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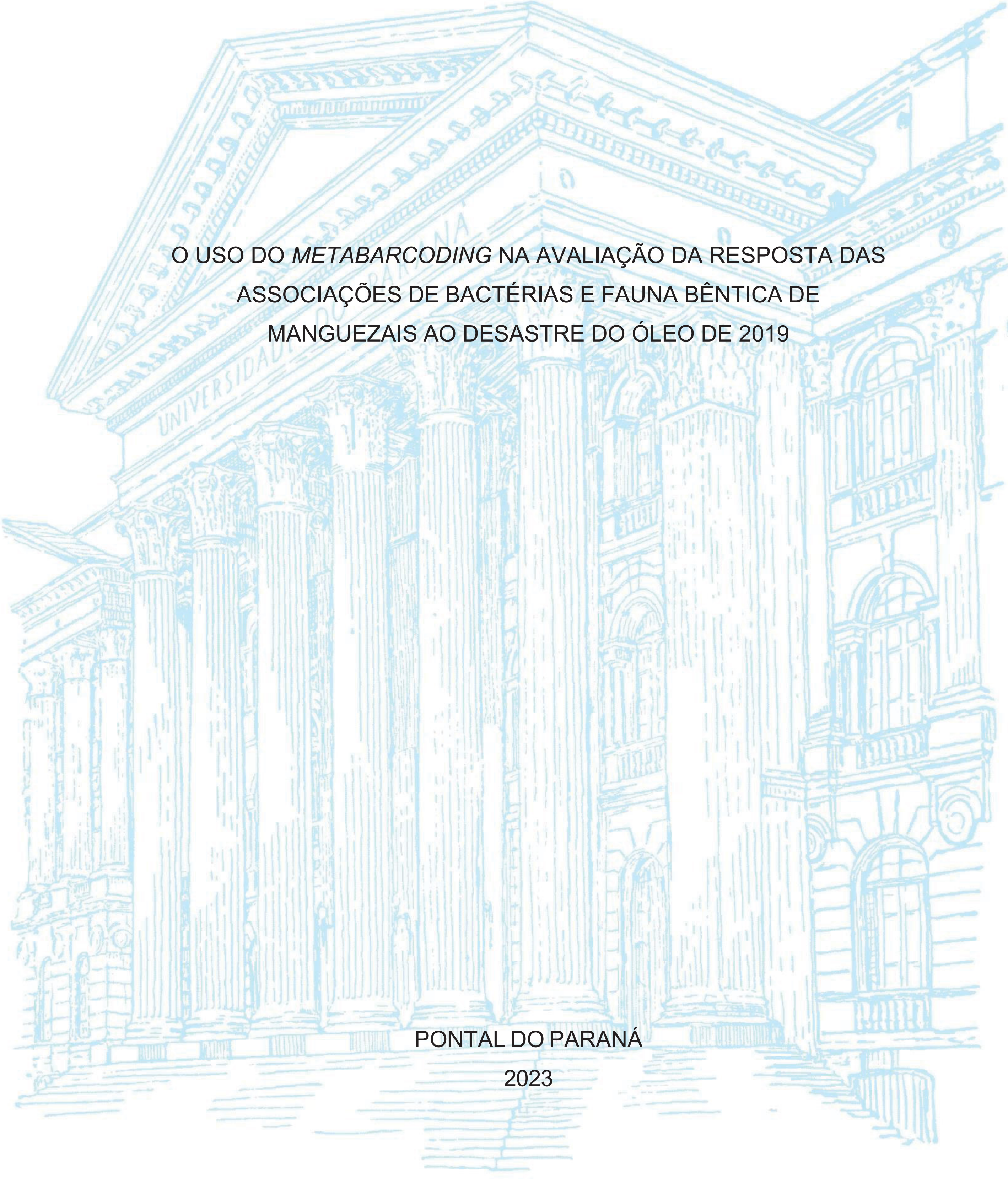

O USO DO *METABARCODING* NA AVALIAÇÃO DA RESPOSTA DAS ASSOCIAÇÕES DE BACTÉRIAS E FAUNA BÊNTICA DE MANGUEZAIS AO DESASTRE DO ÓLEO DE 2019

PONTAL DO PARANÁ

2023

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Dissertação apresentada ao Programa de Pós-Graduação em Sistemas Costeiros e Oceânicos, Campus Pontal do Paraná, Setor Centro de Estudos do Mar, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Sistemas Costeiros e Oceânicos.

Orientador: Prof. Dr. Maikon Di Domenico

Coorientadora: Prof(a). Dr(a). Kátia Cristina Cruz Capel

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação SISTEMAS COSTEIROS E OCEÂNICOS da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **ANA CARLA SANTIN MASSOCATTO** intitulada: **USO DO METABARCODING NA AVALIAÇÃO DA RESPOSTA DAS ASSOCIAÇÕES DE BACTÉRIAS E FAUNA BÊNTICA DE MANGUEZAIS AO DESASTRE DO ÓLEO DE 2019.**, sob orientação do Prof. Dr. MAIKON DI DOMENICO, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestra está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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Dedico aos meus pais e irmã, por serem modelo de simplicidade em suas metas e por me darem todo o suporte, à minha prima Fabiane pelo exemplo de coragem, e ao meu namorado Diego que me deu forças e amor nos momentos difícies.

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Aos meus pais Carlos, Sonia e irmã Laura, por todo o amor e apoio. Obrigada por viabilizarem minha moradia, viagens e tudo mais. Estaremos sempre juntos, longe de casa e perto do coração.

“Eu sou uma subjetividade, mas não sei o que sou a não ser naquilo que faço. Porque quando faço algo, eu me "re-conheço", isto é, eu conheço a mim mesmo de novo.”

Mario Sérgio Cortella

RESUMO

Os manguezais estão entre os mais notáveis ecossistemas costeiros marinhos tropicais e subtropicais devido à sua relevância econômica e ecológica. A fauna bentônica e os organismos da microbiota encontrados em alta densidade e diversidade no sedimento respondem fielmente às mudanças contínuas no solo e são essenciais para o funcionamento desse ecossistema, garantindo a estabilidade dos sedimentos e contribuindo para a remediação quando impactados. Esses organismos reagem rapidamente às mudanças ambientais e podem ser usados como indicadores ambientais. Em 2019, um extenso derramamento de petróleo bruto atingiu a costa brasileira, causando danos agudos e crônicos às funções e serviços de vários ecossistemas costeiros, incluindo os manguezais da Bahia. A presença de óleo foi observada ao longo de toda a região costeira da Bahia, atingindo ecossistemas costeiros críticos, além de algumas das mais importantes áreas turísticas, pesqueiras e aquícolas do país. A grande quantidade de manchas de óleo nos manguezais baianos levantou preocupações sobre os efeitos potencialmente nocivos à fauna desse ambiente. Até agora, apesar de suas altas densidades, as respostas do derramamento de óleo na fauna bentônica e na microbiota não foram avaliadas. Nosso objetivo foi investigar se eles responderam ao desastre e se alguns foram tolerantes à contaminação. Amostramos sete manguezais na porção norte-sul, abrangendo todas as regiões da Bahia afetadas por derramamentos de óleo em diferentes níveis. Fizemos duas campanhas de amostragem; a primeira ~ 12 meses (em 2020) e a segunda ~ 18 meses (em 2021) após o derrame. As amostras foram processadas para obtenção de variáveis abióticas, como características do sedimento, carbonato, teores de matéria orgânica e concentrações de metais pesados e HPAS, e DNA biótico da fauna e microbiota bentônica. Para isso, foi realizado pela primeira vez um levantamento genético de toda a comunidade bentônica avaliada utilizando *metabarcoding*. Para acessar nossos objetivos, criamos uma escala de contaminação usando as concentrações de cada contaminante e avaliamos os dados bióticos com base nessa escala. Análises de diversidade e análises Discriminantes Lineares (LDA) e do Tamanho do Efeito (LEfSe) foram conduzidos para avaliar a comunidade. Nossos resultados indicam que os locais mais contaminados foram BTS, Boipeba e Itacaré, enquanto o menos contaminado foi Cumuruxatiba. Mn, Cu, Zn, Benzo[b]fluoranteno, Dibenz[a,h]antraceno e Indeno[1,2,3-cd]pireno podem ser os principais indicadores de contaminação por óleo, pois apresentaram as maiores concentrações nos sedimentos avaliados A diversidade alfa não apresentou diferenças significativas na fauna bentônica e no microbioma. Diferenças na composição foram visualizadas em análises de diversidade beta, em 2021, tanto para a fauna bentônica quanto para o microbioma a variabilidade e amplitude de diversidade mais significativas ocorreram em locais classificados como "Médio" contaminados. A fauna potencialmente tolerante, incluí os organismos bentônicos da família Capitellidae e os gêneros microbianos *Thiogranum*, *Ilumatobacter*, *Hoppeia*, *SEEP SRB1* e *Sedimenticola*, os quais também apresentaram as maiores abundâncias relativas em 2020 e 2021. Diferentes fontes antropogênicas mascararam o efeito da contaminação do derramamento de 2019, exigindo uma investigação mais aprofundada.

Palavras-chave: Derramamento de óleo; comunidades microbianas; Assembleias bentônicas; HPA.

ABSTRACT

Mangroves are among the most remarkable tropical and subtropical marine coastal ecosystems due to their economic and ecological relevance. Their habitats have complex detrital organic food webs and serve as a feeding ground and refuge for surrounding microorganisms. Benthic fauna and microbiota organisms found in high density and diversity in the sediment faithfully respond to continuous changes in the soil and are essential for the functioning of this ecosystem by guaranteeing the stability of the sediments and contributing to remediation when impacted. These organisms react quickly to environmental changes and can be used as environmental indicators. In 2019, an extensive crude oil spill hit the Brazilian coast, causing acute and chronic damage to the functions and services of several coastal ecosystems, including the mangroves in Bahia. The presence of oil was observed along the entire coastal region of Bahia, reaching critical coastal ecosystems in addition to some of the country's most important tourist, fishing and aquaculture areas. The high amounts of oil slicks in the Bahian mangroves raised concerns about the potentially harmful effects on the fauna of this environment. So far, despite their high densities, oil spill responses on benthic fauna and microbiota have not been evaluated. Our goals was to investigate if they responded to the disaster and if some were tolerant to contamination. We sampled seven mangroves in the north-south portion, covering all regions of Bahia affected by oil spills at different levels. We did two sampling campaigns; the first ~ 12 months (in 2020) and the second ~ 18 months (in 2021) after the spill. The samples were processed to obtain abiotic variables, such as sediment characteristics, carbonate, organic matter contents and concentrations of heavy metals and HPAS, and biotic DNA of benthic fauna and microbiota. For this, a genetic survey of the entire evaluated benthic community was carried out for the first time using metabarcoding. To access our goals, we created a contamination scale using the concentrations of each contaminant and evaluated the biotic data based on this scale. Analysis of diversity, Linear Discriminant (LDA), and Effect Size (LEfSe) were conducted to assess the community. Our results indicate that the most contaminated sites were BTS, Boipeba, and Itacaré, while the least affected were Cumuruxatiba. Mn, Cu, Zn, Benzo[b]fluoranthene, Dibenz[a,h]anthracene, and Indeno[1,2,3-cd]pyrene can be the leading indicators of oil contamination since they presented the highest concentrations in the evaluated sediments. Alpha diversity did not show significant differences in benthic fauna and microbiome diversity. Differences in composition were visualized in beta diversity analyses, in 2021, both for the benthic fauna and the microbiome the most significant variability and amplitude of diversity occurred in sites classified as "Medium" contaminated. The potentially tolerant fauna, including the benthic organisms from the Capitellidae family and the microbiome from the genera, *Thiogranum*, *Ilumatobacter*, *Hoppeia*, *SEEP SRB1*, and *Sedimenticola*, had the highest relative abundances in 2020 and 2021. Different anthropogenic sources mask the effect of contamination from the 2019 spill, requiring further investigation.

Keywords: Oil spill; microbial communities; Benthonic assemblies; PAH.

RESUMO EM LINGUAGEM ACESSÍVEL

Os manguezais ocorrem em áreas subtropicais na transição entre os biomas terrestre e marinho, é uma zona úmida que proporciona condições ideais para a reprodução, eclosão, criadouro e abrigo de animais com grande valor ecológico e econômico. Seus habitats servem como área de refúgio para muitos microrganismos que utilizam a matéria orgânica em decomposição para se alimentar. A microbiota e os organismos bentônicos encontrados em alta densidade e variedade de espécies no sedimento, respondem fielmente as mudanças contínuas no solo, estes contribuem para a anulação de efeitos nocivos de elementos tóxicos ou contaminantes quando os solos sofrem impactos químicos. Em 2019, um extenso derramamento de óleo cru atingiu a costa brasileira, causando danos aos manguezais do estado da Bahia. As altas quantidades de manchas de óleo nos manguezais baianos, levantaram preocupações sobre os problemas causados na vida animal desse ambiente. Até então, respostas do derrame de óleo sobre os indivíduos da fauna bentônica e da microbiota não foram avaliadas, apesar de suas altas densidades. Nesse sentido, nosso objetivo foi investigar se os organismos em estudo responderam ao desastre e se existem gêneros que são tolerantes a contaminação. Amostramos sete manguezais na porção norte-sul, abrangendo todas as regiões do estado da Bahia afetadas pelo derramamento de óleo em diferentes níveis. Fizemos duas campanhas de amostragem; a primeira ~ 12 meses (em 2020) e a segunda ~ 18 meses (em 2021) após o derrame. As amostras foram processadas a fim de obter variáveis abióticas como teores de carbonato e matéria orgânica e concentrações de metais pesados e HPAS (contaminantes derivados do petróleo) do sedimento; e, o DNA total da fauna bentônica e da microbiota. Para isso, pela primeira vez, foi feito um levantamento genético de toda a comunidade bentônica avaliada, utilizando *metabarcoding*, método rápido de avaliação de biodiversidade. Nossos resultados apontam que os mangues com os maiores níveis de contaminação são BTS, Boipeba e Itacaré; enquanto o Cumuruxatiba apresentou os menores níveis. Os sedimentos avaliados possuíam altos índices de Manganês, Cobre e Zinco, Benzo[b]fluoranteno, Dibenz[a,h]antraceno e Indeno[1,2,3-cd]pireno. Esses contaminantes podem ser os principais indicadores de contaminação do óleo. A diversidade alfa não apresentou diferenças significativas na fauna bentônica e na diversidade da microbiota. Diferenças na composição foram visualizadas em análises de diversidade beta, em 2021, tanto para a fauna bentônica quanto para a microbiota; a variabilidade e amplitude de diversidade mais significativas ocorreram em locais classificados como "Médio" contaminados. A fauna potencialmente tolerante, incluí os organismos bentônicos da família Capitellidae e os gêneros microbianos *Thiogranum*, *Ilumatobacter*, *Hoppeia*, *SEEP SRB1* e *Sedimenticola,* os quais também apresentaram as maiores abundâncias relativas em 2020 e 2021. Diferentes fontes antropogênicas mascararam o efeito da contaminação do derramamento de 2019, exigindo uma investigação mais aprofundada.

Palavras-chave: Derramamento de óleo; Diversidade; Assembleias bentônicas; HPA.

