UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Biociências
Campus do Litoral Paulista

Acute toxicity of water-soluble fraction (WSF) from the oil of mysterious origin that reached Brazilian beaches.

Murilo Vieira Guimarães

São Vicente

2023

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Trabalho de Conclusão de Curso apresentado ao instituto de Biociências da UNESP – Campus do Litoral Paulista para obtenção do título de Bacharel em Ciências Biológicas, modalidade Biologia Marinha.

Orientador: Dr. Denis Moledo de Souza Abessa

Coorientador: Dr. Caio Cesar Ribeiro

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RESUMO

Este estudo investiga as consequências ambientais de derramamentos de óleo, enfatizando especialmente o incidente significativo que ocorreu no Brasil de 2019 a 2020. Ele abrange muitos fatores, incluindo a composição química das frações solúveis em água (WSF) do petróleo e seus impactos ecotoxicológicos na vida marinha. A pesquisa utilizou organismos modelo como *Artemia* sp. e embriões do bivalve *Perna perna* para avaliar a toxicidade de WSF obtido a partir de fragmentos de petróleo coletados em vários locais da costa nordeste do Brasil. Os resultados mostraram níveis variados de toxicidade entre as amostras e espécies, com *Perna perna* exibindo uma sensibilidade notavelmente maior do que *Artemia* sp. Os resultados destacam a necessidade de monitoramento ecotoxicológico dos efeitos dos derramamentos de óleo na saúde dos ecossistemas marinhos. Palavras-chave: Toxicidade de Derramamento de Óleo; Impacto em Organismos Marinhos; Gerenciamento de Desastres Ambientais.

Palavras-chave: Toxicidade de Derramamento de Óleo; Impacto em Organismos Marinhos; Gerenciamento de Desastres Ambientais.

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Type of the Paper (Article)

Acute toxicity of water-soluble fraction (WSF) from the oil of mysterious origin that reached Brazilian beaches.

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Abstract: This study delves into the environmental consequences of oil spills, particularly emphasizing the significant incident that occurred in Brazil from 2019 to 2020. It encompasses many factors, including the chemical composition of water soluble fractions (WSF) of oil and and their ecotoxicological impacts on marine life. The research used model organisms like *Artemia* sp. and embryos of the bivalve *Perna perna* to assess the toxicity of WSF obtained from oil fragments collected at various sites of the Brazilian Northeastern coast. The results showed varied levels of toxicity among samples and species, with *P. perna* exhibiting a notably higher sensitivity than *Artemia* sp. The results highlight the need of ecotoxicological monitoring the repercussions of oil spills on the health of marine ecosystems.

Keywords: Oil Spill Toxicity; Marine Organisms Impact; Environmental Disaster Management.

1. Introduction

Environmental accidents caused by oil spills have various impacts on biota, including mortality, coating, intoxication, and even mutagenic, carcinogenic, and teratogenic effects (Savitz & Andrews, 1997; Baan et al., 2009). Notable examples include the Exxon Valdez incident in 1989, which released 42 million liters of crude oil off the Alaskan coast, resulting in massive mortality among marine life, such as approximately 2800 sea otters (Garrott et al., 1993) and an estimated 250,000 seabirds (Piatt & Ford, 1996). Another significant disaster was the Deepwater Horizon explosion in 2010, which released about 3.19 million barrels of oil in the Gulf of Mexico and severely impacted corals and coastal environments (White et al., 2012).

The International Maritime Organization (IMO), established in 1948, plays a crucial role in promoting cooperation, ensuring maritime safety, and preventing pollution (Marinha do Brasil, 2023). The IMO organized the International Convention for the Prevention of Pollution from Ships (MARPOL) to minimize the consequences of such accidents (Walker et al., 2019).

The chemical composition of crude oil, its derivatives, and weathering by-products pose significant risks to many organisms, including humans. Known substances related to environmental impacts include polycyclic aromatic hydrocarbons (PAHs), aliphatic hydrocarbons, and other substances (Reyes et al., 2014; Stogiannidis & Laane, 2015; Nelson et al., 2016). Their physical and chemical properties influence the distribution of hydrocarbons and their fate and effects have on the environment. (Alford et al., 2015).

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When the oil is released in the environment, it undergoes a weathering process, which affects the oil properties and interactions with organisms and the environment. This includes changes in composition, evaporation, dissolution, biodegradation, and photo-oxidation (Schwarzenbach et al., 2017; Fingas, 2015; Guo et al., 2010; Ali et al., 1995). When the oil is not removed from the environment, all these weathering processes occur, some of its chemical compounds are solubilized to the water column and end up creating another dispersion factor for the contaminants that were previously agglomerated in the main oil portion, such mixture is known as the water soluble fraction (WSF) in relation to petroleum. The WSF is already being well studied in different portions of oils from different sources and it is already known that they can cause toxic responses in different groups of organisms, whether marine or not (Nascimento., 2023; Jung, Hicken et al., 2013).

Oil spills disrupt ecological balance, and the ecological effects may be persistent and last for decades due to PAHs biomagnification throughout the local food chains. This may pose significant public health risks, especially to communities reliant on the affected envi ronment (Loya & Rinkevich, 1980; Hughes et al., 2017; Jackson et al., 1989; Guzmán et al., 1991).

From August 2019 to January 2020, Brazil experienced a significant environmental crisis characterized by the arrival and accumulation of large quantities of oil in coastal ecosystems, primarily along the northeast and northern southeast coastlines. Over 1000 locations across 11 states were affected, making it the most significant tropical ocean spill, not only because of the volume (estimated between 5000 and 12000 m^3) but also because of its vast spread over approximately 2890 km (Escobar, 2019; Disner & Torres, 2020; Magris & Giarrizzo, 2020; Soares et al., 2020a, b). Investigations regarding the disaster's origin considered several possibilities: natural seepage, platform spills, intentional or accidental ship spills, and shipwrecks (Escobar, 2019; Soares et al., 2020a, b). Geochemical analyses linked the oil to Venezuelan sedimentary basins, dismissing the first two hypotheses (Lourenço et al., 2020; Oliveira et al., 2020; Carregosa et al., 2021; Zacharias et al., 2021a, b). The Federal Police later attributed the oil source to a Greek ship, estimating a minimum financial impact of R$ 188 million in terms of the federal government, not accounting for additional losses in fishing and tourism (Polícia Federal, 2021). However, Reddy et al. (2022) suggested a possible link to the SS Rio Grande shipwreck and others from the second world war (WWII), highlighting the need for further investigation.

