molluscs (Table I) sampled from the investigated estuaries ranged from 5.53 ng $g^{-1}$ (w.w.) (*Lucina pectinata* from sampling station E1) to 13.10 ng $g^{-1}$ (w.w.) (oyster, from sampling station E1). Total PAHs for these same samples ranged from 24.86 ng $g^{-1}$ (w.w.) (oyster, from sampling station E3) to 54.13 ng $g^{-1}$ (w.w.) (*Mytella* sp., from sampling station E2).

When comparing these concentrations with those presented in Table II, which compiles PAHs concentrations in biota worldwide, Canavieiras molluscs present the lowest PAHs concentrations for both Total PAHs and the sum of the 16 USEPA PAHs. However, comparisons between data presented in Table II should be performed carefully, as the concentrations detected in each species are due to several synergistic factors, such as exposure, incorporation, metabolism and fat content (Massone et al. 2021)

Considering only oysters, Table II presents studies carried out after oil spills, and it appears that, although PAHs concentrations in oysters from Canavieiras are relatively low, they are of the same order of magnitude, for example, as those reported by Fernando et al. (2019) 18 months after the Deep Horizon spill in the Gulf of Mexico for 121 oyster samples from the states of Louisiana, Alabama and Mississippi, in the US. The concentrations detected in Canavieiras oysters are also comparable to those reported by Kelly et al. (2008) for samples obtained off the coast of Lebanon at the most severely affected area 10 months after an oil spill, whereas concentrations in other studies (Table II) ranged between 20 and 188 ng $g^{-1}$ (w.w.). Again, concerning oysters, the samples analyzed by Loh et al. (2017) are noteworthy due to their very high concentrations, ranging from 627 to 81,000 ng $g^{-1}$, especially samples collected four days after an oil spill in Korea.

Regarding other molluscs from Canavieiras, no studies were found in the literature describing PAHs concentrations in the sampled species,

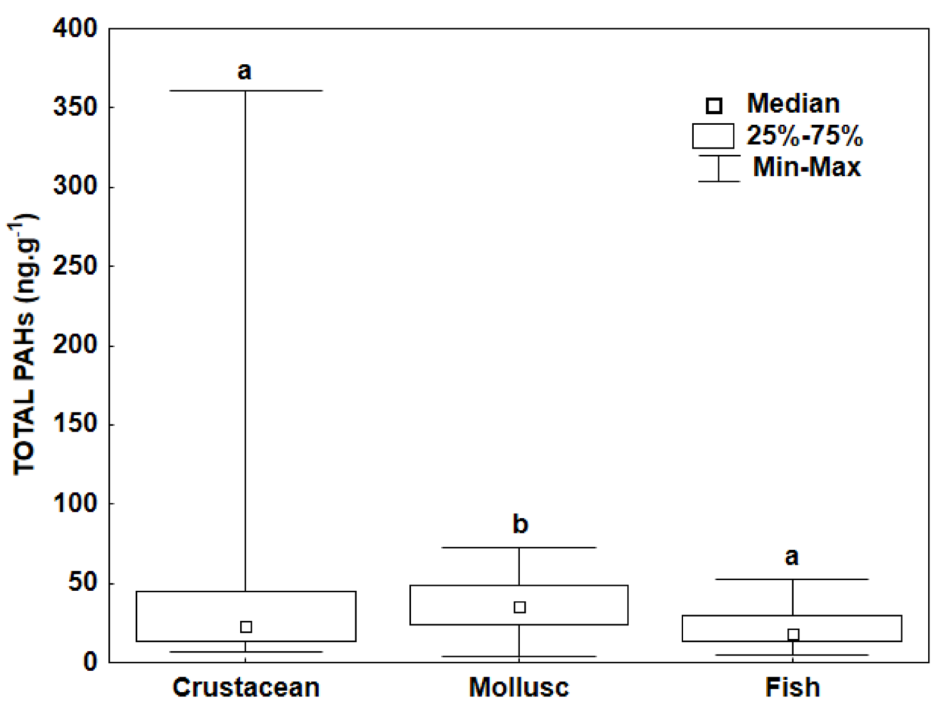


**Figure 4.** Comparison of PAH concentrations determined in organisms collected in the Canavieiras region after the 2019 oil spill in the Brazilian coast. Kuskall-Wallis test result: p = 0.0168. The letters indicate the post-test results.

and therefore, their values were compared with other bivalves (*Mytilus galloprovincialis* and *Perna perna*, Table II). Similarly, to the oysters indicated above, bivalves from Canavieiras contained PAHs concentrations in the same order of magnitude or lower than other studies listed in Table II carried out in chronically impacted areas, especially Guanabara Bay, in Rio de Janeiro, southeastern Brazil.