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CAPÍTULO 1

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CAPÍTULO 1

SUPPLEMENTARY MATERIAL

LISTA DE ABREVIATURAS OU SIGLAS

1-MeNa 1-Methylnaphthalene
2-MeNa 2- Methylnaphtalene
Acy - Acenaphthylene
An - Anthracene
ANOVA - Análise de Variância
AS - Arsenic
ASV - Amplicon sequence variant
BaA - Benz[a]anthracene
BaP - Benzo[a]pyrene
BbF - Benzo[b]fluoranthene
BeP - Benzo[e]pyrene
BghiP - Benzo(ghi)perylene
BkF - Benzo[k]fluoranthene
Chr - Chrysene
CONAMA – Conselho Nacional do Meio Ambiente
Cr - Chromium
Cu - Copper
DBahA - Dibenz[a,h]anthracene
Dib - Dibenzothiophene
Fl - Fluoranthene
Flo - Fluorene
H_2O_2 - Hydrogen peroxide
HCl - Hydrochloric acid
HNO_3 - Nitric acid
HPA - Hidrocarboneto Policíclico Aromático
HPAs - Hidrocarbonetos Policíclicos Aromáticos
Ibama – Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis
IcdP - Indeno[1,2,3-cd]
ICP-OES - Inductively Coupled Plasma Optical Emission Spectrometry
Mn - Manganese
MO - Matéria orgânica
M_Z – Diâmetro médio

Na - Naphthalene

Ni - Nickel

OM - Organic matter

OTU - Operational Taxonomic Unit

PAH - Polycyclic aromatic hydrocarbon

PAHs - Polycyclic Aromatic Hydrocarbones

Pb - Lead

PCoA - Análise de Coordenadas Principais

Per - Perylene

Phe - Phenanthrene

pRDA - Partitioned canonical redundancy analyses

Py - Pyrene

RDA - Redundancy analysis

SIM - Single ion monitoring mode

SD - Standard deviation.

TEL - Threshold Effect Level

Zn - Zinc

CO_3^{-2} - Carbonate

LISTA DE SÍMBOLOS

© - copyright

@ - arroba

® - marca registrada

Σ - somatório de números

™ - trade mark

Φ - Phi

≤ - menor ou igual a

≥ - maior ou igual a

SUMÁRIO

O USO DO *METABARCODING* NA AVALIAÇÃO DA RESPOSTA DAS ASSOCIAÇÕES DE BACTÉRIAS E FAUNA BÊNTICA DE MANGUEZAIS AO DESASTRE DO ÓLEO DE 2019

1 INTRODUÇÃO GERAL

Os manguezais estão entre os mais notáveis ecossistemas costeiros marinhos tropicais e subtropicais devido a sua relevância econômica e ecológica, sendo considerados como um dos ecossistemas naturais mais produtivos da Terra (Pillai & Harilal 2018; Daza et al., 2020). Crescem em áreas úmidas, na zona entre marés, ou seja, na transição entre ecossistemas terrestres e os ecossistemas marinhos (Cao et al., 2008), estima-se que 35% dos os manguezais do mundo estão degradados ou foram perdidos (Polidoro et al., 2010). Esses sistemas são ameaçados por uma série de pressões antropogênicas, incluindo desmatamento, poluição, urbanização, e mudanças climáticas, incluindo secas recorrentes, aumento do nível do mar, ciclones e erosão costeira (Duke, 2017). Dentre as pressões constantes, entre as mais recorrentes, está o aumento na carga de compostos aromáticos provenientes de atividades industriais e acidentes com petróleo (Cabral et al., 2018).

Em sistemas entre marés, o tipo e o papel das interações entre os sedimentos, microrganismos, animais, plantas e fatores abióticos são complexos e ainda pouco compreendidos (Fusi et al., 2022). Os sedimentos são distribuídos no solo formando vários microhabitats, estruturados vertical e horizontalmente e compreendendo diversas propriedades físicas, químicas e biológicas (Totsche et al., 2010). Sabe-se que os habitats de mangue possuem cadeias alimentares orgânicas detríticas complexas e servem como área de alimentação e refúgio para os microrganismos circundantes (Zhu et al., 2012). Organismos da fauna bentônica e da microbiota encontrados em alta densidade e diversidade são importantes para o funcionamento do ecossistema de manguezais por garantirem a estabilidade dos sedimentos (Carugati et al., 2015; Chen et al., 2016; Zhou et al., 2017; Tong et al., 2019; Mare, 1942; Giere, 2009; Brannock et al., 2016). Eles formam associações variadas, apoiando uma complexa cadeia alimentar (Ceccon et al., 2019; Cotta et al., 2019).

Por quase 40 anos, os organismos da fauna bentônica têm sido utilizados com sucesso para entender o efeito da poluição (Raffaelli & Mason, 1981; Boucher, 1985; Lindgren et al., 2012; Fleeger et al., 2015; Zeppilli & Leduc, 2018; Fonseca & Di

Domenico 2018; Gambi et al., 2020; Naidoo et al., 2021). Esses organismos constituem um componente chave e um elo intermediário nas cadeias alimentares marinhas (Fusi et al., 2022; Song et al.,2022). Como consumidores, a fauna bentônica desempenha papéis importantes na reciclagem da matéria orgânica e no fluxo de energia no ecossistema de mangue, principalmente servindo como fonte de alimento para a megafauna (McIntyre, 1969; Murray et al., 2002; Danovaro et al., 2007; Zhang et al., 2017; Wang et al., 2019; Majdi et al., 2020; Zhao & Liu, 2021; Song et al., 2022). Dentre os filos da meiofauna, os nemátodas se destacam por serem os mais dominantes em sedimentos lamacentos (Vieira & Fonseca, 2013). De forma geral, os nemátodas são mais resistentes aos efeitos de derramamento de óleo do que outros filos de meiofauna (Boucher, 1985; Danovaro, 2000; Burgess et al., 2005). Já a macrofauna é dominada por anelídeos poliquetas, crustáceos e moluscos (Soares-Gomes, 2002). A abundância de algumas espécies oportunistas, especialmente poliquetas como *Capitella capitata* (Fabricious, 1780) e *Pseudopolydora cf. paucibranchiata* (Okuda, 1937) geralmente apresentam forte aumento alguns meses após o derramamento; essas rápidas proliferações de oportunistas duram pouco e a população logo recupera os valores normais (Parra e Lopez-Jamar, 1997). Dispersantes usados para combater derramamentos de óleo podem causar declínios populacionais em crustáceos, afetando o tegumento de crustáceos direta ou indiretamente (por meio de comunidades microbianas alteradas), comprometendo a integridade de sua epicutícula, que serve como uma barreira física entre o meio ambiente e as camadas internas mais permeáveis do exoesqueleto (Felder et al., 2014). Os moluscos são bioacumuladores rápidos de poluentes (Araújo et al., 2020). O contato direto deles com o óleo leva à asfixia, resultando na morte e dizimando suas próprias populações e as dos organismos que dependem deles, em um processo que dura décadas (Santos et al., 2016).

Assim como os organismos da fauna bentônica, a microbiota dos manguezais responde fielmente às mudanças contínuas no solo (Schloter et al., 2018). As bactérias reagem muito mais rápido às mudanças ambientais do que os metazoários, portanto, assim como os nemátodas, algumas espécies de microbiota podem ser utilizadas como indicadoras ambientais muito poderosas (Lear et al., 2011). Muitas bactérias são altamente sensíveis às condições ambientais locais, respondendo à anoxia, enriquecimento orgânico, acidificação e poluição química do ambiente (Nogales et al., 2011). As classes de bactérias mais comuns nos sedimentos de áreas subtropicais incluem os filos Proteobacteria (Gammaproteobacteria, Deltaproteobacteria, Betaproteobacteria e Alphaproteobacteria), Bacteroidetes, Chloroflexi e Firmicutes (Clostridia e Bacilli) (Andreote et al., 2012; Ceccon et al., 2019). Já se sabe que as bactérias *Desulfococcus*, *Desulfatibacillum*, *Desulfitobacterium* e *Vibrio* estão ativamente envolvidas na desintoxicação dos sedimentos e são os microrganismos degradadores de compostos aromáticos mais ativos nos sedimentos de manguezais altamente poluídos por óleo (Cabral et al., 2018). A estrutura e a dinâmica da microbiota de mangue são influenciadas por uma miríade de fatores, como variações de maré, flutuações de pH, salinidade, luz, temperatura, precipitação, disponibilidade de nutrientes e por fatores bióticos (Holguin et al., 2006).

A microbiota do solo é altamente diversificada, influenciando as tendências e a magnitude das mudanças, levando a uma homeostase específica alcançada nos ecossistemas (Blagodatskaya,2013; Vestergaard,2017; Bhowmik et al., 2019). Em vários ciclos biogeoquímicos, a microbiota é responsável pela ciclagem e transformação de nutrientes como carbono, nitrogênio, enxofre e fósforo, através das reações redox (Thatoi et al., 2013; Mendes & Tsai, 2018), além de regular a deposição de poluentes antropogênicos, como metais pesados de fluxos terrestres e fluviais, via atividade metabólica (Cao et al., 2011; Li et. al., 2011). Ao longo da evolução, a microbiota de sedimentos de manguezais desenvolveu mecanismos essenciais para remediação de ecossistemas impactados por hidrocarbonetos de petróleo (Sousa et al., 2020). Esses compostos aromáticos servem como fonte de carbono para uma variedade de organismos capazes de mineralizar ou degradar parcialmente em moléculas menos tóxicas (Fathepure, 2014). Os intermediários menos tóxicos podem eventualmente reentrar e serem novamente convertidos dentro dos ciclos geoquímicos globais (Joutey et al., 2013). Com isso, algumas populações de determinados organismos podem realmente se beneficiar da exposição ao óleo, por meio do enriquecimento orgânico da cadeia alimentar ou outros mecanismos (Jewett et al., 1999; Dornberger et al., 2023).

A importância dos ecossistemas dos manguezais vai muito além da defesa costeira e fixação de sedimentos, muitas vezes, os manguezais funcionam como filtros e sumidouros para metais pesados e hidrocarbonetos (Boonsong et al., 2003; Berde et al., 2022). Devido às suas características de baixa solubilidade e alta hidrofobicidade, os HPAs podem ser adsorvidos pelo material particulado e armazenado no sedimento, acumulando facilmente em sedimentos de mangue, causando um desafio ecológico significativo para esse ecossistema (Wise et al., 1995; Fuchs et al., 2011; Lu et al., 2011; Ostling et al., 2009; Cabral et al, 2018). Já, os metais pesados que se acumulam na cadeia alimentar, geram toxicidade para as plantas de mangue e afetam a diversidade microbiana (Muñoz-García, et al., 2022). A contaminação dos sedimentos de manguezais por metais pesados causa mudanças na composição das comunidades microbianas, o aumento da competição entre as espécies por habitats resulta na prevalência de espécies estresse tolerantes (Berde et al., 2022).

Grandes quantidades de petróleo bruto foram encontradas ao longo da costa nordeste e sudeste do Brasil entre agosto de 2019 a janeiro de 2020 (Lourenço et al., 2020). O incidente, ocorrido em algum ponto do oceano e detectado apenas quando as primeiras manchas de óleo chegaram às praias da região nordeste, é reconhecido como um dos mais graves vazamentos de óleo do Brasil (Lobão et al., 2022). Os primeiros estados atingidos na costa brasileira foram especificamente Pernambuco e Paraíba. Quatro semanas após a detecção da primeira mancha de óleo no litoral, novos registros foram feitos na região sudeste do país, no litoral norte do estado do Rio de Janeiro e Espírito Santo (Carmo & Teixeira, 2020; do Nascimento et al., 2022). Ao todo, o derramamento afetou 11 estados (Alagoas - AL, Bahia - BA, Ceará - CE, Espírito Santo- ES, Maranhão - MA, Paraíba - PB, Pernambuco - PE, Piauí - PI, Rio de Janeiro - RJ, Rio Grande do Norte - RN e Sergipe – SE), dos quais nove pertencem a região Nordeste, representando assim a maioria dos locais afetados (>80%) (Soares et al., 2020a, b).

Apesar de não ser o primeiro derramamento de óleo em águas brasileiras, o incidente é considerado um dos mais graves (se não o mais grave) já ocorrido no Brasil, sendo um dos maiores desastres ambientais por derrame de óleo do país e um dos mais extensos do mundo (Araújo et al., 2020; Pena et al., 2020; Magalhães et al.,2021; Lobão et al., 2022). A caracterização química do óleo que atingiu a costa, mostrou que hidrocarbonetos leves ainda estavam presentes, aumentando a probabilidade de efeitos negativos para os organismos e ecossistemas costeiros quando liberados na coluna d'água, causando danos incalculáveis a vida marinha (Lourenço et al., 2020). Os eventos de derramamento de hidrocarbonetos do petróleo na forma de óleo cru alteram as estruturas dos ecossistemas costeiros e causam danos agudos e crônicos às suas funções e serviços (Peterson et al., 2003;

Mendelssohn et al., 2012). Como os sistemas de manguezais apresentam detritos abundantes, carbono orgânico rico e condições anóxicas, a retenção e acúmulo de Hidrocarbonetos Policíclicos Aromáticos (HPAs) nos sedimentos aumenta (Ke et al., 2002). A poluição por óleo não só tem consequências negativas ao nível da população, mas também é uma das ameaças menos controláveis, uma vez que os resíduos persistem no ambiente por várias décadas (Peterson et al., 2003; Culbertson et al., 2008).

O estado da Bahia, foi um dos mais afetados pela chegada das manchas de óleo, com registros em mais de 100 localidades (IBAMA, 2020). A presença do petróleo foi observada ao longo de toda a região costeira da Bahia, atingindo importantes ecossistemas costeiros além de algumas das áreas turísticas, pesqueiras e de aquicultura mais importantes do país, tornando-se por isso de grande interesse para estudos de avaliação da qualidade ambiental (Barbosa, 2022). As altas quantidades de manchas de óleo que chegaram na costa da Bahia, e, mais especificamente, ao longo de diversas áreas de manguezal, levantaram preocupações sobre os efeitos potencialmente negativos na fauna desse ambiente. Até então, respostas do derrame de óleo sobre os indivíduos da fauna bentônica e a microbiota não foram avaliadas nesse período, apesar de suas altas densidades. Dada sua importância para os ecossistemas de manguezais, escolhemos esses dois grupos como nosso objeto de estudo. Nesse sentido, usando a técnica de *metabarcoding*, reunimos e analisamos sequências de DNA de organismos da fauna bentônica e da microbiota dos sedimentos de sete manguezais distribuídos do norte ao sul do Estado da Bahia, considerando os pontos afetados pelo derramamento. Coletamos dados das características sedimentares, das concentrações de HPAs e das concentrações de metais presentes no sedimento em cada ponto, mensuramos quais variáveis afetaram a distribuição das assembleias de fauna bentônica e microbiota em dois períodos distintos, sendo um em 2020 a ~12 meses e o outro em 2021 a ~18 meses após o derrame de óleo atingir a região costeira do estado da Bahia.

1.1 HIPÓTESE

Os sedimentos de manguezais mais impactados por óleo são dominados por organismos tolerantes à poluição e abrigam uma menor diversidade de fauna bentônica e de microbiota.

1.2 OBJETIVOS

1.2.1 Objetivo geral

Avaliar os efeitos do derramamento de óleo ocorrido em 2019 em comunidades da fauna bentônica e da microbiota presentes nos sedimentos de diferentes manguezais da Bahia.

1.2.2 Objetivos específicos

- Avaliar temporalmente, as concentrações e distribuições de HPAs e metais, bem como os teores de matéria orgânica e carbonatos em sete manguezais do estado da Bahia impactados pelo derramamento de óleo de 2019 , identificando quais são os principais elementos indicadores de contaminação.
-Testar se os manguezais altamente contaminados por HPAs e metais abrigam uma menor diversidade quando comparados a manguezais demarcados por contaminação intermediária ou baixa.
-Verificar se manguezais altamente contaminados possuem táxons tolerantes a contaminação e se esses táxons são também os mais abundantes nesses ecossistemas.

2 CAPÍTULO I.

Os resultados do presente capítulo foram estruturados em formato de artigo científico, que será formatado e submetido ao jornal Marine Pollution Bulletin Methods (Electronic ISSN: 0025-326X, fator de impacto: 7.001 (2023), classificação Qualis: A1.