Cleanup efforts saw limited government involvement, with most oil removal carried out by volunteers and NGOs, often lacking proper protection, training or public support. The national government's lack of mobilization and unclear communication further complicated disaster management, leading to misinformation and inadequate response (Brum et al., 2020; Soares et al., 2020; Pena, 2020). As a result, the oil removal was slow and inefficient, increasing the exposure of coastal organisms to oil compounds. Thus, the spill severely affected approximately 144,000 artisanal fishers, whose livelihoods depended on fishing in contaminated coastal areas, including sandy beaches, mangroves, and estuaries, covering approximately 724 areas (Pena & Gomez, 2014; Rêgo et al., 2018).

The uncollected oil remnants likely remain in coastal and shallow marine sediments, potentially re-mobilizing during energetic weather events and threatening local ecosystems, including mesophotic coral reefs (Paixão et al., 2011).

Some chemical analysis studies have already reported that the concentrations of hydrocarbons found are high. Among them, it is possible to observe that naphthalene, anthracene, phenanthrene, fluoranthene, and pyrene represent a significant portion of the compounds present in the oil portions, in addition to usually exceeding CONAMA legislation for class 1 of saline waters (Tongo et al., 2017; Magalhães et al., 2022). For this reason, it is important to know whether the effects of these hydrocarbons above the limit established by legislation are causing any effect on the biota., that's why this study hypothesizes that the oil spill's toxic effects on marine organisms, specifically related to PAHs and hydrocarbons, needed to be adequately addressed by the Federal Government's monitoring group (GAA), underscoring the need for thorough environmental impact analysis.

This study aimed to evaluate the toxicity of WSF obtained from oil fragments collected on several beaches of Northeast Brazil, with a hypothesis rooted in the extensive environmental impact of the 2019-2020 oil spill, as documented in various studies (Escobar, 2019; Soares et al., 2020; Disner & Torres, 2020). The main goal is to assess the oil toxicity using two test organisms: the microcrustacean *Artemia* sp. and the bivalve *Perna perna*. Specifically, the study will focus on evaluating the toxicity of oil from various beaches, including Praia do Leão in Fernando de Noronha, Praia da Coroa do Meio, and Praia Abaís in Sergipe, and several beaches in Bahia such as Massarandupio, Pojuca, and Arraial da Ajuda. To determine the toxic effects caused by the WSF exposure, embryos of *Perna perna* and nauplii of *Artemia* sp. were exposed to the WSF obtained from oil in controlled environments. Additionally, the project aims to compare the observed toxicity with the chemical analyses in the literature, thereby linking empirical data with established scientific findings. This approach seeks to understand the immediate impacts of the oil spill.

2. Materials and Methods

2.1. Chemical analysis

The samples were packaged in glass containers, stored, and transported to the Oceanographic Institute of the University of São Paulo, where the chemical analyzes were conducted. The procedure employed in this process is recognized as a forensic methodology capable of determining the origin of oils resulting from spills (CEN, 2012; Dahlmann and Kienhuis, 2015). The samples were diluted in dichloromethane, with a concentration of 10 mg ml^{-1}, and subjected to analysis by gas chromatography (GC) coupled with a flame ionization detector (FID) and a mass spectrometer (MS). The GC-MS analysis included polycyclic aromatic hydrocarbons (PAHs), non-aromatic cyclic hydrocarbons, and petroleum biomarkers such as hopanes, terpanes, and steranes.

Among the samples, only that from Fernando de Noronha was not analyzed by chromatography, however the analysis with GC-MS was carried out on two samples from Arraial da Ajuda beach and on a sample from Massarandupio. This set of analyzes managed to confirm that Arraial da Ajuda was the only point analyzed that had oil from a different origin than the others, therefore it is possible to state that most samples are from the 2019 spill while the Arraial da Ajuda sample was from another source.

2.2. Sample Characterization

Oil fragments (field samples) were collected from the following beaches: Leão (Fernando de Noronha); Coroa do Meio (Sergipe); Abaís (Sergipe); Massarandupio (Bahia); Pojuca (Bahia); and Arraial da Ajuda (Bahia)(Figure 1). After collection, the samples were stored in glass containers and aluminum foil. Aliquots of these samples were sent to the Oceanographic Institute of the University of São Paulo (IO-USP) for chemical analysis following the protocol to identify the oil's origin (CEN, 2012; Dahlmann & Kienhuis, 2015). At IO-USP, the samples were diluted in dichloromethane and subjected to gas chromatography (GC) analysis coupled with a flame ionization detector (FID) and a mass spectrometer (MS). This analysis identifies and quantifies the presence of PAHs, non-aromatic cyclic hydrocarbons, and petroleum biomarkers such as hopanes, terpanes, and steranes.