In general, the PAHs concentrations determined in shrimp from Canavieiras were low compared to other shrimp species (Table II), such as those from chronically contaminated areas in China (Sun et al. 2016, 2018) and of the same order of magnitude as those determined in samples from the Gulf of Mexico, in studies conducted after the blowout of the Deep Horizon platform (Xia et al. 2012, Fernando et al. 2019), and much lower than those described for the Arabian Gulf (0.02 to 1,029 ng $g^{-1}$ w.w - Fayad et al. 1996), even more so considering that only four PAHs were determined in the latter assessment.

As previously described for shrimp and oysters, crab species from Canavieiras presented Total PAHs concentrations of the same order of magnitude as those reported for the Gulf of Mexico (Table II - Xia et al. 2012, Fernando et al. 2019). However, when compared to other areas known to be contaminated by petroleum residues (Orbea et al. 2002, Sun et al. 2016), the sum values of the 16 USEPA PAHs were much lower, ranging in Canavieiras from 0.47 to 18.05 ng $g^{-1}$ (W.W)..

Finally, considering all fish species sampled from Canavieiras, Total PAHs concentrations ranged from < 0.34 to 21.60 ng $g^{-1}$ (w.w.) and the 16 USEPA PAHs, from 4.46 to 52.82 ng $g^{-1}$ (w.w.). Comparing these values to others listed in Table II, considering the number of compounds analyzed in each study, our findings indicate that the concentrations presented herein are among the lowest reported in the literature.

Regarding food safety, international criteria concerning certain PAHs, especially higher molecular weight PAHs displaying mutagenic and carcinogenic characteristics, are available. The European Standard EC1881, for example, established limit values for fish consumption (EUROPEAN-COMMISSION 2006), although it no longer includes fresh fish from the EC835 standard (EUROPEAN-COMMISSION 2011). Even so, recommended values for benzo(a)pyrene (BaP) are set at 2.0 ng $g^{-1}$ for fresh fish and 5.0 ng $g^{-1}$ for crustaceans and molluscs and can be employed as guide values for fish sample assessments. In this regard, BaP concentrations in all fish from Canavieiras were lower than 2 ng $g^{-1}$ and also lower than the method limit of detection to 1.21 ng $g^{-1}$. Furthermore, this compound was not detectable or was present at concentrations lower than the limit of quantification (0.34 ng $g^{-1}$) in 24% of the samples and the highest concentration (1.83 ng $g^{-1}$, ww) was observed in a *Cynoscion* sp. replica sampled from the marine region. Therefore, BaP values do not suggest contamination in fish samples due to oil spilled and, therefore, do not pose a risk for human consumption.

Concerning the analyzed molluscs and crustaceans, the highest concentrations were observed in *Goniopsis cruentata* samples from the Puxim do Sul estuary (1.5 ± 2.59 ng $g^{-1}$) and for *Litopenaeus schmitti* obtained at the Canavieiras marine area (1.46 ng $g^{-1}$), both well below 5 ng $g^{-1}$ limit recommended by the EUROPEAN-COMMISSION (2006) standard. Therefore, these species do not seem to represent food security risks.

The inclusion of a 30.0 ng $g^{-1}$ (w.w.) limit for the sum of four high molecular weight PAHs (benzo(a)pyrene, benzo(a)anthracene, benzo(b) fluoranthene and chrysene, B(a)Py+B(a)A+B(b) Fl+Ch)) has been established as quality control for marketed fish in the new European Union

**Table II.** **Data compilation for total PAH concentrations in crustaceans, molluscs and fish of Brazilian and worldwide coastal regions.**