Título: Metabarcoding reveals microbiome and benthic fauna response to oil-spill in mangrove ecosystems

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Metabarcoding reveals microbiome and benthic fauna response to oil-spill in mangrove ecosystems

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ABSTRACT

The sediment life is highly sensitive. Many functions performed by microorganisms are currently under threat due to the degradation of marine and estuarine sediments by petroleum-derived products. In August 2019, an extensive oil spill spread along the Northeastern coast of Brazil, affecting beaches, coral reefs, seagrasses, and mangroves. Here we tested the response of the microbial community and benthic fauna from several mangrove ecosystems to the disaster. We sampled seven mangroves covering the Bahia coast 12 and 18 months after the oil spill in 2020 and 2021, and we accessed their DNA through metabarcoding. Changes in the diversity of the microbiome and benthic fauna were investigated by correlating them to abiotic factors (including the presence of polycyclic aromatic hydrocarbons (PAHs), metals, and sedimentary characteristics). The hypothesis tested was that the most oil-impacted mangrove sediments would be dominated by pollution-tolerant organisms, hosting a lower diversity of benthic fauna and microbiome community. Our goals were to access the sites most impacted by PAHs and Metals, test if and how the benthic fauna and microbiome respond to contamination and investigate if tolerant organisms dominate sites with higher contamination. We analyzed the concentrations of contaminants by area, measured two alpha diversity indexes (Chao1 and Shannon), and conducted Linear Discriminant (LDA) and Effect Size (LEfSe) analyses comparing with relative abundances of the taxa to access responses in the community. Our results indicate that the most contaminated sites were BTS, Boipeba, and Itacaré, while the least affected were Cumuruxatiba. Mn, Cu, Zn, Benzo[b]fluoranthene, Dibenz[a,h]anthracene, and Indeno[1,2,3-cd]pyrene can be the leading indicators of oil contamination since they presented the highest concentrations in the evaluated sediments. Alpha diversity did not show significant differences in benthic fauna and microbiome diversity. Differences in composition were visualized in beta diversity analyses, in 2021, both for the benthic fauna and the microbiome; the most significant variability and amplitude of diversity occurred in sites classified as "Medium." The potentially tolerant fauna, including the benthic organisms from the Capitellidae family and the microbiome from the genera, Thiogranum, Ilumatobacter, Hoppeia, SEEP SRB1, and Sedimenticola, had the highest relative abundances in 2020 and 2021. Different anthropogenic sources mask the effect of contamination from the 2019 spill, requiring further investigation. This highlight the urgency of biodiversity baselines along the Brazilian coast.

Keywords: Oil spill; Benthic fauna; Metals; Polycyclic aromatic hydrocarbons; Mangroves; Brazil; microbial communities.

METABARCODING REVEALS MICROBIOME AND BENTHIC FAUNA RESPONSE TO OIL-SPILL IN MANGROVE ECOSYSTEMS

1 INTRODUCTION

In 2019, the Northeastern Brazilian coast endured an oil spill that affected 11 states, the oil spread through marine currents for more than 3,000 km across northeastern reaching the southeastern coast (Escobar, 2019; Disner & Torres, 2020; Magris & Giarrizzo, 2020; Soares et al., 2020; Ibama, 2020). Over 5000 tons of oil was removed from more than 1000 marine protected areas, beaches, coral reefs, mangroves, and seagrasses (Brum et al., 2020; Ibama, 2020; Soares et at., 2020). Therefore, this spill is considered the most extensive and severe environmental disaster ever recorded in the history of Brazil (Magalhães et al., 2021), as well as in the South Atlantic Ocean basin and tropical oceans (Soares et al., 2020). The origin of the spill (company, ship, or shipwreck) remains unknown, and oil tankers in the marine region have not identified any accidents (Câmara et al., 2021; Magalhães et al., 2021). Nevertheless, researchers indicate that the oil's geochemical characteristics are compatible with the Venezuelan sedimentary basin (De Oliveira et al., 2020).

The Bahia State was highly affected by the oil spill in more than 100 locations (Ibama, 2020). Physical models suggest that the spill occurred in international waters in the South Atlantic, where large-scale atmospheric and oceanic circulations defined the latitude at which ocean drifting oil reached the continental margin (~10° S) (Lessa et al., 2021). Northern coastal sites continued to be affected due to mesoscale eddies along the continental margin, thus enabling different drift paths and recurring appearances in several locations (Lessa et al., 2021). As a result, this oil spill increased the load of pollutants and aromatic compounds in various Bahia´s mangrove ecosystems (Cabral et al., 2018). The crude oil can alter the structure of coastal ecosystems promoting acute and chronic damage to their functions and services (Peterson et al., 2003; Mendelssohn et al., 2012). In addition, oil pollution has negative consequences from individual, community and ecosystem levels, persisting in the environment for several decades (Peterson et al., 2003; Culbertson et al., 2008). In mangrove systems, characterized by a high content of debris, organic carbon, and anoxic conditions, there is significant retention and accumulation of Polycyclic Aromatic Hydrocarbons (PAHs) in sediments (Ke et al., 2002).

The importance of mangroves is well-documented (Magris & Barreto, 2010). They are recognized as repositories of marine biodiversity and provide a range of natural resources and ecosystem services to human well-being and survival (World Resources Institute 1996). The ecosystem services provided by mangroves include protection and buffering against tidal and erosion events (Sandilyan & Katthiseran, 2015), tourism and fisheries (Dias et al., 2012), as well as carbon storage (Vo et al., 2012), and maintenance of biogeochemical cycles (Vo et al., 2012). The benthic fauna and microbiota, found in high density and diversity, are important for the functioning of the mangrove ecosystem by ensuring the stability of the sediments (Carugati et al., 2015; Chen et al., 2016; Zhou et al., 2017; Ceccon et al., 2019; Cotta et al., 2019; Tong et al., 2019; Mare, 1942; Giere, 2009; Brannock et al., 2016). The benthic organisms are largest part of coastal ecosystems and play a key function in ecosystem functioning (Snelgrove, 1998). They form an important part in the trophic link by acting as a food for many organisms (Holzhauer et al., 2020). The soil microbiota regulates soil organic matter (OM), decomposition and stabilization, soil nutrient dynamics and rhizosphere function (Strickland et al., 2009; van der Heijden et al., 2008; Mendes et al., 2011; Nelson et al., 2022).

Within the mangrove ecosystem it is expected that the benthic organisms are among the most affected by the oil spill, as the sediment usually represents the final destination of several contaminants in aquatic systems (Eça et al., 2013). In addition, benthic organisms are relatively sedentary, closely related to the sediment characteristics, (Balthis et al., 2005; Egress et al., 2019). Soil microorganisms compared with physical and chemical properties, they are more dynamic and sensitive to soil contamination because their immediate response to natural or anthropogenic changes in the soil (Zhao et al., 2019; Wołejko et al., 2020). Due to their sensibility, the benthic organisms, including invertebrates and microbiome community, is highly used as a model to assess the effect, severity, and extent of damage caused by hydrocarbons (e.g., Cotta et al. 2019; Schratzberger et al., 2003; Dauvin et al., 2010; Patrício et al., 2012). Therefore, soil microorganisms can be used as ecological indicators to monitor soil contamination and provide an integrative biological assessment of soil health (Niemeyer et al., 2012; Zhao et al., 2021).

In this sense, here, the metabarcoding sequencing of sediment samples were used to evaluated the chronic impact of the oil spill on benthic fauna and microbiome community in mangroves at the Bahia Coast, which are of paramount importance for local communities that depend on the exploitation of natural resources and biological aspects of this ecosystem (Alongi, 2014; Holguin et al., 2006; Cotta et al., 2019). The use of environmental samples to extract DNA directly from the sediment provided a rapid assessment to detect the impacts of oil pollution on biodiversity (Valentini et al., 2016; Lekang et al., 2020; Fonseca et al., 2018). The hypothesis tested were that the most oil-impacted mangrove sediments would be dominated by pollution-tolerant organisms, hosting a lower diversity of benthic fauna and microbiome community. Furthermore, the study provided important information on the recovery status of mangroves and the long-term trends of sediment communities after contamination by PAHs and trace metals.

2 MATERIALS AND METHODS

2.1 SAMPLE COLLECTION

Three sediment samples, one meter distant from each other, were collected from seven different mangroves, distributed from north to south in the Bahia State – Brazil (Siribinha, Todos os Santos Bay, Boipeba, Itacaré, Belmonte, Canavieiras and Cumuruxatiba), 12 (September and October 2020) and 18 months (February and April 2021) after the oil spill (Fig.1). Belmonte, Canavieiras and Cumuruxatiba were sampled only in the second campaign. All samples were collected during low tide using a pre-cleaned stainless-steel spatula, stored in sterile bottles in an icebox, and kept frozen at -20°C for biotic and abiotic analyzes. In each campaign, three replicas were collected in each mangrove, taking the first 10 cm of sediment to obtain the DNA of benthic fauna and microbiome. At the same point where these samples were taken, three sediment samples were collected for abiotic analysis (Organic matter, carbonates, granulometry, metals) and three samples to measure PAH concentration.

Sampling sites were hit differently by the oil spill. According to Ibama records, Itacaré (14° 16′ 39.0″ S – 39° 00′ 25.2″ W) (Fig.1) had no signs of oil between 2019 and 2020. The other locations had records of oil within this period. It is unknow if oil stains arrived in the sampled mangroves after march 2020, when reports by Ibama ceased. Baía de Todos os Santos (BTS) (12° 43' 20.9'' S – 38° 32' 13.6'' W) is the only mangrove that historically suffers from high contamination before the 2019 disaster. Several anthropogenic activities currently influence the environmental quality at BTS, such as the influx of domestic and industrial effluents, solid wastes, agriculture, harbors, and mining activities (Hatje et al., 2009). Siribinha (11° 44′ 53.4″ S – 37° 31′ 21.5″ W), Boipeba (13° 38′ 12.0″ S – 38° 53′ 44.5″ W), Canavieiras (15° 43′ 42.5″ S – 38° 55′ 03.0″ W), Belmonte (15° 51′ 01.0″ S – 38° 52′ 05.0″ W) and Cumuruxatiba (17° 00′ 38.6″ S – 39° 10′ 22.6” W) showed acute contamination at different levels (see results). According to Ibama, between 2019 and 2020, the Belmonte, Canavieiras, and Cumuruxatiba sites had oil records in low amounts, while Siribinha presented intermediate amounts of oil and Boipeba was the most affected site.

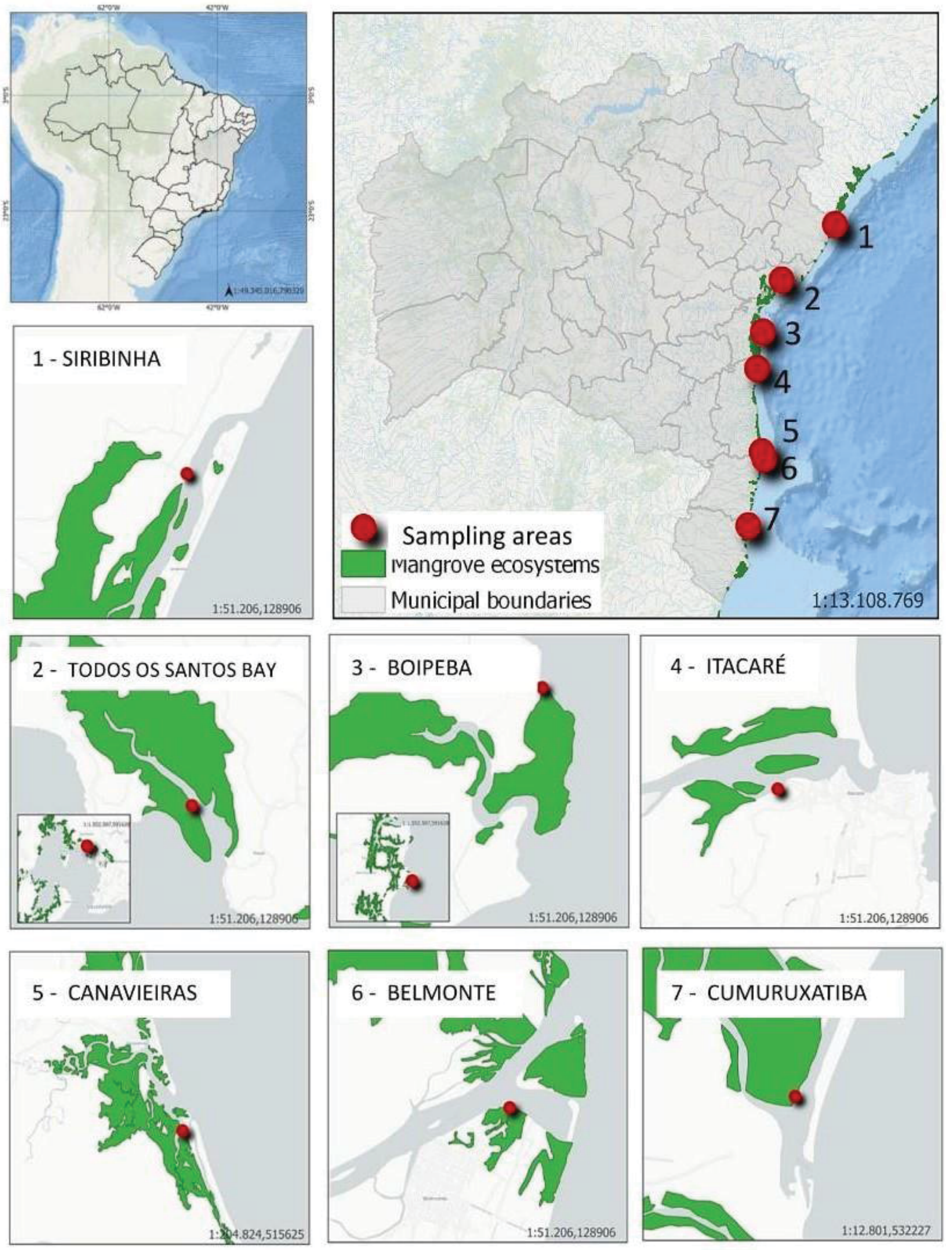


Figure 1 - SAMPLING SITES. The map shows the location of sampling sites in Bahia state, Brazil (1-Siribinha; 2-Todos os Santos Bay (BTS); 3-Boipeba; 4-

Itacaré; 5-Canavieiras; 6-Belmonte, and 7-Cumuruxatiba). Red dots indicate the sampling areas. The green area represents the mangrove ecosystems zones.

2.2 ABIOTIC VARIABLES ANALISYS

The granulometric variables (Organic matter, carbonates and granulometry) were analyzed using 20g of sediment from each sample. Decarbonization was performed with 10% hydrochloric acid (HCl) (v:v) attack and the removal of organic matter (OM) with 10% hydrogen peroxide (H2O2) (v:v), attacking different steps. The carbonate ($CO_3{}^{-2}$) and organic matter samples were washed and sieved after a 24 and 48-h period, respectively. Once dried, each one was weighed on a precision scale (0.0001 g). The initial weights, filters, and filters with dried and attacked samples were registered for later determination of the percentage of carbonates and organic matter present in each sample. The grain size was determined using a laser granulometer (Microtrac Bluewave S54000). The raw data were processed with the FLEX 10.6.2 software, aided in calculating the granulometric parameters, following the Method of Moments (Tanner, 1995), obtaining the percentages of gravel, and sand for each sample of silt and clay.

The metallic elements Copper (Cu), Chromium (Cr), Manganese (Mn), Nickel (Ni), Lead (Pb), Zinc (Zn), and Arsenic (As) were quantified through partial extraction of the elements, following the procedure described in method 3050B of partial acid digestion for sediments (USEPA, 1996). A 1.0 g of dry sediment rate was placed in a 50 ml beaker, and then 5 ml of nitric acid (HNO_3) 65% (V: V), 2.5 ml H_2O_2 30% (V/V), and 5 ML of HCl 37% (V: V) were added. Digestion was performed at 90°C for 4 hours and 20 minutes. Subsequently, all digested samples were filtered at 50 ml with ultra-pit water (Merck-Millipore, Milli-Q®). The trace elements of interest were quantified through the Optical Plasma Emission Spectrometry Technique (ICP-OES) 6010C (Usepa, 2007). The quality control of the analyzes took into account the lowest concentration with a recovery rate of 95% to 99% of confinement (limit of detection of the method - LDM), and the limit of quantification of the method (LQM) is the lowest concentration accurately determined and accuracy. In addition, the sample blanks were submitted to the same analytical procedures as the samples. The confidence interval was within the certified value, with a standard deviation below 20% and recovery values between 75% and 125%, as the USEPA (1996) recommended. These

7 types of metals, were selected for analysis because unlike organic pollutants, they do not decompose, thus proving that they have a high potential for bioaccumulation and biomagnification (Balsamo et al., 2012). Furthermore, these metals are naturally found in fuel oils and crude oils (Burger & Gochfeld 2000; Burger & Snodgrass, 1998; Ruiz-Fernández et al., 2019). Some of these are found in oil slicks as inorganic salts (mainly as Mg chlorides and sulfates) associated with the aqueous phase of crude oil emulsions (Ruiz-Fernández et al., 2019).