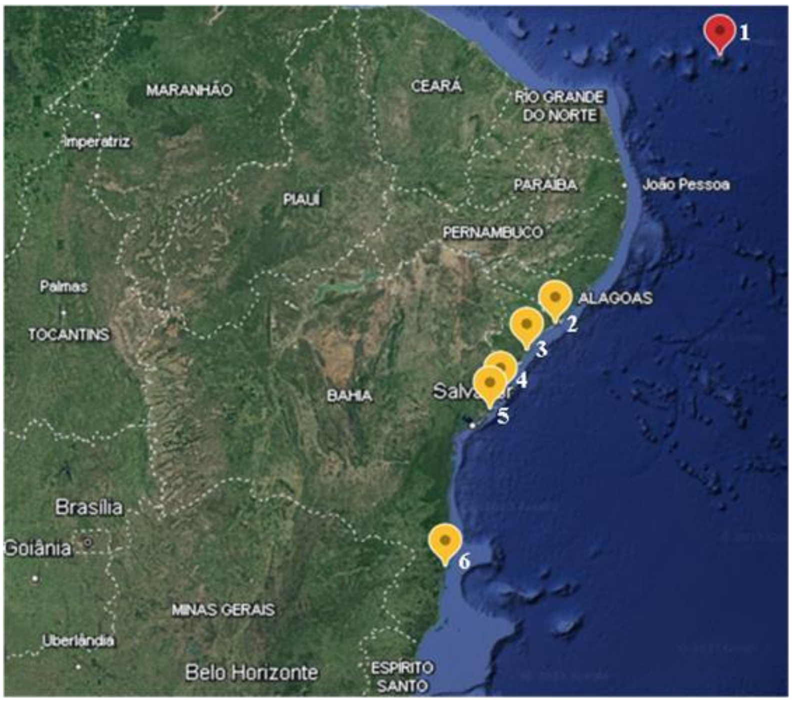


Figure 1: This figure illustrates the beaches from which samples of oil fragments were collected. Their respective names are: 1: Praia do Leão - Fernando de Noronha; 2: Praia da Coroa do Meio – Sergipe; 3: Praia Abaís – Sergipe; 4: Praia de Massarandupio – Bahia; 5: Praia de Pojuca – Bahia; 6: and Praia de Arraial da Ajuda – Bahia.

2.3. Preparation of Concentrations

The stock solution was prepared following the procedures described by Hansen et al. (2013), a method outlined in the "Chemical Response to Oil Spill — Ecological Effects Research Forum (CROSERF)" by Aurand and Coelho (1996) and Singer et al. (2000). According to this standard, a stock solution was prepared for each test based on the sample size collected from the beaches. Each sample was weighed on a precision scale and then transferred to a beaker, which was filled with water (salinity 35) to achieve a ratio of one part oil to nine parts of water.

The beakers with their respective mixtures were placed on a magnetic stirrer and agitated for 20 hours at a low energy agitation of 160 rpm. After this period, the mixtures were allowed to rest for two hours to one some decantation, aiding the subsequent removal of the WSF which represents the Extractable Substance (ES) of each sample (Figure 2).

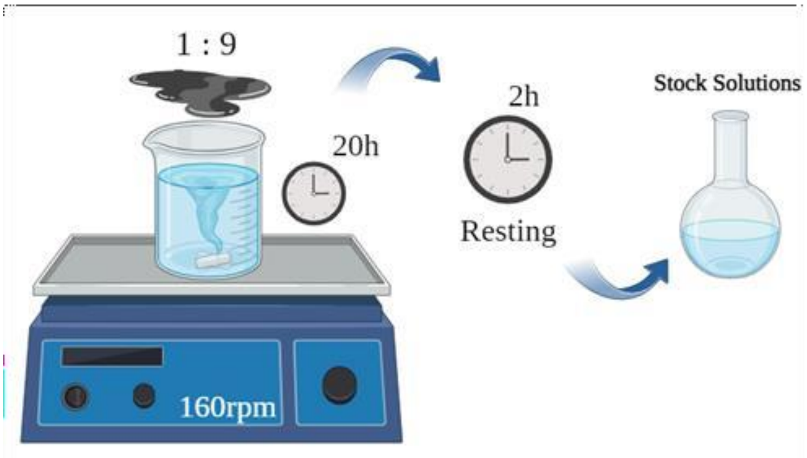
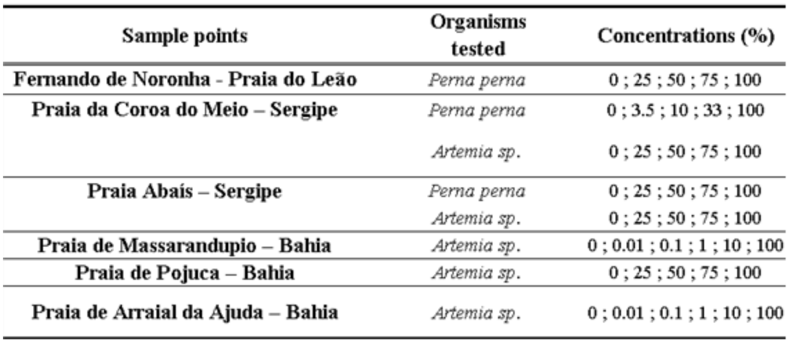


Figure 2: Process for preparing the stock solution. It begins by weighing the oil sample and placing it in a beaker, then adding clean water (1 part oil and 9 parts water). Then heterogeneous mixtures of Water Soluble Fraction are obtained by placing an electromagnetic mixer at 160 rpm for 20 hours to allow for the interaction between oil and water. Then the mixture is left to settle for 2 hours to allow decantation and the collection of the WSF Water-Soluble Fraction). The WSF is the stock solution was used to prepare the test-solutions.

The WSF dilutions of each sample were prepared, and amount of WSF was decisive for defining the concentrations used in each test. The preparation of the WSF from the oil samples was based on a 1:9 ratio (sample to water). Therefore, samples with less quantity of oil collected resulted in a smaller amount of WSF (Table 1).

Table 1. Visualization of all selected concentrations for the toxicity tests conducted in this study. Thus, the first column represents the beaches where oil fragments were collected. The second column indicates the organism species for each test. Finally, the third column shows the range of concentrations (in percentage) for each test.