| Seafood | Organism | Local | Total PAH (ng g$^{-1}$, w.w.) | No of Compounds | References |
|---|---|---|---|---|---|
| Shrimp | *Metapenaeopsis palmensis)* | Zhanjiang Harbor, China | 86.7 | 16 | Sun et al. 2018 |
| | *Oratosquilla nepa*<br>*Oratosquilla interrupt*<br>*Oratosquilla oratoria* | Daya Bay, China | 110 to 300 | 16 | Sun et al. 2016 |
| | *Farfantepenaeus aztecus*<br>*Litopenaeus setiferus* | Gulf of Mexico (Louisiana, Alabama and Mississipi) | 13.1 ± 2.2 to<br>107.9 ± 24.3 | 38 | Fernando et al. 2019 |
| | *Macrobrachium felicinum* | Imo River, SE Nigeria | 509.39 ± 354.2 | 22 | Dosunmu et al. 2016 |
| | *Litopenaeus schmitti* (Estuarine area) | Canavieiras, Southern Bahia, Brazil | 4.10<br>33.45 | 16<br>38 | This study |
| | *Litopenaeus schmitti* (Marine area) | | 1.49 and 7.27<br>21.45 and 31.14 | 16<br>38 | |
| | Xiphopenaeus kroyeri | | 2.50 to 30.81<br>18.76 to 360.75 | 16<br>38 | |
| Crab | *Eucrate crenata*<br>*Portunus sanguinolentus* | Daya Bay, China | 110 to 210 | 16 | Sun et al. 2016 |
| | *Callinectes sapidus* | Gulf of Mexico (Louisiana, Alabama and Mississipi) | 8.3 ± 5.6 to<br>77.2 ± 18.5 | 38 | Fernando et al. 2019 |
| | Goniopsis cruentata | Canavieiras, Southern Bahia, Brazil | 0,47 – 18,05<br>6,80 – 52,80 | 16<br>38 | This study |
| | Ucides cordatus | | 12.49 – 17.18<br>47.55 – 136.34 | 16<br>38 | |
| | Callinectes exasperatus | | 0.95 – 12.16<br>6.53 – 45.03 | 16<br>38 | |
| | Callinectes danae | | 3.47 – 12.79<br>10.53 – 59.45 | 16<br>38 | |
| Oyster | *Crassostrea gigas* | Gwangyang Bay, Korea | 627 to 110.000 | 37 | Loh et. al. 2017 |
| | *Crassostrea virginica* | Gulf of Mexico (Louisiana, Alabama and Mississipi) | 13.6 ± 4.9 to 71.8 ± 20.0 | 38 | Fernando et al. 2019 |
| | *Crassostrea sp.* | Canavieiras, Southern Bahia, Brazil | 0.58 to 30.74<br>3.53 to 54.21 | 16<br>38 | This study |
| Mussels | *Perna perna* | *Guanabara Bay, Brazil* | 76.29 to 118.97 | 38 | Ramos et al. 2017 |
| | Lucina pectinata | Canavieiras, Southern Bahia, Brazil | 4.33 to 6.18<br>34.89 to 49.07 | 16<br>38 | This study |
| | *Anamalocardia brasiliensis* | | 2.20 to 11.94<br>26.86 to 54.63 | 16<br>38 | |
| | Mytella sp. | | 3.20 to 11.94<br>12.73 to 72.66 | 16<br>38 | |

**Table II.** Continuation.

| | | | | | |
|---|---|---|---|---|---|
| Fish | *Lepidorhombus whiffiagonis*<br>*Lophius piscatorius*<br>*Raja miraletus* | Mediterranean Sea | 104 to 232 | 11 | Storelli et al.2013 |
| | 19 species | Daya Bay, China | 110 to 520 | 16 | Sun et al. 2016 |
| | *Cynoglossus senegalensis*<br>*Drapane Africana*<br>*Pomadasys perotet* | Ghana (West Africa) | 94.7 to 641 | 28 | Bandowe et al. 2014 |
| | *Sardinella brasiliensis* | Southern Brazilian Shelf, Brazil | 6.02 to 4074 | 37 | Massone et al. 2021 |
| | *Several species*[*] | Canavieiras, Southern Bahia, Brazil | < 0.34 – 21.60<br>4.46 – 52.82 | 16<br>38 | This study |

***Mugil curema, Cynoscion sp., Lutjanus synagris, Rajidae sp., Larimus breviceps, Paralonchurus brasiliensis, Anchoa sp., Odonthognathus mucronatus.**

regulation (EUROPEAN-COMMISSION, 2011). Considering this recommendation, all 72 samples from Canavieiras presented values below this guide value. Considering all samples, B(a)Py+B(a)A+B(b)Fl+Ch ranged from less than the limit of quantification of these four compounds to 4.59 ng $g^{-1}$ in one *Cynoscion* sp. sample from marine Canavieiras region, still lower than the limit proposed by the EC835/2011 standard.