The analytical procedure for PAH analysis was based on Almeida et al. (2018) and Guimarães et al. (2020), with minor modifications. Briefly, 3 g of sediment was extracted in an ultrasonic system (15 min, 50 °C, 35 Hz) using 25 mL of n-hexane and dichloromethane (1:1, v:v). This procedure was repeated three times to ensure the total extraction of PAHs and the extracts were combined. First, activated copper was used to remove the sulfur. Next, concentrated extracts were purified using column chromatography with 3 g of activated silica (140°C for 3 hours) and anhydrous sodium sulfate and eluted with 20 mL of n-hexane and dichloromethane (1:1, v:v). Finally, the resultant extract was concentrated to 1 mL under a gentle gas stream of nitrogen and added p-terphenyl as an internal standard.

PAH analyses were performed using an Agilent technologies instrument 7890B GC System/5977B GC/MSD. The separation occurs by a capillary column (Agilent 19091S-433 HP-5ms 30 m x 250 µm x 0.25 µm) with high-purity helium at a constant flow rate of 1.0 mL min-1. The following conditions of GC were used: the starting temperature for column 18 was 95 °C, then raised to 180 °C at four °C min-1, 220 °C at six °C min-1, and then to 300 °C where it was kept for 3 minutes. The mass spectrometer was operated in a full scan mode from 35 to 550 m/z. The transfer line to the mass spectrometer was heated to 240 °C and the quadrupole at 150 °C. Quantification and identification of target PAH were performed in both the scan and single ion monitoring mode (SIM). The analytical curves for the PAH ranged from 0.1 to 20 µg L^{-1}, while the surrogates' standards were between 100 to 600 µg L-1 and the internal standard (p-terphenyl-d14) at a concentration of 500 µg L^{-1}. The data related to the detection of all compounds showed an excellent correlation coefficient, with values above 0.99. Method accuracy was assessed using the matrix (n = 7) and blank (n = 3) spikes. For this purpose, sediment samples were spiked with a known concentration of PAHs standard (CRM 47543, Sigma Aldrich) containing the following compounds: naphthalene (Na), 2- Methylnaphtalene (2-MeNa), 1-Methylnaphthalene

(1-MeNa), acenaphthylene (Acy), phenanthrene (Phe), fluorene (Flo), dibenzothiophene (Dib), anthracene (An), fluoranthene (Fl), pyrene (Py), benz[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[e]pyrene (BeP), benzo[a]pyrene (BaP), perylene (Per), dibenz[a,h]anthracene (DBahA), indeno[1,2,3-cd] pyrene (IcdP), and benzo(ghi)perylene (BghiP). Mean recoveries were 74 ± 22.7% and 103.3 ± 26.8% for matrix and blank spikes, respectively.

2.3 MOLECULAR ANALYSIS

2.3.1 DNA EXTRACTION AND SEQUENCING

Samples were homogenized with an electric hand blender with a stainless-steel arm (method outlined in Wangensteen et al., 2018) and stored at − 20 °C before DNA extraction. The blender was previously cleaned with bleach and ethyl alcohol 70% and sterilized in the flame of a Bunsen burner between each sample processing to avoid cross-contamination.

For DNA extractions, 1,5 g of each homogenized sediment sample was processed with the DNeasy® PowerSoil® DNA Isolation Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions, and DNA was resuspended in a final volume of 50 µl. After amplification, each sample was quantified with a Nanodrop 2000 and a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific).

Library preparation for microbiome analyses followed the Illumina protocol "16S Metagenomic Sequencing Library Preparation: Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System". The primers Bakt_341F (5′-CCTACGGGNGGCWGCAG-3′) and Bakt_805R (5′-GACTACHVGGGTATCTAATCC-3') (Klindworth et al., 2013) with Illumina adapters were used to amplify the V3 and V4 regions of the 16S gene. DNA extraction was sent to a third-party company for Library preparation and sequencing.

PCR conditions were 5 min initial denaturation at 95°C, followed by 25 cycles consisting of denaturation (95°C for 40 s), annealing (2 min) and extension (72°C for 1 min) and a final extension step at 72°C for 7 min. PCR products were purified with a QiaQuick PCR purification kit (QIAGEN, Hilden, Germany). The quantity and quality of the extracted DNA were analyzed by spectrophotometry using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and by agarose gel electrophoresis. The PCR products were stored at −20°C for sequencing.

For benthic analyses, primers SSU_FO4 (5'- GCTTGTCTCAAAGATTAAGCC-3') and SSU_R22 (5'- GCCTGCTGCCTTCCTTGGA - 3') (Fonseca et al., 2010), targeting the V1 and V2 regions of the 18S gene of ribosomal DNA (rDNA) were used. Libraries were quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and sequenced on a MiSeq Illumina platform (2x250 bp). DNA extraction was sent to a third-party company for Library preparation and sequencing. PCR conditions were 2 min denaturation at 95 °C, followed by 35 cycles of 1 min at 95 °C, 45 s at 57 °C, 3 min at 72 °C and a final extension of 10 min at 72 °C (Fonseca et al., 2010). First, PCR products were purified using Illustra™ GFX PCR DNA and Gel Band Purification kit, according to the company protocols. Afterward, we measured the concentration of PCR product in each sample using Qubit 3.0.

2.4 DATA ANALYSIS

2.4.1 BIOINFORMATIC ANALYSES

For the analyzes of the benthic fauna, the “Bioinformatic Methods for Biodiversity Metabarcoding” (https://learnmetabarcoding.github.io/) was followed. Briefly, sequences were filtered to remove low-quality sequences, grouped into operational taxonomic units (ZOTUs) using the VSEARCH software (Rognes et al., 2016), and compared to the NCBI libraries (https://www.ncbi.nlm.nih.gov/) using GenBank BLAST (Sayers et al., 2019) for taxonomic assignment.

For the microbiome, sequences were filtered and normalized using the DADA2 pipeline (https://benjjneb.github.io/dada2/tutorial.html). The final product obtained with DADA2 was an amplicon sequence variants (ASVs) table, a high-resolution analog of the traditional OTU table, which records the number of times each exact amplicon sequence variant was observed in each sample. Taxonomic assignment was obtained by comparison to the 16S SILVA database (https://www.arb-silva.de/).

2.4.2 STATISTICAL ANALYSES

Descriptive statistics, such as absolute and relative frequency (percentage), mean and standard deviation, were used to compare the abiotic variables (OM and $CO_3{}^{-2}$; PAHs; and Metals) among sites and years.

A workflow (Fig.2) was developed to order the measured abiotic data (metals and PAH concentrations) within a contamination scale. First, four ranges of concentration were defined for each contaminant. For the PAHs: level 3 = (>21.48 ng g^{-1}); level 2 = (21.47 ng g^{-1} to 13.41 ng g^{-1}); level 1 = (13.40 ng g^{-1} to 03.08 ng g^{-1}) and level 0 = (<3.8 ng g^{-1}). For metals: level 3 = (>16.67 mg/Kg); level 2= (16.50 mg/Kg to 6.15 mg/Kg); level 1= (6.14 mg/Kg to 2.49 mg/Kg) and level 0 = (<2.49 mg/Kg). After establishing each measured value within this scale, the Expected value was calculated for each sample. The calculation was made for a second scale. This total contamination scale considers the average between the Expected value of the PAHs and the Expected value of the metals for each sample. The contamination scale gradient was assumed to be "High = averages between 2.25 to 1.49"; "Medium = averages between 1.35 to 1.20" and "Low = averages between 1.18 to 1.13". This total contamination scale was used to determine which sites were most impacted by the 2019 oil spill.

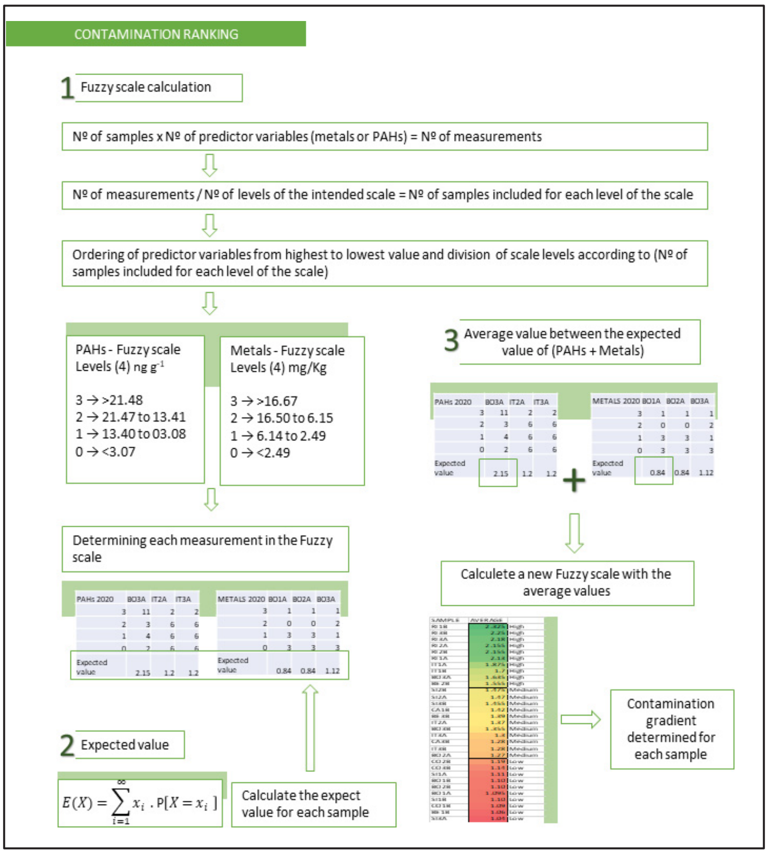


Figure 2 - PRISMA. Flow diagram showing the procedure used for the selection of the *Gradient Contamination*, based on the establishment of a first *Fuzzy scale*, the calculation of *Expect value*, averages and a second *Fuzzy scale*, used in data analysis.

The statistical and visual analyses of the ZOTUs (for benthic fauna) and ASVs (for microbiome) have been performed by the *Marker Data Profiling* tool of *MicrobiomeAnalis*t (Chong et al., 2020). Data filtering was performed to remove low-quality features, ZOTUs, and ASVs for which at least 20 % of their counts in the different samples contained at least four reads have been excluded. In addition, ZOTUs and ASVs with lower variance than 10% throughout the experimental conditions were discarded.

To evaluate the benthic fauna diversity among the contamination gradient and the differences in their composition, we plot both the alpha and beta diversity indices. To observe how the microbiome and benthic fauna diversity varies according to the gradient of the total contamination scale, “Alpha diversity” was calculated; Moreover, with “Beta-diversity” analysis, the community composition indices were outlined. Differences in the alpha diversity at the feature (ZOTUs/ASV) level classes associated with the variables (year, site, and total contamination scale) were assessed by calculating the Chao1 and Shannon indices, with significant differences evaluated with t-tests “ANOVA.” “Chao1” estimate taxa richness by accounting for undetected features because of low abundance. “Shannon” considers species richness and evenness, with varying weight given to evenness (Chong et al., 2020). Beta-diversity analysis was used to investigate, for both benthic fauna and microbiome, the differences in the community composition among total contamination scale using the Principal Coordinates Analysis (PCoA) based on Jensen Shannon Divergence. “Jensen-Shannon divergence” assesses the distance between two probability distributions that account for the presence and abundance of microbial and benthic fauna features. Permutational multivariate analysis of variance (PERMANOVA) was used to compare the differences among treatments.

Linear Discriminant Analysis (LDA) Effect size (LEfSe), a non-parametric statistical method, was applied to identify benthic fauna and microbiome taxa that significantly differ between groups when they respond to the total contamination scale. LEfSe first uses the Kruskal–Wallis’s test to identify taxa whose relative abundances are significantly different between groups. LDA is then applied to taxa meeting the significance threshold to estimate their effect size. This approach outputs a ranked list of taxa based on their LDA scores (Chong et al., 2020). A significance level of $p < 0.05$ and an LDA score of 2 were used to determine taxa that best characterize each contamination level. It is expected that with the analysis (LEfSe), it will be possible to identify which taxa of the benthic fauna and microbiome tolerate the high contaminations caused by the 2019 oil spill. LEfSe scores can be interpreted as the degree of consistent difference in relative abundance between features in the two levels “Medium and High” in 2020 and in the three levels “High”, “Medium” and “Low” in 2021 of analyzed benthic fauna communities or microbiome communities. The histogram thus identifies which features among all those detected as statistically and biologically differential explain the greatest differences between communities. Comparing the (LEfSe) with a relative abundance for ZOTUs and ASVs, it is possible to check if the most abundant taxa are also the most tolerant to oil contamination. The relative abundances were visually inspected with a Pie Chart plot.

3 RESULTS

3.1 ENVIRONMENTAL CHARACTERISTICS

The highest percentage of the samples presented sediment classified as sand (77,2%), ranging from very fine to very coarse (with the grain size parameter (Mz) ranging from 1013 μm to 64.68 μm) and present in all sites (Table 1). Mud fractions represented 22,8% of the samples, just beyond coarse silt (with the Mz parameter ranging from 61.88 μm to 32.00 μm), the sites that report this granulometry were Belmonte, BTS and Itacaré (Table 1). In 2020, 88,8% of the samples (BTS, Boipeba, Itacaré and Siribinha) exhibited coarse and very fine sand. The other 11,2% (including samples from BTS and Itacaré) are composed by coarse silt, measured at the sites. In 2021, 77,2% of the samples displayed very coarse sand and very fine sand (found in Siribinha, BTS, Boipeba, Itacaré, Canavieiras, Belmonte and Cumuruxatiba), and only 22,8% were coarse silt, which for this year, was measured in BTS, Itacaré and Belmonte (Table 1). Supplementary Material 1 presents the mean and standard deviation of the granulometry results – Organic matter content (MO) (%), and carbonates content ($CO_3{}^{-2}$) (%) according to the location and year of sampling. BTS had a higher average of organic matter than other sites. However, it is worth mentioning that Siribinha, presented a high variability among the collected samples, showed by the high standard deviation. Regarding CO_3^{-2} content (%), Boipeba presented the highest average.

Year	Site	Sample replica	MZ - Micron (µm)	Granulometric class
2020	BELMONTE	1	Not measure	Not measure
		2	Not measure	Not measure
		3	Not measure	Not measure
	BOIPEBA	1	227.20	Fine sand
		2	565.00	Coarse sand
		3	170.50	Fine sand
	ITACARÉ	1	61.88	Coarse silt
		2	84.21	Very fine sand
		3	32.00	Coarse silt
	BTS	1	58.40	Coarse silt
		2	48.51	Coarse silt
		3	64.68	Very fine sand
	SIRIBINHA	1	259.00	Medium sand
		2	143.00	Fine sand
		3	573.00	Coarse sand
2021	BELMONTE	1	68.10	Very fine sand
		2	61.77	Coarse silt
		3	116.30	Very fine sand
	BOIPEBA	1	1013.00	Very coarse sand
		2	942.10	Coarse sand
		3	1010.00	Very coarse sand
	CANAVIEIRAS	1	68.01	Very fine sand
		2	Not measure	Not measure
		3	70.45	Very fine sand
	CUMURUXATIBA	1	178.70	Fine sand
		2	110.00	Very fine sand
		3	125.60	Fine sand
	ITACARÉ	1	54.34	Coarse silt
		2	Not measure	Not measure
		3	68.51	Very fine sand
	BTS	1	80.70	Very fine sand

		2	38.91	Coarse silt
		3	77.90	Very fine sand
	SIRIBINHA	1	616.30	Coarse sand
		2	135.30	Fine sand
		3	539.20	Coarse sand

Table 1 – PARTICLE SIZE CLASSIFICATION OF WENTWORTH. Table showing the particle size distribution (µm) and the Granulometric class of the samples of studied mangroves. The name of each mangrove was indicated in the column “Site”. The table is divided by year of sampling campaign (2020 and 2021).