Sample points	Organisms tested	Concentrations (%)
Fernando de Noronha - Praia do Leão	*Perna perna*	0 ; 25 ; 50 ; 75 ; 100
Praia da Coroa do Meio – Sergipe	*Perna perna*	0 ; 3.5 ; 10 ; 33 ; 100
	Artemia sp.	0 ; 25 ; 50 ; 75 ; 100
Praia Abaís – Sergipe	*Perna perna*	0 ; 25 ; 50 ; 75 ; 100
	Artemia sp.	0 ; 25 ; 50 ; 75 ; 100
Praia de Massarandupio – Bahia	*Artemia sp.*	0 ; 0.01 ; 0.1 ; 1 ; 10 ; 100
Praia de Pojuca – Bahia	*Artemia sp.*	0 ; 25 ; 50 ; 75 ; 100
Praia de Arraial da Ajuda – Bahia	*Artemia sp.*	0 ; 0.01 ; 0.1 ; 1 ; 10 ; 100

2.4. Artemia sp.

One of the test-organisms in this study was *Artemia* sp., a cosmopolitan microcrustacean (phylum: Arthropoda; class: Crustacea; order: Anostraca; family: Artemidae (Leach, 1819)) found on all five continents in saline lakes, highly adaptable to temperature variations. It has been a model organism in various locations affected by the environmental disaster caused by oil spills. Its cosmopolitan characteristic and the ease of cyst collection have made this organism an ideal model for toxicological tests in pesticides, petrochemicals, dispersants, heavy metals, and others since the 1950s. It is currently used as a comparative organism for other microcrustaceans like mysids and copepods due to its lower sensitivity to contaminants (Veiga & Vital, 2002).

2.5. Acute Toxicity Test with Artemia sp.

These tests were conducted based on the method described by Veiga & Vital (2002). Each concentration had four replicates, consisting of 10 ml glass test tubes containg the test solutions and 10 metanauplius II phase organisms (Figure 3). Physical-chemical parameters were monitored, and included temperature (mercury thermometer), salinity (refractometer), pH (pH meter), and dissolved oxygen (oximeter). The water used in the dilutions was saline 35 reconstituted with RedSea brand marine salt, aerated for 24 hours to ensure oxygenation above 5mg.L^{-1} of O_2. Mortality/mobility of each organism was checked after 24 and 48 hours.

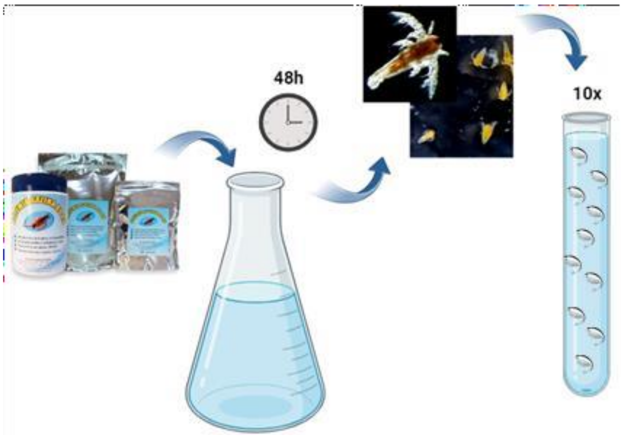


Figure 3: This figure illustrates the process from the hatching of *Artemia* sp. eggs to the placement of organisms in the Metanauplius II stage at the tested concentrations. The process begins by placing the eggs to hatch in a 2L Erlenmeyer flask under constant aeration for a period of 48 hours. Afterward, 10 organisms are separated for each test tube to be tested at each concentration.

2.6. Short-term Sub-chronic Toxicity Test with Perna perna

These tests were based on the ABNT – NBR16456 method (ABNT, 2022). For this test, four replicates were used per test-concentration, and each received a number of 400 to 500 fertilized eggs.

Initially, adult mussels spawning was induced by thermal shock; organisms were exposed to waters at different temperatures. When this primary stimulus was insufficient, stimulation was done through the injection of KCl (maximum of 5ml per organism) into the mantle using a syringe. Upon spawning, the gametes were collected with glass pipettes, with male gametes kept on ice to reduce activity.

After inserting the eggs into the test tubes, the tests were kept in an incubator (25±2 °C) for 48 hours with a 12h:12h light-dark photoperiod. Next, a clean control was read to confirm larval development (over 70% normality, see Zaroni et al., 2005)). Then, for the test completion, 500 µL of 10% buffered formaldehyde were added to each replicate. Subsequentially, larvae were counted under microscope, to assess normal development in each concentration.

2.7. Statistical analyses

The data were processed using the statistical software PAST (Hammer et al., 2001). The calculation of the lethal concentration for 50% of organisms (LC50) at 24 and 48 hours was performed in the R Studio program. Normality was assessed by the Shapiro-Wilk test, and homoscedasticity was examined using the Levene test. For parametric data, the t-Student test was applied, while for non-parametric data, the Mann-Whitney test was employed to determine the Concentration of Observed Effect (CEO) and Concentration of Unobserved Effect (CENO), when applicable.