The Brazilian ANVISA (National Health Surveillance Agency from Brazil) has recently published a Technical Note (ANVISA 2019) regarding risks to human health due to seafood consumption from beaches contaminated by crude oil in northeastern Brazil. This is the first Brazilian guiding document concerning intake limits for PAHs-contaminated seafood products and provides a calculation to assess the level of concern for genotoxic carcinogenic compounds. For PAHs, it considers the weighted sum in relation to the toxicity of B(a)Py and another eight PAHs (benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indene(1,2,3-cd)pyrene and benzo(g,h,i)perylene) among the 16 priority USEPA PAHs, accounting for a consumption of 180 g per day of seafood, an average body weight of 60 kg, life span of 70 years and exposure period (consumption) of 5 years, resulting in a level of concern of 6 ng $g^{-1}$ BaP equivalents for fish and 18 ng $g^{-1}$ for molluscs and crustaceans. In our study, PAHs values below the limits of the ANVISA Technical Note were observed for all fish, crustaceans and molluscs from Canavieiras, varying between below the detection limit (various organisms analyzed) and 7,36 ng $g^{-1}$ BeP equivalents (*Goniopsis cruentata*). However, despite the low toxic potential of the samples, it should be noted that this widely applied risk assessment methodology can underestimate risks by not considering some important PAHs, especially those associated with a petrogenic oil origin, such as alkylated compounds, as previously recommended by Pampanin (2017), Fernando et al. (2019), Farrington (2020) and Massone et al. (2021).

## CONCLUSIONS

The PAHs contamination detected in Canavieiras biota is low/modest, possibly due to the nature of the spilt heavy fuel oil. However, due to the lack of baseline information, this study cannot determine to what extent seafood PAHs concentrations may have been altered following the oil contamination.

Unlike other spills, the oil that affected northeastern Brazilian beaches, similarly to Deepwater Horizon blowout, was transported over long distances and reached the mainland, allowing for greater oil weathering processes. This results in the loss of lighter fractions, the most soluble and, therefore, the most bioavailable, which may partially explain the low concentrations determined in the biota samples reported herein.

As function of the small concentrations obtained and the natural biological processes regarding the assimilation and liberation of hydrocarbons by biota, was difficult to associate the compounds assemblage determined in samples with the oil spilled, even in more contaminated samples. None of the fish, mollusc or crustacean samples from Canavieiras presented risks to human consumption, as PAHs concentrations were below food safety limits established by both national and international agencies. However, it is important to note that other potentially toxic PAHs were not included in this risk assessment.

It should also be noted that this investigation focused only on the edible portions of fish, crabs, shrimp, and oysters and did not include nonedible portions (e.g., fish entrails) or attempt to determine whole-body PAHs residues. Thus, caution is required concerning result extrapolation regarding potential ecological effects.

The results of the 72 analyzed samples did not allow for the unequivocal detection of petrogenic hydrocarbons. Several of the analyzed organisms, such as fish and crustaceans, can metabolize PAHs, resulting in their detoxification.

Although the PAHs concentrations in fish samples from Canavieiras are not indicative of consumption risks, oyster and shrimp levels were similar to those reported for hydrocarbon-contaminated regions. Furthermore, new oil slicks have appeared along the Brazilian coast after the sampling date of the present study. Thus, we strongly recommend further assessments focusing on the systematic monitoring of filter-feeding and benthic species, in order to monitor PAHs contamination evolution in the region.

Finally, the partnership developed herein between fishers, shellfish and crab gatherers, environmental agencies, protected area managers and university comprises an example of successful participatory and citizen science based on the dialogue between different forms of knowledge, proving very promising and adequate for the assessment concerning species of interest to local communities and ensuring contributions to fishing activities and seafood item sales in a short period of time.

**Acknowledgments**

The authors would like to thank FUNBIO for financial support, ICMBio, Canavieiras Extractive Reserve managers and agents, Canavieiras Extractive Reserve fishermen and fisherwomen and the AMEX - Canavieiras Extractive Reserve Association. We are also grateful to Dr. Rodrigo Leão de Moura for the support in the identification of the organisms sampled in Canavieiras.

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**How to cite**

HAMACHER C, FARIAS CO, ARAÚJO MP, PITTA JPMP, SANTOS CAP & SOARES MLG. 2022. Community-based assessment of marine resources contamination after a large-scale oil spill. An Acad Bras Cienc 94: e20211392. DOI 10.1590/0001-3765202220211392.

*Manuscript received on October 25, 2021;*
*accepted for publication on June 4, 2022*

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