The PAHS with the highest average concentration percentage, is Benzo[b]fluoranthene (BbF), Dibenz[a,h]anthracene (DBahA) and Indeno[1,2,3-cd]pyrene (IcdP). The BbF was the most abundant individual PAHs in all sites (Supplementary Material 2). On average, the concentration of all PAHs increased from 2020 (ranging from 422.1 to 212.6 ng g^{-1} with an average of 291.8 ± 75.1 ng g^{-1}) to 2021 (ranging from 1051.4 to 194.3 ng g^{-1} with an average of 309.5 ± 114.9 ng g^{-1}). According to TEL (Threshold Effect Level), which defines limits for each chemical levels analyzed, the concentrations of Acenaphthylene and Dibenz[a,h]anthracene showed ecological risk. Mean acenaphthylene concentrations in Boipeba were above the TEL (5.87 ng g-^{1}). Dibenz[a,h]anthracene showed levels almost five-fold higher than the TEL in 95% of the analyzed samples. In general, the other PAHs concentrations did not exceed the TEL.

BTS had the highest average amount (mg/Kg) of all evaluated metals. From 2020 to 2021, the concentration consistently increased in this site, contrasting with the main pattern observed for other sites (Supplementary Material 3). We identified Cu as the only element with concentrations above those considered "natural", as established by CONAMA Resolution N° 454/ 2012. (CONAMA, 2012). When observing the concentration of metals by region/year (Table 3), the highest values concentration was Mn (ranging from 319 to 35.27 mg/Kg).

3.2 CONTAMINATION INDICATORS

Considering metals and PAHs together within a single contamination scale, the sites with the highest scores of contaminations by the oil spill were BTS, Boipeba and Itacaré; while the less affected was Siribinha, in the first campaign (Table 2). During the second campaign, the higher score of contamination was found in BTS and Itacaré, and the lowest values were from Cumuruxatiba (Table 2). All sites showed sediments enriched more severely by (Mn, Cu, and Zn) and by the PAHs (Benzo[b]fluoranthene, Dibenz[a,h]anthracene, and Indeno[1,2,3-cd]pyrene). These contaminants are, therefore, the leading indicators of oil contamination in the evaluated mangroves. The contamination indicators Ni, Pb, 2-Methylnaphtalene and Perylene were the ones with the lowest concentrations. However, there were a large variance within the same site for several PAHs and metals. A decreasing order in terms of concentration of inorganic forms of trace metals was Mn > Cu > Zn > Cr > As > Ni > Pb. Moreover, a decreasing order in terms of PAH concentration was BbF > DBahA > IcdP > BkF > Phe> BeP > An > Dib > BaA > BghiP > Acy > BaP > Fl > Na > Flo > Py > Chr > 1-MeNa > 2-MeNa > Per. The contamination scale showed an increase in the concentration of contamination indicators from one year to the next in BTS, suggesting the accumulation of these elements and a possible arrival of oil stains after the first sampling.

Fuzzy scale	First campaign (2020)		Second campaign (2021)	
	Site	Average	Site	Average
High	BTS	2.15	BTS	2.25
	BOIPEBA	1.62	ITACARÉ	1.49
	ITACARÉ	1.51		
Medium	SIRIBINHA	1.20	CANAVIEIRAS	1.35
			SIRIBINHA	1.34
			BELMONTE	1.30
Low			BOIPEBA	1.18
			CUMURUXATIBA	1.13

Table 2 – GRADIENT CONTAMINATION. Table showing the *Gradient Contamination* in each site, divided by *Fuzzy scale* “High”, “Medium” and “Low”. In the first sampling campaign (2020) the sites were grouped in two levels of the Fuzzy scale and in the second sampling campaign (2021) grouped the sites in the three levels.

3.3 BENTHIC FAUNA DIVERSITY ANALYSIS

For alpha diversity, neither the Chao 1 nor the Shannon index showed a significant difference between the contamination gradient levels (Fig.3 A, B, C, and D).

In both 2020 and 2021 samples, sites classified as "Medium" contaminated had higher variance measures (Fig.3).

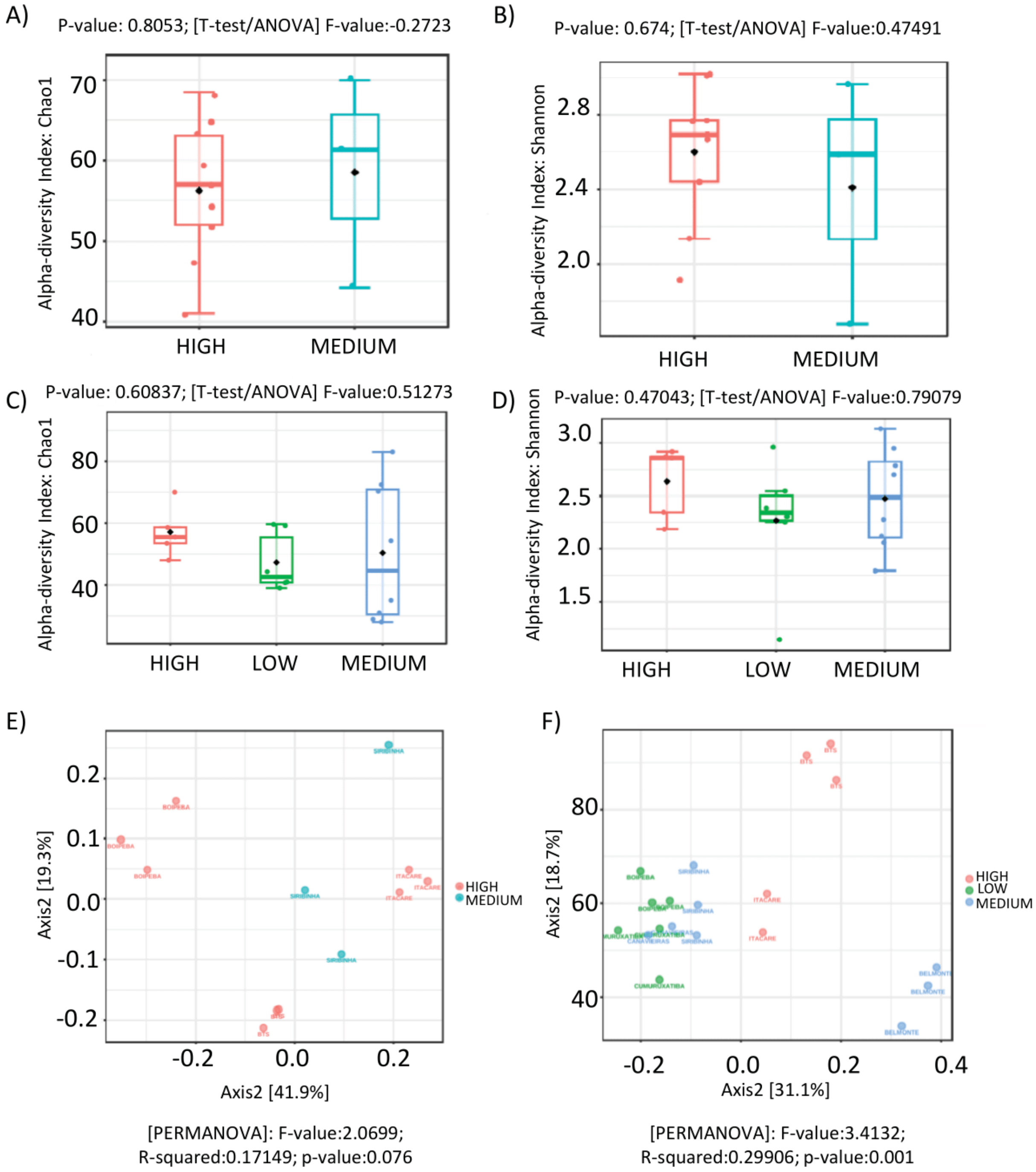


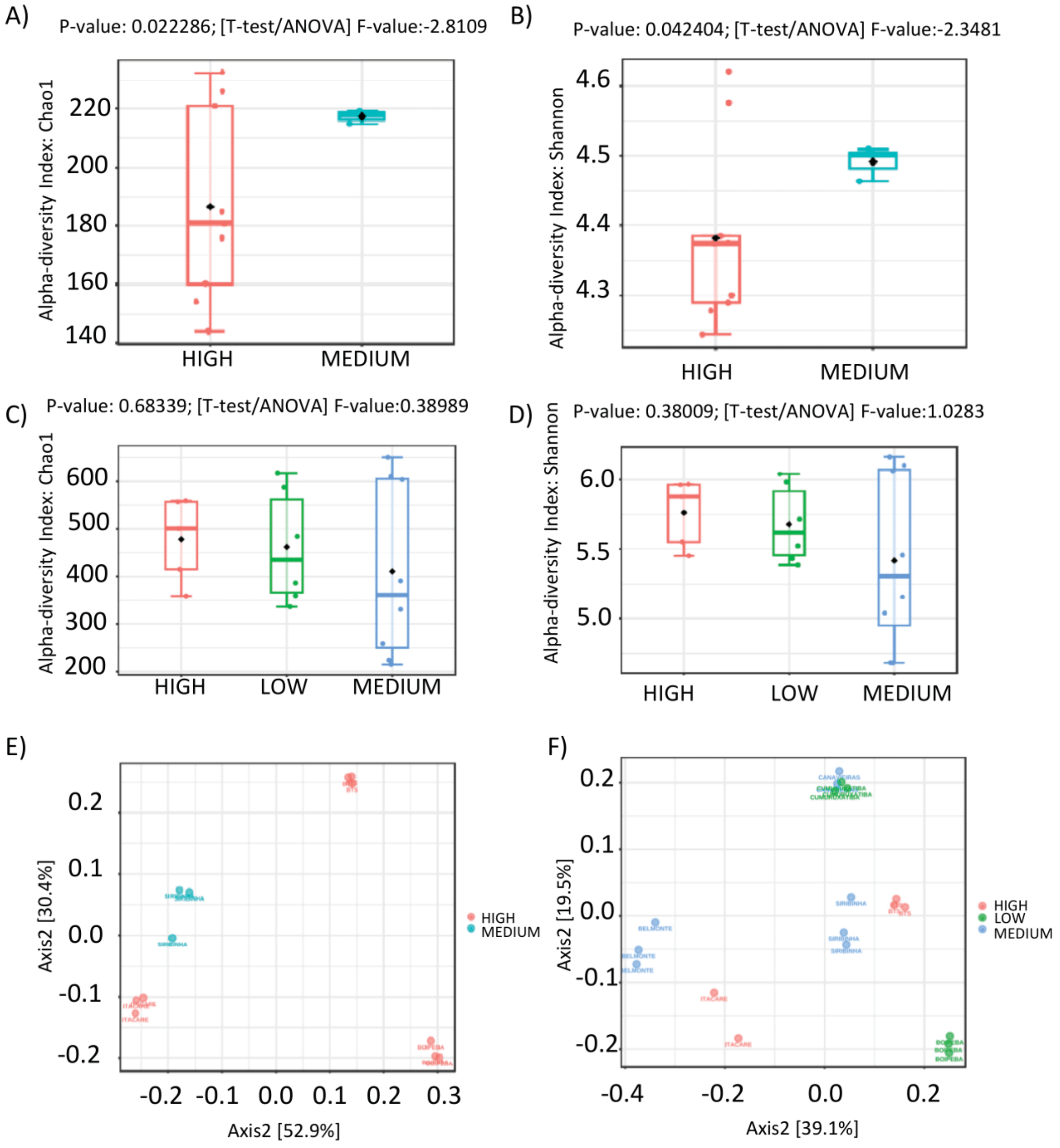
Figure 3- BENTHIC FAUNA DIVERSITY ANALYSIS. Box and whiskers plot of benthic fauna alpha-diversity: A) Chao 1 index (2020); B) Shannon index (2020); C) Chao 1 index (2021) D) Shannon index (2021). The feature-level alpha-diversity associated with each contamination level has been calculated according to Chao1 and Shannon diversity metrics with the following parameters: data input: filtered, experimental factor: contamination level, taxonomic level: feature-level, statistical method T-test: ANOVA. PcoA (Principal coordinates analysis) plot of benthic fauna beta diversity: E) PcoA (2020). F) PcoA (2021). The beta-diversity of benthic fauna assemblages among the different contamination levels was estimated by a PCoA analysis based on Jensen-Shannon Divergence, with the following parameters: taxonomic level: feature-level, statistical method: PERMANOVA, experimental factor: Contamination Level. Dots represent all samples; Different colors represent different levels of contamination; Axis.1 and Axis.2 represent two eigenvalues that cause largest difference between

samples, they represent the degree of major influence in the form of a percentage. The closer the distance, the higher is the similarity.

The benthic fauna beta diversity revealed that the composition was different between the sites classified as "High," "Low," and "Medium" for 2021 (p-value=0.001 in 2021) (Fig.3, F). During 2020, axes 1 and 2 explained 61.2%, and during 2021, the sum of the axes explained 49.8% of the observed variability (Fig.3, E, and F).

3.4 MICROBIOME DIVERSITY FAUNA DIVERSITY ANALYSIS

For microbiome alpha diversity, Chao 1 and Shannon index showed significant differences among the levels of contamination gradient in 2020 (see the p-values < 0.05 in Fig.4 A and B), with higher variance measures in sites classified with "medium" contamination (Fig.4 A and B). In 2021, the Shannon and Chao index did not show significant differences, but the highest variances of these indexes were observed on sites classified as "medium" contaminated. For the microbiome, the composition was different between the "Medium," "low," and "high" contaminated sites for 2020 and 2021 (Fig 4. E and F); The main components, axis1 and axis 2, together explain in 2020 a total of (83.3%) and in 2021 (58.6) (Fig 4. E and F).



[PERMANOVA]: F-value:2.6963;
R-squared:0.21237; p-value:0.049

[PERMANOVA]: F-value:3.1264;
R-squared:0.28099; p-value:0.005

Figure 4- MICROBIOME DIVERSITY ANALYSIS. Box and whiskers plot of microbiome alpha-diversity: A) Chao 1 index (2020); B) Shannon index (2020); C) Chao 1 index (2021) and D) Shannon index (2021). The feature-level alpha-diversity associated with each contamination level has been calculated according to Chao1 and Shannon diversity metrics with the following parameters: data input: filtered, experimental factor: contamination level, taxonomic level: feature-level, statistical method T-test: ANOVA. PcoA (Principal coordinates analysis) plot of microbiome beta diversity: E) PcoA (2020). F) PcoA (2021). The beta-diversity of microbiome assemblages among the different contamination levels was estimated by a PCoA analysis based on Jensen-Shannon Divergence, with the following parameters: taxonomic level: feature-level, statistical method: PERMANOVA, experimental factor: Contamination Level. Dots represent all samples; Different colors represent different levels of contamination; Axis.1 and Axis.2 represent two eigenvalues that cause largest difference between samples, they represent the degree of major influence in the form of a percentage. The closer the distance, the higher is the similarity.

3.5 ROLES OF CONTAMINATION LEVELS ON BENTHIC FAUNA AND MICROBIOME COMMUNITY

With the discriminant analysis effect size (LEfSe), differently abundant taxa between the levels of contamination and the estimative of the effect size of each significant taxa for benthic fauna and microbiome were shown in Figures 5 and 6. The taxas represented in the LEfSe histogram have statistically significant differences and consistent biological differences.