3. Results

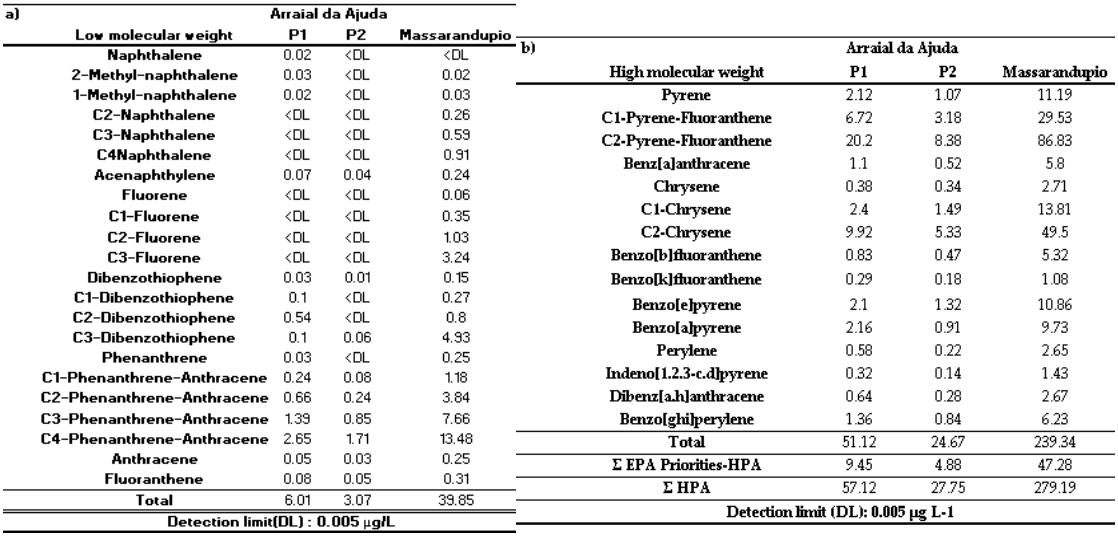
3.1. Chemical analysis

When examining the results of the chemical analyzes (Tables 2 and 3) that were first analyzed by Santana(2021) and comparing the concentrations measured in the samples versus the standards for saline waters according to CONAMA Resolution nº 357, of March 17, 2005 (Brazil, 2005), it was found that it is clear that the legal standard for PAHs in water is 0.018 µg L-1 has been exceeded. Thus, the results indicate that the oil has the potential to cause water contamination with hydrocarbons solubilized in the water-soluble fraction (WSF).

Table 2. Levels of aliphatic hydrocarbons (AH) were measured in all water-soluble fractions (WSF) and these compounds were present in the three analyzed oil samples. (When values were below the detection limit, they were indicated as <DL).

	Arraial da Ajuda		
Concentration: µg L-1	**P1**	**P2**	**Massarandupio**
n-C13	<DL	3.43	<DL
n-C19	<DL	<DL	4.93
n-C23	3.31	3.67	7.02
n-C24	<DL	<DL	5.9
n-C25	<DL	<DL	8.35
n-C28	<DL	<DL	5.79
n-C29	4.58	4.86	4.37
n-C30	<DL	<DL	17.85
ΣAHs	7.89	11.96	0
Detection limit(DL) : 2.5 µg/L			

Table 3. The concentrations of low ("a") and high ("b") molecular weight polycyclic aromatic hydrocarbons (PAHs) were measured in the water-soluble fractions (FSH) of the three oil samples analyzed, expressed in µg L^{-1}. (Values <DL indicate concentrations below the limit of detection).



a)

	Arraial da Ajuda		
Low molecular weight	**P1**	**P2**	**Massarandupio**
Naphthalene	0.02	<DL	<DL
2-Methyl-naphthalene	0.03	<DL	0.02
1-Methyl-naphthalene	0.02	<DL	0.03
C2-Naphthalene	<DL	<DL	0.26
C3-Naphthalene	<DL	<DL	0.59
C4Naphthalene	<DL	<DL	0.91
Acenaphthylene	0.07	0.04	0.24
Fluorene	<DL	<DL	0.06
C1-Fluorene	<DL	<DL	0.35
C2-Fluorene	<DL	<DL	1.03
C3-Fluorene	<DL	<DL	3.24
Dibenzothiophene	0.03	0.01	0.15
C1-Dibenzothiophene	0.1	<DL	0.27
C2-Dibenzothiophene	0.54	<DL	0.8
C3-Dibenzothiophene	0.1	0.06	4.93
Phenanthrene	0.03	<DL	0.25
C1-Phenanthrene-Anthracene	0.24	0.08	1.18
C2-Phenanthrene-Anthracene	0.66	0.24	3.84
C3-Phenanthrene-Anthracene	1.39	0.85	7.66
C4-Phenanthrene-Anthracene	2.65	1.71	13.48
Anthracene	0.05	0.03	0.25
Fluoranthene	0.08	0.05	0.31
Total	6.01	3.07	39.85
Detection limit(DL) : 0.005 µg/L			

b)

	Arraial da Ajuda		
High molecular weight	**P1**	**P2**	**Massarandupio**
Pyrene	2.12	1.07	11.19
C1-Pyrene-Fluoranthene	6.72	3.18	29.53
C2-Pyrene-Fluoranthene	20.2	8.38	86.83
Benz[a]anthracene	1.1	0.52	5.8
Chrysene	0.38	0.34	2.71
C1-Chrysene	2.4	1.49	13.81
C2-Chrysene	9.92	5.33	49.5
Benzo[b]fluoranthene	0.83	0.47	5.32
Benzo[k]fluoranthene	0.29	0.18	1.08
Benzo[e]pyrene	2.1	1.32	10.86
Benzo[a]pyrene	2.16	0.91	9.73
Perylene	0.58	0.22	2.65
Indeno[1.2.3-c.d]pyrene	0.32	0.14	1.43
Dibenz[a.h]anthracene	0.64	0.28	2.67
Benzo[ghi]perylene	1.36	0.84	6.23
Total	51.12	24.67	239.34
Σ EPA Priorities-HPA	9.45	4.88	47.28
Σ HPA	57.12	27.75	279.19
Detection limit (DL): 0.005 µg L-1			

3.2. Oil Toxicity

Figure 4 shows the results of the three tests conducted with *Artemia* sp. nauplii. In graph "a" (test with the WSF of the oil of Massarandupio Beach – Bahia), it is demonstrated that the *Artemia* sp. Metanauplii II showed no response at any concentration, allowing the conclusion that this sample was not toxic to this organism. In contrast, tests "b" (WSF of the oil of Pojuca Beach – Bahia) and "c" (WSF from the oil of Arraial da Ajuda Beach – Bahia) show different results. In the first, *Artemia* sp. Metanauplii II showed significant responses at the first concentration (25 mg/L). In a general view, it is observed that at all concentrations of the WSF oil, there was a response in the ecotoxicological test when compared to the control (0 mg/L concentration). However, the mortality/mobility of the organisms at all concentrations did not reach 50%, making it impossible to calculate the EC50. In the second test, *Artemia* sp. Metanauplii II showed significant responses from the concentration of 10 mg/L. Again, the concentration of WSF oil and the mortality/mobility of *Artemia sp*. are directly proportional, meaning that the higher the concentration of the WSF, the higher the rate of mortality/mobility of *Artemia* sp. The EC50 was calculated as 72.19 (68.16, 76.23) %. However, for a more precise EC50, a test with intermediate concentrations between 10 and 100% should be done in the future.