For the benthic fauna, in 2020, taxa enriched in the “Medium” contaminated sites are indicated with a positive LDA score (blue), and taxa enriched in the “High” contaminated sites have a negative score (red). LEfSe analysis detected 2 benthic fauna families in “Medium” contaminated sites, and 7 in “High” contaminated sites, with an LDA score higher than 2.0. The families Spirina, Anoplostomatidae, Syllidae, Echinoderidae, Rhabdolaimidae, Haliplectoidea, and Chromadoridae of the genus *Chromadora* all had negative scores in the sites classified how “High contamination” while Capitellidae and Spionidae have positive scores in the sites classified how “Medium” (Fig 5, B). In 2021, all taxa were enriched in the “Low” (blue), “Medium” (green) and “High” (red) contaminated sites and are indicated with a positive LDA scores. LEfSe detected 6 benthic fauna families in “Low” contaminated sites and 3 in “Medium” contaminated sites, and 7 in “High” contaminated sites, with an LDA score higher than 2.0. Placorhynchidae, Spirina, Oxystominidae, Syllidae, and Echinoderidae had positive scores in the sites classified how “Low” contaminated sites; Loxoconchidae, Chaetonotidae, and Leptolaimoidea had positive scores in “Medium” contaminated sites, while Capitellidae, Promesostomidae, and Naididae has positive scores in “High” contaminated sites (Fig 5, D). Capitellidae the third most abundant taxa in 2020 and the second most abundant in 2021, in particular is detected by LEfSe with a very high LDA score - more than five orders of magnitude (LDA score>4.0, $p<0.05$); this demonstrates that is the only taxa who showed a strong positive association with “Medium contamination” and “High contamination,” which is why it can be considered as potentially tolerant to metals and PAHs contamination (Fig 5).

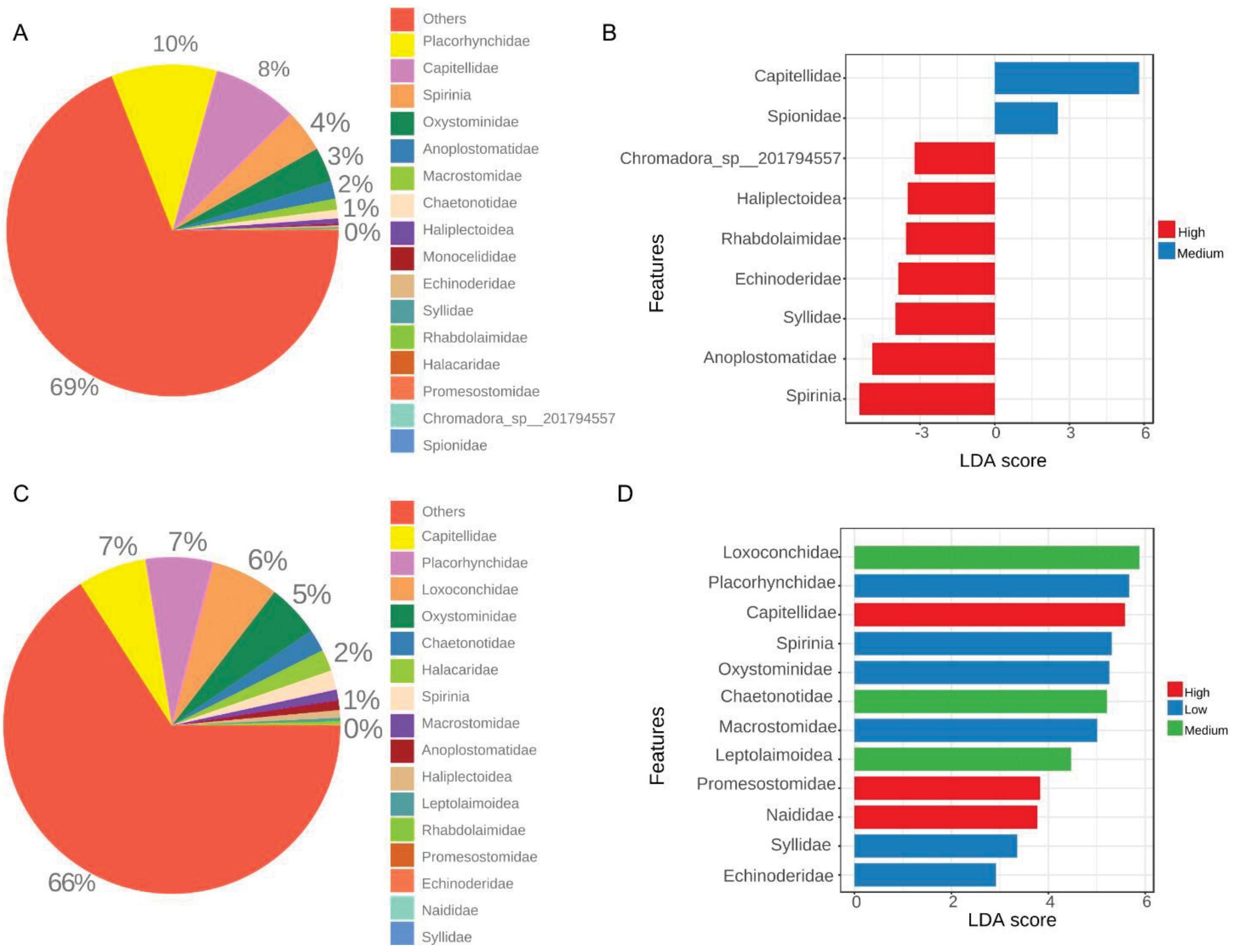
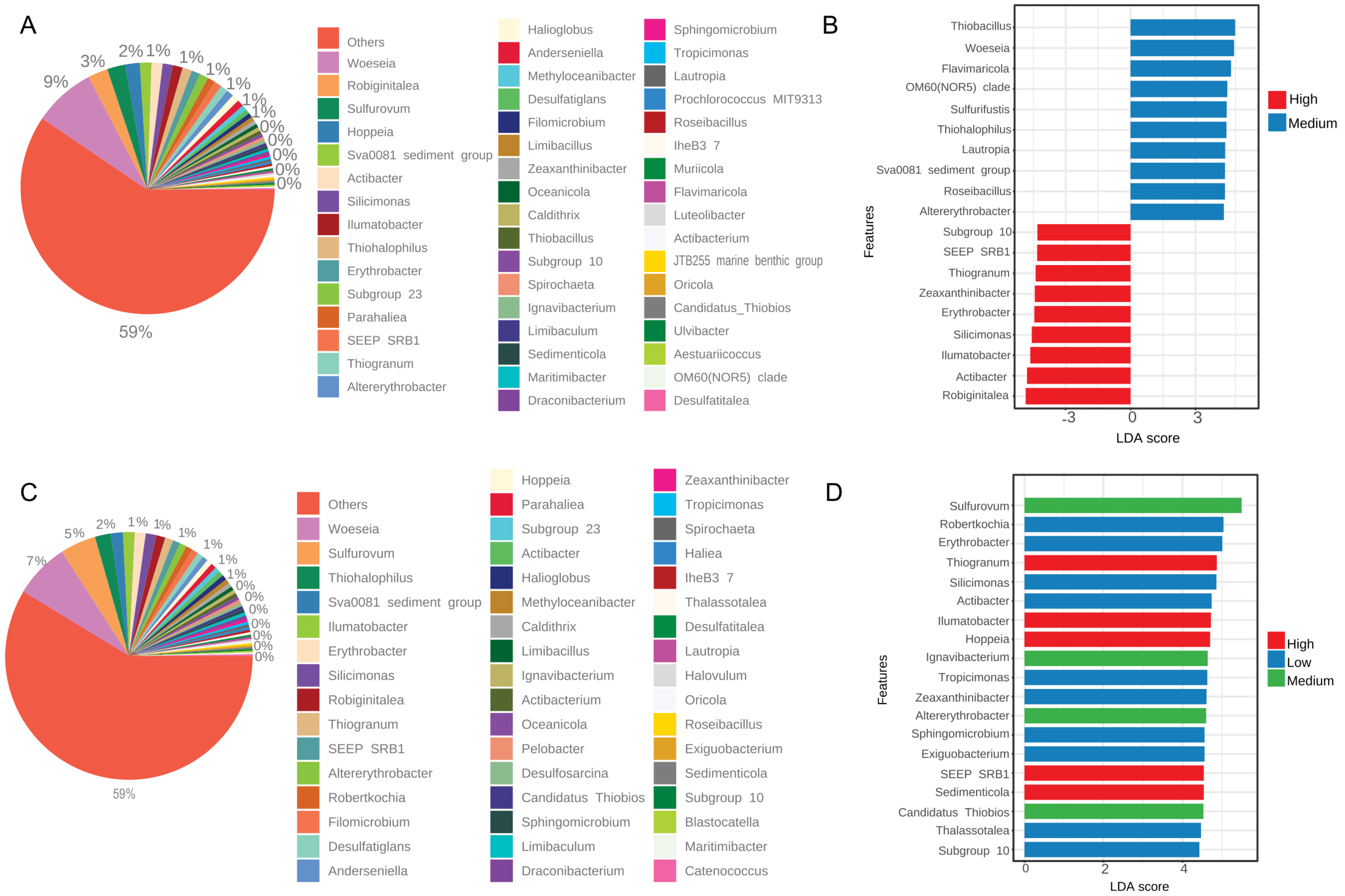


Figure 5- PIE CHART AND LINEAR DISCRIMINANT ANALYSIS EFFECT SIZE. A) and C) show the relative abundances in the "Family" taxonomic level, showing top n taxa, with n =50. In the Pie chart the relative abundances were represented by different colors. The identification of each taxon can be seen in the legend on the right. Linear discriminant analysis effect size (LEfSe) was applied to identify the benthic fauna taxa with different abundances in three different levels of contamination, "High," "Low," and "Medium" groups, represented by three different colors. B) and C) Show the families whose LDA score is higher than the set value (the default is 4.0). The histogram length represents the impact of different species (i.e., LDA Score), and each bar represents different ZOTUs at family levels. A) and B) are plots representing the first sample campaign, 2020, and C) and D) represent the second sample campaign, 2021.

For the microbiome in 2020, taxa enriched in the “Medium” contaminated sites are indicated with a positive LDA score (blue), and taxa enriched in the “High” contaminated sites have a negative score (red). LEfSe analysis detected 10 microbiome genera in “Medium” contaminated sites, and 9 in “High” contaminated sites, with an LDA score higher than 2.0. The taxa *Thiobacillus*, *Flavimaricola*, *Sulfurifustis*, *Thiohalophilus*, *Sva0081_sediment_group*, and *Roseibacillus* (LDA score>4.0, ρ<0.05) had positive scores with “Medium” contamination sites. No taxa showed positive scores concerning “High” contaminated sites (Fig 6 B). In 2021, all microbime taxa were enriched in the “Low” (blue), “Medium” (green) and “High” (red) contaminated sites and are indicated with a positive LDA scores. LEfSe detected 10 microbiome genera in “Low” contaminated sites and 4 in “Medium” contaminated sites, and 5 in “High” contaminated sites, with an LDA score higher than 2.0. *Thiogranum*, *Ilumatobacter*, *Hoppeia*, *SEEP SRB1*, and *Sedimenticola* genus (Fig 6 C and D) showed positive scores associated with “High” contaminated sites; these taxa have LDA scores >4.0 and p<0.05, and can be considered as a family potentially tolerant to metals and PAHs contamination.


A
9%
3%
2%
1%
1%
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1%
1%
1%
0%
0%
0%
0%
0%
59%
Others
Woeseia
Robiginitalea
Sulfurovum
Hoppeia
Sva0081 sediment group
Actibacter
Silicimonas
Ilumatobacter
Thiohalophilus
Erythrobacter
Subgroup 23
Parahaliea
SEEP SRB1
Thiogranum
Altererythrobacter
Halioglobus
Anderseniella
Methyloceanibacter
Desulfatiglans
Filomicrobium
Limibacillus
Zeaxanthinibacter
Oceanicola
Caldithrix
Thiobacillus
Subgroup 10
Spirochaeta
Ignavibacterium
Limibaculum
Sedimenticola
Maritimibacter
Draconibacterium
Sphingomicrobium
Tropicimonas
Lautropia
Prochlorococcus MIT9313
Roseibacillus
IheB3 7
Muriicola
Flavimaricola
Luteolibacter
Actibacterium
JTB255 marine benthic group
Oricola
Candidatus_Thiobios
Ulvibacter
Aestuariicoccus
OM60(NOR5) clade
Desulfatitalea
B
Features
Thiobacillus
Woeseia
Flavimaricola
OM60(NOR5) clade
Sulfurifustis
Thiohalophilus
Lautropia
Sva0081 sediment group
Roseibacillus
Altererythrobacter
Subgroup 10
SEEP SRB1
Thiogranum
Zeaxanthinibacter
Erythrobacter
Silicimonas
Ilumatobacter
Actibacter
Robiginitalea
-3
0
3
LDA score
High
Medium
C
7%
5%
2%
1%
1%
1%
1%
1%
1%
0%
0%
0%
0%
0%
0%
0%
59%
Others
Woeseia
Sulfurovum
Thiohalophilus
Sva0081 sediment group
Ilumatobacter
Erythrobacter
Silicimonas
Robiginitalea
Thiogranum
SEEP SRB1
Altererythrobacter
Robertkochia
Filomicrobium
Desulfatiglans
Anderseniella
Hoppeia
Parahaliea
Subgroup 23
Actibacter
Halioglobus
Methyloceanibacter
Caldithrix
Limibacillus
Ignavibacterium
Actibacterium
Oceanicola
Pelobacter
Desulfosarcina
Candidatus Thiobios
Sphingomicrobium
Limibaculum
Draconibacterium
Zeaxanthinibacter
Tropicimonas
Spirochaeta
Haliea
IheB3 7
Thalassotalea
Desulfatitalea
Lautropia
Halovulum
Oricola
Roseibacillus
Exiguobacterium
Sedimenticola
Subgroup 10
Blastocatella
Maritimibacter
Catenococcus
D
Features
Sulfurovum
Robertkochia
Erythrobacter
Thiogranum
Silicimonas
Actibacter
Ilumatobacter
Hoppeia
Ignavibacterium
Tropicimonas
Zeaxanthinibacter
Altererythrobacter
Sphingomicrobium
Exiguobacterium
SEEP SRB1
Sedimenticola
Candidatus Thiobios
Thalassotalea
Subgroup 10
0
2
4
LDA score
High
Low
Medium

Figure 6-. PIE CHART AND LINEAR DISCRIMINANT ANALYSIS EFFECT SIZE. A) and C) show the relative abundances in the "Genus" taxonomic level, showing top n taxa, with n =50. In the Pie chart the relative abundances were represented by different colors. The identification of each taxon can be seen in the legend on the right. Linear discriminant analysis effect size (LEfSe) was applied to identify the bacterial taxa with different abundances in three different levels of contamination, "High," "Low," and "Medium" groups, represented by three different colors. B) and C) Show the genus whose LDA score is higher than the set value (the default is 4.0). The histogram length represents the impact of different species (i.e., LDA Score), and each bar represents different ASVs at genus levels. A) and B) are plots representing the first sample campaign, 2020, and C) and D) represent the second sample campaign, 2021.

4 DISCUSSION

Both benthic fauna and microbiome responded to oil contamination. Although diversity was not lower in the most contaminated sites, changes in the composition of the benthic fauna and the microbiome according to the established contamination gradient were observed and suggests a species/taxon-specific response, which was evident in the LDA-LfSe analyses.

The oil-impacted mangrove sediments affected the benthic fauna and microbiota, mainly explained by the beta diversity. The results of alpha diversity on species richness (Chao1) and the measurement of the effects of each species for each level of contamination (LfSe) showed from one year to the next a tendency less tolerant species to be replaced by more stress-tolerant species. This may have occurred mainly because the 2019 oil spill caused the destruction of the habitat of many species, as a consequence, competition for both space and food resources increases, if they are unable to migrate to other places where conditions are ideal for their survival, these taxa may disappear and be replaced by others. Some pollutant-tolerant species may also increase their dominance at the expense of sensitive species through immigration from unaffected areas (Afli et al., 2008; Amaro et al., 2018). The loss of species with low redundancy and critical ecological functions in the community can cause significant changes in the functional profile of the community (Moya & Ferrer, 2016). Pollutant-sensitive species' abundance is likely to plummet on exposure to oil pollution, whereas the pollutant-tolerant species tend to survive and proliferate, presenting a different community structure (Mulik et al., 2023).