The black target represents the LOEC (Lowest Observed Effect Concentration), where in "b" the LOEC is at a concentration of 25% and in "c" at a concentration of 10%.

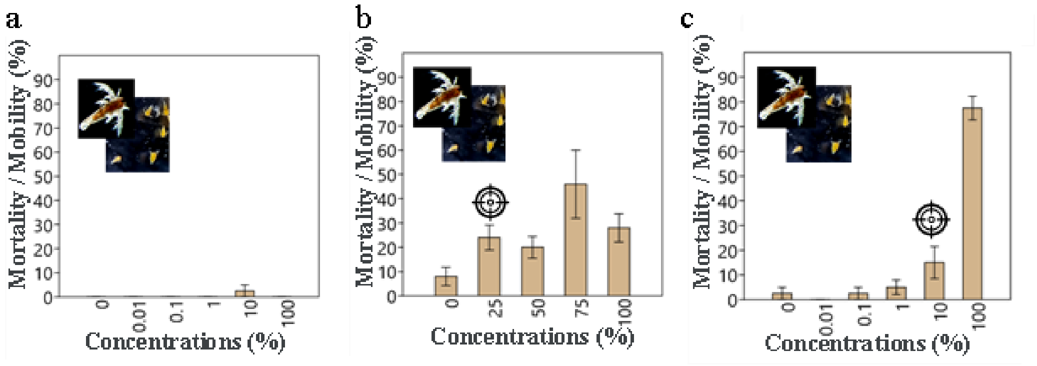


Figure 4. This figure is the graphical representation in boxplot format of the three tests conducted on *Artemia* sp. The graphs "a," "b," and "c" respectively represent the tests with WSF of oil from Massarandupio – Bahia; Pojuca – Bahia; and Arraial da Ajuda – Bahia. The x-axis represents the concentrations in percentage of WSF, and the y-axis represents the test endpoint, which was the mobility/mortality of the organisms.

Figure 5 illustrates tests conducted with both *P. perna* embryos and *Artemia* sp. nauplii with samples from Coroa do Meio Beach ("a" and "b") and Abaís Beach ("c" and "d"). In graph "a," it can be observed that *P. perna* larvae showed significant response at the second concentration (3.5 mg/L), these data, when compared to the EC50 found through the calculation of this result (2.24 (0.16, 24) %), confirms that a subsequent test with lower concentrations of the WSF of this oil could reveal a better precision of the actual EC50 value. This is because the first concentration above the control (0%) already shows a response with larval abnormalities above 50%.

. The *P. perna* embryos exhibited high sensitivity to the WSF at all concentrations, with abnormality rates exceeding 70% from the first concentration and over 90% at higher concentrations. Through numerical analysis, the EC50 was calculated to be 2.24 (0.16, 24) %. Meanwhile, in graph "b," *Artemia* sp. Metanauplii II showed a significant response from the first concentration (25 mg/L), and mortality exhibited an increase trend together with the WSF concentration. The calculated EC50 was 37.5 (27.5 - 47.50) %. In graph "c," *P. perna* larvae also exhibited significant toxicity from the first concentration (25 mg/L), with larval abnormality rates close to 100% in all WSF concentrations. It was not possible to calculate the EC50. Similarly, in graph "d," the EC50 could not be estimated, in general view, all WSF concentrations of the oil caused significant ecotoxicological effects, compared to the negative control (clean water).

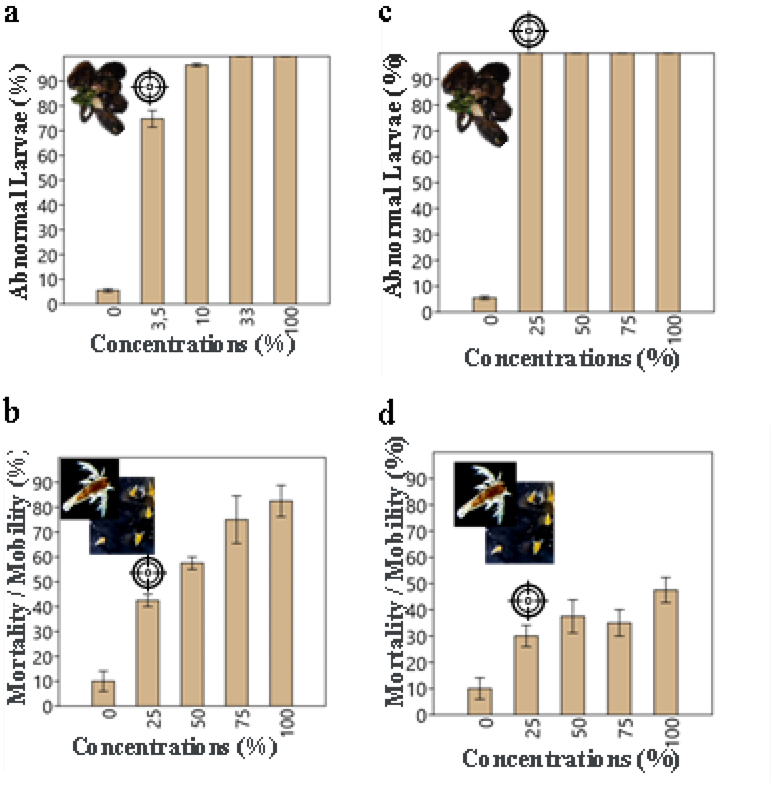


Figure 5. This figure is the graphical representation in boxplot format of four tests conducted on *Artemia* sp. and *Perna perna* embryos. Graphs "a" and "b" represent the tests with P. *perna* and *Artemia* sp., respectively, with the WSF from the oil sampled at Coroa do Meio Beach - Sergipe. Meanwhile, graphs "c" and "d" are the respective tests with *P perna* embryos and *Artemia* sp. with the WSF from the oil sampled at Abais Beach – Sergipe. The x-axis represents the concentrations (in % of WSF), and the y-axis represents the test endpoints, which were mobility/mortality in the tests with *Artemia* sp. tests, and the normality in the tests with *P. perna* embryos.