A high variability was observed for the alpha diversity for the microbiome and benthic fauna for sites classified as "Medium" contaminated (Fig 3 C and D; Fig 4 C and D). The higher variability observed in sites classified as "Medium" contamination can be explained by the intermediate perturbation hypothesis, described by (Connel, 1978), which assumes that local diversity depends on local extinction rates (caused by predators, diseases, competitive exclusion, or by unpredictable disturbances). This hypothesis predicts that the diversity in a community subjected to an intermediate level of disorder would be greater than that under large or small disturbances, therefore, "High" and "Low" tend to present smaller diversities. "Low," according to this hypothesis, due to the low level of disturbance, allows competition within habitats to reduce diversity. Numerous works of benthic ecology apply this concept (e.g., Pearson & Rosenberg, 1978; Weston, 1990; Rosenberg et al., 2004; Begon et al., 2007). Through evolution, benthic species have adjusted to cope with predicted environmental variations and interspecific competition (Rosenberg et al., 2004). Intermediate disturbances increase species richness compared to extreme degrees of disturbance (small and large) (Begon et al., 2007). A significant disturbance introduces changes in the species composition, abundance, and biomass (Rosenberg et al., 2004). Such successional changes in benthic community structure are often predictable, and with increased perturbation, the diversity, abundance, and biomass will show a general decline (Pearson & Rosenberg, 1978). A benthic community subject to pollution will exhibit a decrease in mean species size, with infauna occupying shallow sediment depths (Weston, 1990). Although the intermediate perturbation hypothesis seems consistent with the results of this research, it is worth noting that in 2020 no site was classified as "Low" contaminated, therefore, the high variability of diversity observed in sites classified as "Medium" may be due to an analytical artifact used in the Fuzzy scale for the contamination gradient.

The acute impact caused by the oil spill in Bahia added to chronic impacts of contamination that were not previously measured for the sampled mangroves, which could cause even more abrupt changes in the community composition over time, while metals may be present in the sediment through natural sources, PAHs are mostly the result of anthropic action. Polycyclic aromatic hydrocarbons are a class of persistent organic compounds widely distributed in the soil, river, groundwater, and ocean (Abdel-Shafy & Mansour, 2016). The PAH distribution in the sediment samples varied significantly among different sites, what draws attention is that the largest variances were observed in Boipeba and in BTS. Our BTS collection point was located adjacent to the Rio São Paulo estuary, which according to IBAMA reports did not receive high amounts from the 2019 accident, therefore this site cannot have been affected by the spill in high proportions as our results indicated. The high concentrations of benzo[b]fluoranthene, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene recorded in BTS, reflect the influence of different anthropogenic inputs and sources of PAHs and the proximity of the collection point with a densely populated and industrialized area and is subject to long-term hydrocarbon contamination from industrial, port, and petroleum exploitation activities (Milazzo et al., 2020; Miranda et al., 2017; Barbosa, 2022). Boipeba was another site that showed a high discrepancy, in the first sampling campaign (2020) it was considered a highly contaminated site according to our Fuzzy scale, because compared to other sites it showed high concentrations of fluoranthene, pyrene, and benzo[a]anthracene found, however, in the second sampling campaign in 2021, it was considered a low contamination site. This decrease in contamination from 2020 to 2021 in Boipeba does not mean that the mangrove "recovered" naturally. Thus, on this site, large amounts of oil were removed by the cleaning efforts of the community volunteers (Barbosa, 2022). Furthermore, we cannot rule out that in Boipeba the high contamination values measured in 2020 may be a recurrence of the entry of contaminants into the environment by other anthropic activities in this period. Boipeba is located on a well-preserved coastal island without direct sources of contamination, except for the occasional traffic of small boats around the island (Barbosa, 2022). In general, the contamination indicators persisted from 2020 to 2021 in sediments; this is a long period which indicates the recovery of these mangroves might be a slow process or that the mangroves continued to receive input of contaminants in 2021. In our diagnostic, the influence of different anthropogenic inputs and sources of PAHs masked the effect of PAHs and metals contamination from the 2019 spill.

Our data indicate that pyrogenic sources (e.g., biomass, coal, petroleum combustion, and industrial waste) are the main contributor to the PAH and metal concentrations detected in the analyzed sediments, primarily related to biomass combustion. Although the influence of this oil spill has not been observed in surface sediments analyzed at impacted sites, more studies using additional tools (e.g., analysis of alkylated PAHs to the identification of petrogenic sources, analysis of sedimentary cores to define the baseline levels of PAHs at impacted sites) are needed for a better understanding of the impacts related to the oil spill. Our study highlights the importance of having past measurements of contaminating elements in oil spill assessments, mainly to understand the level of impact and monitor ecosystem recovery. Recovery of an ecosystem occurs when it re-establishes to a close approximation of its original structure and related Physico-chemical characteristics prior to the disturbance (Kingston, 2002; Dufour & Piégay, 2009). So, accurately diagnosing community changes in benthic fauna and microbiome without these previous measurements is challenging. In regions lacking historical data on anthropogenic perturbations, a reference site can help differentiate human-induced alterations in biological community structure from natural variations (Mulik et al., 2023). Mainly in terms of diversity, it is challenging to investigate the combined contamination of heavy metals and polycyclic aromatic hydrocarbons (PAHs). Although most of the evaluated metals and PAHs are not found in the study area at values considered toxic to organisms, it does not mean that their "synergistic" effect is not causing damage to soil fauna. On the contrary, the synergy observed between the spatial distribution of metals and PAHs exacerbates adverse effects on human and ecological health (Fazeli et al. 2019). With the development of society and technological advances, highly developed industrialization and urbanization are the primary sources of Heavy metals and polycyclic aromatic hydrocarbons in sediments (Hung et al., 2022 a; b). Generated in significant quantities by industrial processes, such as steel production and petroleum processing, they are often co-contaminants in aquatic and terrestrial environments (Amodio-Cocchieri, 1993; Wey, 1996; Hung et al., 2022 a). Nevertheless, little is known about the extent or mechanisms of their co-toxicity. When PAHs and metals co-exist in the environment, their harmful effect is aggravated (Wang et al., 2022). Moreover, these compounds' physiological mechanisms of action, alone or together, are not well understood (Babu et al., 2001; Plomp et al., 2020; Wang et al., 2022).

In the soil system, heavy metals and polycyclic aromatic hydrocarbons (PAHs) are two contaminants with different properties (Chen et al., 2016). Both are trace and persistent environmental pollutants, which are stable in the soil and readily adsorbed in soil organic matter (Yuesuo et al., 2017). In fact, due to their similar chemical properties and sources, more studies in recent years have focused on the coexistence of these two types of pollutants in the environment (Gulan et al., 2017; Thavamani et al., 2012; Wang et al., 2018; Lu et al., 2023). Our study demonstrated that using a contamination scale can be valuable to measure the synergistic effect on the affected fauna when there is difficulty in considering which site is more contaminated. On the other hand, a scale can mask the observed response since each species, in addition to responding to the spatial gradient, also responds differently to specific contaminants and may have beneficial interactions or/and suffer toxic or lethal effects. In addition, they may impair the quality of ecological interactions, leading to diversity fluctuations and increasing competition in the habitat. Oil spill-associated reduction in diversity and abundance can be attributed to various reasons, such as loss of habitat, clean-up tasks, or oil toxicity (Shigenaka, 2014).

The potential fauna tolerant to highly contaminated mangroves were organisms from the Capitellidae family of the benthic fauna and organisms from the genus Thiogranum, Ilumatobacter, Hoppeia, SEEP SRB1, and Sedimenticola. Capitellidae is one of the most abundant groups of marine soft-bottom sediment polychaetes. Moreover, it is an opportunistic family known to increase in abundance in the presence of petroleum hydrocarbons, showed some resistance, and is abundant in sediments recorded with high concentrations of heavy metals such as Cu, Pb, and Cd (Hyland et al., 1985; Olsgard & Gray, 1995; Einav et al., 2002; Avramidi et al., 2022; Al Solami & Satheesh, 2022). However, LEfSe results revealed that Chromadoridae of the genus Chromadora have adverse effects in the sites classified as "High contamination. In the literature, some species of this genus were identified as tolerant species (e.g., Daptonema fallax (Mahmoudi et al., 2005), Spirina parasitifera (Louati et al., 2015)). Thiogranum is a genus of Proteobacteria identified as sulfur-oxidizing bacteria involved in N, P, and S cycling. (Sorokin et al. 2007; Mori et al.2015; Li et al., 2022). The members of Thiogranum of the class Gammaproteobacteria. Members of the Gammaproteobacteria are well-known hydrocarbon-degrading bacteria found in marine environments (Gutierrez, 2015; Mishamandani et al., 2014; Tiralerdpanich et al., 2018). However, more evidence on the hydrocarbon-degrading ability of Thiogranum is needed (Tiralerdpanich et al., 2021). OTUs representing the Ilumatobacter genus Actinobacteria were previously found in marine and freshwater sediments (Fang et al., 2015; Gugliandolo et al., 2016), and also found in high abundance in Republic Creosoting Industries (RCI) fish gut libraries (7.2% of all sequences) (Redfern et al., 2021). A survey involving the shifts in the commensal microbiome of Atlantic killifish (Fundulus heteroclitus) in the Elizabeth River, VA, reveals that Iluminobacter has a prevalence in the relative abundance in the RCI sediment libraries samples, suggesting they are potentially a part of a microbiome adaption to the contaminated environment by PAHs, but the results of the description provide few clues as to what role they may play (Matsumoto et al., 2013; Redfern et al., 2021). The genus Hoppeia is a member of the family Flavobacteriaceae, phylum Bacteroidetes (Kwon et al., 2014). The only species described in this genus (namely, *Hoppeia youngheungensis*) was isolated from flat tidal sediment collected from an island in the West Sea of Korea (Kwon et al., 2014), which evidences that marine environments are typical habitats for members of this genus (Zhang et al., 2014). The current status of west sea fisheries resources and utilization in Korea's fishery management context. Ocean & coastal management, 102, 493-505.). However, no current literature proves that this genus is tolerant to oil spills. The SEEP-SRB1 is a diverse clade of sulfate-reducing bacteria affiliated with the deltaproteobacterial family Desulfobacteraceae (Knittel et al., 2003; Schreiber et al., 2010). The SEEP-SRB1 clade comprises six subgroups, SEEP-SRB1a – SEEP-SRB1e (Schreiber et al., 2010). they have been shown to predominate in seep environments where residual oil and aromatic hydrocarbons were observed (Kleindienst et al., 2012; Vigneron et al.,2017). While these subgroups are thought to play a role in non-methane hydrocarbon degradation, little genomic information is available to confirm their metabolic function (Petro et al., 2019). Within Proteobacteria, some Gammaproteobacteria were also found in the contaminated marine sediment, such as *Sedimenticola* spp. (1% of total ZOTUs), most of them known as sulfur-oxidizing bacteria in marine environments capable of coupling the oxidation of elemental sulfur and sulfide to autotrophic growth and producing sulfur inclusions as metabolic intermediates (Flood et al., 2015; Matturro et al.,2017).

Our results suggest the need for further efforts to shed light on the identity of novel microorganisms involved in petroleum hydrocarbon degradation in mangrove environments. We conclude that even with all this evidence in the literature corroborating our results that these organisms are possible tolerant taxa, laboratory and *in situ* tests need to be carried out to confirm our findings. However, it is worth mentioning that a large part of the microbiota we indicate as possible tolerant are still not cultivable, which requires technological advancement.

5 CONCLUSION

The sites with the higher concentrations of the PAHs and metals compounds were BTS, Boipeba, and Itacaré, while the less contaminated site was Siribinha in 2020. In 2021, sites with higher concentrations of the PAHs and metals compounds were BTS and Itacaré, and fewer concentrations were found in Cumuruxatiba. Boipeba was the only site that showed a decrease in contamination concentration between 2020 and 2021. All sites showed sediments enriched more severely by (Mn, Cu, and Zn) and by the PAHs (Benzo[b]fluoranthene, Dibenz[a,h]anthracene, and Indeno[1,2,3-cd]pyrene). These contaminants are the leading indicators of oil contamination in the evaluated mangroves. Although we could not find out if all taxa respond to all PAHs and Metals, we assume that those with higher concentrations are responsible for most of the observed effects. However, studies to assess their levels of interaction and co-contamination are necessary to confirm this. Alpha diversity did not show significant differences for benthic fauna and microbiome among contaminated sites. Differences in composition were visualized in beta diversity analyses, in 2021, both for the benthic fauna and the microbiome; the most significant variability and amplitude of diversity occurred in sites classified as "Medium." We cannot discover whether this result is a consequence of the sample artifact or explained by the intermediate perturbation hypothesis.

Results from our study demonstrate that PAH and metals mixture caused significant changes in benthic fauna and microbiome. The dominance of opportunistic species (the Capitellidae family of the benthic fauna, and organisms from the genus Thiogranum, Ilumatobacter, Hoppeia, SEEP SRB1, and Sedimenticola;) can be used as a bioindicator for monitoring mangroves impacted by PAHs and metals. The fauna potentially tolerant to highly contaminated mangroves were also the taxa with the highest relative abundances in highly contaminated sites. Even with all these taxa being possibly tolerant, laboratory and *in situ* tests must confirm our findings. However, it is worth mentioning that a large part of the microbiota that we indicate as possible tolerant still needs to be cultivable, which requires technological advancement. There are some limitations of this study. The effects of oil spills on benthic fauna and microbiome groups require investigation since it was impossible to know which and what percentage of analyzed PAHs and metals were involved with the 2019 oil spill.

The lack of monitoring before the disaster, added to the input of different anthropogenic sources of PAHs and metals in the collected mangroves, masked the effect of PAHs and metals contamination from the 2019 spill. Our data indicate that pyrogenic sources are the main contributors to the PAH and metal concentrations detected in the analyzed sediments, primarily related to biomass combustion. In future analysis, we suggest implementing additional data using additional tools to understand the oil spill impacts.

Author contributions

Both authors contributed to the review concept, data interpretation, and the writing of the manuscript. PD performed the literature search and statistical analysis.
Both authors contributed intellectually to this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approvals

This review article does not contain any studies with human participants performed by any of the authors. This review article does not contain any studies with animals performed by any of the authors.

Consent for publication

All authors have contributed to the preparation and editing of this manuscript and have approved it for publication in its current form.

Supplementary data

After the REFERENCES topic.

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SUPPLEMENTARY MATERIAL

Supplementary material 1: Mean ± standard deviation of the abiotic data of Organic matter (OM) content (%) and CO_3^{-2} content. (%) according to the site and year of sampling.

Variable	Local	Year	
		2020	2021
OM (%)	Belmonte	--------	1,89 ± 0,28
	Boipeba	10,33 ± 2,21	10,34 ± 4,99
	BTS	15,00 ± 4,13	17,19 ± 3,73
	Canavieiras	--------	2,67 ± 0,70
	Cumuruxatiba	--------	3,90 ± 0,78
	Itacare	7,85 ± 0,94	10,42 ± 4,06
	Siribinha	7,44 ± 2,93	11,59 ± 10,02
CO_3^{-2} (%)	Belmonte	--------	3,08 ± 2,01
	Boipeba	32,97 ± 5,74	37,33 ± 5,05
	BTS	9,35 ± 3,00	12,44 ± 1,96
	Canavieiras	--------	3,90 ± 1,36
	Cumuruxatiba	--------	13,27 ± 1,74
	Itacare	3,62 ± 1,92	5,80 ± 0,74
	Siribinha	5,58 ± 1,51	6,74 ± 2,75

Supplementary material 2: Mean ± standard deviation of the abiotic data of PAHs ng g^{-1} (%) Metals content (%) according to the site and year of sampling.