Figure 6 shows that *P. perna* larvae was significantly affected from the first concentration (25 mg/L). The WSF concentration and the embryolarval abnormality were directly proportional, meaning that the higher the concentration of the WSF, the higher the rate of larval abnormality. Thhe EC50 was calculated to be 45.21 (41.21, 49.21) %.

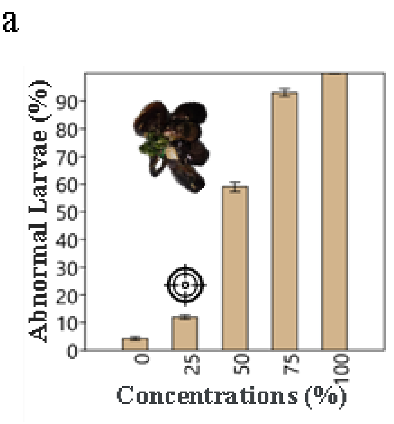
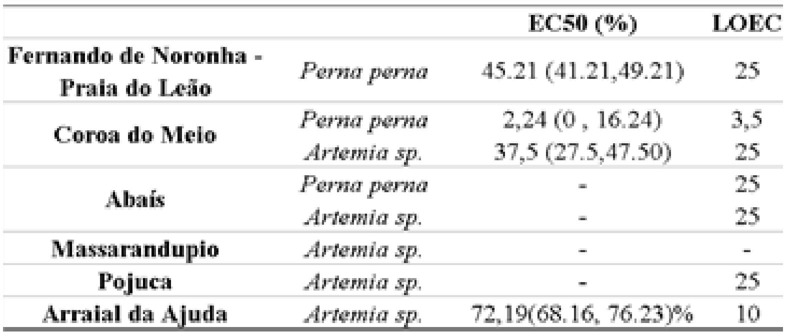


Figure 6. This figure is the graphical representation in boxplot format of the test conducted with embryos of *Perna perna*, with the graph representing the results obtained from the WSF sample from Leão Beach – Fernando de Noronha. The x-axis represents the concentrations in percentage of WSF, and the y-axis represents the test endpoint, which was the mobility/mortality of organisms.

Table 5 allows a comprehensive view of all EC50s and LOECs from tests organized by beach (oil sampling sites and organism (*P. perna* and *Artemia* sp.). Comparing the results, the higher sensitivity of *P. per*na larvae compared to *Artemia* sp. nauplii II is evident. This is demonstrated in the two tests with the most extreme responses: *P. perna* test from Coroa do Meio (EC50 = 2.24 (0.16, 24) %) and Abaís Beach, which were so sensitive that all concentrations resulted in 100% larval abnormality, preventing EC50 calculation. In contrast the less sensitive tests were those with *Artemia* sp., in which the WSF of oils from Abais, Pojuca, and Massarandupio beaches. In the first two, the mortality rates did not reach 50% in any concentration tested, and in the last one, no significant differences were observed between the concentrations and the control.

Table 5. This table consolidates information from all ecotoxicological tests conducted on Northeast beaches. The first column indicates the beach names, the second column specifies the tested organism, the third column provides the EC50 for each test where calculation was possible, and the fourth column displays the LOEC for each test where calculation was possible.



		EC50 (%)	LOEC
Fernando de Noronha - Praia do Leão	*Perna perna*	45.21 (41.21,49.21)	25
Coroa do Meio	*Perna perna*	2,24 (0 , 16.24)	3,5
	Artemia sp.	37,5 (27.5,47.50)	25
Abaís	*Perna perna*	-	25
	Artemia sp.	-	25
Massarandupio	*Artemia sp.*	-	-
Pojuca	*Artemia sp.*	-	25
Arraial da Ajuda	*Artemia sp.*	72,19(68.16, 76.23)%	10

4. Discussion

Our results suggest that the FSM obtained from oil fragments that reached the NE coast of Brazil present potential toxicity to marine life depending on the proximity to the oil portion and also the time that the oil portions are exposed to the environment, and other authors corroborated our findings. Costa et al. (2023) analyzed the presence of polycyclic aromatic hydrocarbons (PAHs) in seafood, distinguishing between non-carcinogenic (e.g., naphthalene, fluorene, anthracene) and carcinogenic PAHs (e.g., benz[a]anthracene, chrysene, benzo[a]pyrene) as classified by the NOAA-FDA (2010). And they observed higher concentrations of all analyzed PAHs in shellfish *Anomalocardia brasiliana* on the northern coast compared to the southern coast, with significant differences in both non-carcinogenic and carcinogenic hydrocarbons. In contrast, for *C. rhizophorae*, no significant variation was found between the two locations.

In another research carried out by Tongo et al., (2017) found out that naphthalene and anthracene as the main contributors to the total concentration of 16 PAHs in both species, In this work, the presence of naphthalene was observed only in a sample from Arraial da Ajuda at a lower concentration (0.02µg L^{-1}) when compared to the concentration of anthracene, which was observed in the three samples analyzed, having a greater impact on the sample. of Massarandupio, with a concentration of 0.25µg L^{-1}(table 3). Their results align with those of Soares et al. (2021) but differ from Magalhães et al. (2022), who reported phenanthrene, fluoranthene, and pyrene as frequent compounds, which corroborates what was found in this work, where the three compounds appeared in the analyzed samples (table 3), with pyrene being the most representative PAHs, as it is present in the three samples and is in higher concentrations (11.19 µg L^{-1}) while fluoranthene and phenanthrene had a lower concentration of approximately 0.28. These concentrations of naphthalene, anthracene, phenanthrene, fluoranthene, and pyrene are above the standards determined by CONAMA Resolution No. 357, of March 17, 2005. The findings suggest that animals exposed to oil spills are more likely to exhibit higher levels of low molecular weight PAHs (Romero et al., 2018). Such analysis of these compounds was carried out only with crude PAHs, that is, without measuring their derivatives or larger PAHs that are part of

From an animal health perspective, PAHs can cause various lesions by binding to animal tissues and disrupting biological processes (Neff et al., 1987; Martins et al., 2013). Mollusks, which can bioaccumulate PAHs, may experience DNA damage and oxidative effects leading to histopathological changes in tissues due to PAH interaction (Piazza et al., 2016; Sarkar et al., 2017.These findings align with previous research on the impact of PAH exposure on bivalve gills (Al-Hashem, 2017; Al-Hashem and Behbehani, 2016). The higher PAH concentrations on the northern coast likely contributed to the more severe histopathological alterations in bivalves there, potentially disrupting essential biological processes such as detoxification, respiration, and osmoregulation (Au, 2004).