Variable	Local	Year	
		2020	2021
Na (ng g^{-1})	Belmonte	--------	1,98 ±3,13
	Boipeba	14,70 ± 4,30	0,44 ± 0,38
	BTS	2,55 ± 1,68	4,89 ± 6,10
	Canavieiras	--------	27,02 ± 1,96
	Cumuruxatiba	--------	11,49 ± 4,42
	Itacare	7,37 ± 5,82	8,46 ± 6,39
	Siribinha	13,49 ± 8,68	17,62 ± 15,94
2-MeNa (ng g^{-1})	Belmonte	--------	0,00 ± 0,00
	Boipeba	6,31 ± 6,54	1,08 ± 0,39
	BTS	6,31 ± 0,51	3,65 ± 0,36
	Canavieiras	--------	3,62 ± 0,49
	Cumuruxatiba	--------	1,79 ± 0,72
	Itacare	0,83 ± 1,11	1,00 ± 0,97
	Siribinha	0,04 ±0,07	0,01 ± 0,01
1-MeNa(ng g^{-1})	Belmonte	--------	0,57 ± 0,40
	Boipeba	4,60 ± 4,03	2,05 ± 1,44
	BTS	2,76 ± 0,29	1,87 ± 0,37
	Canavieiras	--------	3,64 ± 0,69
	Cumuruxatiba	--------	2,06 ± 0,31
	Itacare	3,16 ± 0,50	0,91 ± 0,07
	Siribinha	2,15 ± 0,91	1,69 ± 1,25

Acy (ng g^{-1})	Belmonte	--------	8,84 ± 1,13
	Boipeba	59,47 ±9,54	9,77 ± 2,30
	BTS	26,71 ± 19,74	6,98 ± 2,06
	Canavieiras	--------	10,97 ± 2,25
	Cumuruxatiba	--------	11,21 ± 7,44
	Itacare	9,08 ±4,59	9,70 ± 2,46
	Siribinha	10,49 ± 9,28	12,52 ± 7,13
Flo (ng g^{-1})	Belmonte	--------	4,73 ± 1,41
	Boipeba	3,16 ± 2,96	12,52 ± 14,73
	BTS	5,70 ± 0,55	10,60 ± 2,49
	Canavieiras	--------	5,08 ± 0,76
	Cumuruxatiba	--------	1,50 ± 2,60
	Itacare	5,40 ± 1,09	5,14 ± 0,42
	Siribinha	3,24 ± 0,11	8,75 ± 5,55
Dib (ng g^{-1})	Belmonte	--------	18,42 ± 1,74
	Boipeba	18,55 ± 0,60	18,86 ± 0,29
	BTS	18,71 ± 0,79	20,22 ± 0,49
	Canavieiras	--------	18,42 ± 3,68
	Cumuruxatiba	--------	21,75 ± 1,75
	Itacare	18,93 ± 1,93	18,45 ± 0,50
	Siribinha	17,73 ± 0,99	17,40 ± 0,75
Phe (ng g^{-1})	Belmonte	--------	17,82 ± 1,40
	Boipeba	22,80 ± 4,46	45,61 ± 47,20
	BTS	25,99 ± 0,62	28,06 ± 1,19
	Canavieiras	--------	20,38 ± 3,50
	Cumuruxatiba	--------	17,39 ± 1,04
	Itacare	22,14 ± 7,13	17,08 ± 0,21
	Siribinha	18,68 ± 1,08	21,07 ± 4,31
An (ng g^{-1})	Belmonte	--------	16,80 ± 0,16
	Boipeba	19,41 ± 1,34	23,96 ± 9,50
	BTS	24,77 ± 0,47	30,25 ± 1,59
	Canavieiras	--------	16,76 ± 0,23
	Cumuruxatiba	--------	16,52 ± 0,10
	Itacare	19,40 ± 3,45	17,04 ± 0,01
	Siribinha	17,31 ± 0,64	17,80 ± 0,90
Fl (ng g^{-1})	Belmonte	--------	2,70 ± 0,11
	Boipeba	13,87 ± 10,16	55,67 ± 79,48
	BTS	14,53 ± 1,17	19,93 ± 3,22
	Canavieiras	--------	3,57 ± 0,51
	Cumuruxatiba	--------	3,20 ± 0,38
	Itacare	8,29 ± 6,51	4,36 ± 0,80
	Siribinha	4,16 ± 2,22	6,50 ± 2,89
Py (ng g^{-1})	Belmonte	--------	0,00 ± 0,00
	Boipeba	4,55 ± 7,78	36,64 ± 63,47
	BTS	2,81 ± 1,93	9,64 ± 3,75
	Canavieiras	--------	0,00 ± 0,00
	Cumuruxatiba	--------	0,00 ± 0,00
	Itacare	0,17 ± 0,29	0,00 ± 0,00
	Siribinha	0,00 ±	0,00 ± 0,00
BaA (ng g^{-1})	Belmonte	--------	10,44 ± 0,07
	Boipeba	17,56 ± 6,63	43,26 ± 47,3
	BTS	23,98 ± 0,95	29,04 ± 2,97
	Canavieiras	--------	10,59 ± 0,14

	Cumuruxatiba	--------	10,63 ± 0,10
	Itacare	13,88 ± 4,97	11,43 ± 0,59
	Siribinha	7,97 ± 7,05	13,34 ± 1,98
Chr (ng g^{-1})	Belmonte	--------	0,00 ± 0,00
	Boipeba	0,87 ± 0,66	4,04 ± 3,67
	BTS	2,30 ± 3,99	6,55 ± 1,53
	Canavieiras	--------	0,00 ± 0,00
	Cumuruxatiba	--------	0,00 ± 0,00
	Itacare	0,39 ± 0,40	0,48 ± 0,13
	Siribinha	11,85 ± 10,28	18,42 ± 1,63
BbF (ng g^{-1})	Belmonte	--------	16,55 ± 0,22
	Boipeba	26,93 ± 10,43	56,45 ± 52,69
	BTS	55,37 ± 7,17	66,87 ± 5,65
	Canavieiras	--------	16,82 ± 0,44
	Cumuruxatiba	--------	17,17 ± 0,27
	Itacare	20,80 ± 5,80	17,74 ± 0,36
	Siribinha	17,25 ± 1,27	20,27 ± 2,31
BkF (ng g^{-1})	Belmonte	--------	21,15 ± 0,04
	Boipeba	27,48 ± 5,71	42,99 ± 27,71
	BTS	35,64 ± 0,89	41,82 ± 3,21
	Canavieiras	--------	21,24 ± 0,12
	Cumuruxatiba	--------	21,48 ± 0,10
	Itacare	23,81 ± 3,28	22,14 ± 0,37
	Siribinha	21,48 ± 0,57	23,04 ± 1,32
BeP (ng g^{-1})	Belmonte	--------	12,86 ± 0,08
	Boipeba	19,35 ± 6,13	33,97 ± 26,40
	BTS	32,87 ± 1,17	41,08 ± 2,70
	Canavieiras	--------	13,00 ± 0,16
	Cumuruxatiba	--------	13,04 ± 0,07
	Itacare	15,49 ± 3,04	13,85 ± 0,23
	Siribinha	13,66 ± 0,88	15,31 ± 1,48
BaP (ng g^{-1})	Belmonte	--------	10,04 ± 0,02
	Boipeba	16,22 ± 6,80	34,71 ± 33,28
	BTS	20,19 ± 0,59	28,31 ± 3,72
	Canavieiras	--------	10,03 ± 0,05
	Cumuruxatiba	--------	10,10 ± 0,03
	Itacare	5,64 ± 9,50	11,27 ± 0,40
	Siribinha	10,38 ± 0,56	12,08 ± 1,21
Per (ng g^{-1})	Belmonte	--------	5,63 ± 5,92
	Boipeba	1,09 ± 1,06	3,44 ± 4,24
	BTS	0,00 ± 0,00	0,00 ± 0,00
	Canavieiras	--------	3,48 ± 4,91
	Cumuruxatiba	--------	0,00 ± 0,00
	Itacare	1,30 ± 0,40	3,94 ± 0,79
	Siribinha	0,00 ± 0,00	0,00 ± 0,00
IcdP (ng g^{-1})	Belmonte	--------	21,28 ± 0,04
	Boipeba	27,22 ± 6,44	42,01 ± 25,39
	BTS	42,01 ± 1,58	44,98 ± 3,72
	Canavieiras	--------	21,40 ± 0,11
	Cumuruxatiba	--------	21,45 ± 0,04
	Itacare	23,80 ± 2,98	22,48 ± 0,05
	Siribinha	21,94 ± 0,92	22,96 ± 1,11

DBahA (ng g^{-1})	Belmonte	--------	28,58 ± 0,05
	Boipeba	30,96 ± 2,37	33,94 ± 6,49
	BTS	33,06 ± 0,39	34,88 ± 0,99
	Canavieiras	--------	28,70 ± 0,06
	Cumuruxatiba	--------	28,67 ± 0,11
	Itacare	29,22 ± 0,73	28,87 ± 0,06
	Siribinha	28,70 ± 0,19	29,10 ± 0,34
BghiP (ng g^{-1})	Belmonte	--------	10,17 ± 0,13
	Boipeba	16,46 ± 7,25	27,53 ± 19,33
	BTS	29,05 ± 0,97	34,08 ± 4,44
	Canavieiras	--------	10,36 ± 0,35
	Cumuruxatiba	--------	10,28 ± 0,09
	Itacare	13,09 ± 3,15	12,12 ± 0,20
	Siribinha	10,63 ± 0,93	11,93 ± 1,13

Supplementary material 3: Mean ± standard deviation of the abiotic data of Metals content (Mg/Kg) (%) according to the site and year of sampling.

Variable	Local	Year	
		2020	2021
Cu (mg/Kg)	Belmonte	--------	9,54 ± 1,91
	Boipeba	3,40 ± 1,45	1,98 ± 0,79
	BTS	46,29 ± 6,52	53,96 ± 7,21
	Canavieiras	--------	7,76 ± 0,77
	Cumuruxatiba	--------	4,40 ± 0,18
	Itacare	9,18 ± 0,55	8,99 ± 0,52
	Siribinha	6,81 ± 1,02	6,65 ± 0,90
Cr (mg/Kg)	Belmonte	--------	13,14 ± 4,78
	Boipeba	5,37 ± 2,44	2,83 ± 0,70
	BTS	18,86 ± 3,69	25,45 ± 2,39
	Canavieiras	--------	10,65 ± 2,57
	Cumuruxatiba	--------	10,98 ± 1,05
	Itacare	14,67 ± 2,85	15,04 ± 3,69
	Siribinha	8,13 ± 2,77	8,22 ±3,23
Mn (mg/ kg)	Belmonte	--------	102,72 ± 44,98
	Boipeba	105,21 ± 19,16	115,12 ±22,46
	BTS	123,41 ± 27,85	256,23 ± 69,12
	Canavieiras	--------	126,01 ± 41,27
	Cumuruxatiba	--------	57,59 ± 5,67
	Itacare	123,93 ± 65,45	142,08 ± 107,40
	Siribinha	46,38 ± 13,42	65,08 ± 25,27
Ni (mg/ kg)	Belmonte	--------	3,94 ± 1,96
	Boipeba	0,28 ± 0,48	0,00 ± 0,00
	BTS	8,76 ± 1,53	11,69 ± 1,67
	Canavieiras	--------	2,59 ± 0,97
	Cumuruxatiba	--------	0,00 ± 0,00
	Itacare	4,04 ± 0,84	4,34 ± 1,20
	Siribinha	0,88 ± 0,97	1,14 ± 0,99
Pb (mg/ kg)	Belmonte	--------	3,89 ± 1,37
	Boipeba	1,16± 0,62	0,33 ± 0,31
	BTS	7,96 ± 1,23	9,92 ± 1,86
	Canavieiras	--------	2,92 ± 0,94
	Cumuruxatiba	--------	1,15 ± 0,47
	Itacare	2,78 ± 0,91	2,77 ±0,31

	Siribinha	2,58 ± 1,57	1,87 ±0,80
Zn (mg/ kg)	Belmonte	--------	13,49 ± 7,18
	Boipeba	0,46 ± 0,80	0,00 ± 0,00
	BTS	44,80 ± 7,41	57,69 ± 9,11
	Canavieiras	--------	9,65 ± 6,79
	Cumuruxatiba	--------	0,31 ± 0,54
	Itacare	9,74 ± 6,18	11,16 ± 8,64
	Siribinha	0,98 ± 1,70	0,77 ±0,77
As (mg/ Kg)	Belmonte	--------	3,74 ± 1,93
	Boipeba	5,25 ± 1,29	3,43 ± 0,28
	BTS	5,17 ± 1,35	6,99 ± 1,31
	Canavieiras	--------	5,46 ±1,75
	Cumuruxatiba	--------	6,92 ± 0,11
	Itacare	5,22 ± 2,66	5,00 ± 3,67
	Siribinha	4,83 ± 2,08	5,90 ± 2,65

3 CONCLUSÃO GERAL

Em conclusão, esta dissertação teve o objetivo geral de avaliar se as comunidades de fauna bentônica e da microbiota presentes nos sedimentos de manguezais respondem ao misterioso derramamento de petróleo de 2019. Pela primeira vez, foi feito um levantamento genético de toda a comunidade de fauna bentônica e da microbiota ao longo de manguezais da Bahia, impactados em diferentes níveis por petróleo e metais. Nossos dados sugerem que a presença de metais e HPAs em sedimentos impactados pelo derrame de petróleo representa baixo risco de toxicidade para os organismos, exceto algumas espécies de poluentes, como o metal Cu, que excede os níveis permitidos pela legislação CONAMA e os HPAs acenaftileno e dibenz[a,h]antraceno em níveis que excedem o TEL.

Essa pesquisa demonstrou os organismos mais abundantes em locais altamente contaminados pertencem a família Capitellidae da fauna bentônica, e aos gêneros *Thiogranum*, *Ilumatobacter*, *Hoppeia*, *SEEP SRB1* e *Sedimenticola*, estes são também os possíveis organismos tolerantes ao desastre de 2019. A diversidade alpha apesar de não mostrar nenhuma diferença significativa entre os níveis de contaminação, demonstrou que fauna avaliada em 2021 apresentou as maiores variabilidades em relação a contaminação de HPAs e Metais nos manguezais classificados como "Médio" contaminados. Já para a diversidade beta foram observadas diferenças significativas na composição, também ficou evidente que há influência espacial dos locais na composição.

O manguezal da Bahia de Todos os Santos, localizado adjacente ao Rio São Paulo, que historicamente sofre contaminação crônica, demonstrou, segundo nossos resultados, entre todos os manguezais ser o local mais contaminado. A contaminação medida em BTS, demonstra que ambientes que sofrem contaminações crônicas acumulam mais compostos no sedimento, do que locais que sofrem contaminações agudas.

Existem algumas limitações nesse estudo. Os efeitos dos derramamentos de óleo na fauna bentônica e nos grupos de microbiota requerem investigação, uma vez que não foi possível saber quais e qual a porcentagem de PAHs e metais analisados estão envolvidos com o derramamento de óleo de 2019. A falta de monitoramento antes do desastre somado à entrada de PAHs e metais nos manguezais coletados por diferentes fontes antropogênicas mascarou o efeito da contaminação do derramamento de 2019. Acreditamos que as fontes pirogênicas (por exemplo, biomassa, carvão, combustão de petróleo e resíduos industriais) são as principais contribuintes para as concentrações de PAH e metais detectadas nos sedimentos analisados, especialmente relacionadas à combustão de biomassa. Sugerimos a implementação de dados adicionais em investigações futuras, para melhor compreensão dos impactos relacionados ao derramamento de óleo. Detalhes destes resultados podem ser encontrados no capítulo 1, onde realizamos uma avaliação cuidadosa dos impactos sobre a microfauna bentônica do sedimento e uma discussão que correlaciona a ecologia das comunidades bentônicas e da microbiota frente a derramamento de óleo em diferentes manguezais da Bahia.

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