Recent research indicates that even after three years, the oil spill on along the northern and southern coast of Pernambuco indicates a continued presence of oil residues, as evidenced by the detection of low molecular weight PAHs like naphthalene and anthracene (Costa, 2023). Although these concentrations of PAHs are lower than previously reported, the histological changes in bivalves' gills suggest ongoing adverse health effects due to PAH contamination, particularly on the northern coast. This highlights the long-term ecological impact of oil spills and underscores the importance of continued monitoring and assessment of affected marine life.

In addition to studies with bivalves, embryolarval tests were carried out (whose concentrations used were 1.65; 3.3; 6.6; 13.2; and 26.4 µgL^{-1}) with *Danio rerio* by Nascimento (2023) in which the sublethal effects such as biochemistry, morphology, and behavioral changes of *D. rerio* exposed to WSF were observed, which all had significant responses, In some of the parameters he analyzed, significant responses were observed at the first concentration, which is equivalent to 0.00165% in the measurement unit used in this work, which shows that the toxicity potential of WSF can reach concentrations much lower than those found in this work. Such as an increase in the rate of PAH in the hearts of the organisms, which was observed opposed by Li, Xiong et al. (2019), who exposed zebrafish larvae to crude oil WSF Furthermore, regarding behavioral parameters, it was observed that contact with WSF caused an effect on the autonomic nervous system of these organisms, which was associated with the inhibition of acetylchosterase, consequently causing behavioral changes in the organisms, making them more susceptible. to be preyed upon. Notably, the decrease in average swimming speed was observed only at the highest exposure concentration, aligning with findings of hypoactivity in zebrafish larvae exposed to phenanthrene (Vergauwen, Schmidt et al., 2015).

The judicial system's understanding of the impacts of oil spills is often limited, focusing mainly on short-term effects while ignoring long-term consequences such as the loss of species or impacts on fish reproduction (Silva, 2019). After the spill, the oil goes through several transformations such as spreading, evaporation and emulsification, the latter being capable of transporting petroleum hydrocarbons over significant distances. Taking into account that all this weathering under the oil creates an area of WSF around it, it is noted that even after years the oil still has the potential to continue contaminating its surroundings, see the results of this work, that is, this shows that the The judicial system ends up not solving the complete problem, focusing only on short-term measures, and this means that these affected areas continue to have consequences, increasingly increasing the quality of water to the surrounding animal and people communities.

The toxicity of weathered oils is mainly due to polycyclic aromatic hydrocarbons (PAHs), causing prolonged damage to marine life and ecosystems (GESAMP, 1993; NRC, 2003; Lopes et al., 1997; Hoff, 2002; Burns et al. , 1993). , 1994; Rodrigues et al., 1990; Lee et al., 1985; Paul et al., 2013).Ecotoxicological methods, including toxicity tests, offer crucial insights into the impacts of oil spills, providing a comprehensive assessment of both lethal and sub-lethal effects on marine organisms. These approaches have been effectively used in environmental impact assessments, consisting of important tools in environmental management and policy formulation (Chapman & Long, 1983; Lamberson et al., 1992; Adams et al., 1992; ASTM, 1992; USEPA, 1998; Environment Canada, 1999; Fonseca et al., 2011; Abessa et al., 2013, 2017; Abessa, 2023). In Brazil, the environmental impact assessments of oil spills normally do not include ecotoxicologival monitoring (Abessa, 2023), despite the numerous episodes of oil spill reported for the national coastline, and the existence of a national legislative framework to deal with oil spills, which include federal laws (Brasil 2000), and international protocols (IPIECA/IOGP, 2015; IMO/UNEP, 2009; Abessa et al., 2017). However, the response actions can be improved by the incorporation of ecotoxicological tools to assess the environmental impacts, as proposed by Abessa (2023) and demonstrated in the present study.

5. Conclusions

This work concludes that WSF obtained from oil fragments collected in the Brazilian NE coast during the arrival of oil of mysterious origins can cause water column contamination and noticeable ecotoxicological effects in both *P. perna* embryos and *Artemia* sp. nauplii. The embryos of *P. perna* showed were more sensitive to the WSF than *Artemia* sp.

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Instituto de Biociências
Câmpus do Litoral Paulista

unesp IB-CLP

PARECER FINAL DO TRABALHO DE CONCLUSÃO DE CURSO

Discente: **MURILO VIEIRA GUIMARÃES**

Título: *"Acute toxicity of water-soluble fraction (WSF) from the oil of mysterious origin that reached Brazilian beaches."*

Orientador: Prof. Dr Denis Moledo de Souza Abessa

Curso/Habilitação: Bacharelado em Ciências Biológicas/Gerenciamento Costeiro

COMISSÃO EXAMINADORA	CONCEITO
Prof. Dr Denis Moledo de Souza Abessa	APROVADO
Dr. Lucas Buruem Lucas Buruem	APROVADO

PARECER: O aluno deverá fazer as correções propostas pela banca

CONCEITO FINAL:

A Comissão Examinadora abaixo assinada conclui que o discente **Murilo Vieira Guimarães** obteve o seguinte conceito:

☒ APROVADO ☐ REPROVADO

São Vicente, 06 de dezembro de 2023.

Prof. Dr Denis Moledo de Souza Abessa

Dr. Lucas Buruem
Lucas Buruem